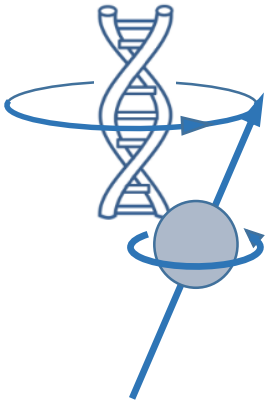
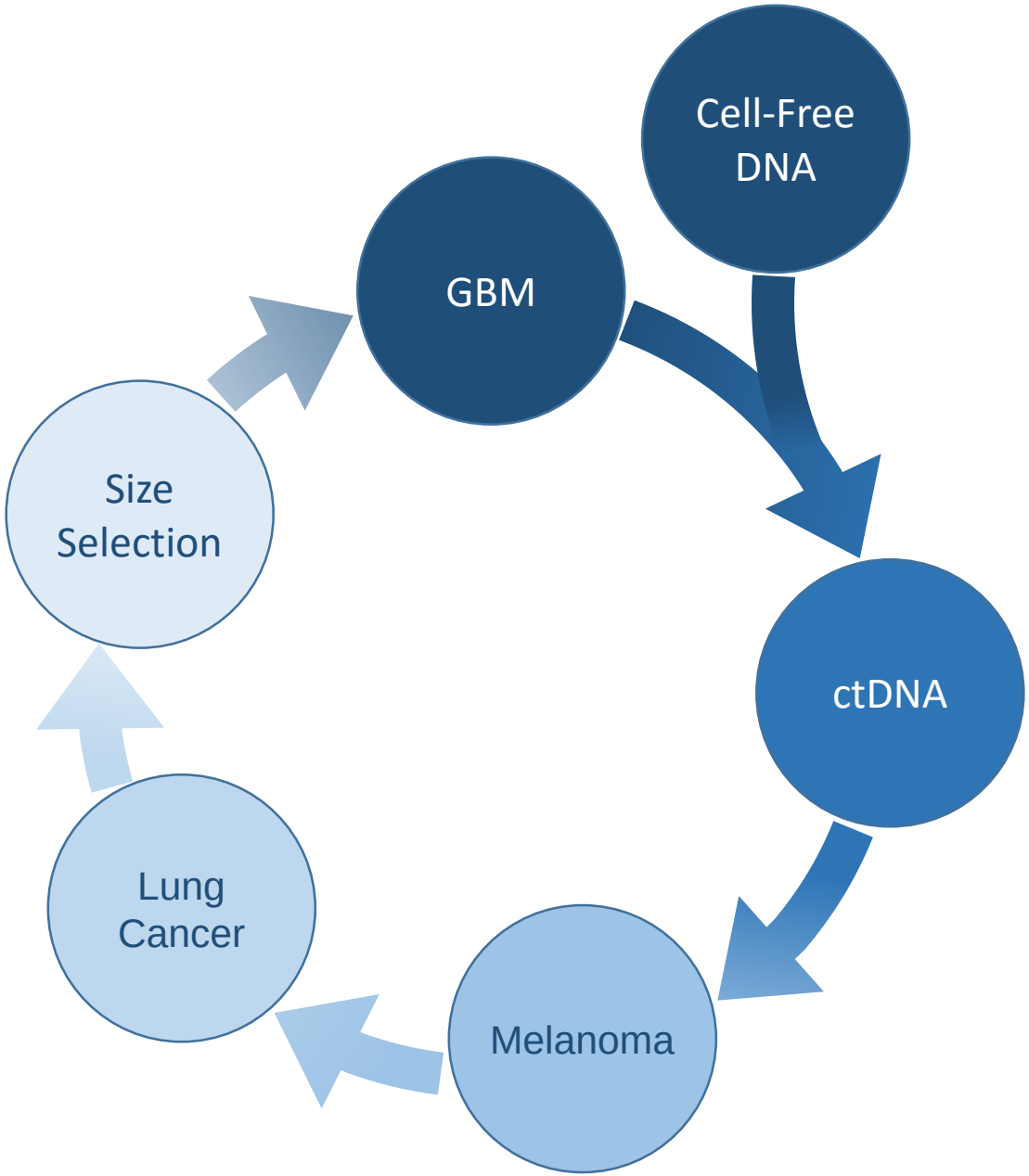
Sizing Up Cancer in Cell-Free DNA

(a series of happy accidents)

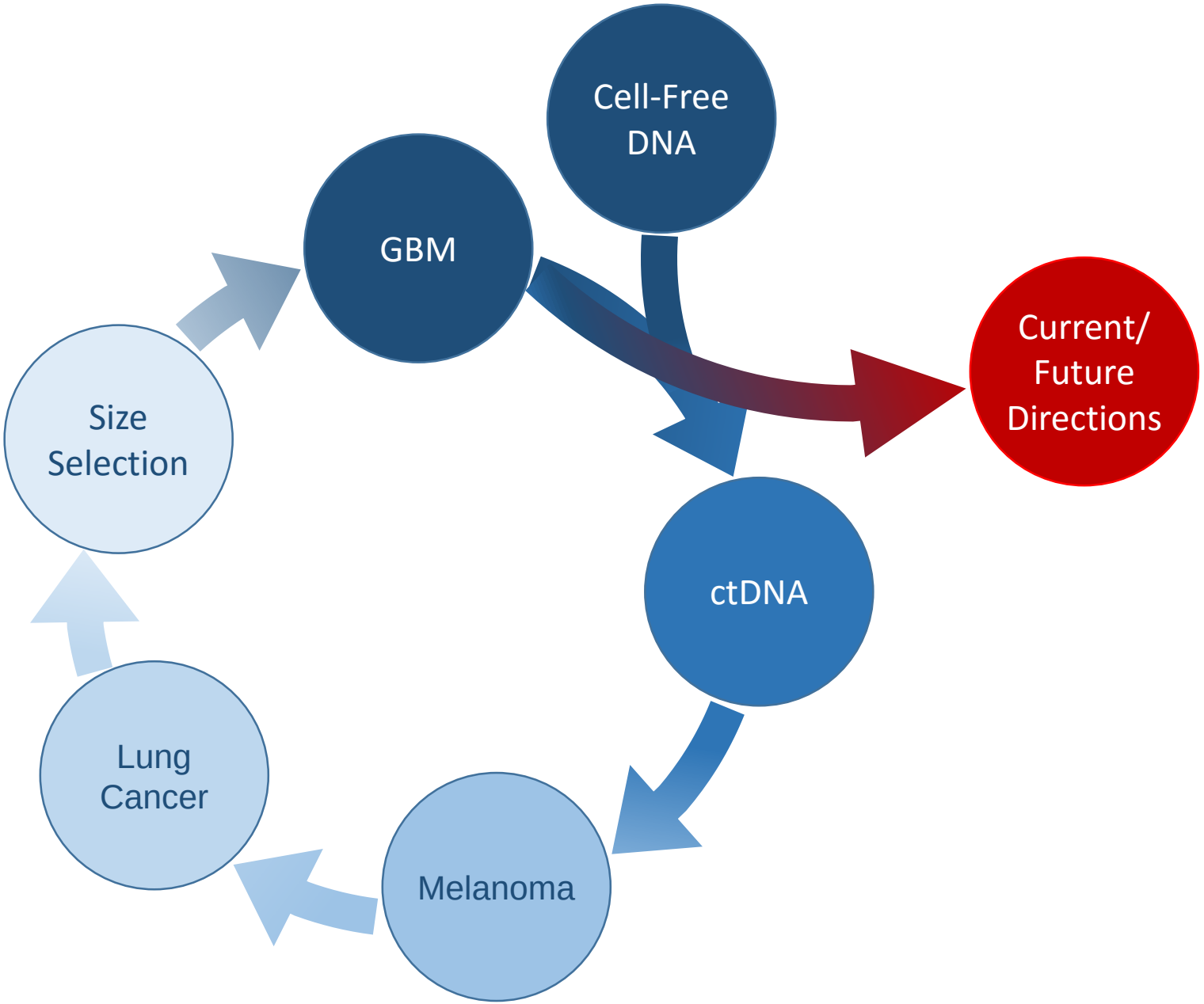
Hunter Underhill
Division of Medical Genetics
Department of Pediatrics
University of Utah
December 15, 2016



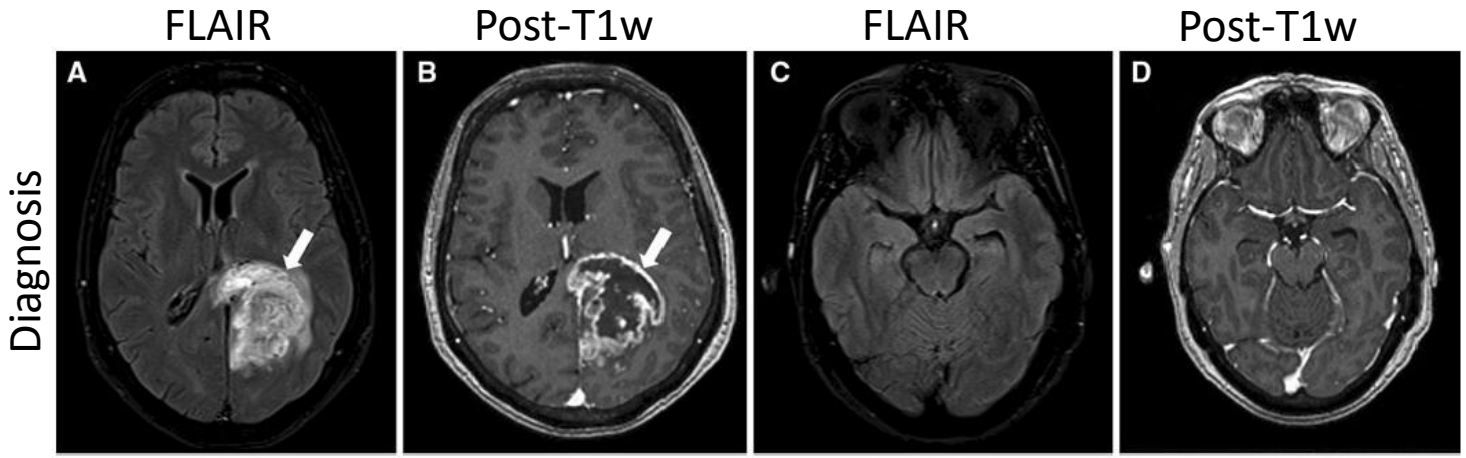
Roadmap


Cell-Free DNA
GBM
Size Selection
ctDNA
Lung Cancer
Melanoma

Roadmap

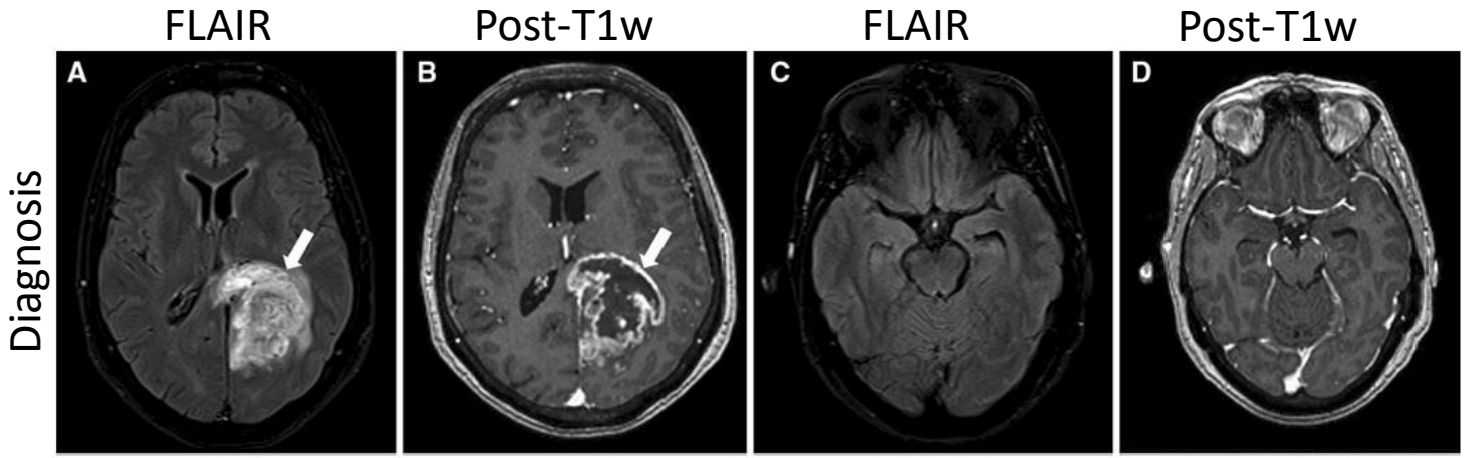

Cell-Free DNA
GBM
Current/ Future Directions
Size Selection
ctDNA
Lung Cancer
Melanoma

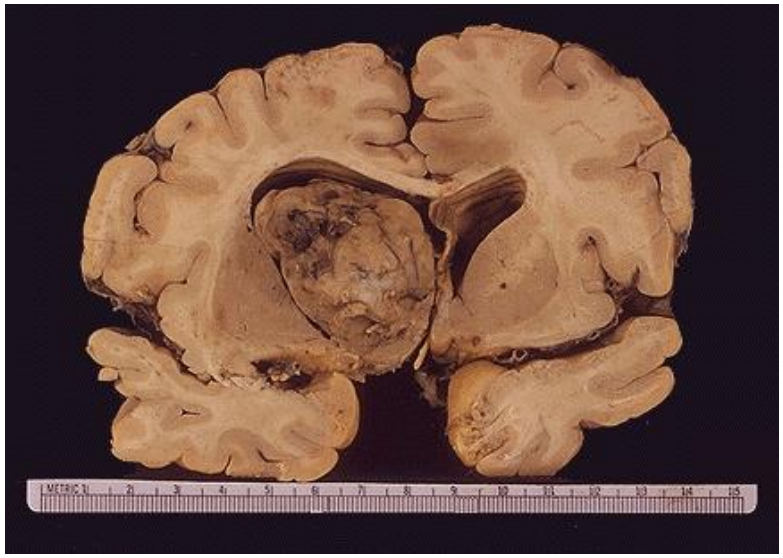
Glioblastoma Muliforme - Background



http://library.med.utah.edu/WebPath/CNSHTML/CNSIDX.html#13

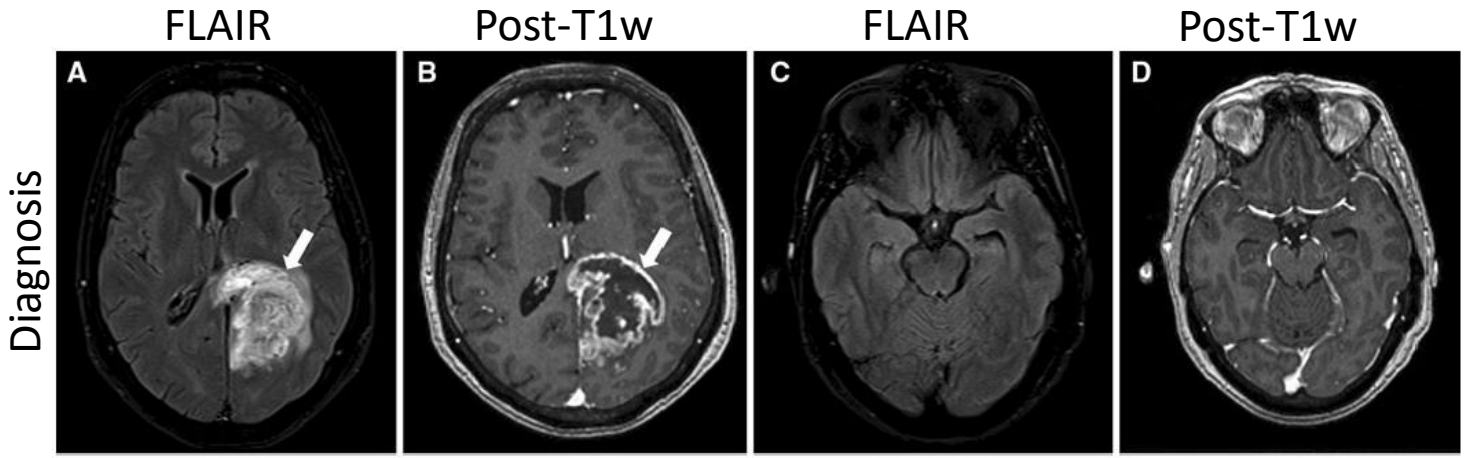
Glioblastoma Muliforme - Background

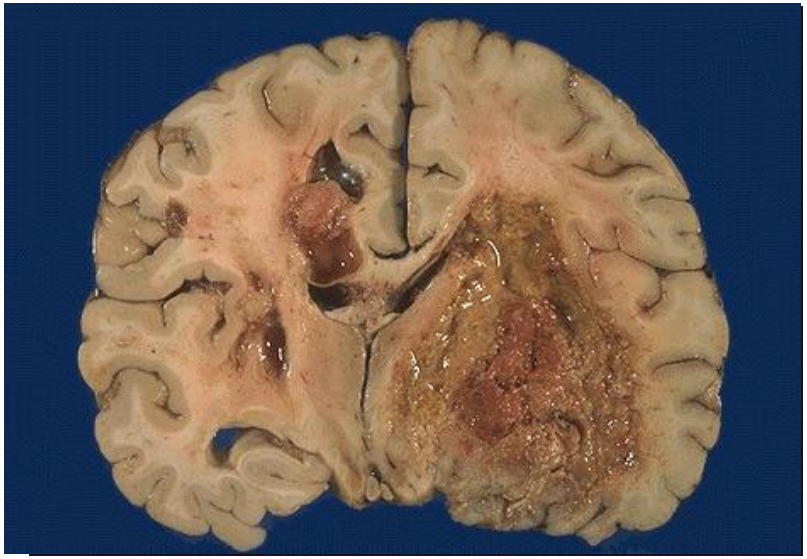




http://library.med.utah.edu/WebPath/CNSHTML/CNSIDX.html#13

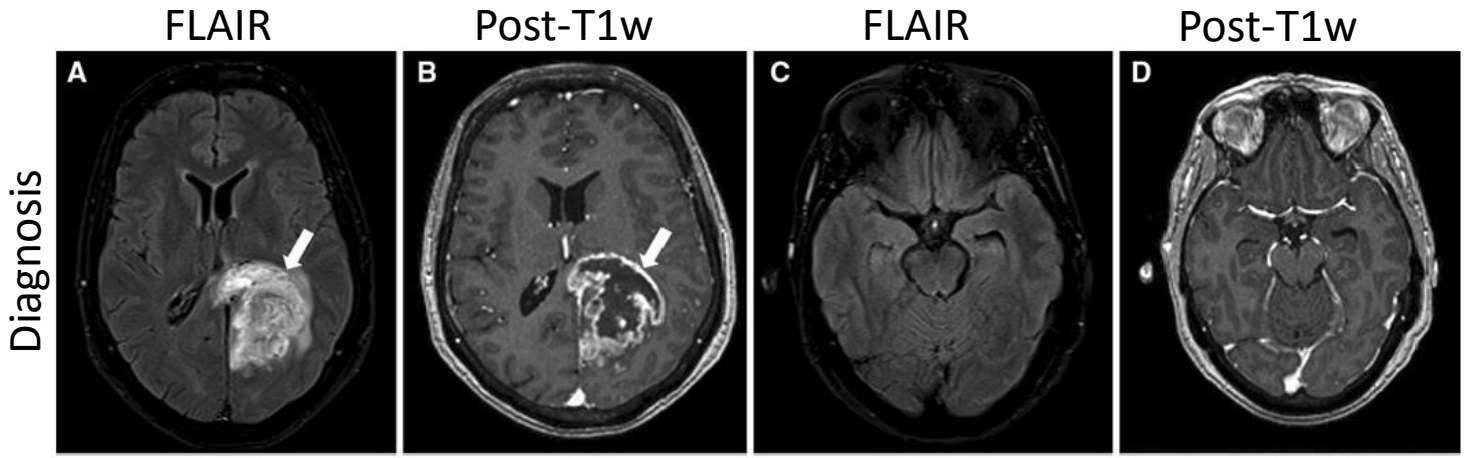
Glioblastoma Muliforme - Background

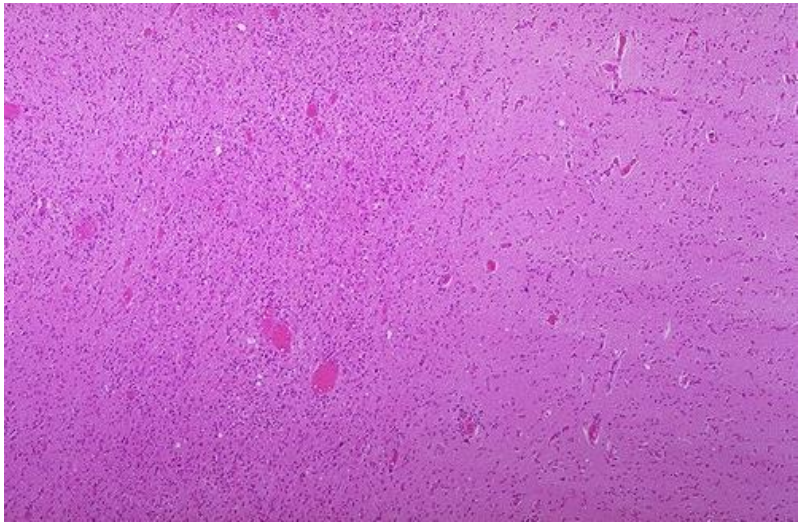




http://library.med.utah.edu/WebPath/CNSHTML/CNSIDX.html#13

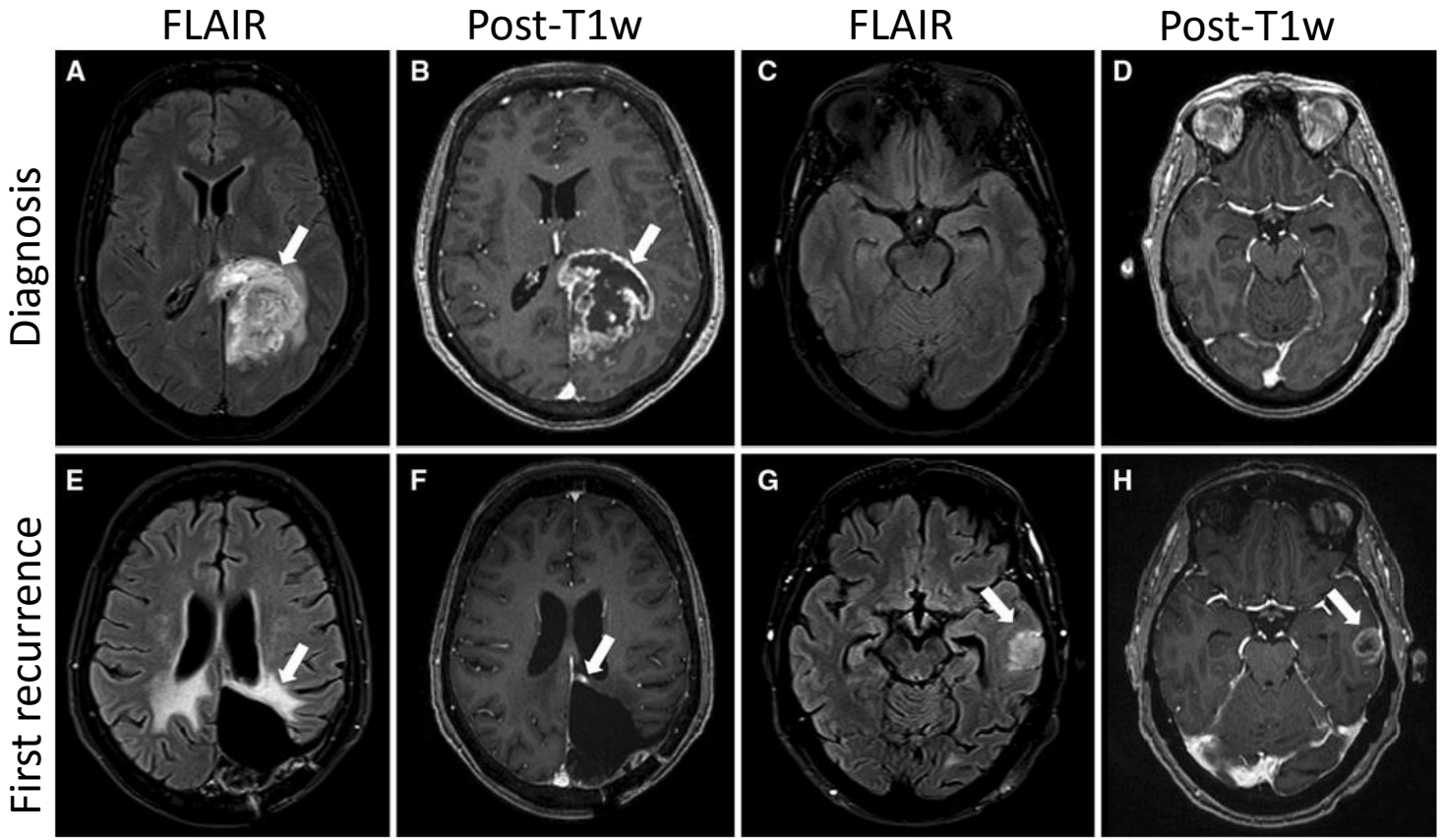
Glioblastoma Muliforme - Background





http://library.med.utah.edu/WebPath/CNSHTML/CNSIDX.html#13

Glioblastoma Muliforme - Background



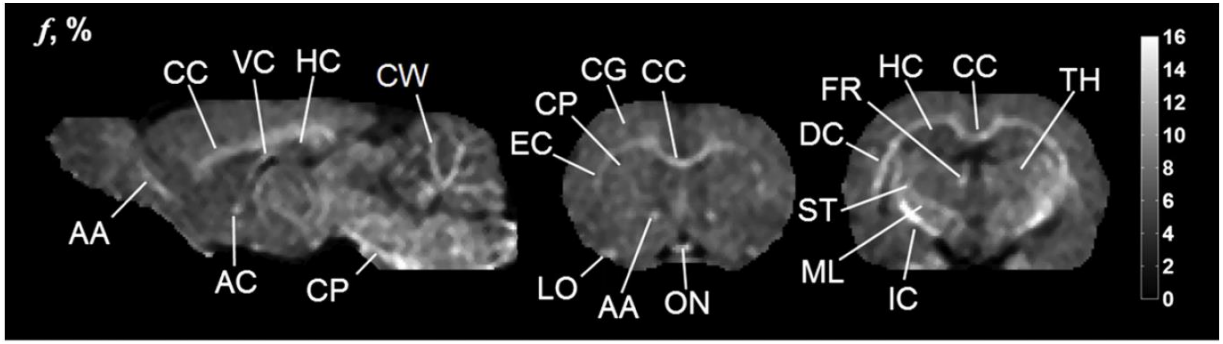
Tejada et al., *J Neurooncol*, 2013;116:169-175

Regardless of therapy, median survival remains <15 months after the initial diagnosis (Stupp et al., *Lancet Oncol*, 2009;10:459-66)

http://library.med.utah.edu/WebPath/CNSHTML/CNSIDX.html#13

GBM – Imaging Invasion

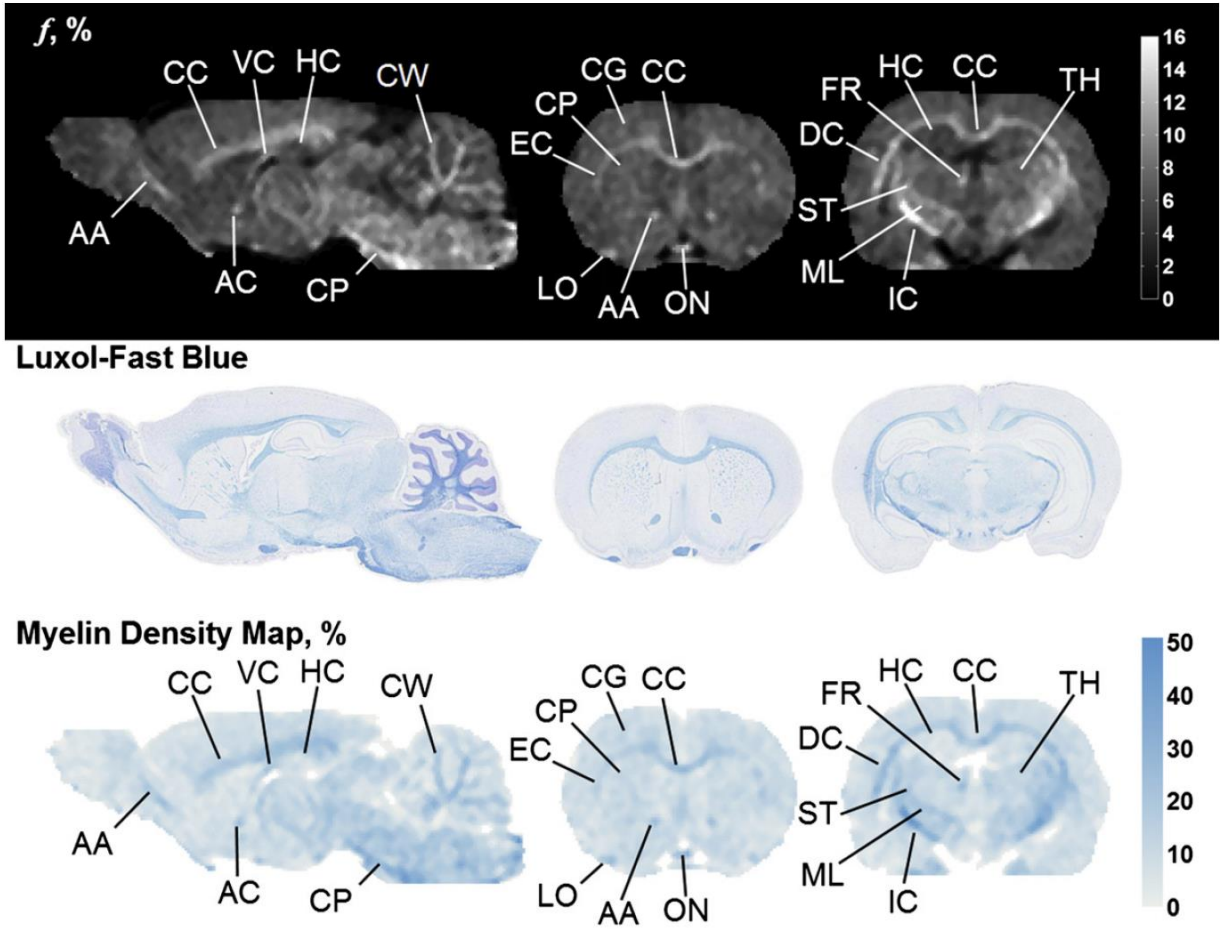
Fast Bound-Pool Fraction Imaging (FBFI) vs. Histology



Underhill et al., *NeuroImage,* 2011;54:2052-65

GBM – Imaging Invasion

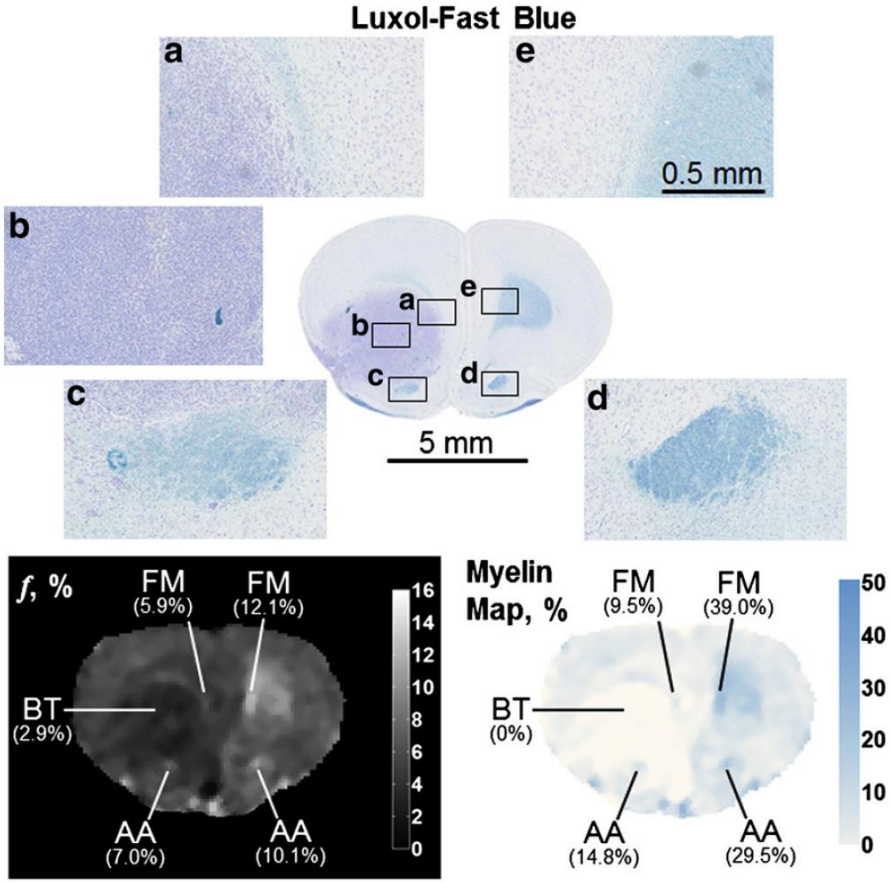
Fast Bound-Pool Fraction Imaging (FBFI) vs. Histology



Underhill et al., *NeuroImage,* 2011;54:2052-65

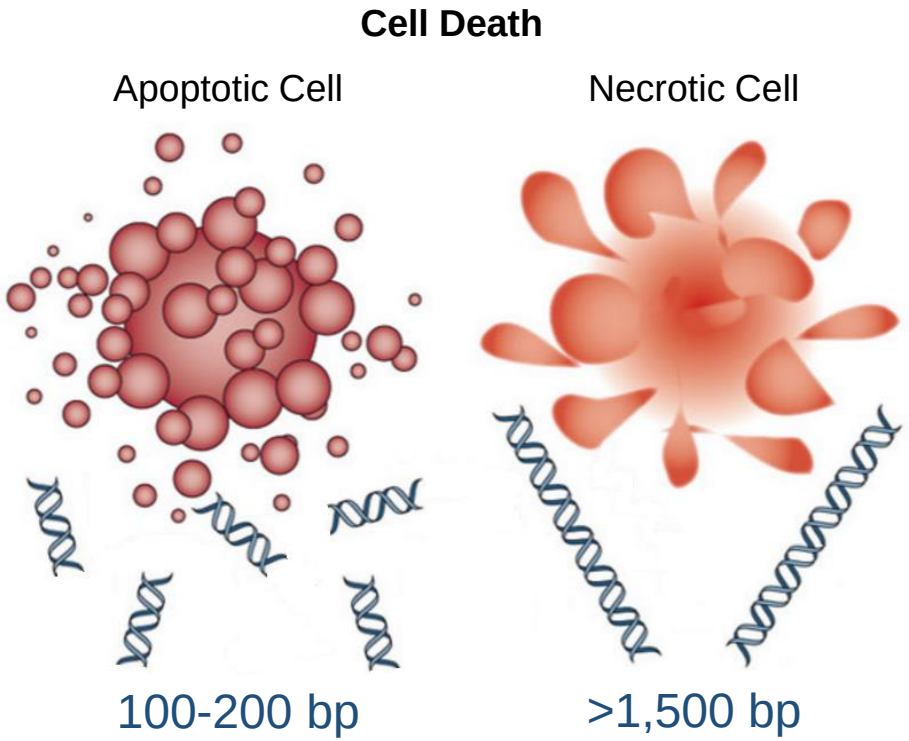
GBM – Imaging Invasion

Fast Bound-Pool Fraction Imaging (FBFI) vs. Histology



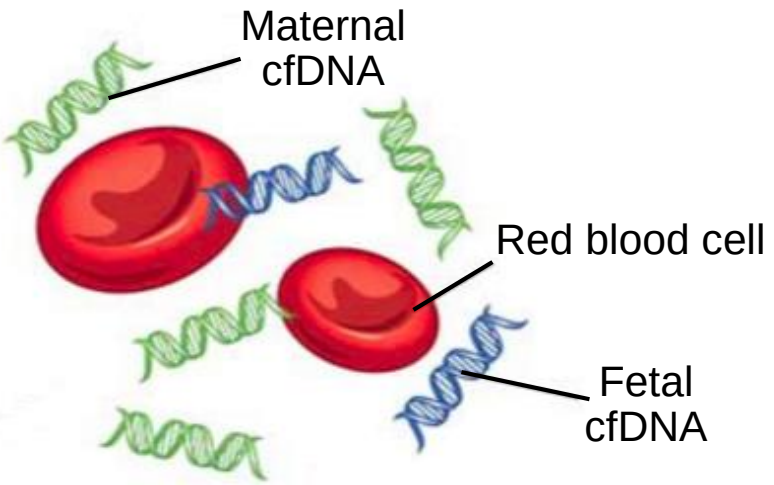
Underhill et al., *NeuroImage,* 2011;54:2052-65

Cell-Free DNA



Cell-free DNA trivia:

1. Half-life is ~10-15 minutes
2. Primary source (~80%) is circulating cells
3. 2-20 ng/mL plasma in healthy adults



1. Schwarzenbach et al., *Nature Rev Clinical Oncol,* 2014;11:145-56
2. http://www.ultrasoundcare.com.au/services/nipt.html

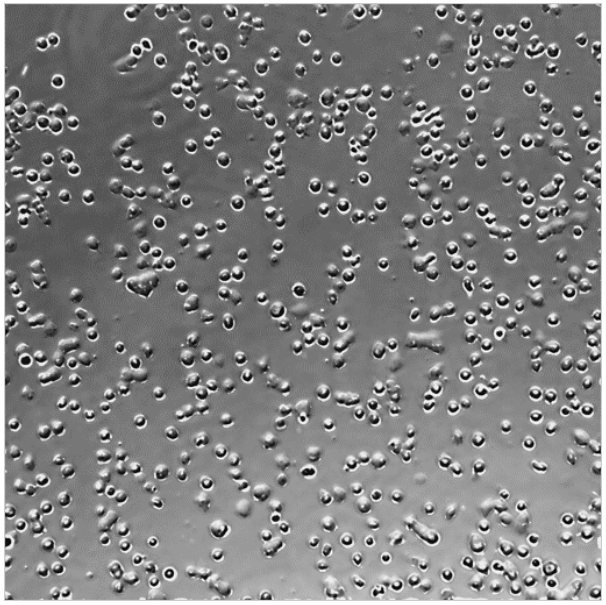
Circulating Tumor DNA – Accident #1

Human Stem Cell-Like Lines: GBM4 and GBM8

Wakimoto et al., *Cancer Research,* 2009;69:3472-81

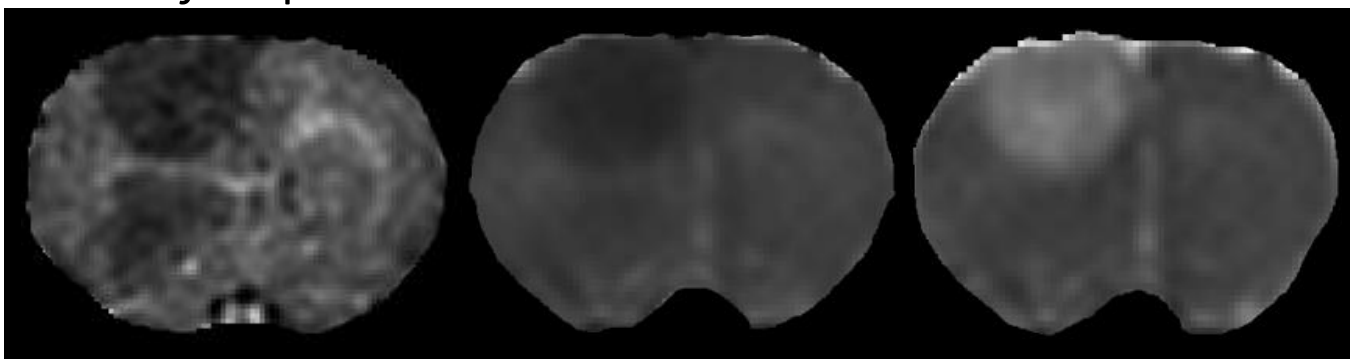
No Serum

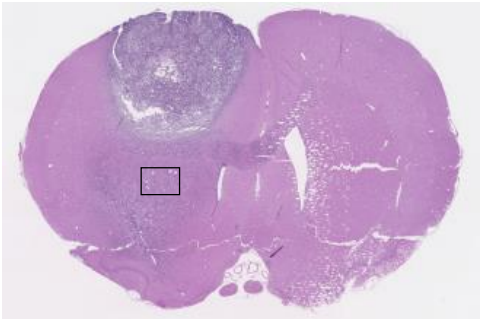
Yes Serum

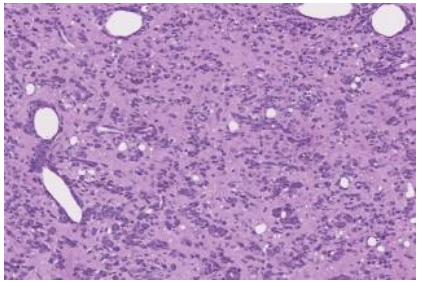


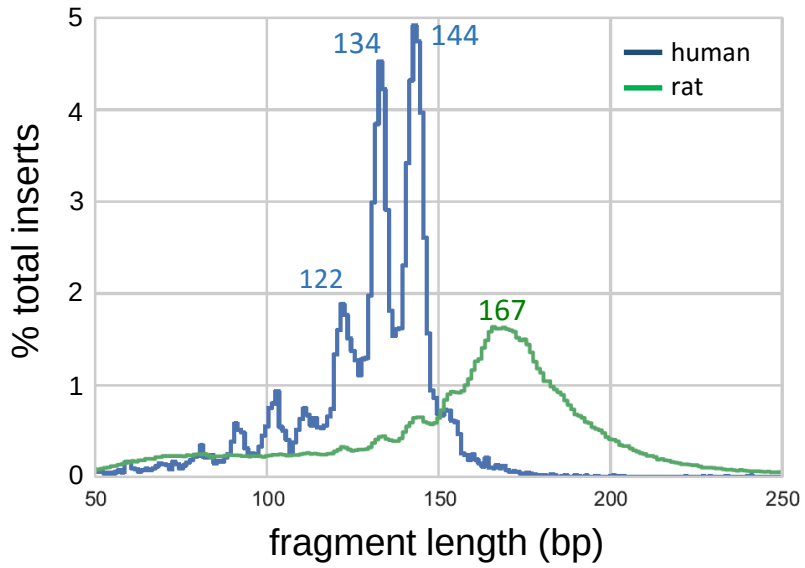
Circulating Tumor DNA

Xenograft Model: Rat Brain – Human $GBM8_1$





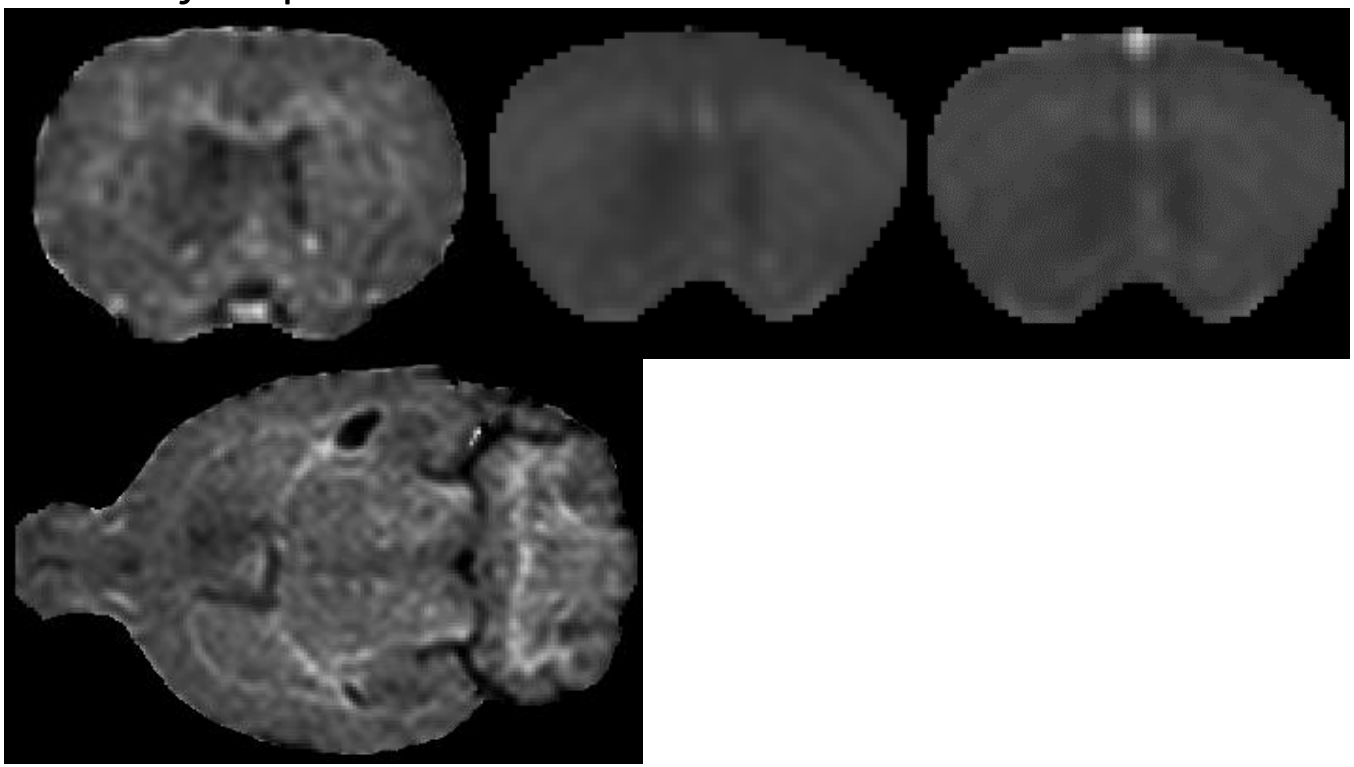


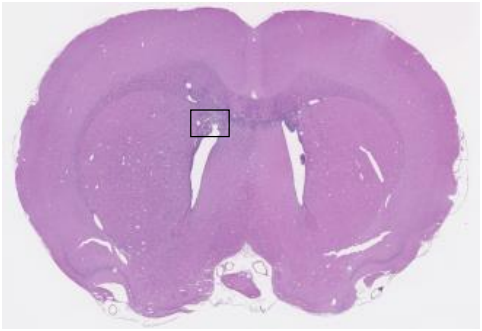


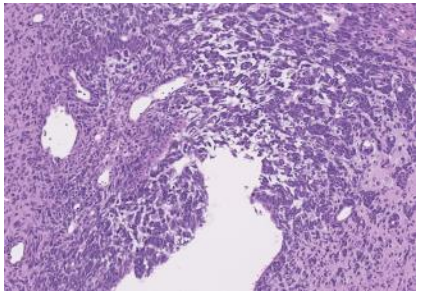
Circulating Tumor DNA

Xenograft Model: Rat Brain – Human $GBM8_3$

f map | Pre-Contrast | Post-Contrast

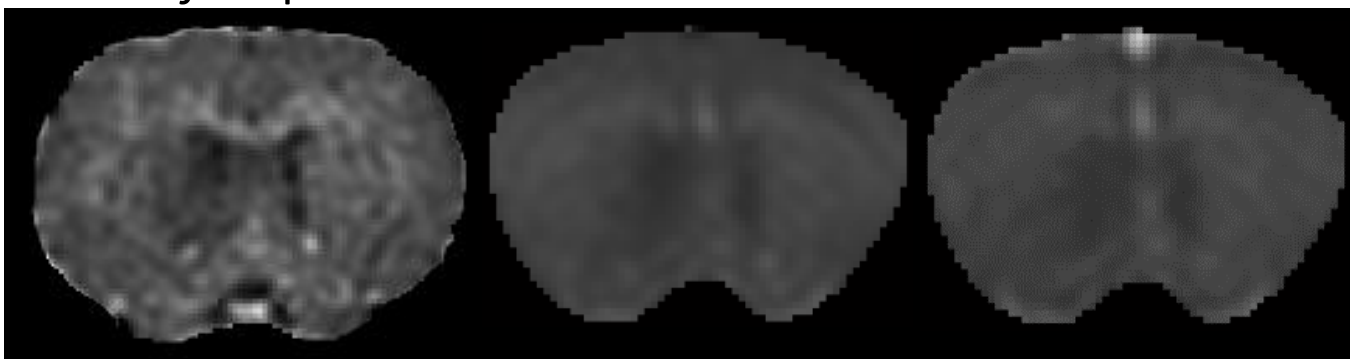


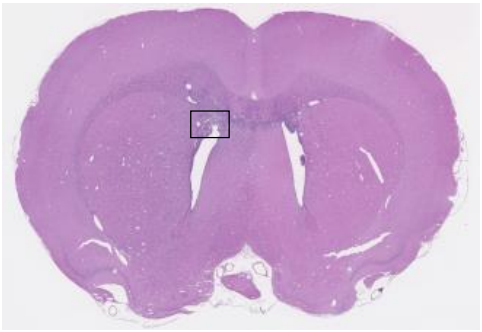


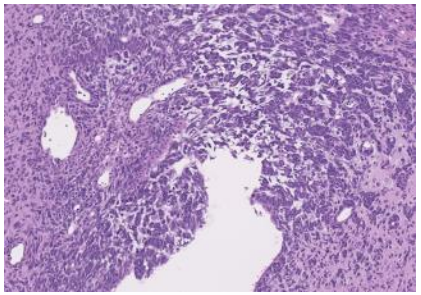


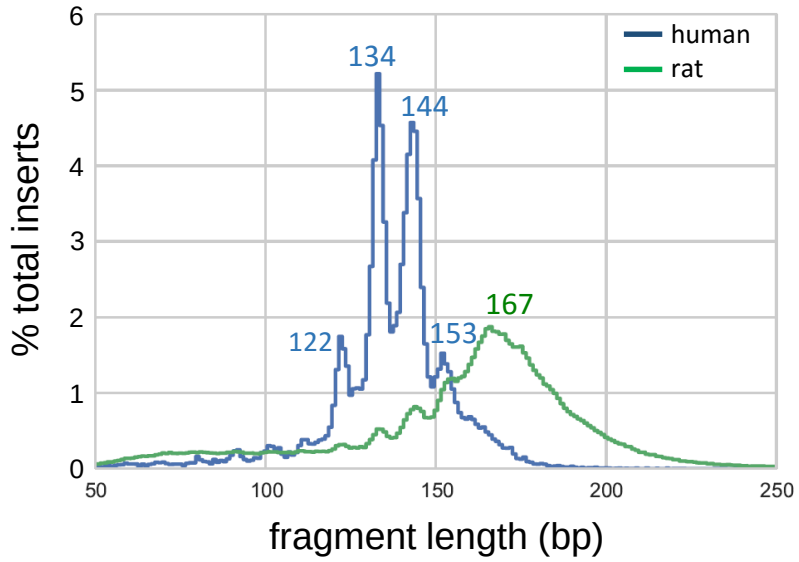
Circulating Tumor DNA

Xenograft Model: Rat Brain – Human $GBM8_3$



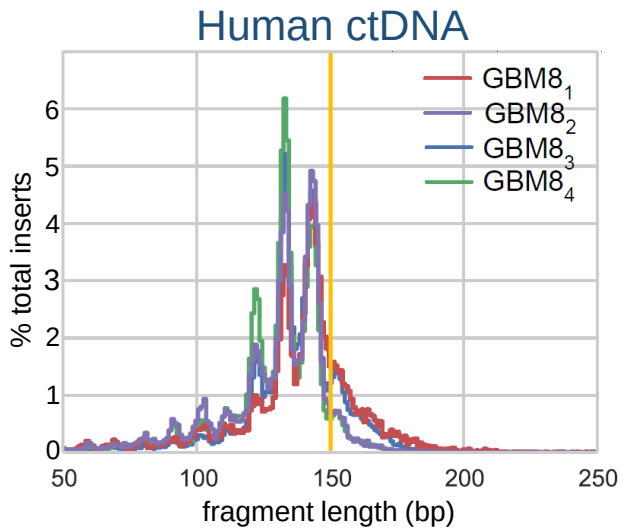


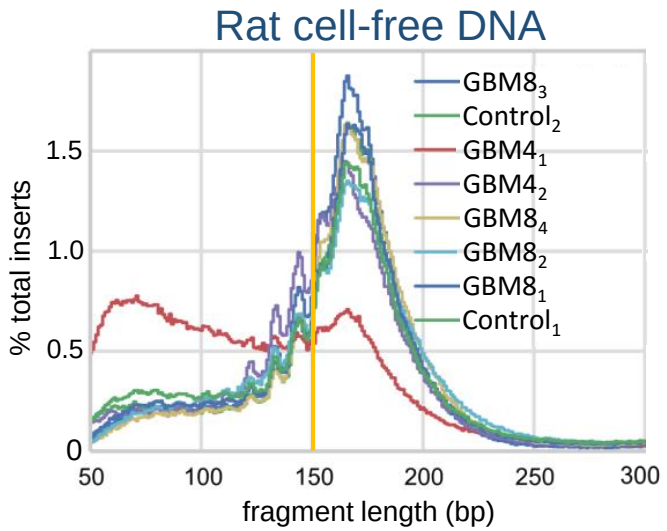


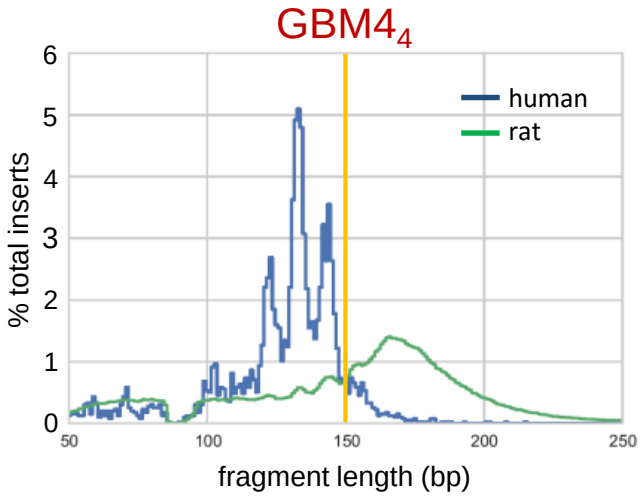


Circulating Tumor DNA

Xenograft Model: Rat Brain – Human GBM

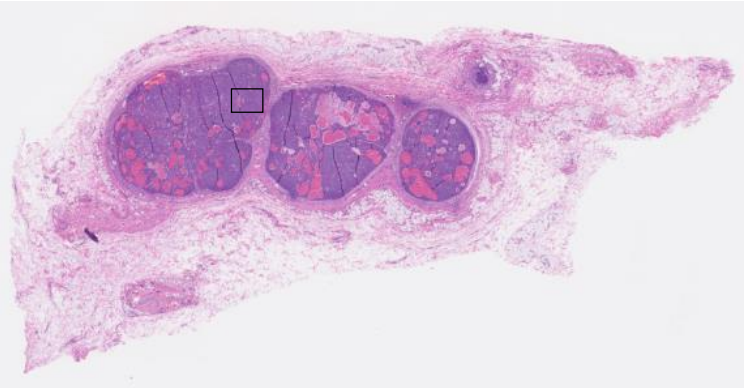


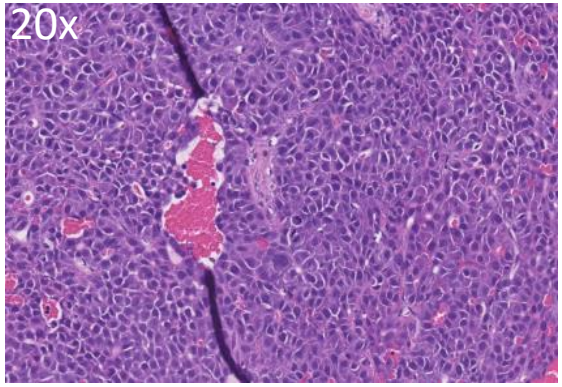


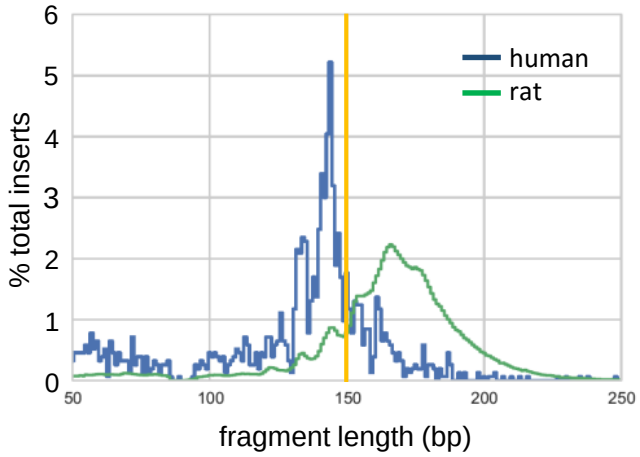


Circulating Tumor DNA

Xenograft Model: Rat Flank – Human HCC

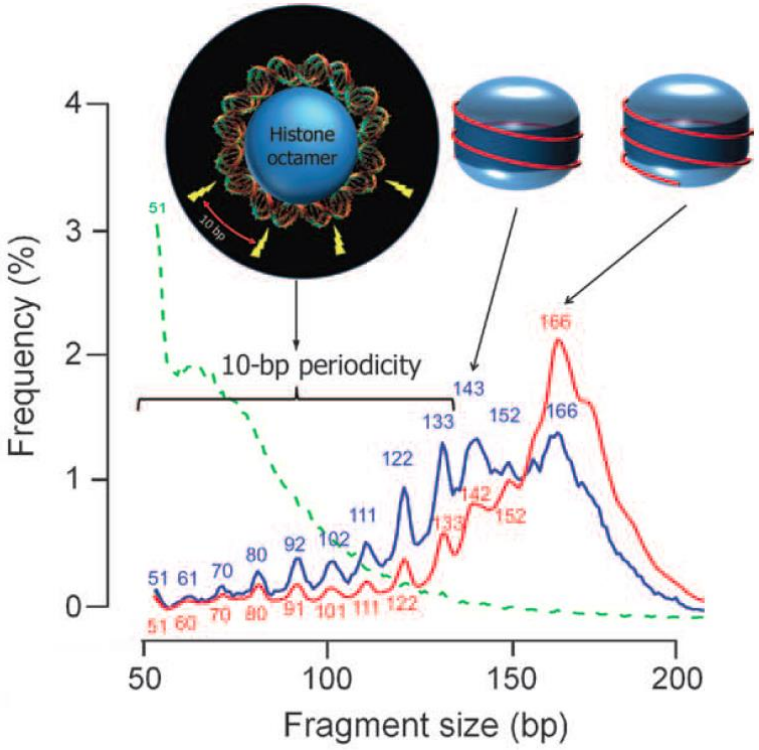




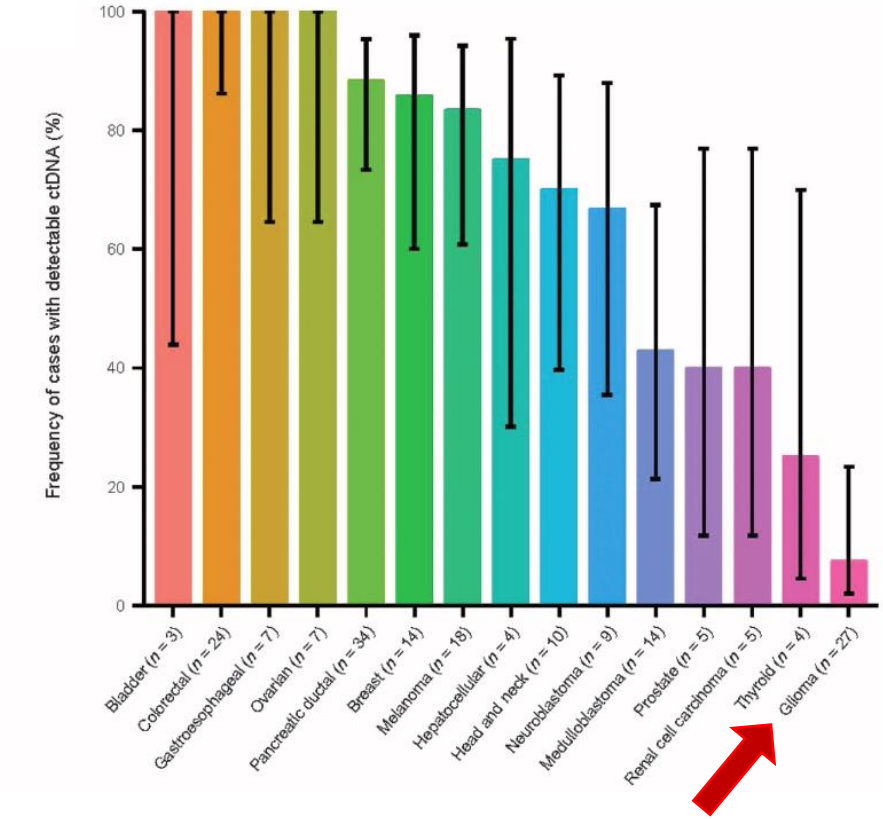


Is the shift a xenograft effect?

Circulating Tumor DNA



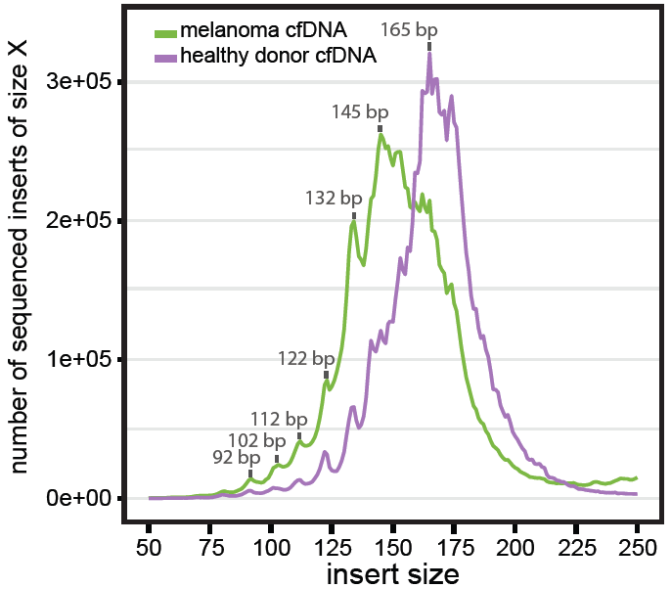
Lo et al., *Sci Transl Med* 2010;61ra91

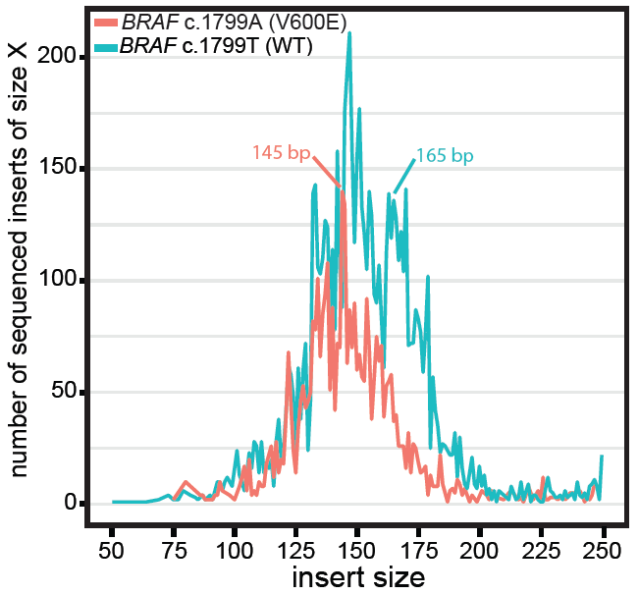


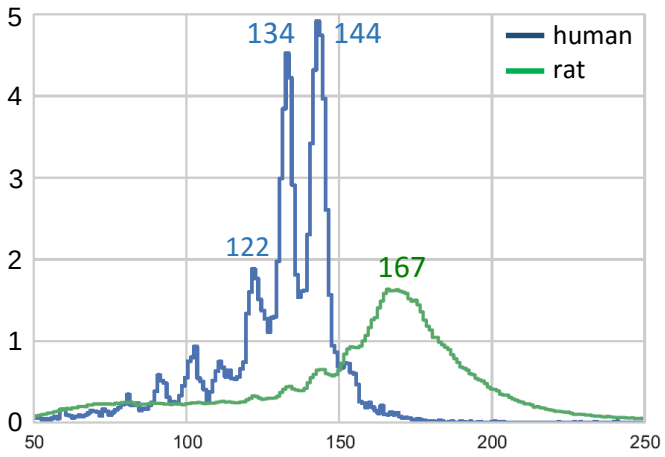
Bettegowda et al., *Sci Transl Med* 2014;6:224ra24

Circulating Tumor DNA – Accident #2

Human Melanoma

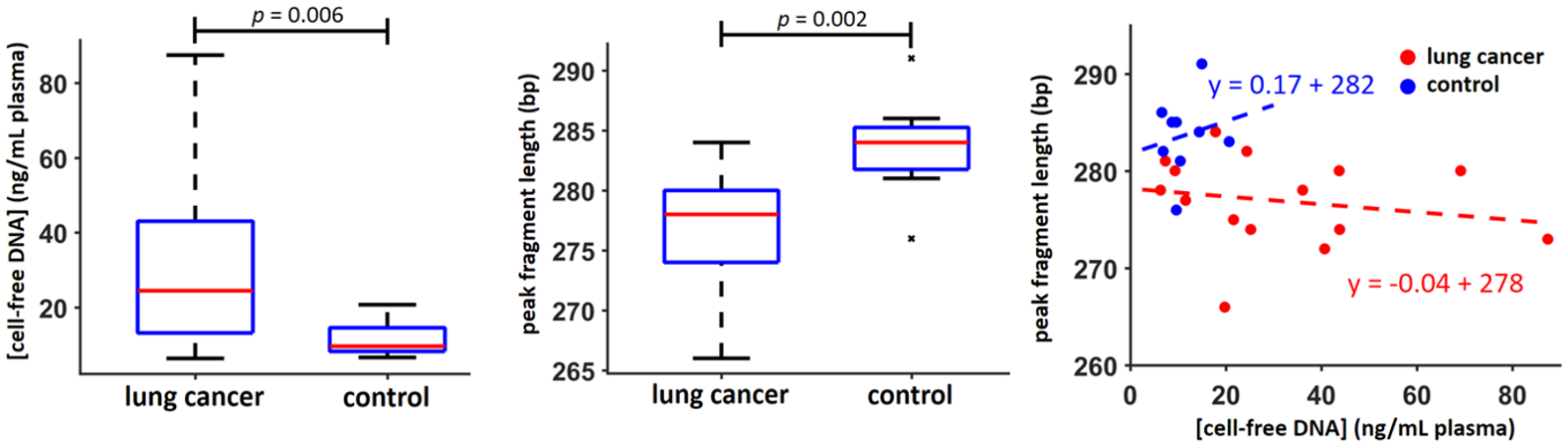






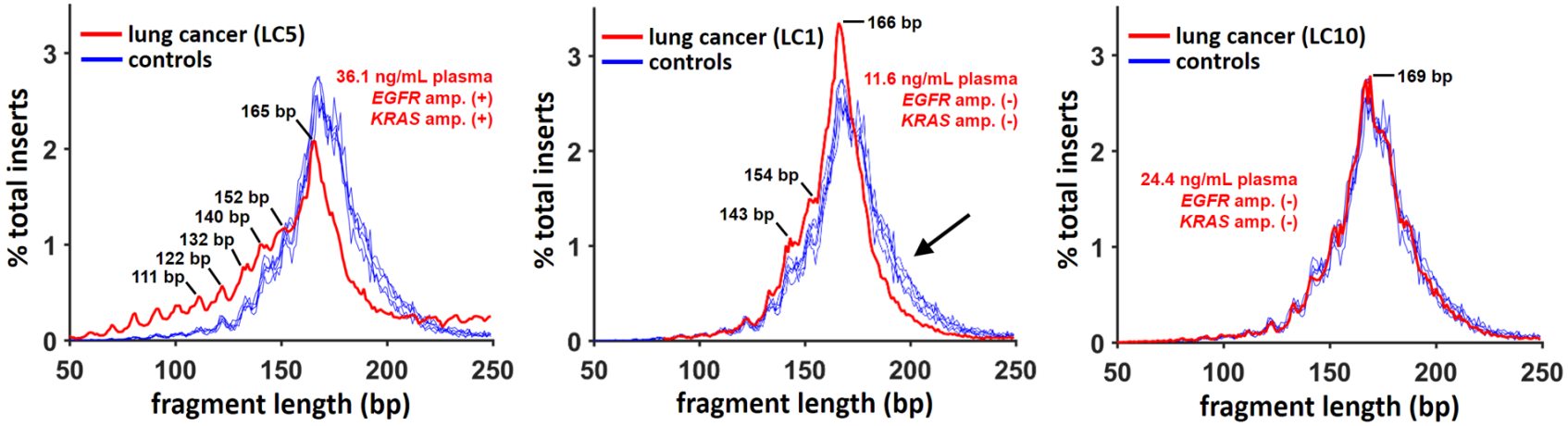
Circulating Tumor DNA

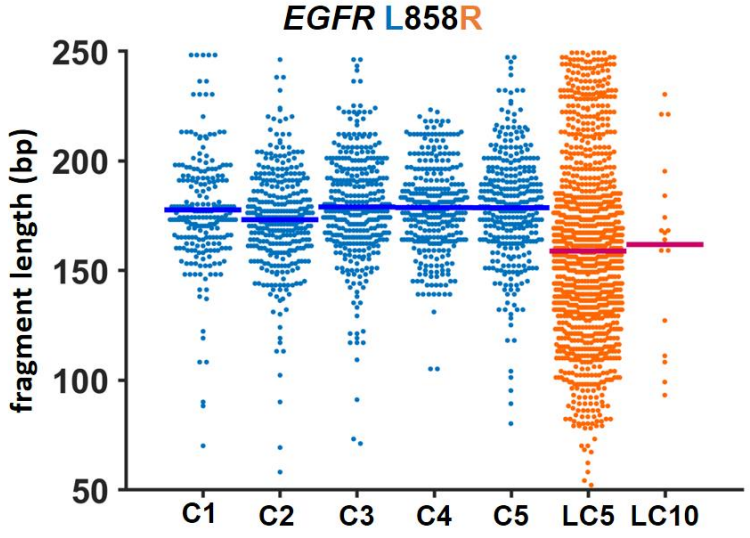
Human Lung Cancer – Cell-Free DNA

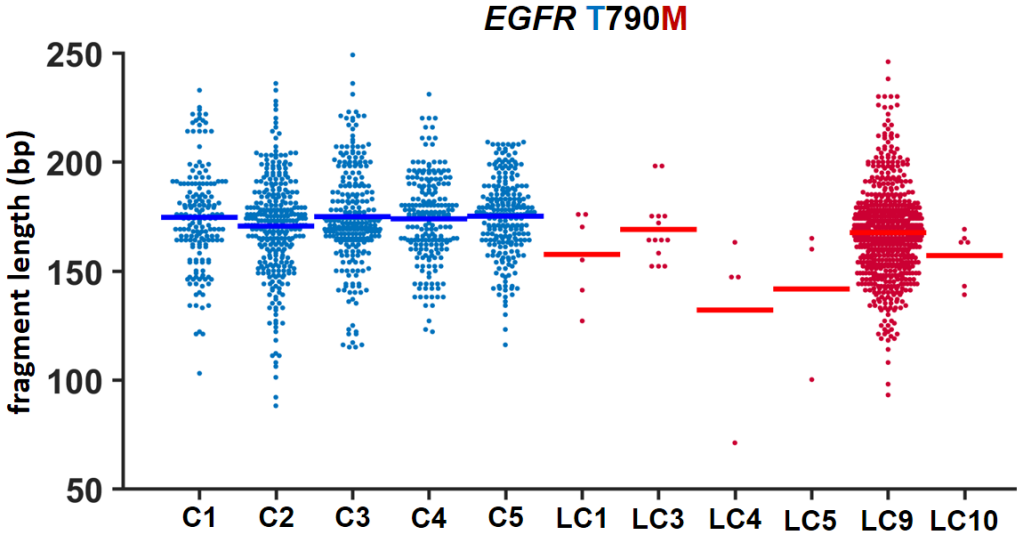


Circulating Tumor DNA

Human Lung Cancer – Sequencing Data

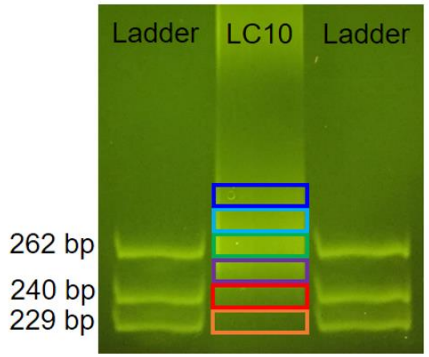


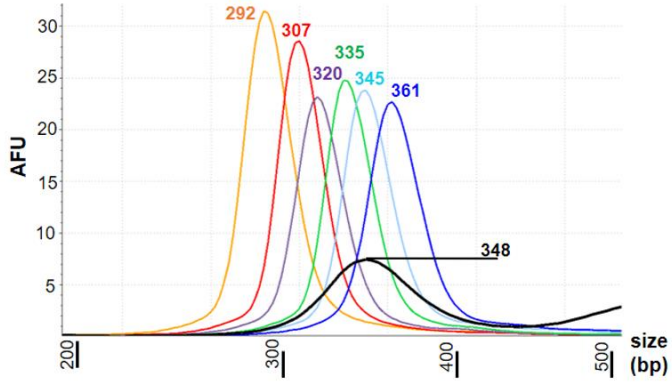


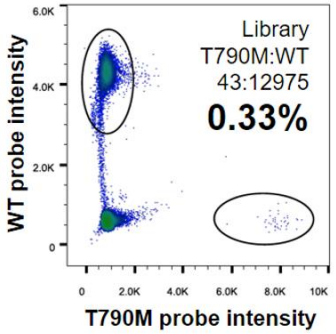


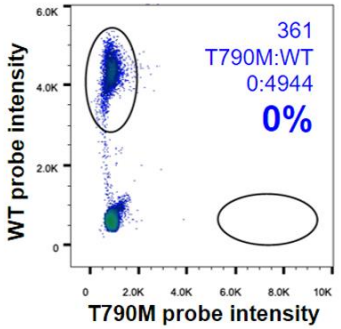
Circulating Tumor DNA

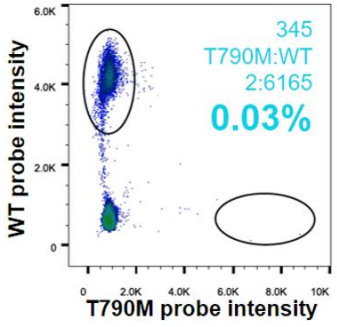
Human Lung Cancer – ddPCR

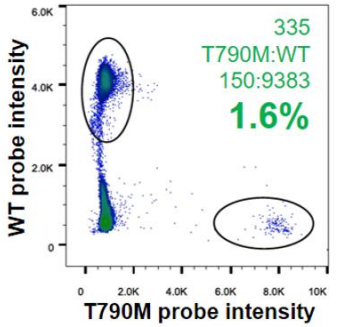


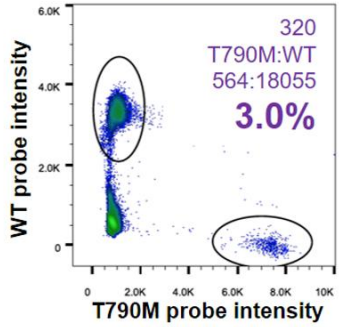


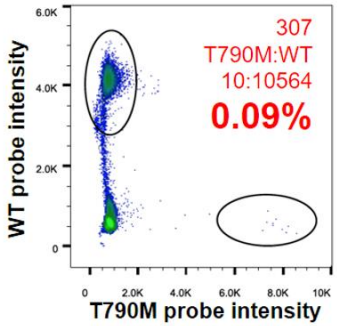


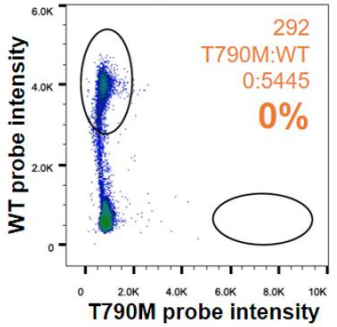






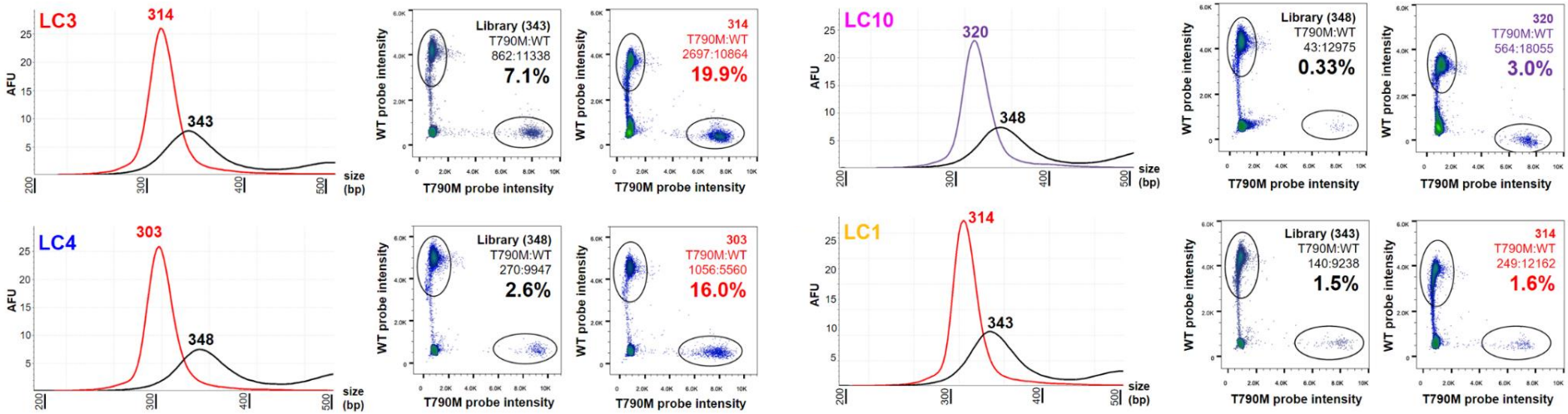


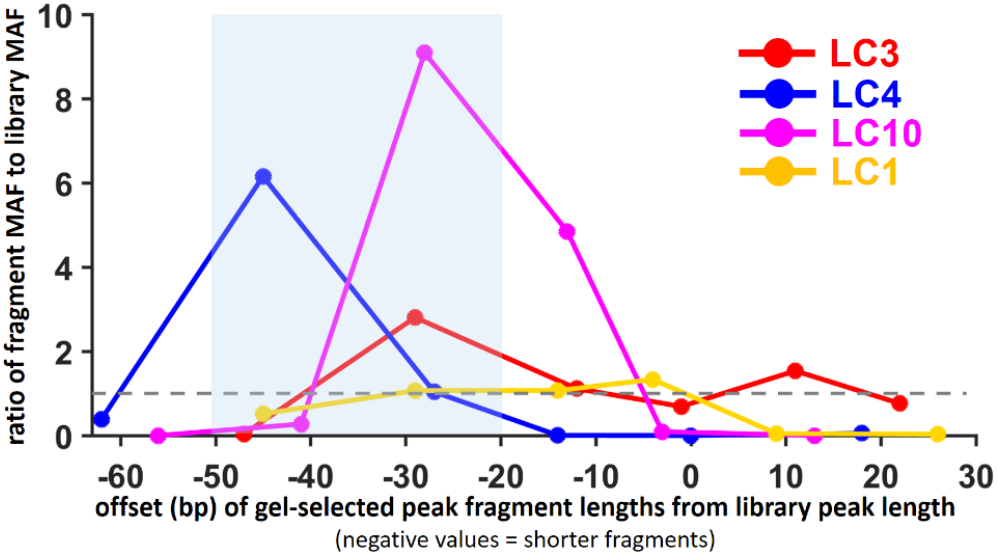




Circulating Tumor DNA

Human Lung Cancer – Fraction Selection





Circulating Tumor DNA

PLOS | GENETICS

PLOS Genetics, 2016; 18:e1006162

RESEARCH ARTICLE

Fragment Length of Circulating Tumor DNA

Hunter R. Underhill[1,2,3]*, Jacob O. Kitzman[4,5], Sabine Hellwig[6], Noah C. Welker[6], Riza Daza[4], Daniel N. Baker[6], Keith M. Gligorich[6,7], Robert C. Rostomily[3], Mary P. Bronner[6,7], Jay Shendure[4]

1 Department of Pediatrics, Division of Medical Genetics, University of Utah, Salt Lake City, Utah, United States of America, **2** Department of Radiology, University of Utah, Salt Lake City, Utah, United States of America, **3** Department of Neurological Surgery, University of Washington, Seattle, Washington, United States of America, **4** Department of Genome Sciences, University of Washington, Seattle, Washington, United States of America, **5** Department of Human Genetics, University of Michigan, Ann Arbor, Michigan, United States of America, **6** ARUP Laboratories, Salt Lake City, Utah, United States of America, **7** Department of Pathology, University of Utah, Salt Lake City, Utah, United States of America

Key points:

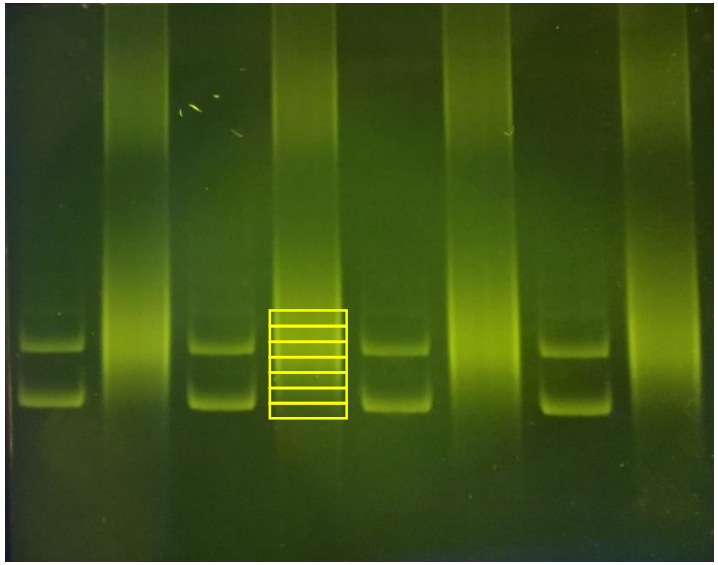
1. Cell-free DNA derived from tumor cells has a shorter fragment length distribution in plasma compared to healthy cell-free DNA
2. Sub-fraction selection of smaller cell-free DNA fragments appears to enrich for circulating tumor DNA

Size Selection

PAGE

T790M gBlocks (130 bp)

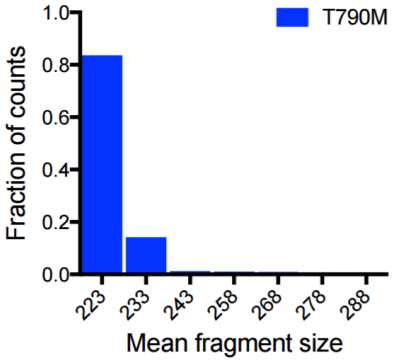
V600E gBlocks (165 bp)

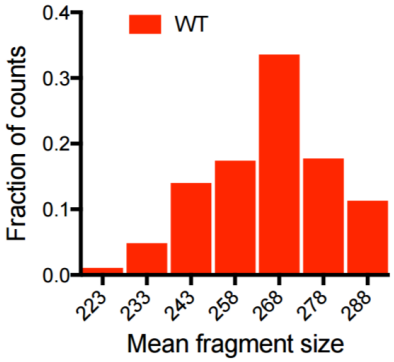


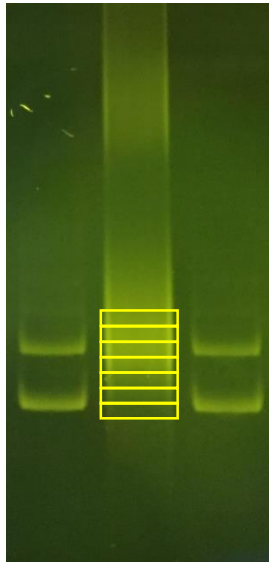
Size Selection

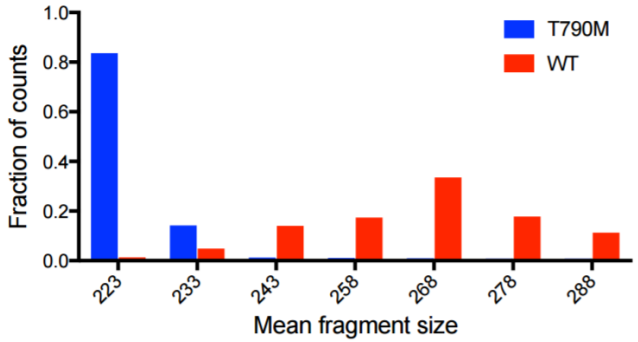
PAGE

T790M gBlocks (130 bp)

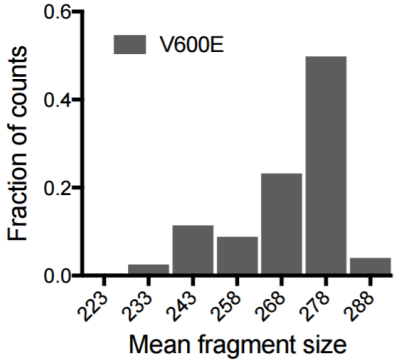


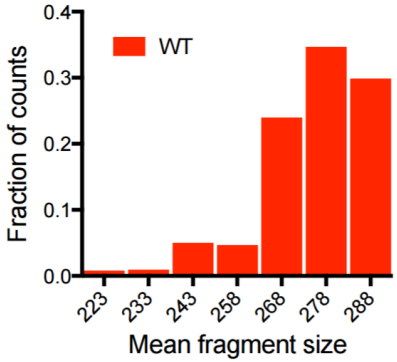


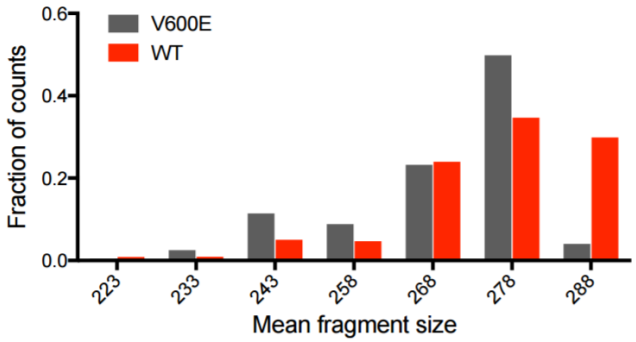




V600E gBlocks (165 bp)

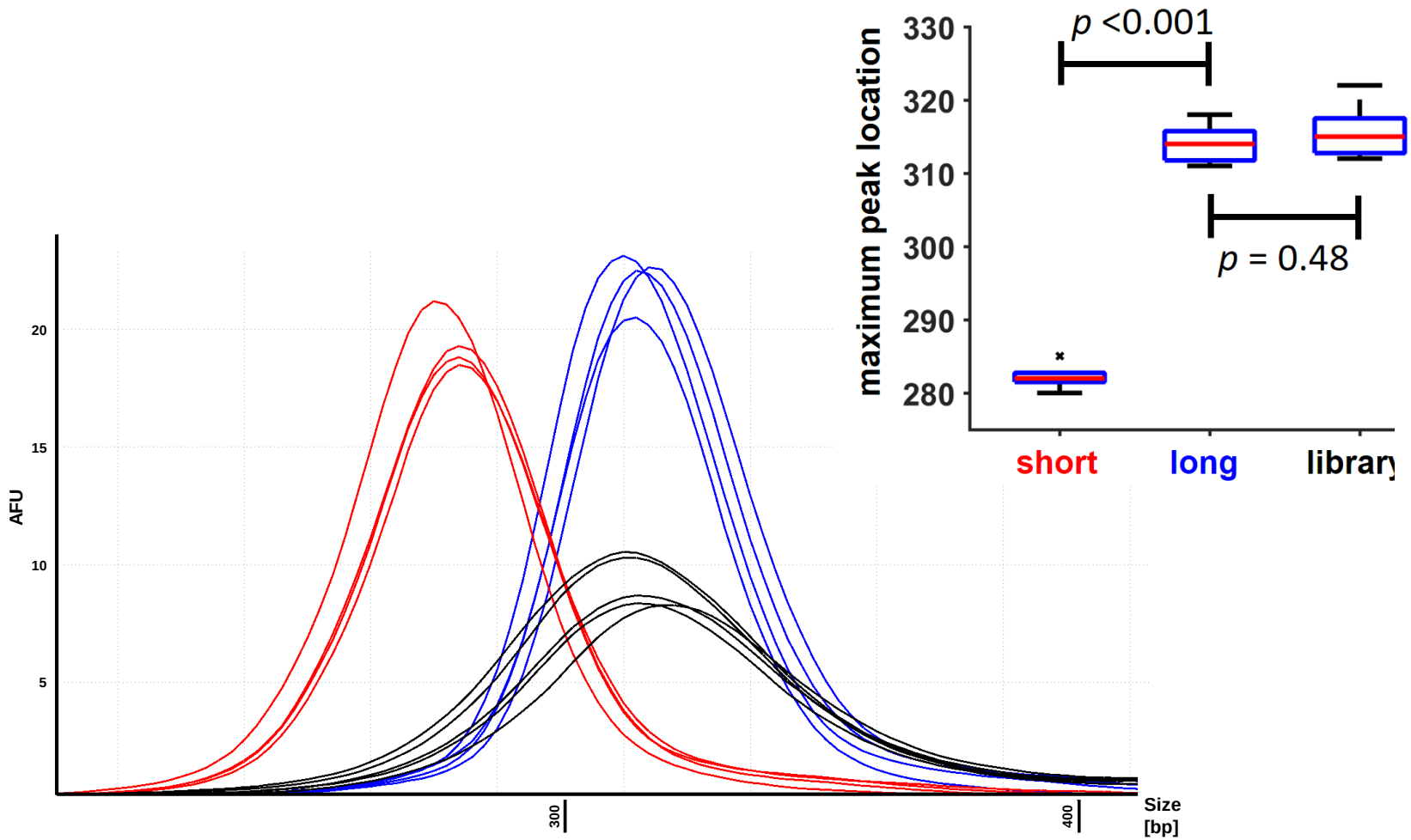






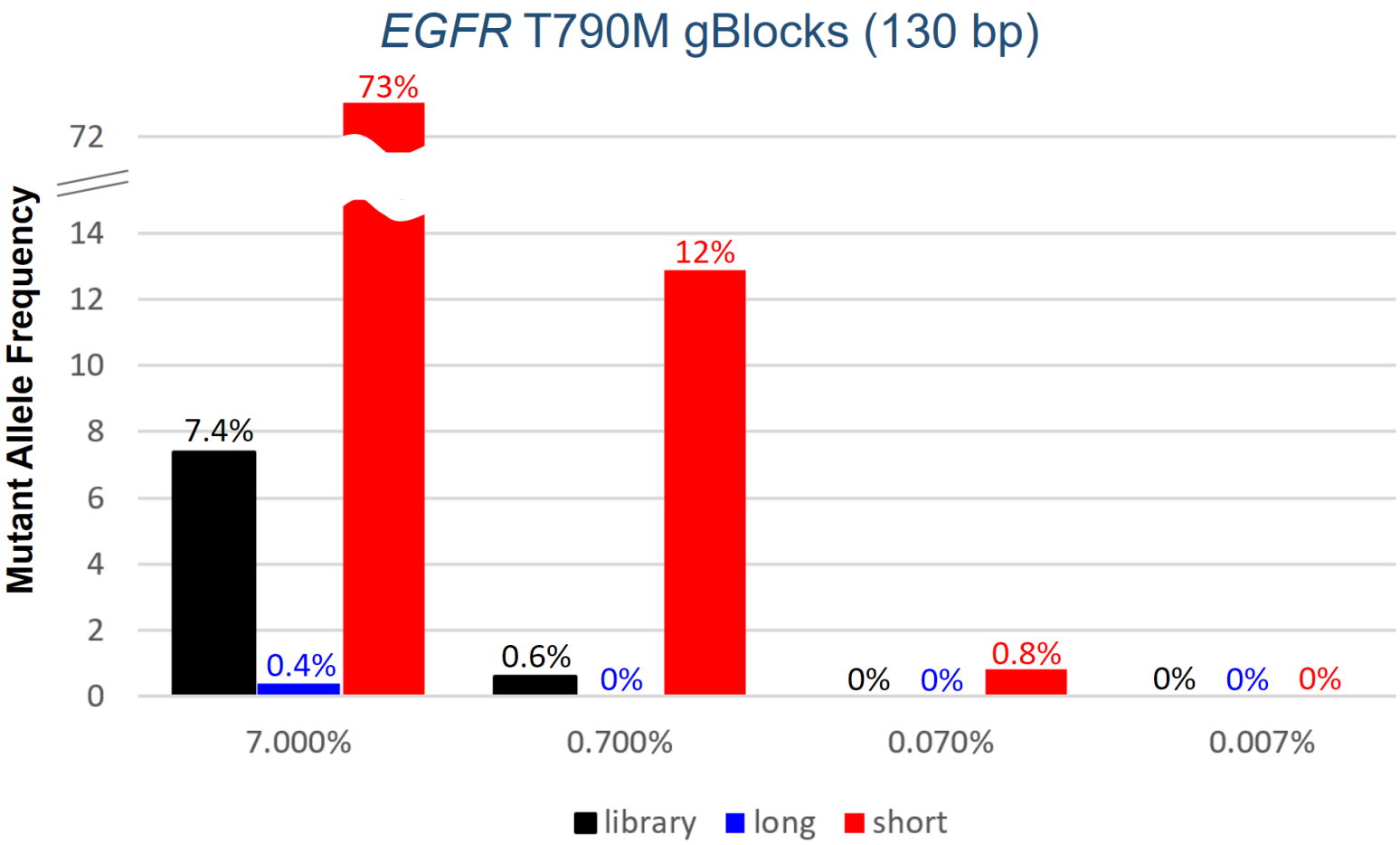
Size Selection

CoastalGenomics – Nimbus Ranger



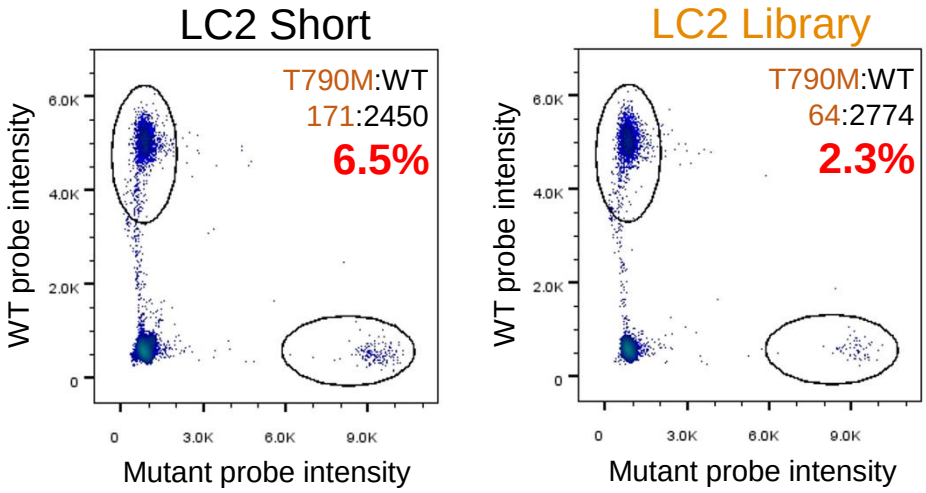
Size Selection

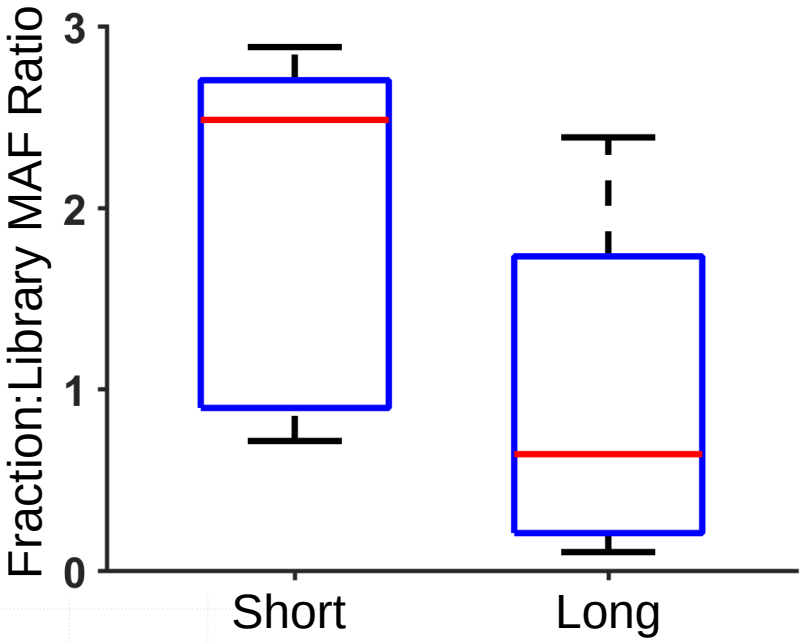
CoastalGenomics – Nimbus Ranger

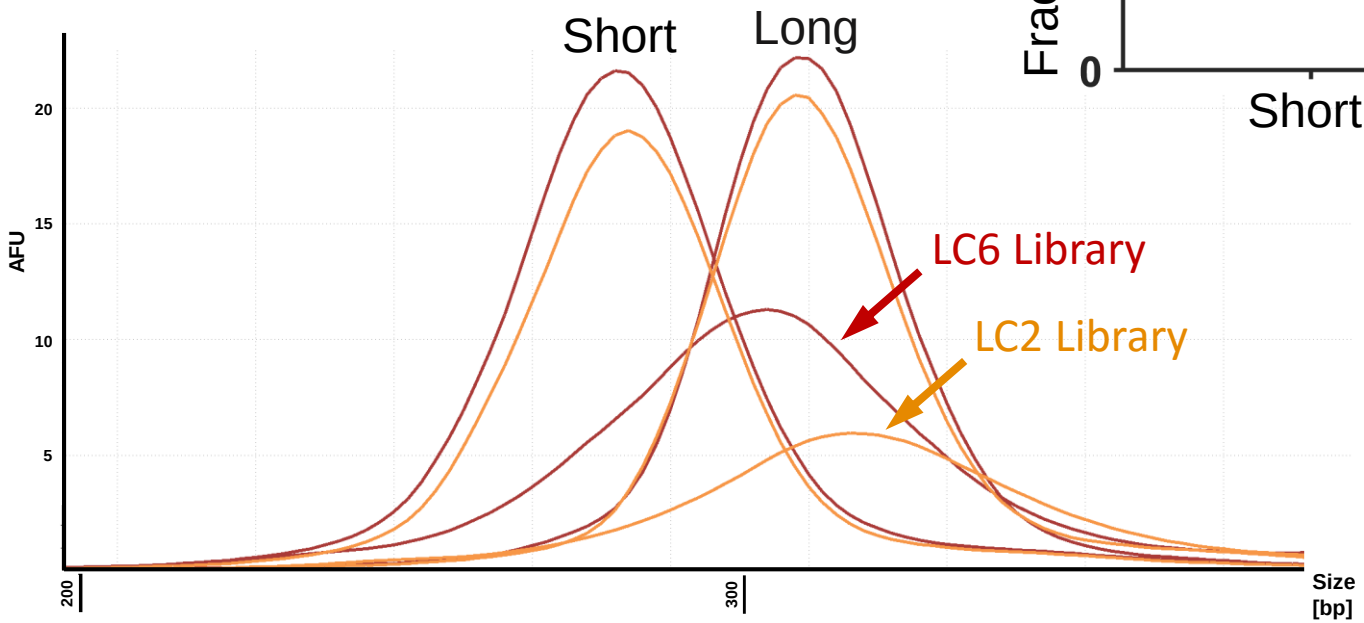


Size Selection

Nimbus Ranger – Lung Cancer T790M (*N*=5)

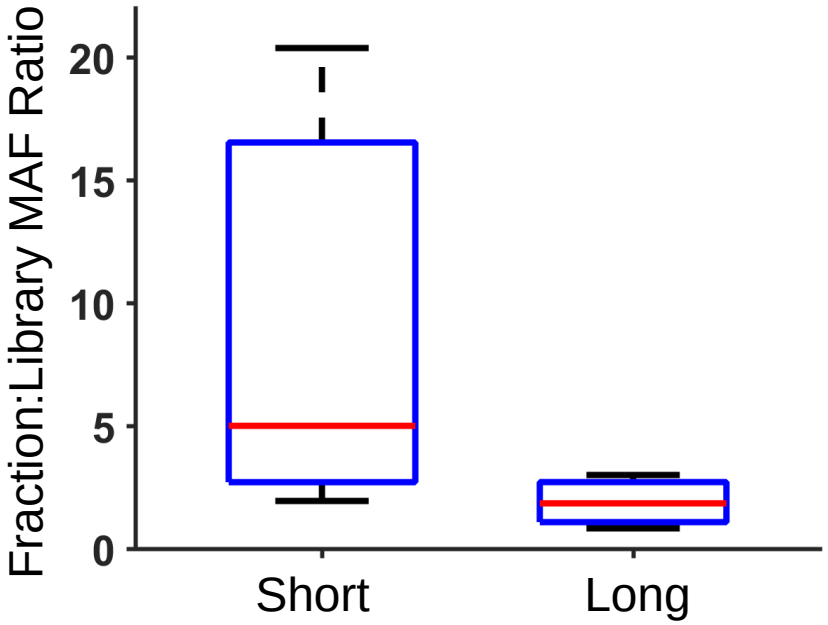


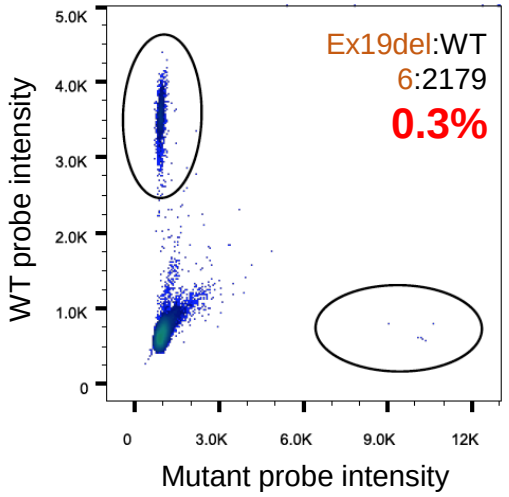


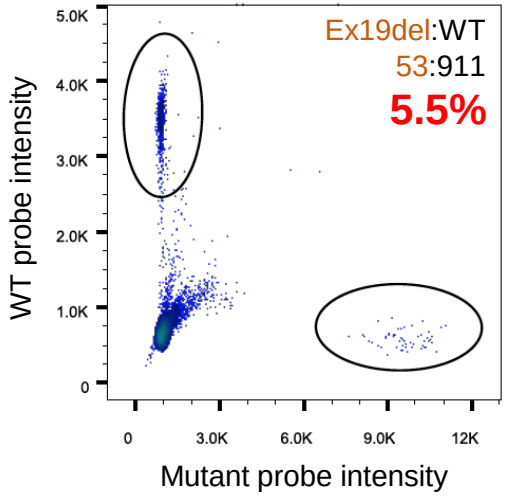


Size Selection

Nimbus Ranger – Lung Cancer Exon19Del (*N*=3)







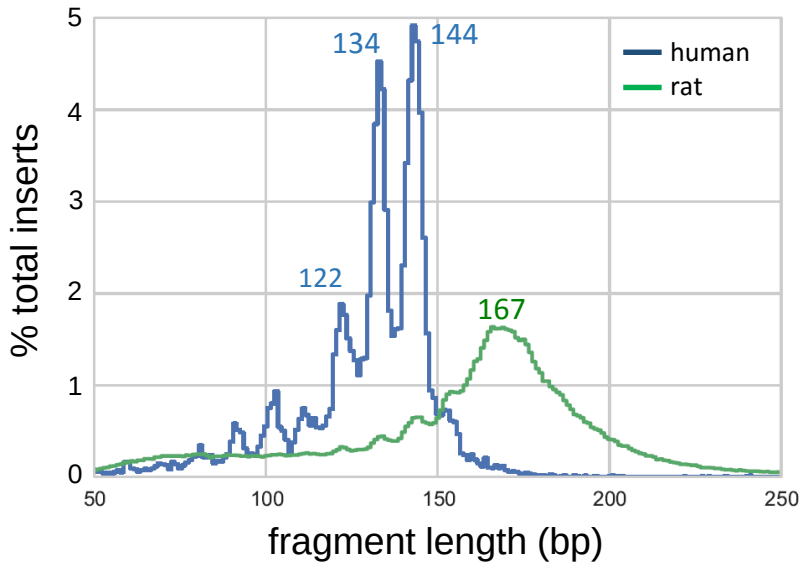
Size Selection

Summary

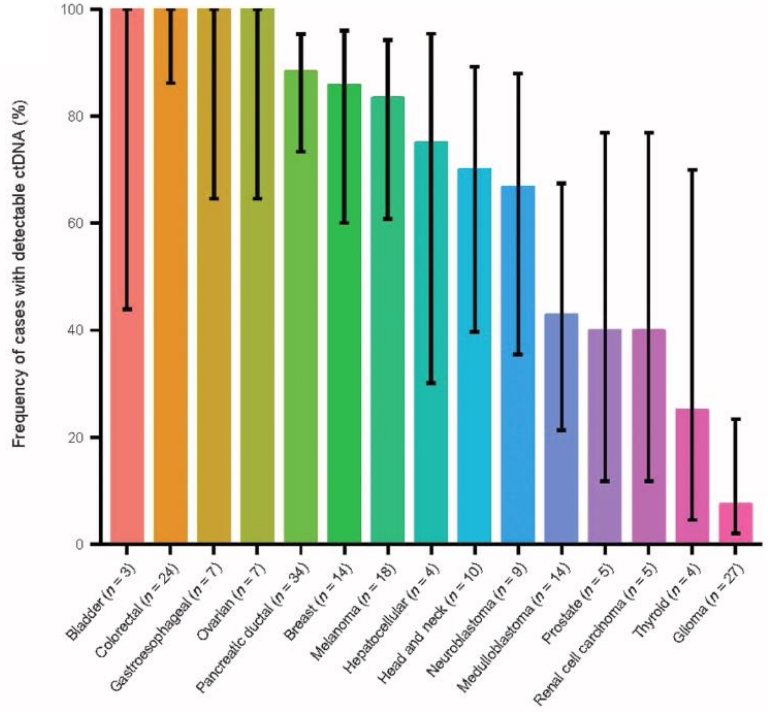
- PAGE affords selection of multiple adjacent fractions with high resolution, but is SLOW!
- Nimbus Ranger provides rapid (<6 hours) collection of 2 non-adjacent fractions with good recovery in up to 96 samples
- Selection of shorter cell-free DNA fragments may enrich for circulating tumor DNA in some samples, while not negatively impacting MAF in other samples

GBM

Reminders



GBM-associated cell-free DNA is present in plasma from a xenograft brain model of GBM

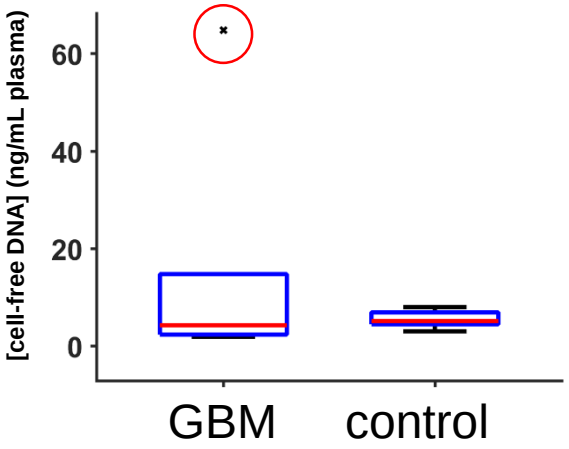


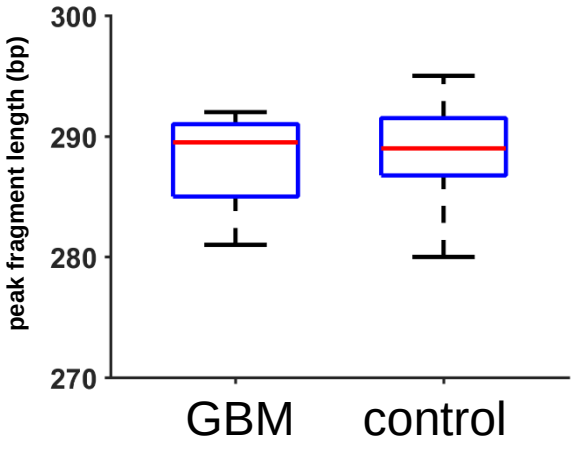
GBM-associated cell-free DNA has not been previously detected in humans

Bettegowda et al., *Sci Transl Med* 2014;6:224ra24

GBM

Cell-Free DNA Characteristics

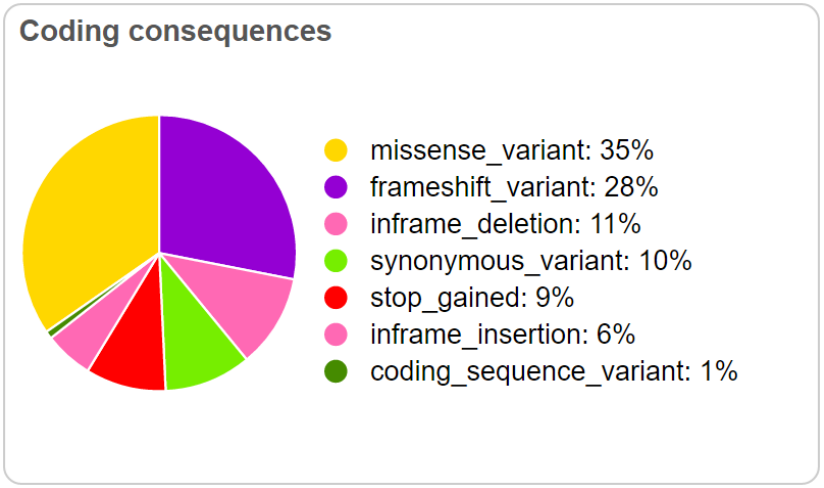




GBM – Accident #3

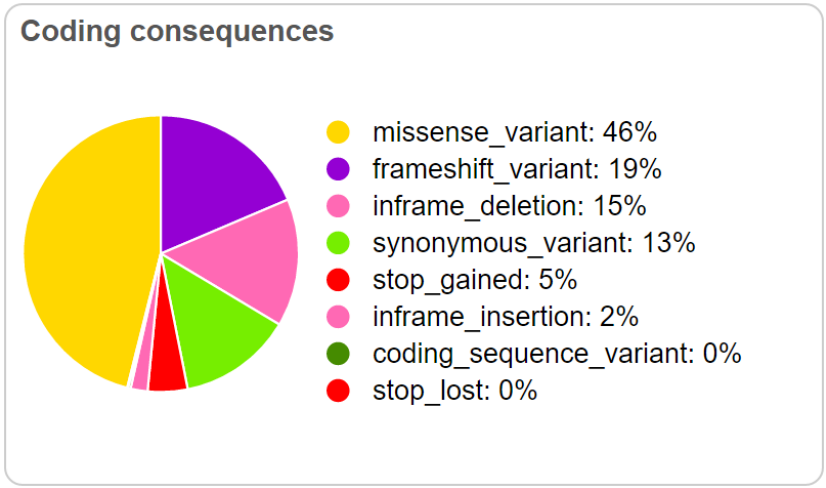
Tumor/Normal Whole Exome Sequencing (WES)

Tumor1/Normal1



Variants (novel/existing): 1470 (70.5%/29.5%)

Tumor2/Normal2



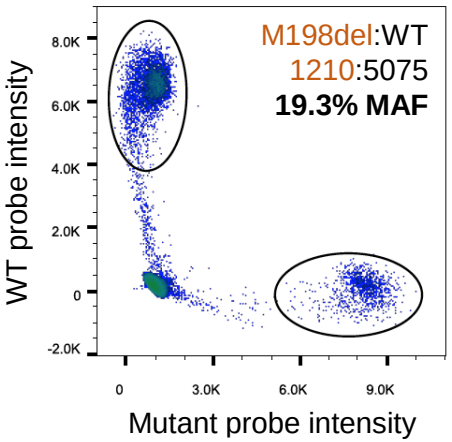
Variants (novel/existing): 1108 (61.1%/38.9%)

- *PTEN* p.Met198del custom-designed Taqman assay for ddPCR

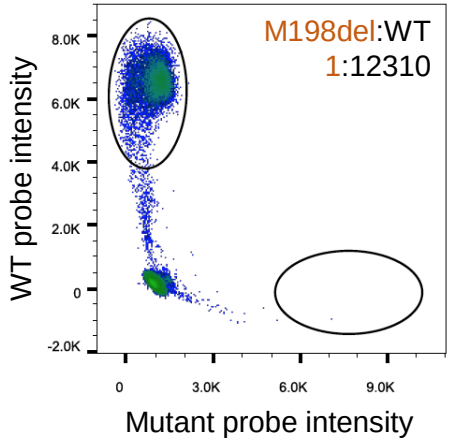
GBM – Accident #3

PTEN p.Met198del ddPCR

PAXgene tumor DNA

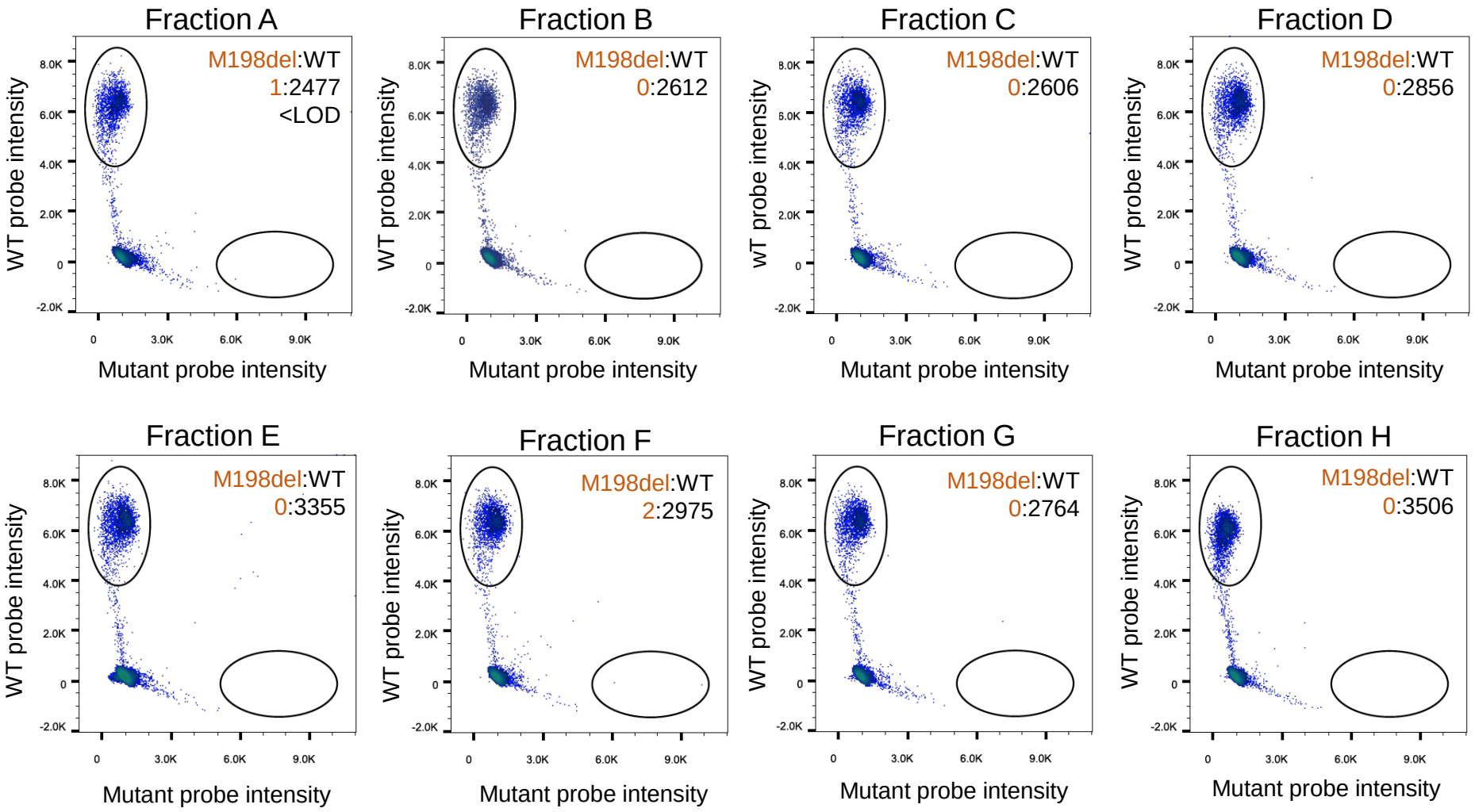


Buffy coat DNA



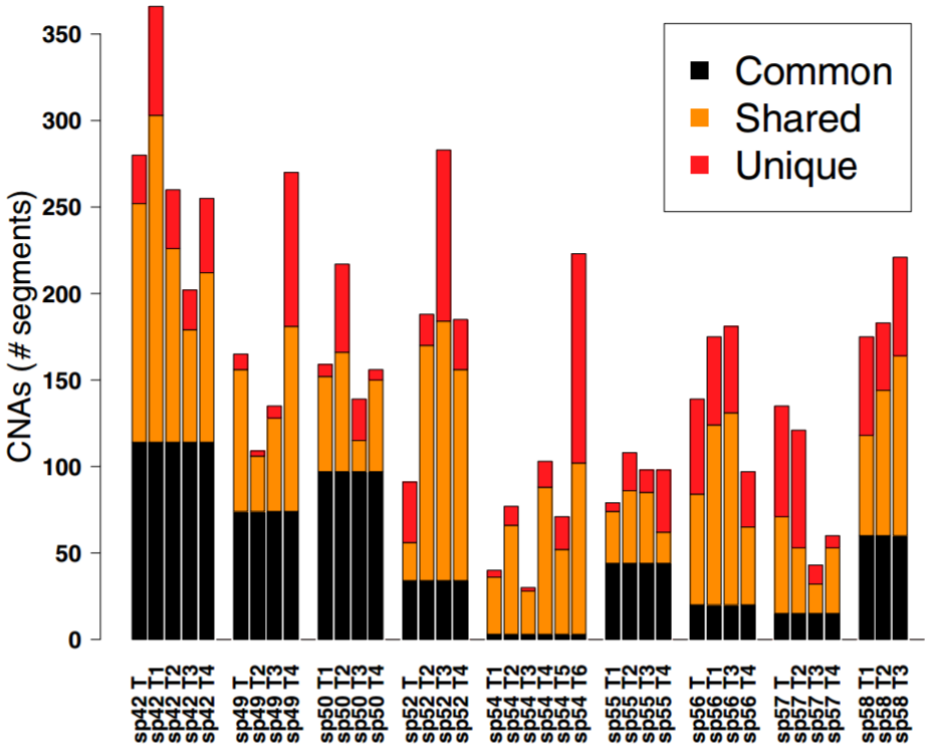
GBM – Accident #3

PTEN p.Met198del ddPCR



GBM

Intratumor Genetic Heterogeneity



Sottoriva et al., *Proc Natl Acad Sci,* 2013;110:4009-14

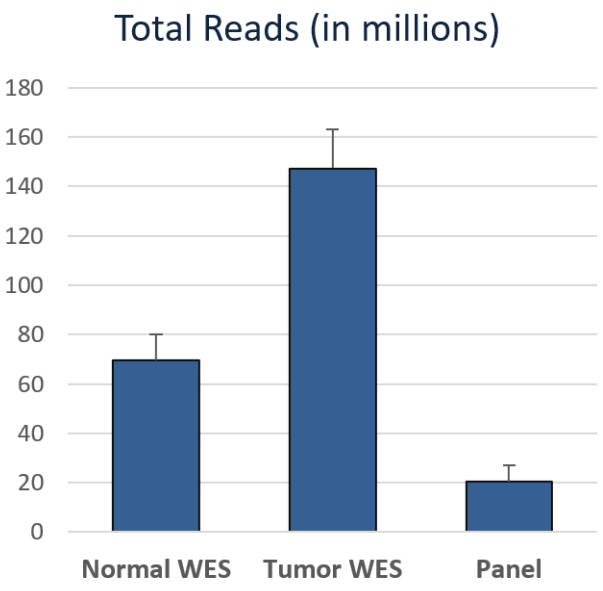
GBM

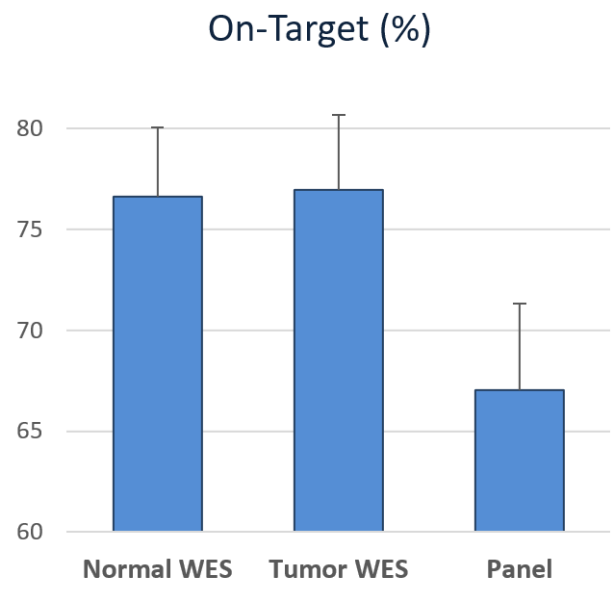
Custom Panel (128 genes; 128 kb)

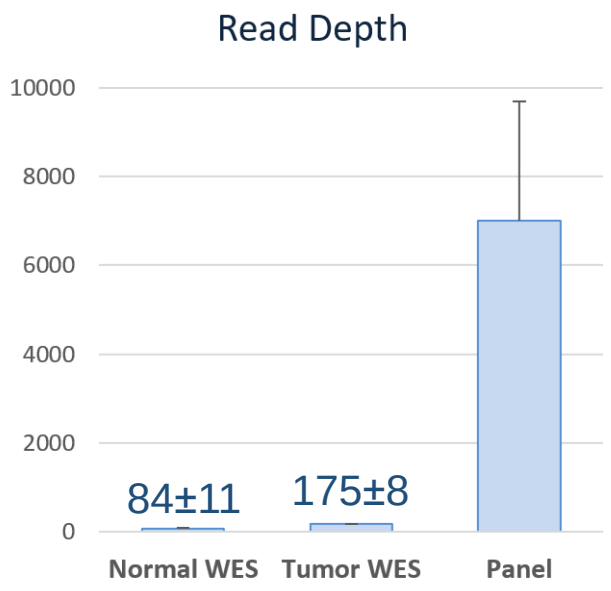
ABCB1	CDKN2A	FGFR3	HRAS	MET	QKI	TERT
ABCC9	CDX4	FHL2	IDH1	MMP13	RB1	TMEM147
ABL1	CIC	FIP1L1	IDH2	MROH2B	RET	TP53
ADAM29	COL1A2	FLT3	IL18RAP	MSH6	RFX6	TPTE2
AFM	CTNNB1	FOXR2	IL1R2	MTOR	RPL5	TRAF7
AIFM3	CXorf22	FRMD7	JAK2	NF1	SCN9A	TRIM51
AKT1	CDAF12L2	FUBP1	JAK3	NF2	SEMA3C	TRIM51BP
ALK	DDR2	FZD7	KCNC2	NLRP5	SIGLEC8	TRIM51EP
ANKRD36	DRD5	GABRA1	KDR	NOTCH1	SLC26A3	TRPV6
APC	DYNC1I1	GABRA6	KEL	NOVA1	SMAD4	UGT2A3
ATM	EDIL3	GABRB2	KIT	NRAS	SMG5	VHL
ATRX	EGFR	GCSAML	KLF4	ODF4	SMO	WNT2
BRAF	ERBB2	GNA11	KRAS	PARD6B	SPO11	ZNF844
CALCR	ERBB4	GNAQ	KRTAP20-2	PDGFRA	SPTA1	ZNF99
CARD6	ERCC1	GNAS	LCE4A	PIK3CA	STAG2	
CDH1	FBXW7	GOLGA5	LRRC55	PIK3R1	STK11	
CDH18	FGA	GPX5	LUM	PLCH2	SULT1B1	
CDH9	FGFR1	H3F3AP4	LZTR1	PODNL1	SYT14	
CDHR3	FGFR2	HIST1H3B	MAP2K1	PTEN	TCHH	

GBM

Sequencing Metrics (*N*=6)

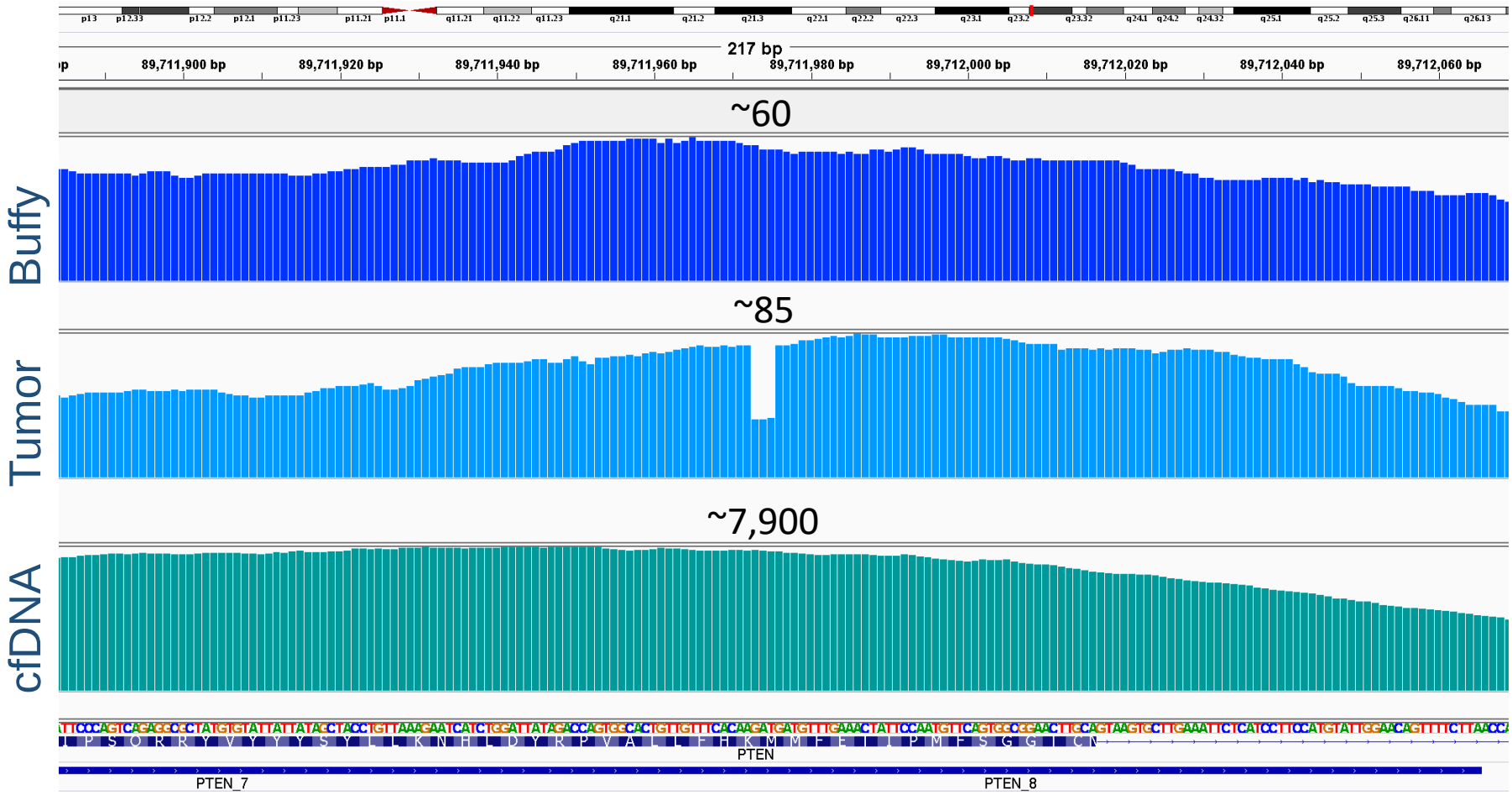






GBM

PTEN sequencing: p.Met198del



Potential GBM Variants in Cell-Free DNA

GBM

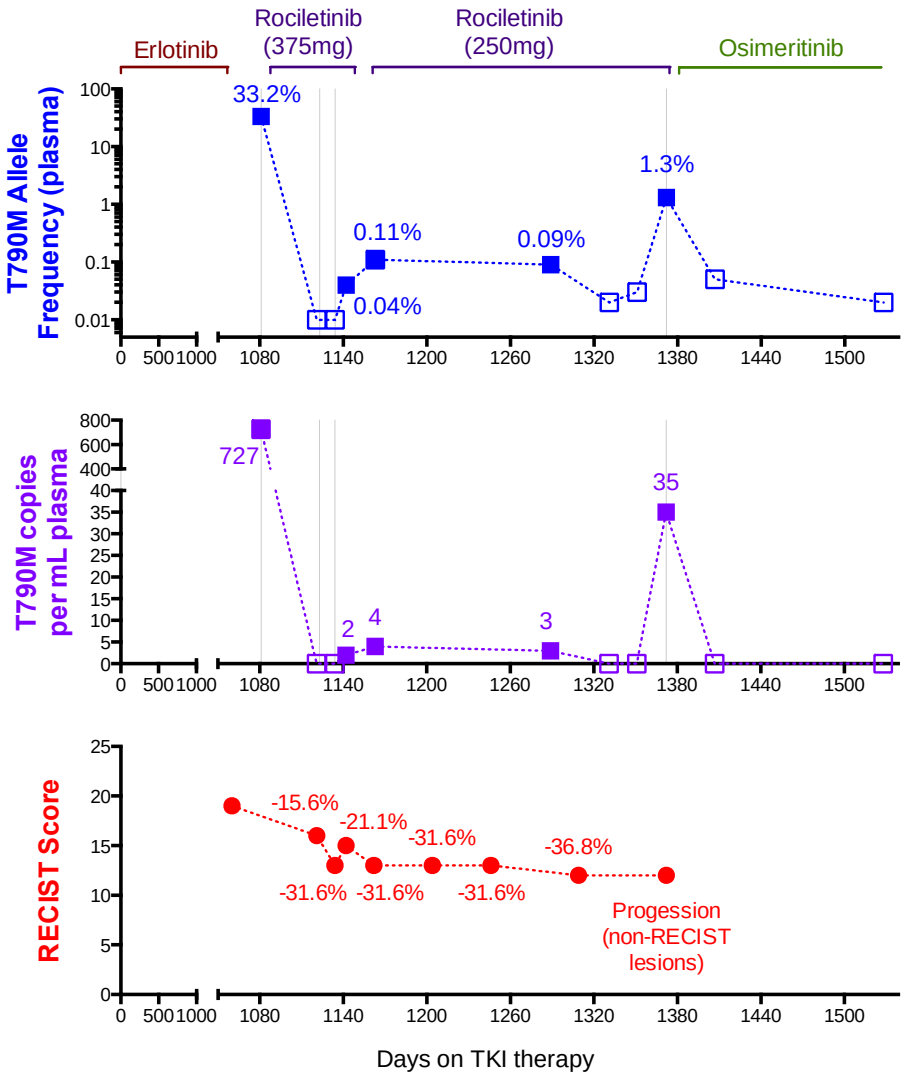
Summary

- Inter-tumor genetic heterogeneity requires a personalized approach for detecting circulating tumor DNA
- Intra-tumor genetic heterogeneity coupled with the non-metastatic nature of GBM requires an approach with high-sensitivity for detection of variants in cell-free DNA with a frequency <1%

Slide courtesy of Sabine Hellwig, PhD

Current/Future Directions

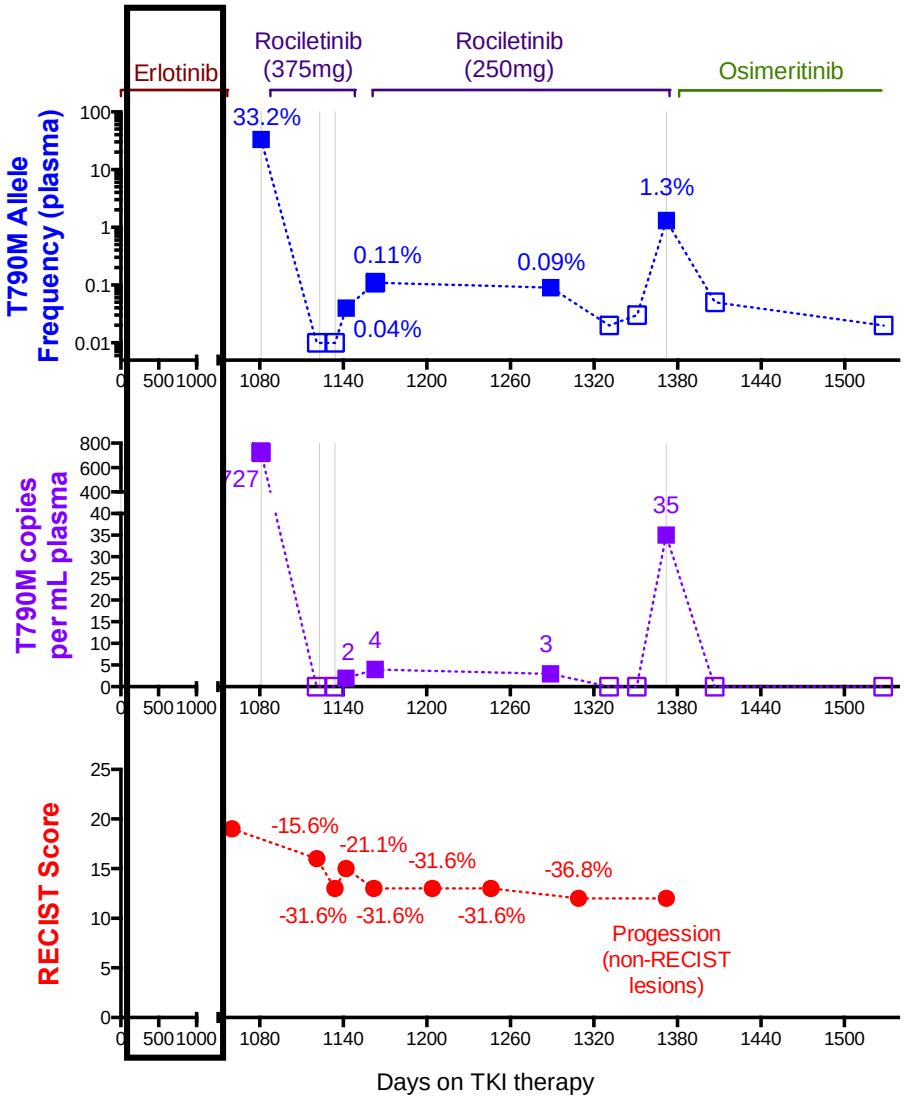
NSCLC Serial Monitoring (*EGFR* T790M)



Slide courtesy of Sabine Hellwig, PhD

Current/Future Directions

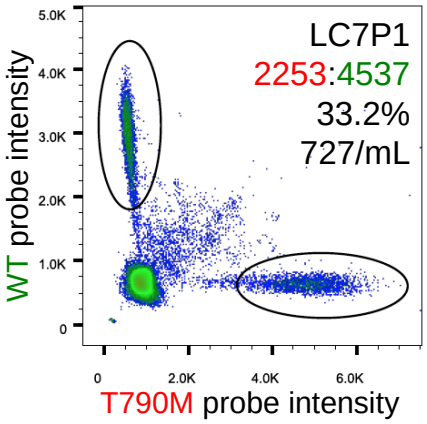
NSCLC Serial Monitoring (*EGFR* T790M)

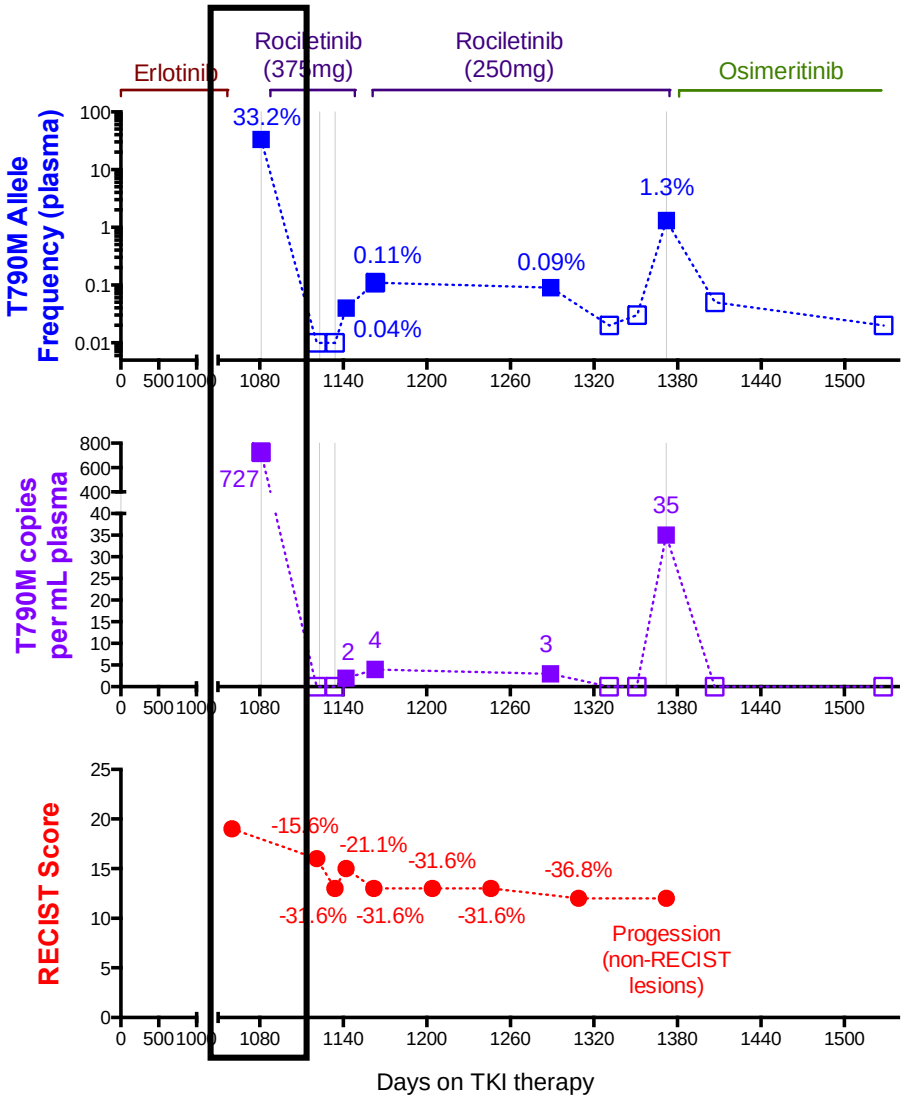


Slide courtesy of Sabine Hellwig, PhD

Current/Future Directions

NSCLC Serial Monitoring (*EGFR* T790M)

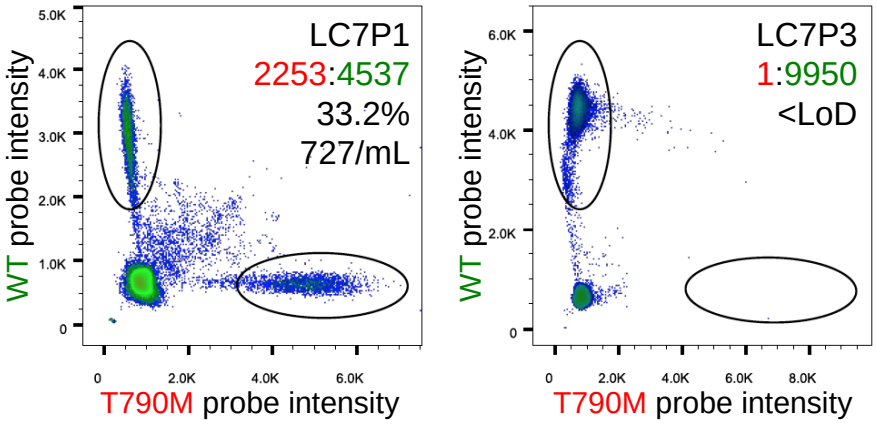


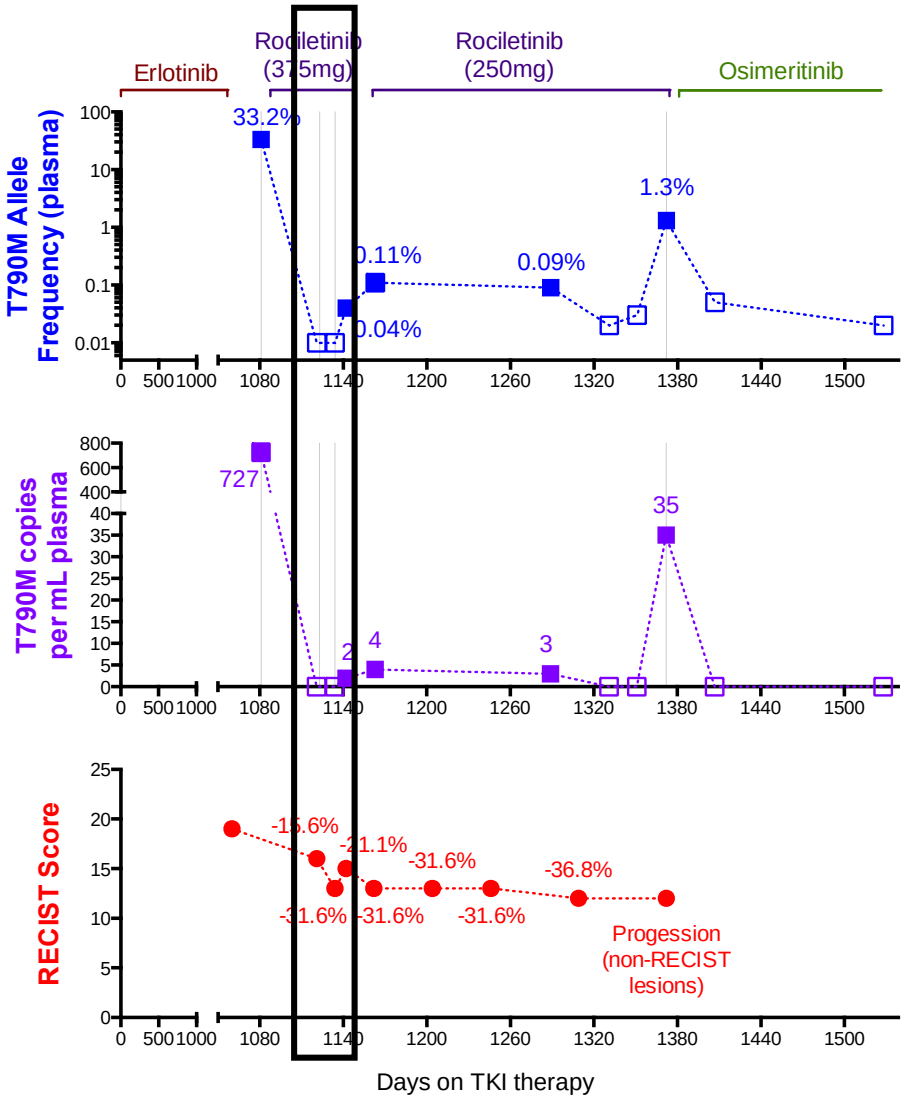


Slide courtesy of Sabine Hellwig, PhD

Current/Future Directions

NSCLC Serial Monitoring (*EGFR* T790M)

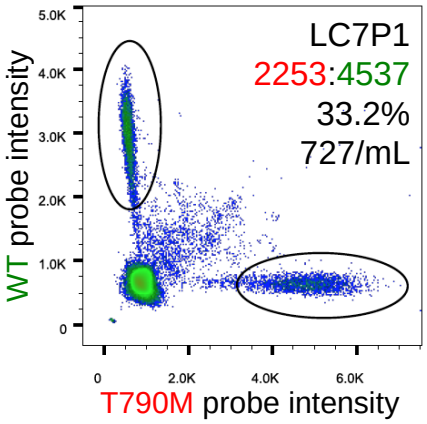


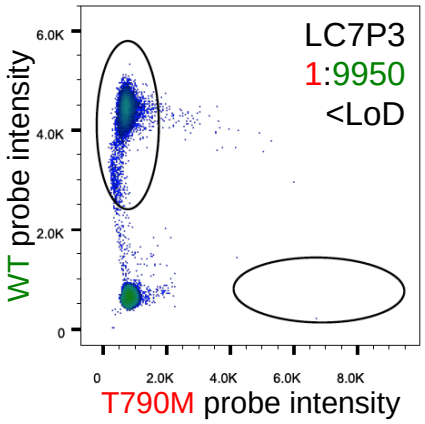


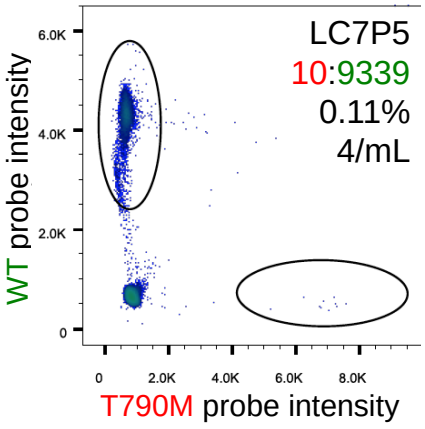
Slide courtesy of Sabine Hellwig, PhD

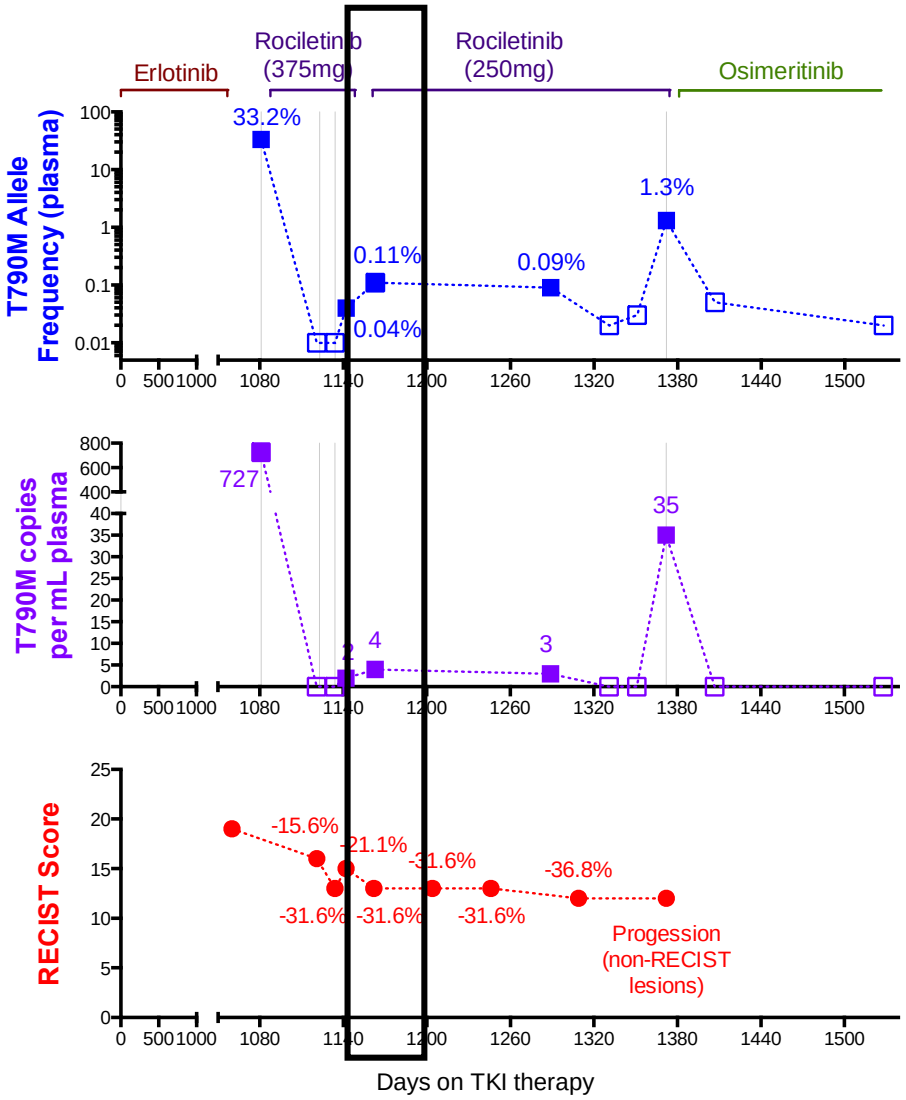
Current/Future Directions

NSCLC Serial Monitoring (*EGFR* T790M)





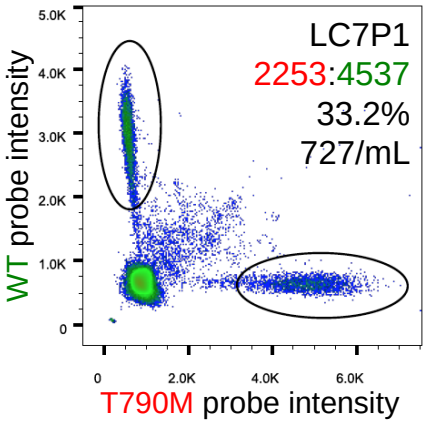


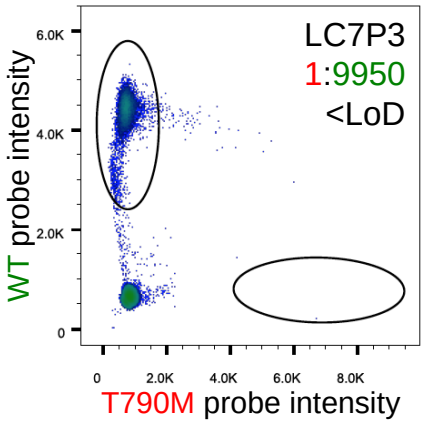


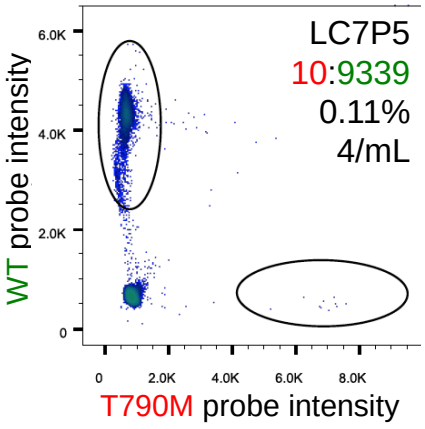
Slide courtesy of Sabine Hellwig, PhD

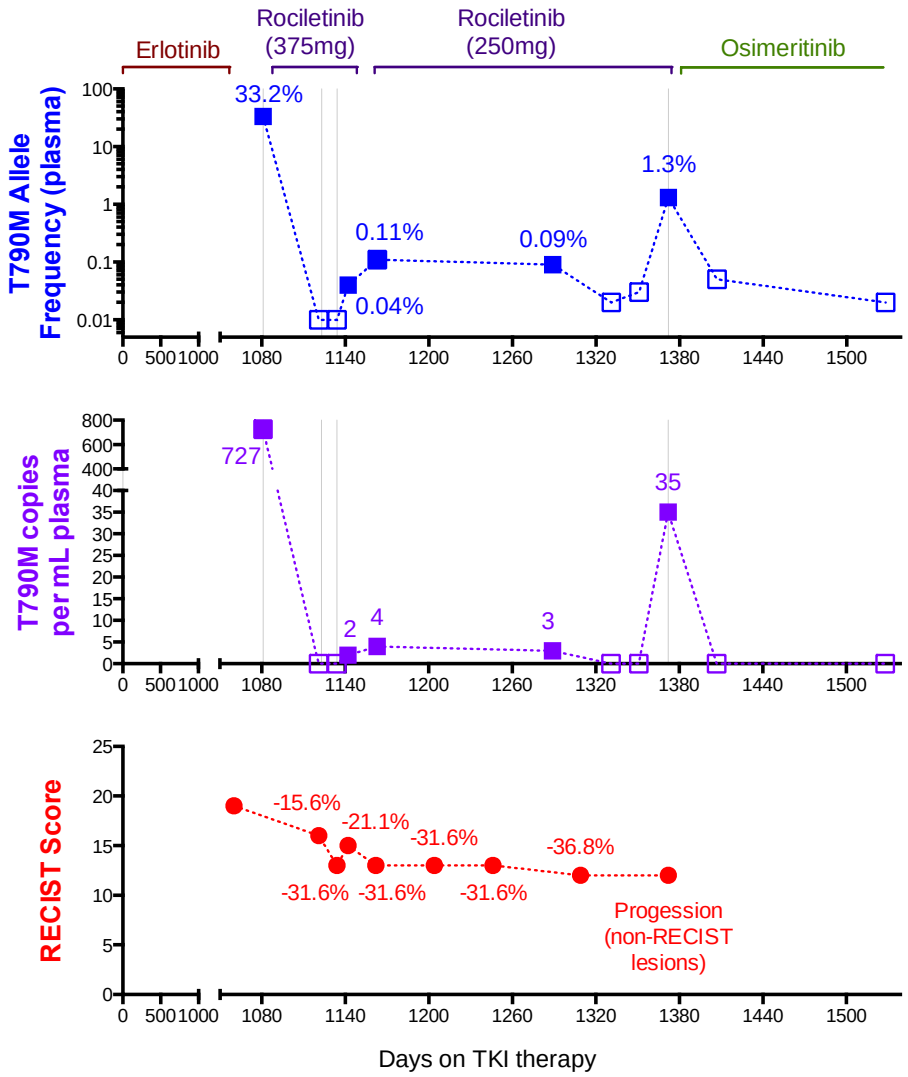
Current/Future Directions

NSCLC Serial Monitoring (*EGFR* T790M)





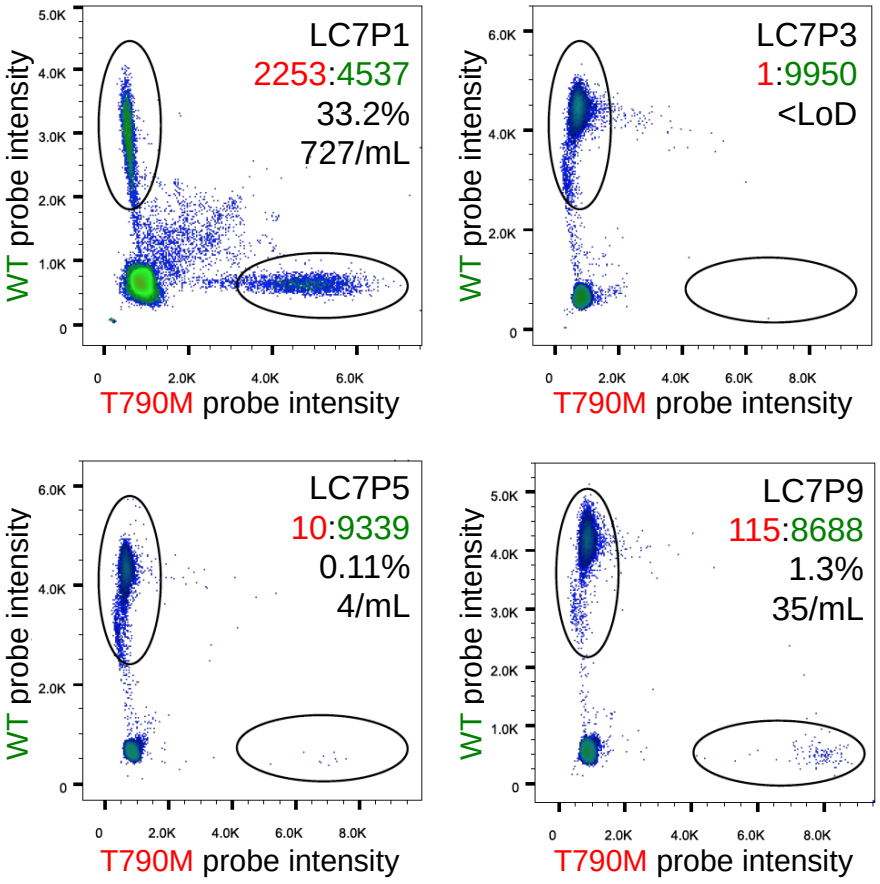


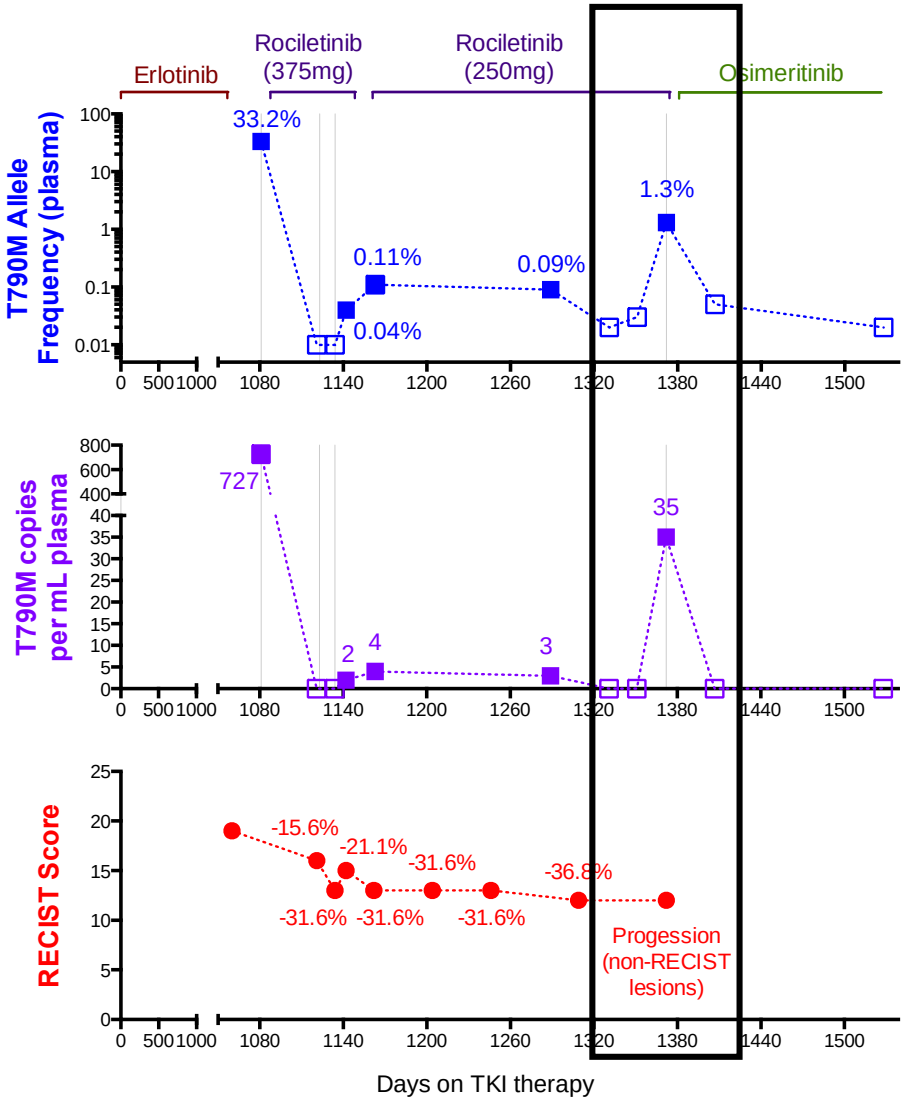


Slide courtesy of Sabine Hellwig, PhD

Current/Future Directions

NSCLC Serial Monitoring (*EGFR* T790M)

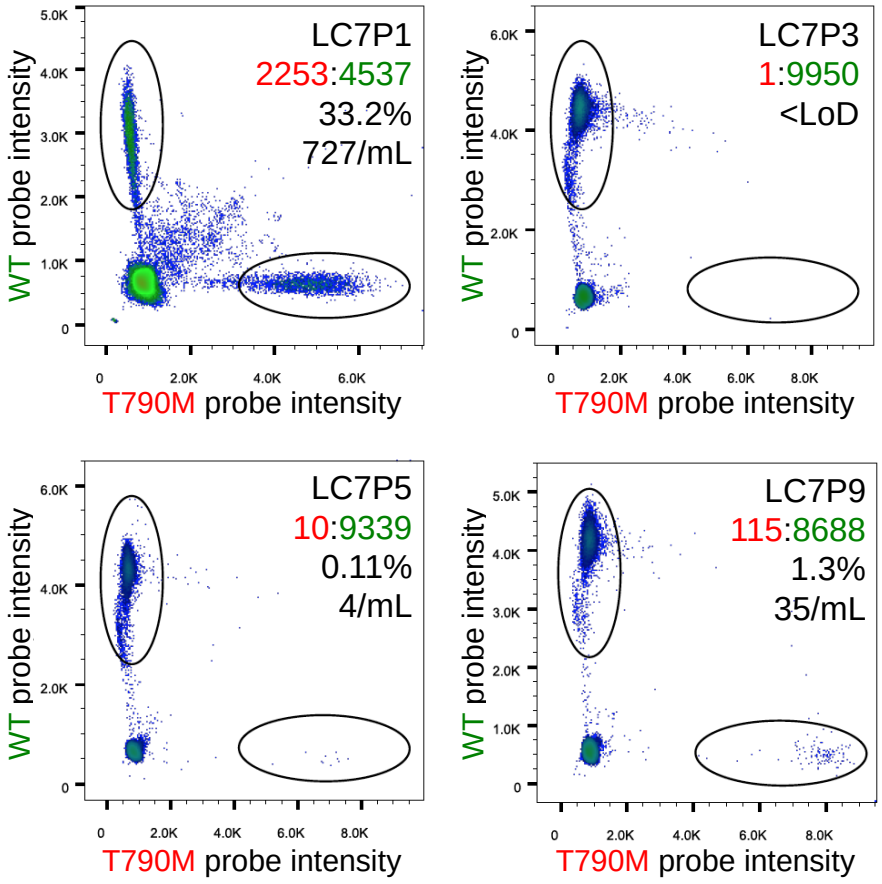


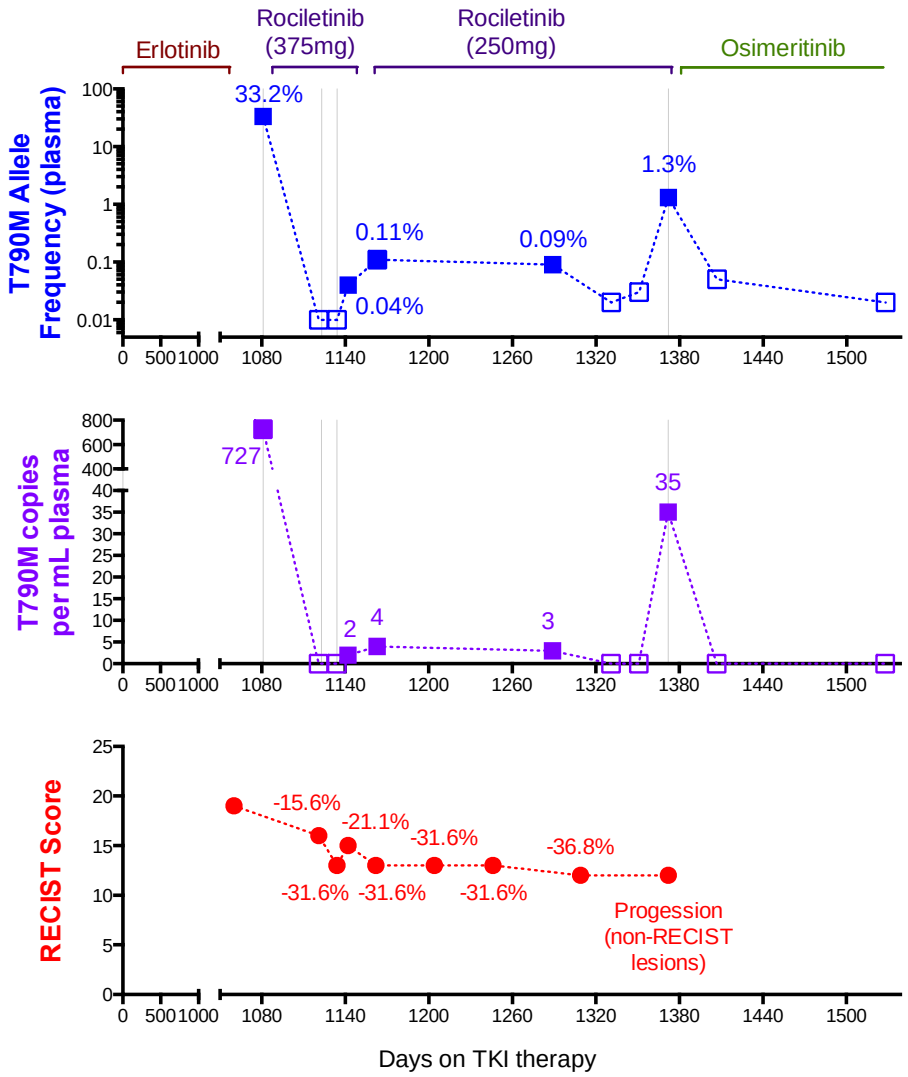


Slide courtesy of Sabine Hellwig, PhD

Current/Future Directions

NSCLC Serial Monitoring (*EGFR* T790M)

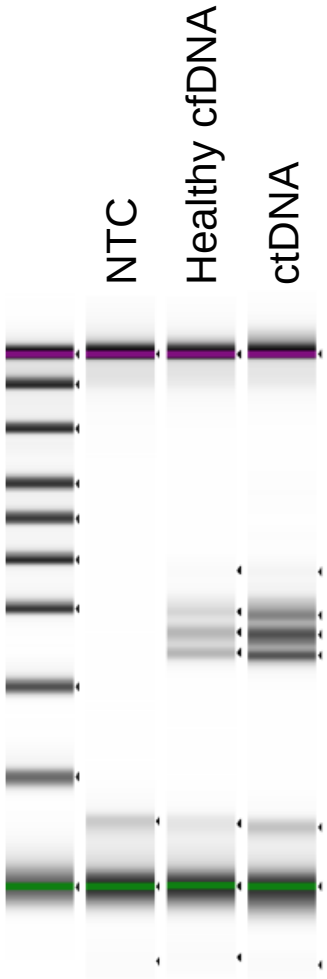




Slide courtesy of Sabine Hellwig, PhD

Current/Future Directions

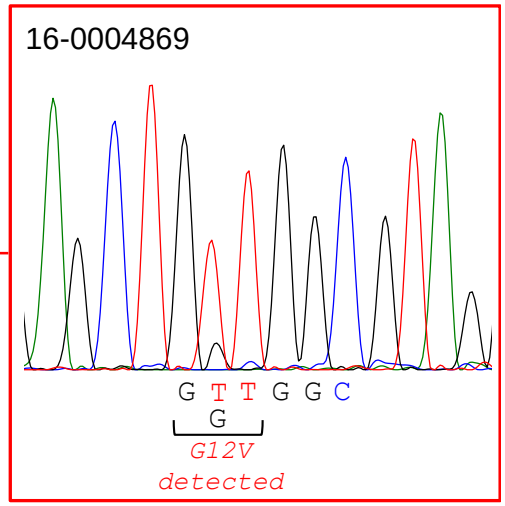
Pancreatic Cancer – *KRAS* exon 2 ice-COLD-PCR



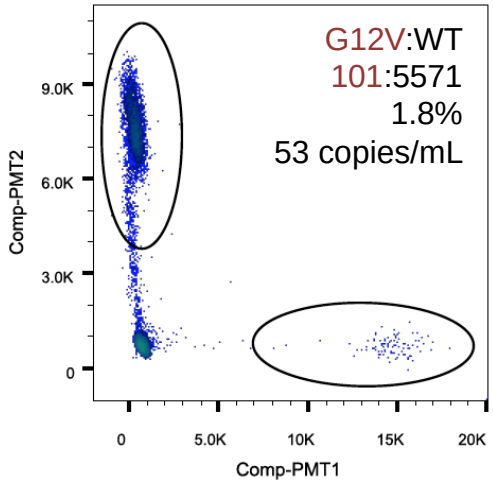
Multiplex PCR products

KRAS exon2 ICP products

Qualitative analysis by Sanger



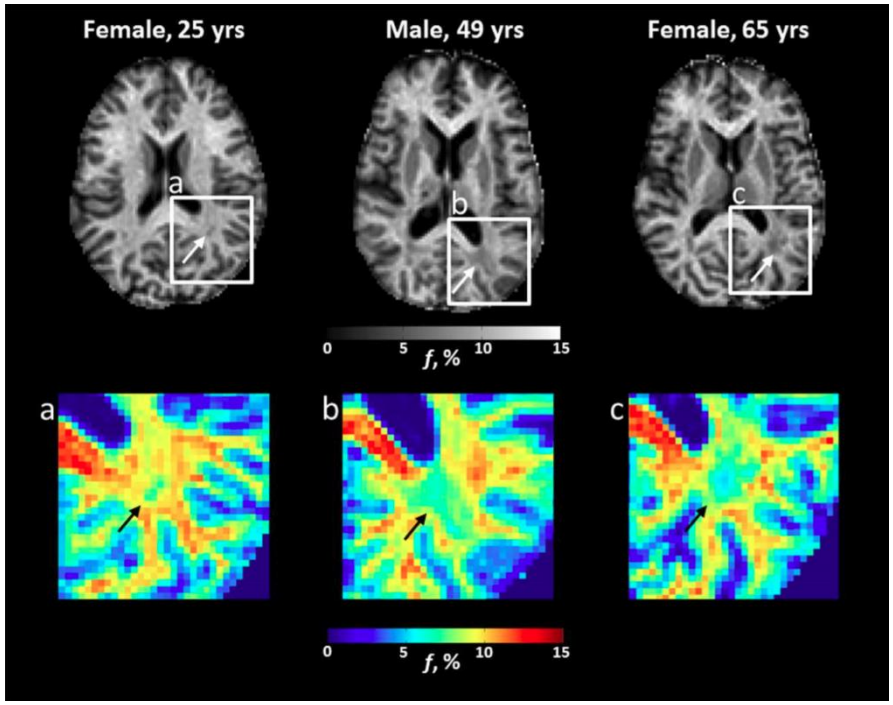
Quantitative analysis by ddPCR:



Current/Future Directions

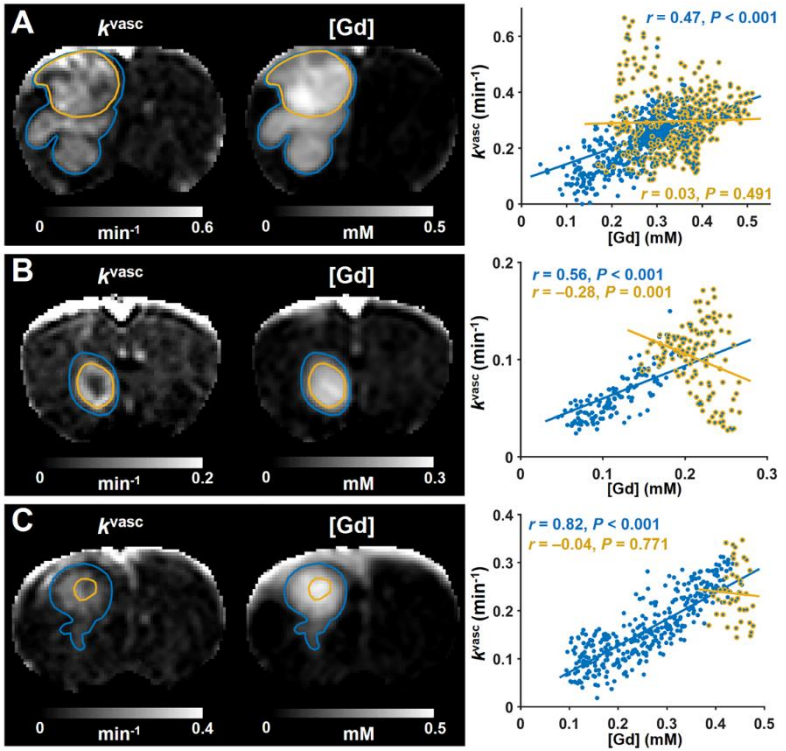
Genotype/Phenotype Associations

Myelin Density Imaging



Underhill et al., *J Magn Reson Imaging,* 2015;42:1611-22

Dynamic MRI



Underhill, *Magn Reson Med,* 2016; In press

Conclusions

- Fragment size is important in cell-free DNA
- Overcoming challenges associated with detection of cell-free DNA derived from GBM has profound implications for the “liquid biopsy”

Acknowledgments

Sabine Hellwig, PhD
Keith Gligorich, PhD
Mary Bronner, MD

Carrie Fuertes, CRC
Amy Hall, MB (ASCP) MLS (ASCP)

David Nix, PhD
Brett Milash, MS

ARUP
- Brett Kennedy, PhD
- Daniel Baker, MS
- Elaine Gee, PhD
- Brendan O'Fallon, PhD
- Ashini Bolia, PhD

Randy Jensen, MD, PhD
Howard Colman, MD, PhD

BMP-Core
- John O'Shea, PhD
- Katy Phillips
- Kevin Lee
- James Kline
- Andy Lee

Funding
- NIH K99CA168943
- Department of Pediatrics research support funds
- Shameless begging