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Circulating Tumor DNA in GIST

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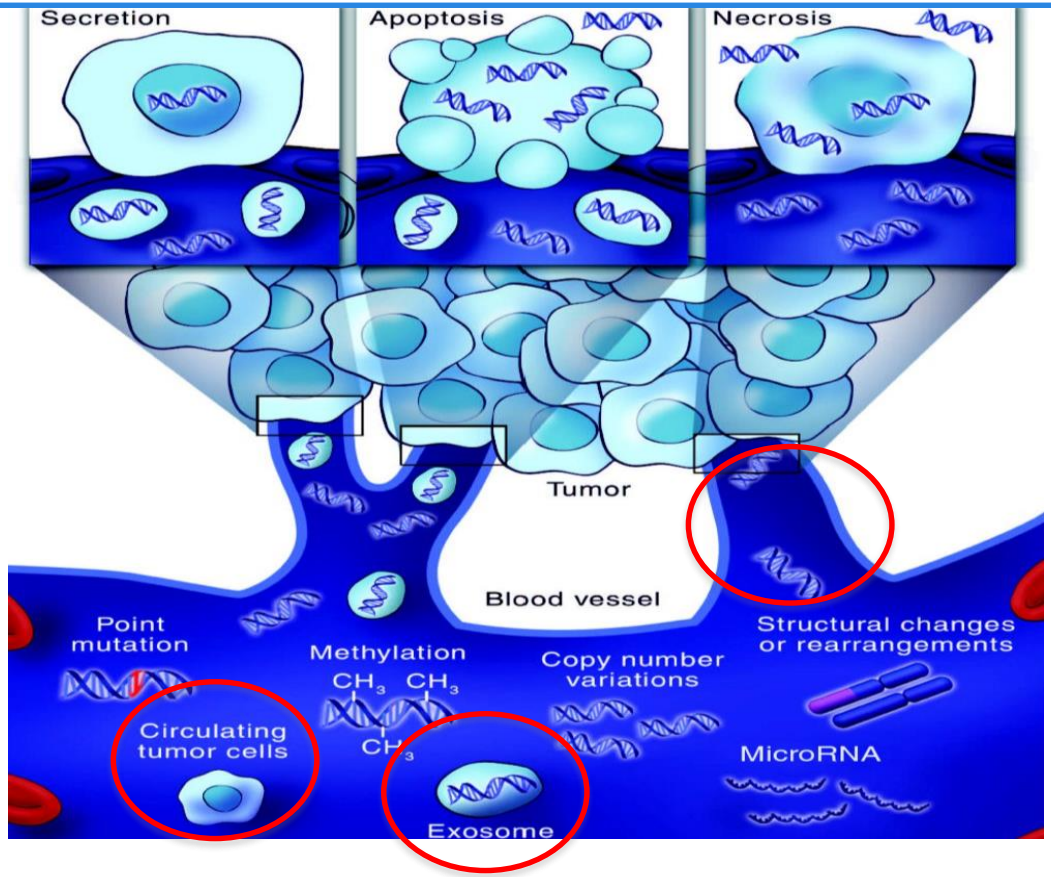
Objectives

- Background – Liquid biopsy & ctDNA
- Methodology of extraction and downstream analysis of ctDNA
- Utility of ctDNA in GIST & current evidence available
- Future Directions

Background- Liquid biopsies

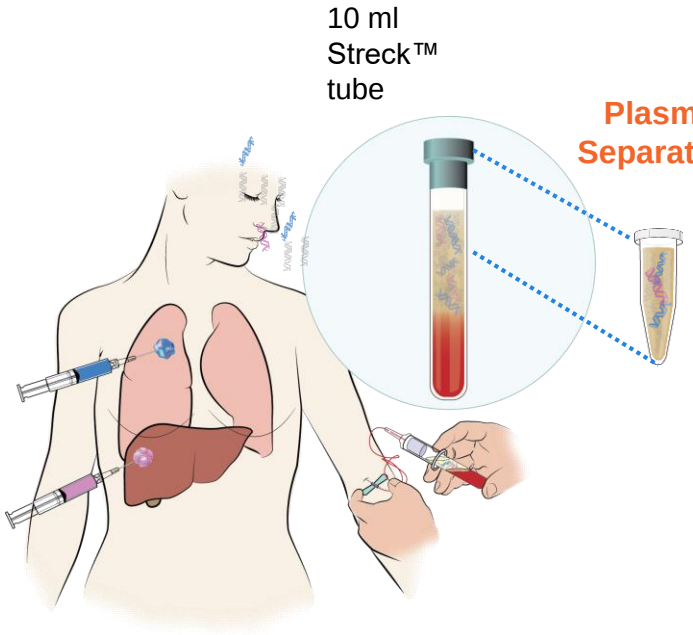


Diaz and Bardelli, JCO 2014

Circulating Tumor DNA

- ctDNA is a component of cell free DNA (cfDNA)
- cfDNA – fragments of normal and cancer cells shed into the blood stream
- ctDNA- tumor derived
- Sources of ctDNA: blood, urine, csf, respiratory secretions

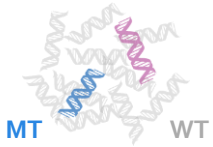
Sample Collection



Plasma Separation

cfDNA Extraction

cfDNA Quantification



DOWNSTREAM ANALYSIS of Tumors Genomic Landscape

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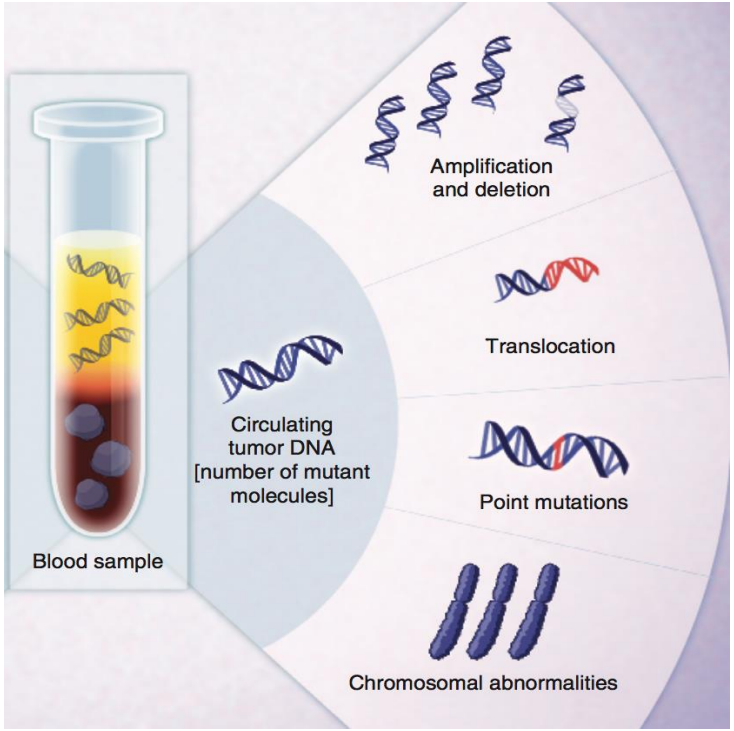
Downstream analysis

Downstream analysis of ctDNA facilitates sequencing and detection of the tumor's genomic landscape



Haber, Cancer Disc 2014

Downstream Analysis Methods

Underlying technology	Mutation detection approach	Type of alteration	Example alterations
Real-time or end-point PCR	ARMS-Scorpion PCR PCR-SSCP Mutant allele–specific PCR Mass spectrometry Bi-PAP amplification	Known point mutations	*KRAS*, *EGFR* hotspot changes
Digital PCR	BEAMing Droplet-based digital PCR Digital droplet PCR	Known point mutations	*KRAS*, *EGFR* hotspot changes
Gene sequencing	SafeSeqs OnTarget TamSeq	Point mutations in gene regions	*PIK3CA*, *EGFR*, *TP53* coding mutations
Whole-genome sequencing	Digital karyotyping	Genome-wide copy-number changes	Personalized amplifications
Whole-genome sequencing	PARE	Genome-wide rearrangements	Personalized rearrangements
Targeted sequencing	Digital karyotyping/PARE	Structural alterations in gene regions	*MET*, *ERBB2* amplification

Abbreviations: SSCP, single-strand conformational polymorphism; BEAM, Beads, Emulsions, Amplification, and Magnetics; PARE, Personalized Analysis of Rearranged Ends.

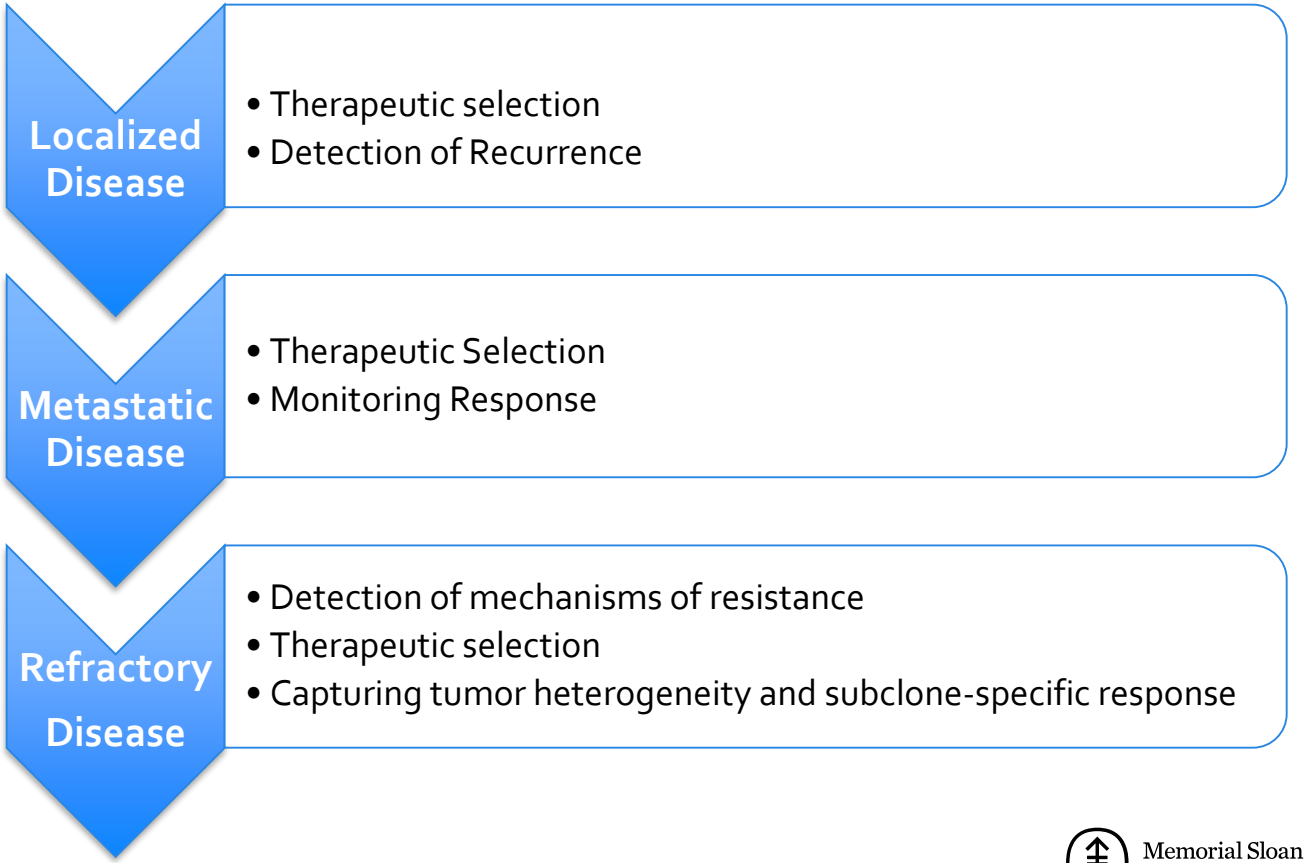
ctDNA as a Biomarker: Biomarker Categories

TYPE	DEFINITION	EXAMPLE
Diagnostic	Identifies presence of malignancy	Tissue biopsy
Prognostic	Characteristic that categorizes pts by degrees of risk for disease recurrence/progression	ECOG PS/KPS
Predictive	Characteristic that categorizes pts based on their likelihood to respond to a given therapy	KIT ex 11 mut – imatinib
Pharmacodynamic	Provides dynamic assessment showing biological response has occurred after a therapeutic intervention	Radiographic imaging
Discovery	Intended to identify previously unknown aberrations that promote tumorigenesis or resistance to therapy	Genomic analyses – secondary KIT mutations
Surrogate	Substitute for clinical efficacy endpoint	Progression free survival

Quality Control

- Accurate detection of somatic mutations
  - Exclude noise of surrounding cells
  - Germline alterations detectable in both normal and ctDNA
  - Collect and sequence normal reference germline DNA
  - Compare sequenced ctDNA and germline DNA
  - Allows for unambiguous detection of tumor specific DNA
- Further evaluate sequenced ctDNA samples that fail to identify somatic mutations
  - Determine if adequate DNA present for analysis
  - Accuracy of test improves if a QC step is used to identify and eliminate samples with insufficient DNA that yield inconclusive results

ROLE OF CTDNA IN GIST



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Therapeutic Selection

What Informs Therapeutic Choices Patients with GIST?

PERSONAL GENOMICS

PHARMACO-GENOMICS

ITS PERSONAL

TUMOR GENOMICS

CLINICAL CHARACTERISTICS

< 15% of patients with GIST have their tumors genotyped

Barrios et al, Eur J Cancer, 2015

Concordance

- Several studies have shown the ability to detect somatic mutations in ctDNA collected from patients with GIST[1,2,3,4]

- Few studies have reported on the concordance rate between the molecular spectrum detected by sequenced ctDNA and tumor DNA from biospy/surgical specimens
  - Detection of primary KIT mutations – high concordance rate (84%)[3]
  - Secondary KIT mutations – poor concordance[3]
    - Plasma superior at detecting secondary mutations 47% vs 12% in tissue

1. Bauer S, et al, ASCO annual meeting 2015
2. Heinrich M, et al, ASCO annual meeting 2015
3. Demetri G, et al, ASCO annual meeting 2013
4. Janku F, et al, AACR annual meeting 2017

Companion Diagnostics, Pharmacogenomic, and Cancer Biomarkers

Molecular Cancer Therapeutics

Clinical Application of Circulating Tumor DNA in the Genetic Analysis of Patients with Advanced GIST

Hao Xu[1], Liang Chen[1], Yang Shao[2], Dongqin Zhu[2], Xiaofei Zhi[3], Qiang Zhang[1], Fengyuan Li[1], Jianghao Xu[1], Xisheng Liu[4], and Zekuan Xu[1]

Objectives:

- Evaluate feasibility of ctDNA detection by NGS
- Determine concordance between ctDNA and tissue DNA detection by NGS

Methods:

- Retrospective analysis of prospectively collected tumor tissue and ctDNA
- 32 patients with advanced GIST
- NGS: Hybridization-based target enrichment was performed using GeneseeqOne™ 416-gene panel
- HiSeq 4000 (Illumina) was employed as a sequencing platform

Xu et al, Mol Cancer Ther, 2018

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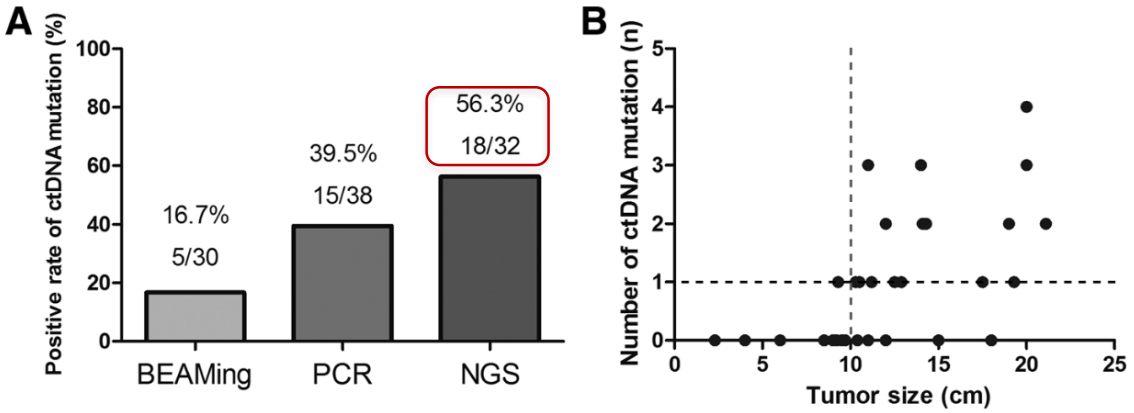
Results

ctDNA Detection Rate by NGS



A) ctDNA detection rate by NGS vs previous reports using BEAMing and PCR

B) The number of ctDNA mutation is positively correlated with tumor size. Almost minimal ctDNA detection when tumor size <10cm

Univariate analysis of influence factors of positive rate of ctDNA detection

Factors	Patients	ctDNA Positive	ctDNA Negative	*P*
Gender				0.341
Male	19	12	7	
Female	13	6	7	
Age				0.712
>70	10	5	5	
≤70	22	13	9	
Primary tumor site				0.178
Stomach	18	12	6	
Non-stomach	14	6	8	
Tumor size				0.004[a]
>10 cm	23	17	6	
≤10 cm	9	1	8	
Mitotic figure				0.358
>5/50 HPF	12	8	4	
≤5/50 HPF	20	10	10	
Risk level				0.182
Very low/Low	5	1	4	
Intermediate	6	3	3	
High	21	14	7	
History of medicine				0.631
Taking imatinib	5	2	3	
None	27	16	11	
Ki-67				0.002[a]
≤5%	13	3	10	
>5%	19	15	4	
Morphology				0.609
Spindle	27	16	11	
Epithelioid	1	0	1	
Mixed	4	2	2	
Total	32	18	14	

NOTE: Tumor size and Ki-67 were the significant influence factors of positive rate of ctDNA detection.

[a]*P* < 0.05.

Results: Concordance

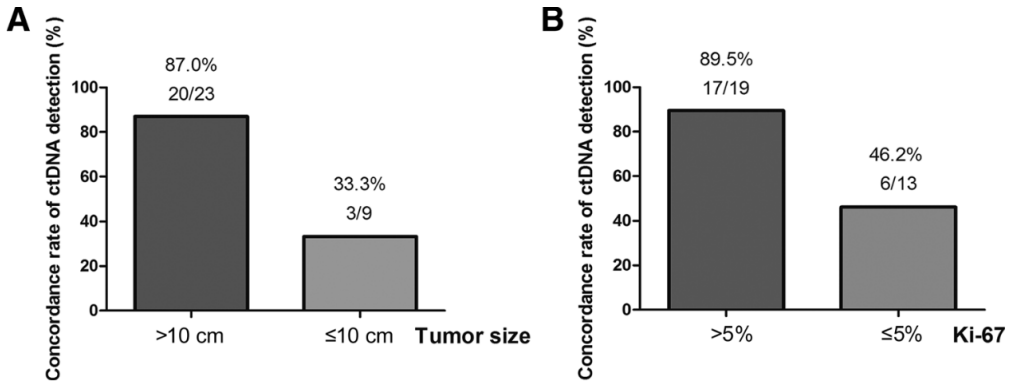
Table 2. Concordance analysis between ctDNA and tissue DNA detections in genetic analysis (Kappa concordance test, $n = 32$)

	Tissue DNA mutation					
ctDNA mutation	**KIT exon 9**	**KIT exon 11**	**KIT exon 14**	**PDGFRA 18**	**WT**	**Total**
KIT exon 9	2	0	0	0	0	2
KIT exon 11	0	14	0	0	0	14
KIT exon 14	0	0	0	0	1	1
PDGFRA 18	0	0	0	1	0	1
WT	3	4	0	1	6	14
Total	5	18	0	2	7	32

NOTE: In all patients, the concordance was moderate (weighted Kappa = 0.489, $P < 0.001$).

Concordance rate: 72% (23/32)
Moderate Concordance

Concordance higher in individuals with larger tumors (>10cm) and ki67 index >5%



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Monitoring response to therapy

Monitoring response to therapy

- Radiological response assessment criteria
  - RECIST
  - CHOI
  - PERCIST

- Tumor Markers
  - Prostate Cancer – PSA
  - Ovarian Cancer – Ca125
  - Long half lives
  - Not always available for each cancer type – e.g., GIST

Monitoring response to therapy - ctDNA

- Advantages
  - ctDNA – good potential biomarker of response
    - Short half life
    - High specificity
    - Accurate
- Setting
  - Neoadjuvant setting
    - Assess response to imatinib
    - Optimal time of resection
  - Metastatic setting
    - Facilitate treatment decisions in timely fashion

Monitoring response to therapy

- Prospective studies have shown that changes in levels of mutational burden detected by sequenced ctDNA in GIST has been shown to correlate with
    - Tumor volume
        - Higher levels with progressive disease
    - Response to treatment
        - Lower levels with response to treatment[1, 2, 3]

1. Meier S, et al, Clin Cancer Res 2013
2. Heinrich M, et al, ASCO annual meeting 2015
3. Janku F, et al, AACR annual meeting 2017

Correlation of ctDNA and Response in PDGFRα D842 GIST Treated with Avapritinib

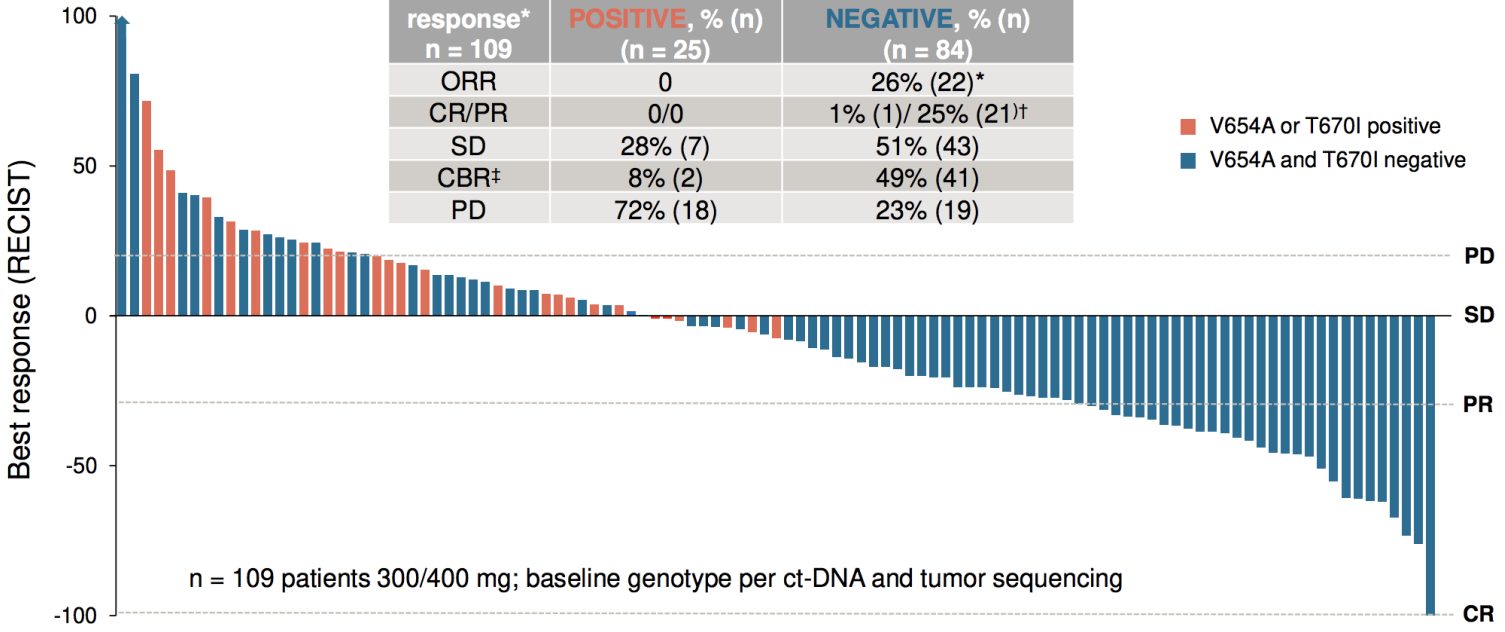
- Navigator Trial - evaluated
  - baseline ctDNA levels
  - changes in ctDNA during treatment with avapritinib
  - relationship to clinical outcomes
- ESMO 2018 poster presentation of this data focused on D842V mutant GIST cohort
- Majority of patients, ctDNA levels fell below the limit of detection after 2 months
- Lower baseline ctDNA levels were predictive of prolonged PFS
- Large reductions in ctDNA on treatment were associated with high baseline ctDNA, but were not predictive of prolonged PFS
- Baseline ctDNA levels may have utility as a predictive biomarker; however, changes in on-treatment ctDNA levels should be interpreted with caution and in the context of baseline ctDNA

George S. et al, ESMO 2018

Phase I study BLU 285

Best response by mutational profile detected by ctDNA in ≥4L GIST

Best response* n = 109	V654A or T670I POSITIVE, % (n) (n = 25)	V654A and T670I NEGATIVE, % (n) (n = 84)
ORR	0	26% (22)*
CR/PR	0/0	1% (1)/ 25% (21)†
SD	28% (7)	51% (43)
CBR‡	8% (2)	49% (41)
PD	72% (18)	23% (19)



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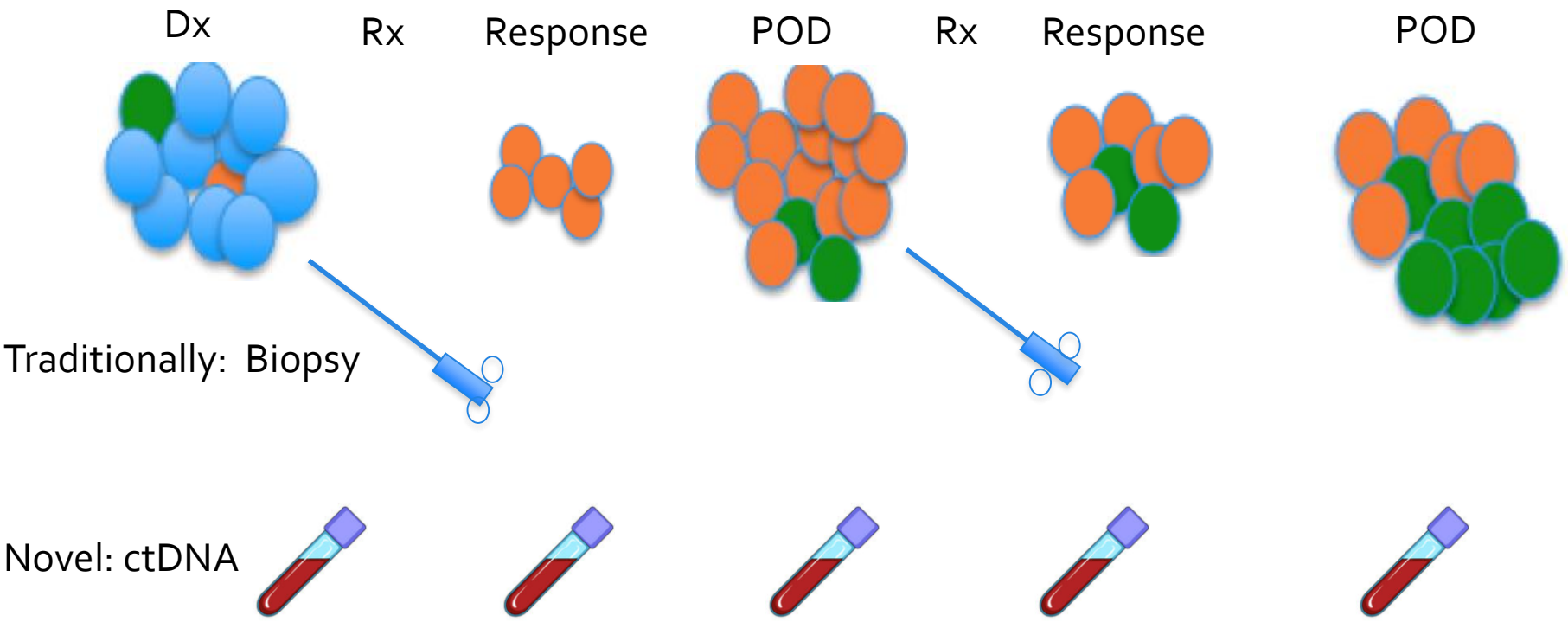
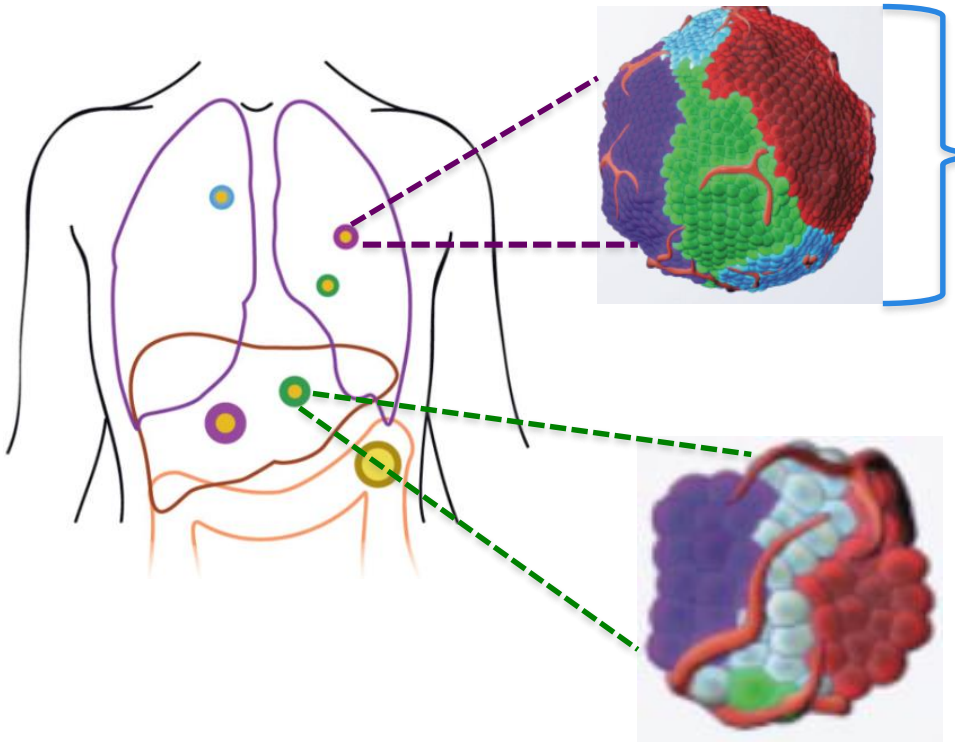
Detection of Resistance to Therapy

Detection of Resistance - Polyclonal



Capture Tumor Heterogeneity



Intra-lesional

Inter-lesional

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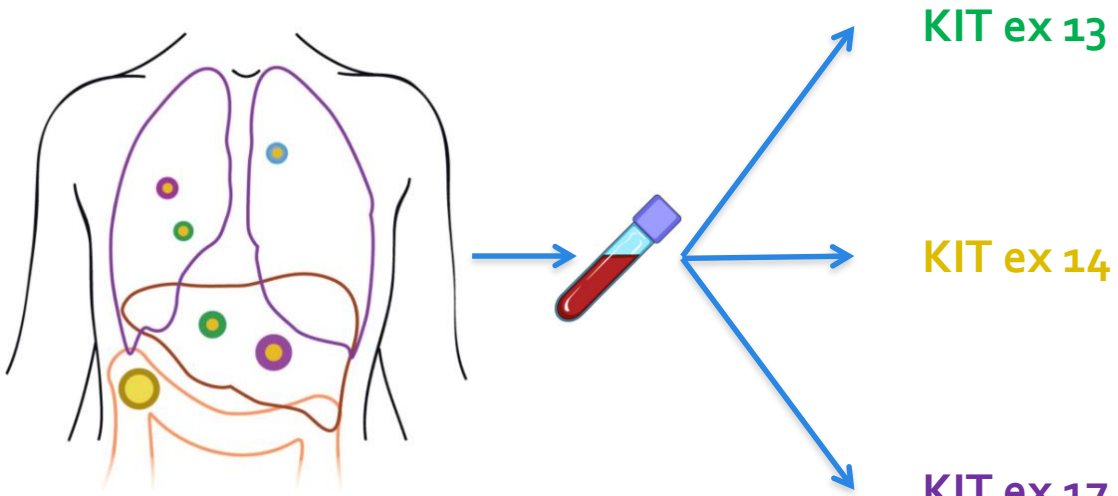
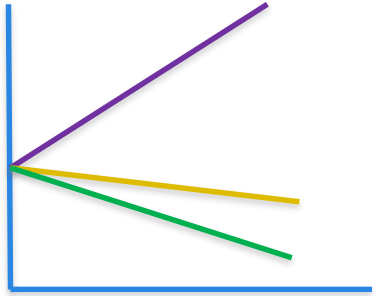
Monitoring response of tumor specific subclones to therapy





Assess response at a molecular level

IMPORTANCE IN PATIENT SELECTION AND DESIGN OF FUTURE CLINICAL TRIALS

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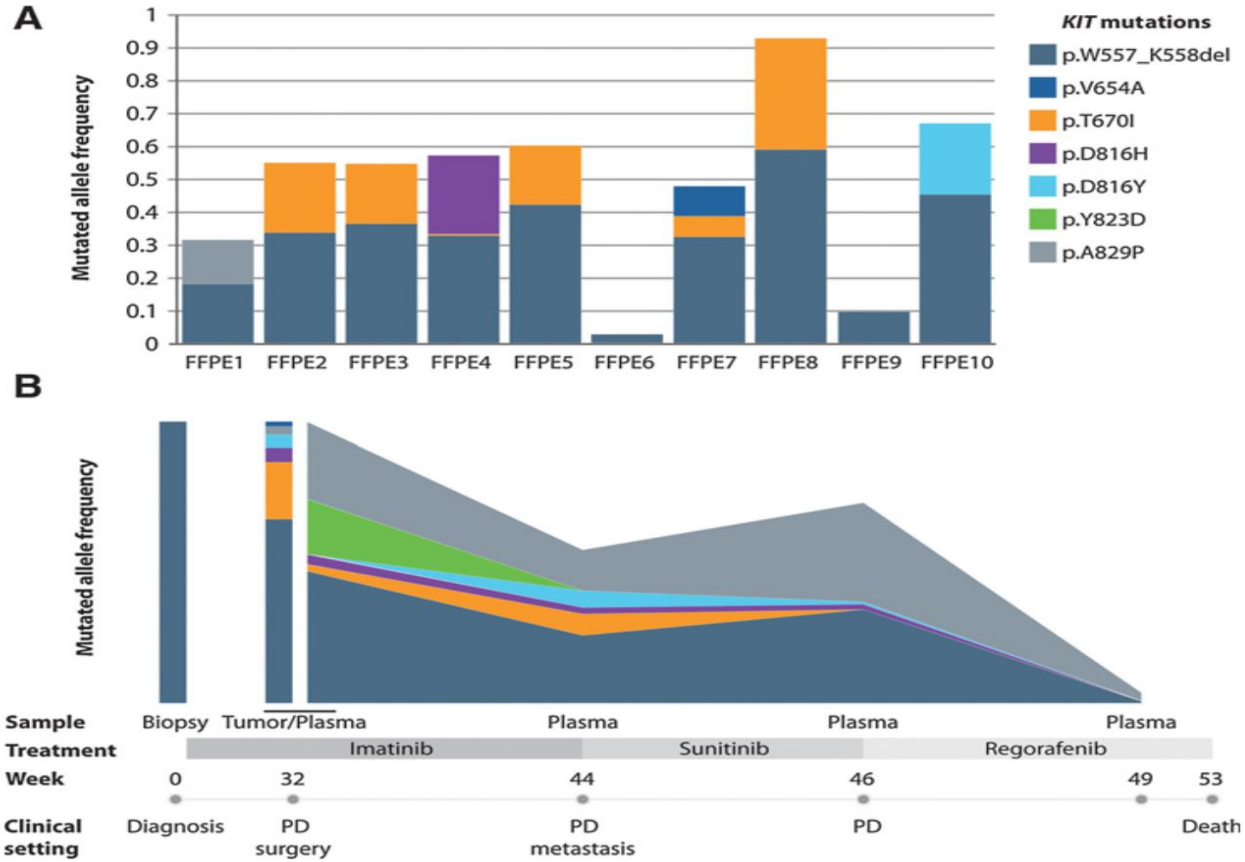
Detection of tumor heterogeneity by ctDNA



Namløs HM et al, Mol Cancer Ther, 2018

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Context of Use – Factors to Consider in Developing sequenced ctDNA technology in GIST

- What clinical factors influence tumor shedding and the ability to detect ctDNA
  - Sites of disease
    - Does the predominant site of disease influence the detection rate of ctDNA
    - Liver > peritoneum
  - Primary tumor in situ/resected
  - Clinical status of disease
    - Progressive state – more likely to capture ctDNA
    - Low tumor burden – high false positive rates (noise : tumor ratio rises)
  - Treatment ongoing at time of ctDNA collection
    - Do certain treatments reduce tumor shedding more than others
- These factors may influence the sensitivity of the assay used to sequence ctDNA in order to accurately detect the molecular landscape of GIST
- Effective tool when used in the right patients at the right time

Further development of ctDNA in GIST

- Prospective correlative studies are the ideal to obtain data
- A bigger NGS panel is not necessarily better
  - A focused targeted assays could allow for maximal sensitivity and specificity
  - Especially reasonable in GIST where limited number of genes have been shown to be recurrently mutated in NGS analysis
- Plasma genomic sequencing is aided by a QC step – improves performance of the test
  - Eliminate samples with insufficient DNA for analysis
  - False negatives
    - Revert to gold standard – molecular assessment of tumor tissue

- Determine concordance rate for detecting molecular spectrum of GIST between plasma derived ctDNA and tumor tissue
- Understand how clinical factors impact the analysis of ctDNA
- Clinical utility is hard to prove
  - Prospective clinical trial
  - Sequenced ctDNA – diagnostic biomarker – selects pts for rx
  - Therapeutic phase to assess the impact of the diagnostic biomarker of the efficacy of treatment

Economics of sequenced ctDNA in GIST

- Short term – additional cost
  - ctDNA extraction
  - Expertise
  - Sequencing technology
- Long-term - cost saving
  - Replace invasive tissue biopsies
  - Companion diagnostic test - optimize therapeutic selection
    - Minimize use of ineffective therapies
  - Better selection of pts requiring adjuvant therapy

CONCLUSION

- ctDNA – potential blood biomarker of clinical and molecular behavior of GIST
  - Sequencing technology is evolving
  - Optimize assay to improve sensitivity of detection

- Routine collection of ctDNA in prospective clinical trials in GIST is necessary to move advance this technology forward

Conclusion

- Integration of ctDNA in to clinical trial design – importance:
  - Determine concordance rate between detection of molecular spectrum of GIST in seqeunced ctDNA and tumor tissue
  - Develop sequenced ctDNA as a companion diagnostic test and predictive biomarker for novel agents
  - Complementary method of response evaluation
  - Guide therapeutic selection – more efficient manner
  - Describe the plasticity of GIST cells during mets process
  - Identifying mechanisms of resistance
  - Tracking tumor specific subclones – molecular basis of response
  - Identify novel therapeutic strategies to overcome resistance