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Circulating Tumor DNA in GIST and its Implications on Treatment

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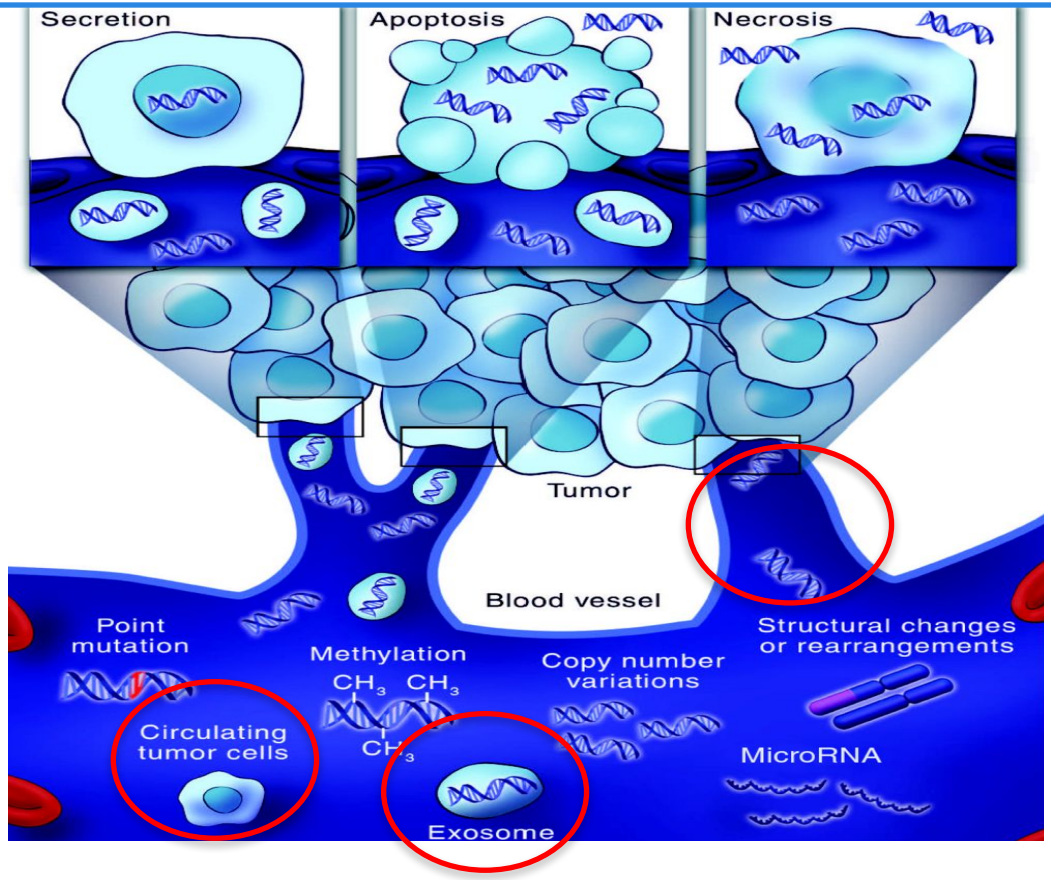
Objectives

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

Background- Liquid biopsy

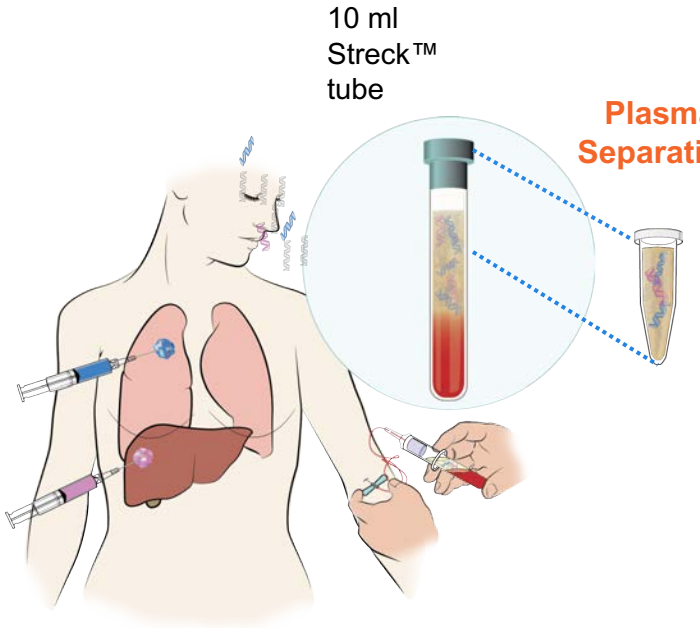


Diaz and Bardelli, JCO 2014

Circulating Tumor DNA vs Cell Free 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

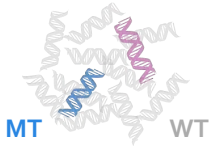
Sample Collection



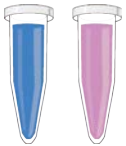
Plasma Separation

cfDNA Extraction

cfDNA Quantification



DNA Extraction From matched normal specimens +/- tumor FFPE



DOWNSTREAM ANALYSIS of Tumors Genomic Landscape

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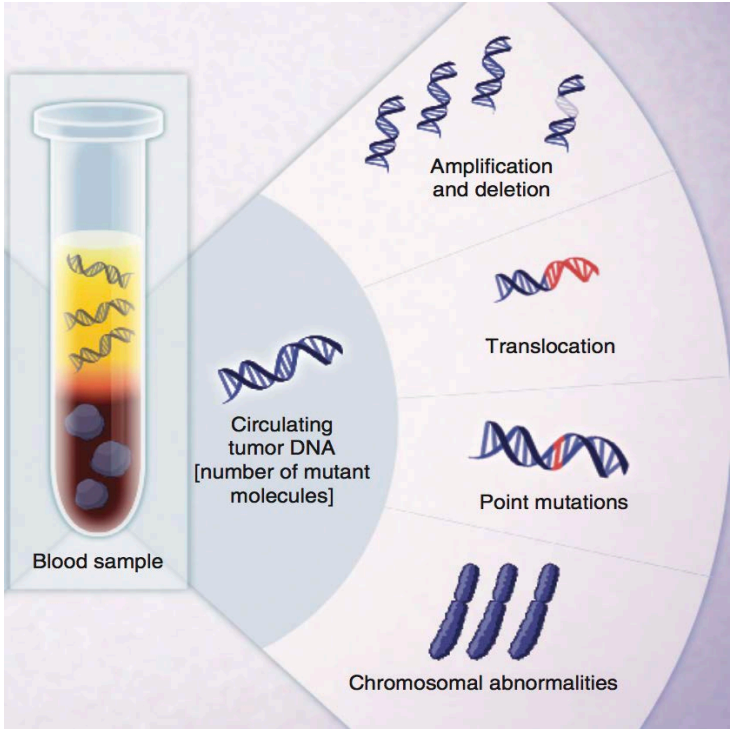
Downstream analysis

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.

Haber, Cancer Disc 2014

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 sample
  - Compare sequenced ctDNA and germline sample
  - Allows for unambiguous detection of tumor specific DNA
- Further evaluate sequenced ctDNA samples that fail to identify somatic mutations
  - Determine if adequate ctDNA present for analysis
  - Accuracy of seqeuncing ctDNA improves if a QC step is used to identify and eliminate samples with insufficient DNA that yield inconclusive results

FDA Approval of Liquid Biopsy test in Lung Cancer

June 1, 2016, the U. S. FDA approved **cobas** EGFR Mutation Test v2 using plasma specimens as a companion diagnostic test to detect specific EGFR mutations to identify patients with metastatic non-small cell lung cancer (NSCLC) eligible for treatment with Tarceva® (erlotinib).

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FDA Approval of Liquid Biopsy test in Lung Cancer

2013

Molecular Testing Guideline for Selection of Lung Cancer Patients for EGFR and ALK Tyrosine Kinase Inhibitors

Guideline from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology

Neal I. Lindeman, MD, Philip T. Cagle, MD, Mary Beth Beasley, MD, Dhananjay Arun Chitale, MD, Sanja Dacic, MD, PhD, Giuseppe Giaccone, MD, PhD, Robert Brian Jenkins, MD, PhD, David J. Kwiatkowski, MD, PhD, Juan-Sebastian Saldivar, MD, Jeremy Squire, PhD, Erik Thunnissen, MD, PhD, and Marc Ladanyi, MD

FDA Approval of Liquid Biopsy test in Lung Cancer

Annals of Oncology 26: 1883–1889, 2015
doi:10.1093/annonc/mdv270
Published online 23 June 2015

First-line erlotinib versus gemcitabine/cisplatin in patients with advanced *EGFR* mutation-positive non-small-cell lung cancer: analyses from the phase III, randomized, open-label, ENSURE study†

Y.-L. Wu[1*], C. Zhou[2], C.-K. Liam[3], G. Wu[4], X. Liu[5], Z. Zhong[6], S. Lu[7], Y. Cheng[8], B. Han[7], L. Chen[9], C. Huang[10], S. Qin[11], Y. Zhu[12], H. Pan[13], H. Liang[14], E. Li[15], G. Jiang[16], S. H. How[17], M. C. L. Fernando[18], Y. Zhang[19], F. Xia[19] & Y. Zuo[19]

- 99% of the 217 pts enrolled had tumor tissue and plasma samples available for analysis
- High concordance between Cobas test detecting EGFR mutations in tissue and plasma
  - 77% of tissue positive specimens - plasma detected EGFR mutation
  - 98% of tissue negative specimens – plasma was also negative

ROLE OF ctDNA IN GIST

Localized Disease

- Detecting minimally residual disease - ?Risk of recurrence
- Therapeutic selection
- Detection of Recurrence

Metastatic Disease

- Therapeutic Selection
- Monitoring Response

Refractory Disease

- Detection of mechanisms of resistance
- Appropriate therapeutic selection in timely fashion
- Capturing tumor heterogeneity and subclone-specific response

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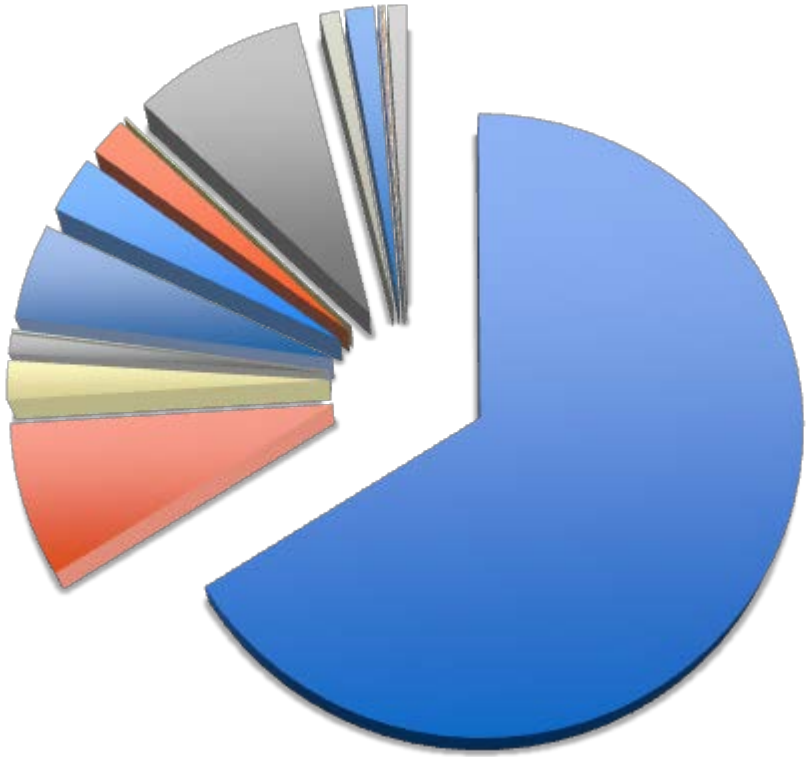
Therapeutic Selection

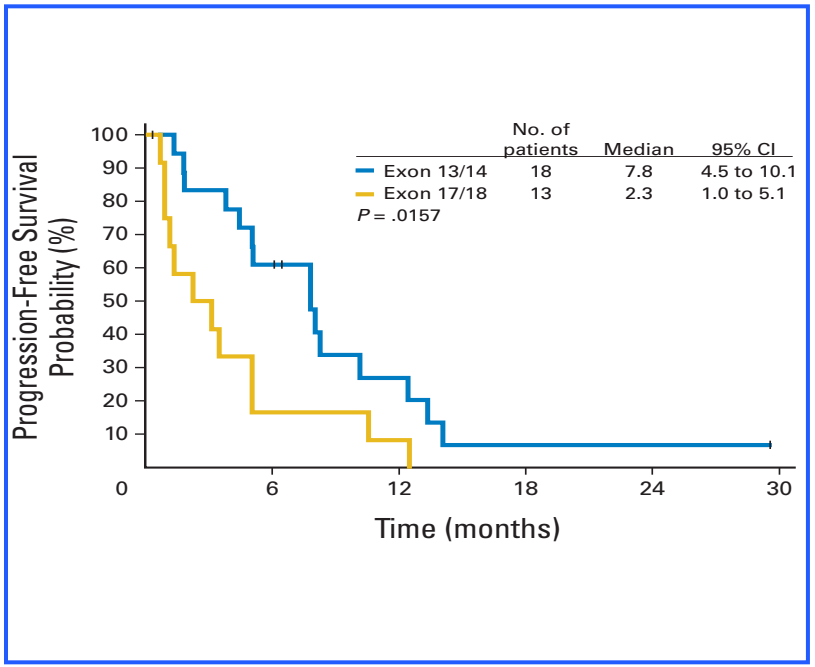
Molecular Classification of GIST



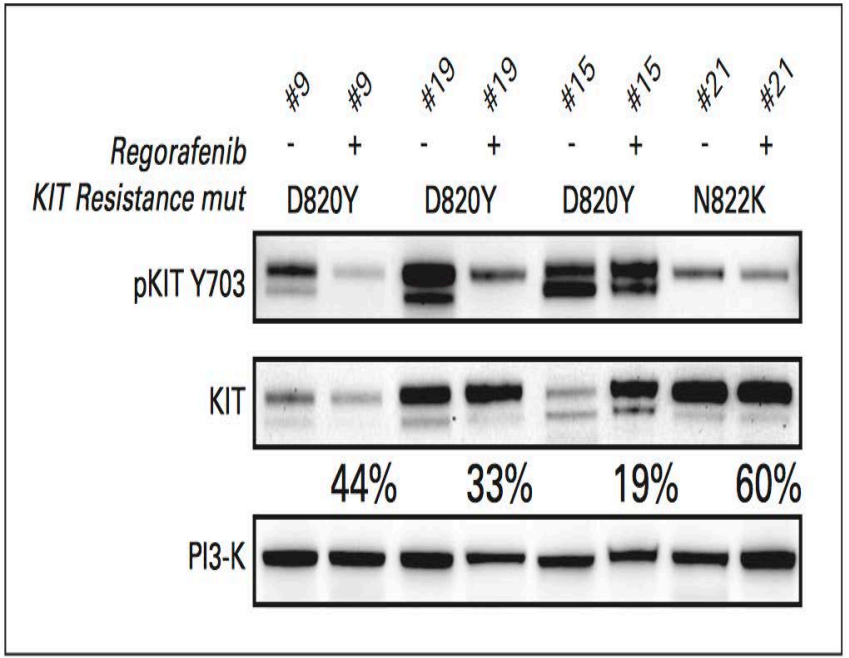
Adapted from Bannon AE et al, Expert Rev Mol Diagn 2017

Therapeutic Selection – TKI refractory setting

Sunitinib - Inhibitory Activity Against ATP Binding Pocket Mutations



Regorafenib – Inhibitory Activity Against Activation Loop Kinase Mutations



Heinrich et al, J Clin Oncol, 2008
George et al, J Clin Oncol, 2012

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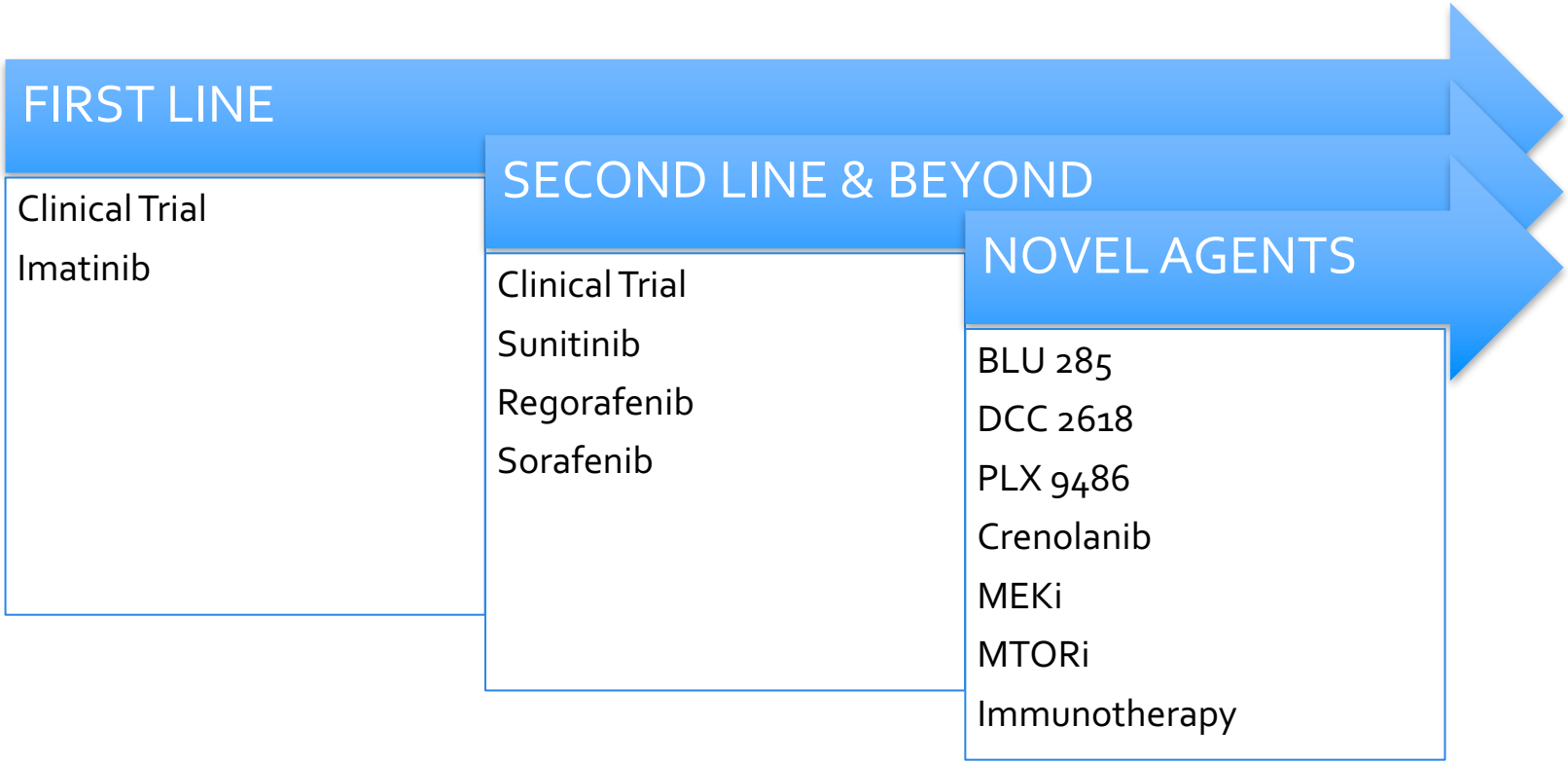
Therapeutic Landscape for Advanced GIST



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What Informs Therapeutic Choices Patients with GIST?

TUMOR GENOMICS

< 15% of patients with GIST have their tumors genotyped

CLINICAL CHARACTERISTICS

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 tissue specimens
  - Detection of primary KIT mutations-high concordance rate (>80%)[3,5]
  - 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
5. Boonstra P, et al, AACR annual meeting 2016

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Monitoring response to therapy

Methods to monitor 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
    - Optimal time of resection
  - Adjuvant setting
    - Effectiveness of adjuvant imatinib
  - 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

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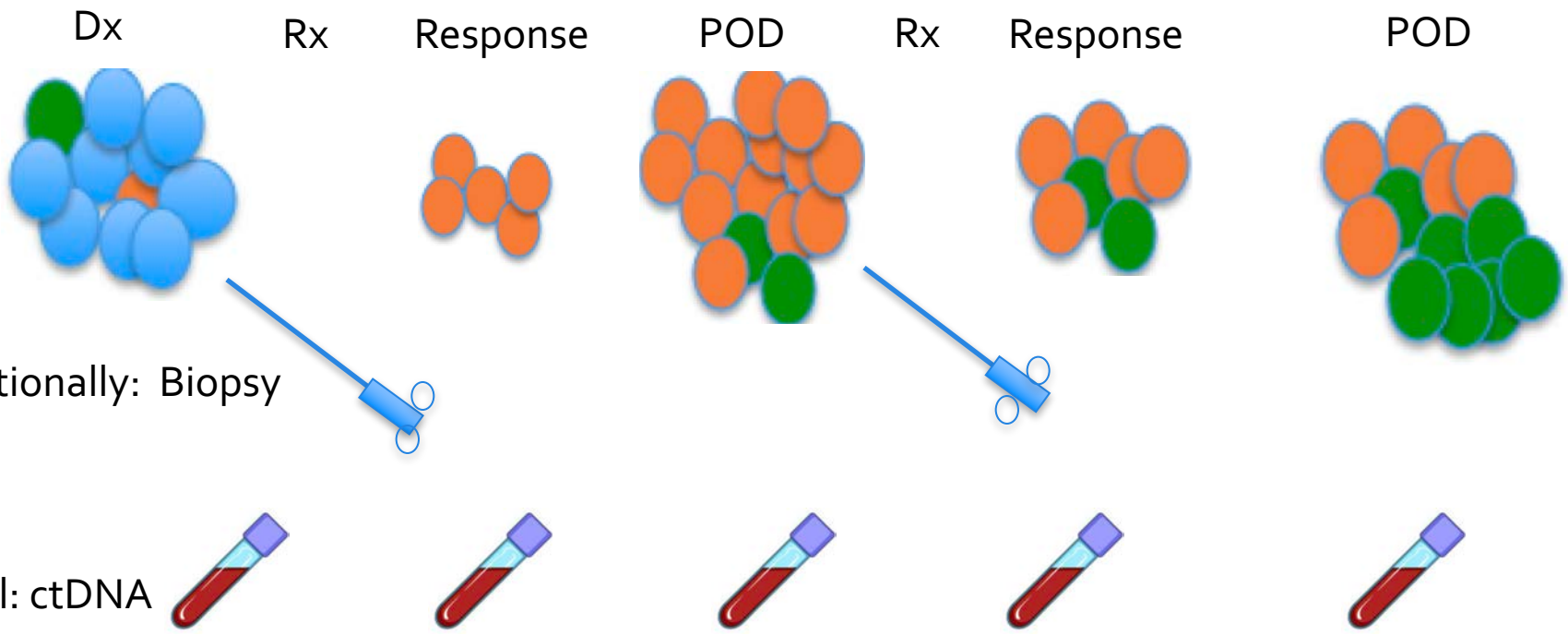
Detection of Resistance to Therapy

Detection of Resistance - Polyclonal



Advantages of ctDNA over tissue biopsy: non-invasive, less expensive, capture heterogeneity, monitor response at a molecular level

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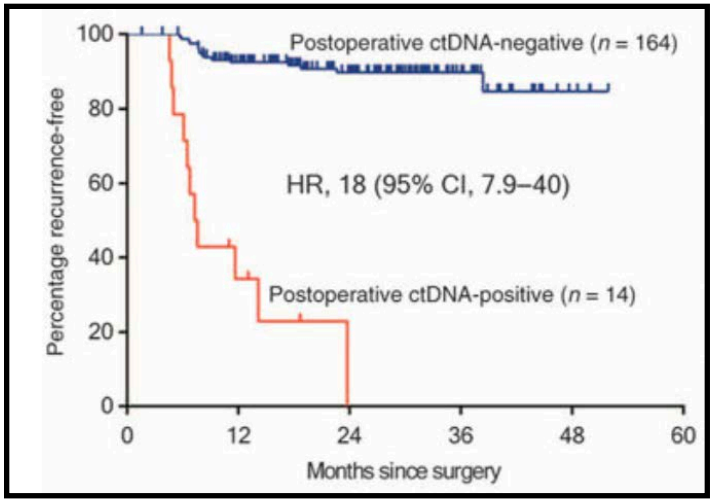
Detection of Minimal Residual Disease

Detection of Minimal Residual Disease

- Adjuvant setting
  - Studies including early stage breast and CRC pts have shown that ctDNA detection post-operatively correlated with risk of relapse



Prospective study: n=230, stage II CRC

Post-op: ctDNA detected in 8%

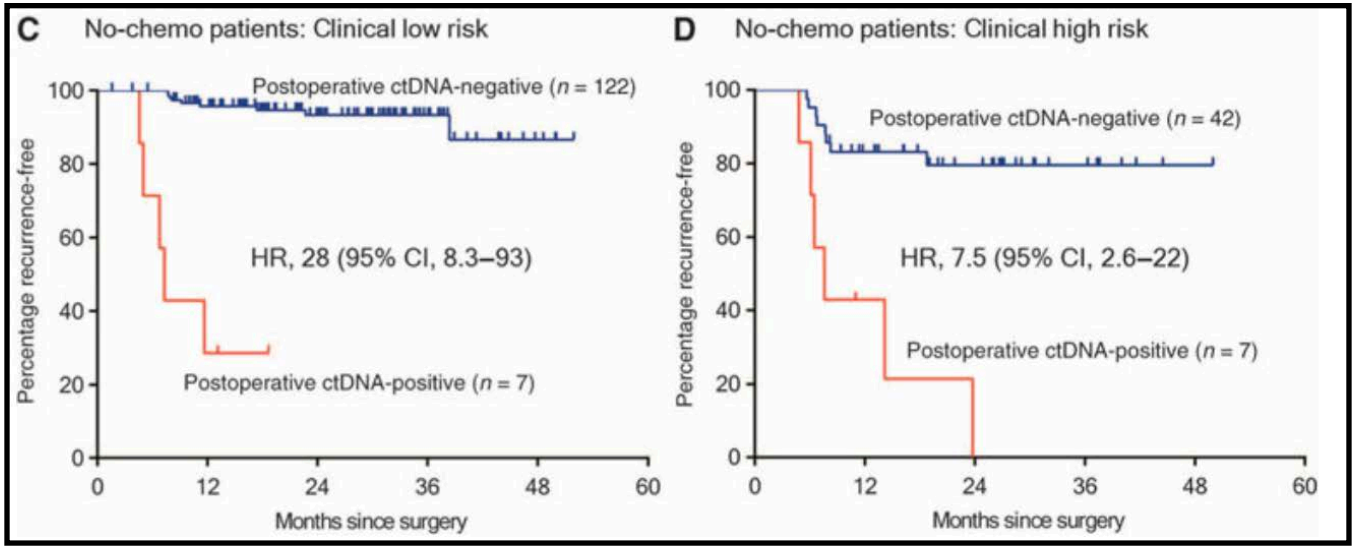
ctDNA +ve - recurrence rate 79%

ctDNA –ve – recurrence rate 10%

Tie J, et al, Sci Transl Med, 2017
Garcia-Murillas I, et al, Sci Transl Med, 2015

Detection of Minimal Residual Disease

- Prognostic impact of post-op ctDNA was independent of individual clinicopathological risk features and improved RFS estimates for both high and low risk groups



Tie J, et al, Sci Transl Med, 2017

Detection of Minimal Residual Disease

- Potential utility of ctDNA in this setting in GIST
  - Assist in risk stratification of pts in post-op setting
  - Molecularly characterize residual disease – therapeutic selection
  - Monitor impact of adjuvant therapy
  - Detect recurrence earlier than imaging

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Future Directions

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 assay 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

Development of ctDNA in GIST

- 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 trials are warranted
  - Incorporating a diagnostic and therapeutic phase
  - Diagnostic phase - molecular genotyped determined by sequenced cfDNA
  - Therapeutic stratification based on this result
  - Therapeutic phase - assess impact of therapeutic stratification by sequenced cfDNA on clinical outcomes

Unanswered Questions: Context of Use

- 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
  - 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)
  - Ongoing treatment 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

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
  - Further development required
  - Sequencing technology is evolving
    - Improve sensitivity of detection

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

Conclusion

- Integration of sequenced ctDNA into clinical trial design – importance:
  - Determine **concordance rate** between detection of molecular spectrum of GIST in sequenced ctDNA vs 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 metastatic process**
  - Identifying **mechanisms of resistance**
  - Track tumor specific sub-clones – **molecular basis of response**

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Thank You