Liquid Biopsies As Guides to Therapy Selection in Genitourinary Cancers

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Disclosures

- Employee of Foundation Medicine and Equity owner in Roche Holdings
- Consultant and Equity owner of Tango Therapeutics
- Consultant and equity owner of Celsius Therapeutics

The Rise of Biomarker-Driven Treatment

- Solid tumor
- Hematologic malignancy
- Tumor-agnostic
- Immunotherapy

1998-2002

- Trastuzumab Breast 1998
- Imatinib CML, 2001 GIST, 2002
- Imatinib GIST 2002

2003-2007

- Gefitinib Lung 2003
- Erlotinib Lung 2004
- Sunitinib GIST 2006
- Dasatinib CML 2006
- Nilotinib CML 2007

2008-2012

- Lapatinib Breast 2008
- Vemurafenib Melanoma 2011
- Crizotinib Lung 2011
- Vandetanib Thyroid 2011
- Ruxolitinib MF 2011
- Bosutinib CML 2012
- Pertuzumab Breast 2012

2013-2016

- TDM-1 Breast 2013
- Afatinib Lung 2013
- Dabrafenib Trametinib Melanoma 2014
- Olaparib Ovarian 2014
- Nivolumab Melanoma 2014
- Pembrolizumab Lung 2014
- Ceritinib Lung 2014
- Vemurafenib Cometinib Melanoma 2015
- Alectinib Lung 2015
- Ibrutinib CLL 2015
- Atezollzumab Bladder 2016
- Venetoclax CLL 2016
- Rucaparib Ovarian 2016

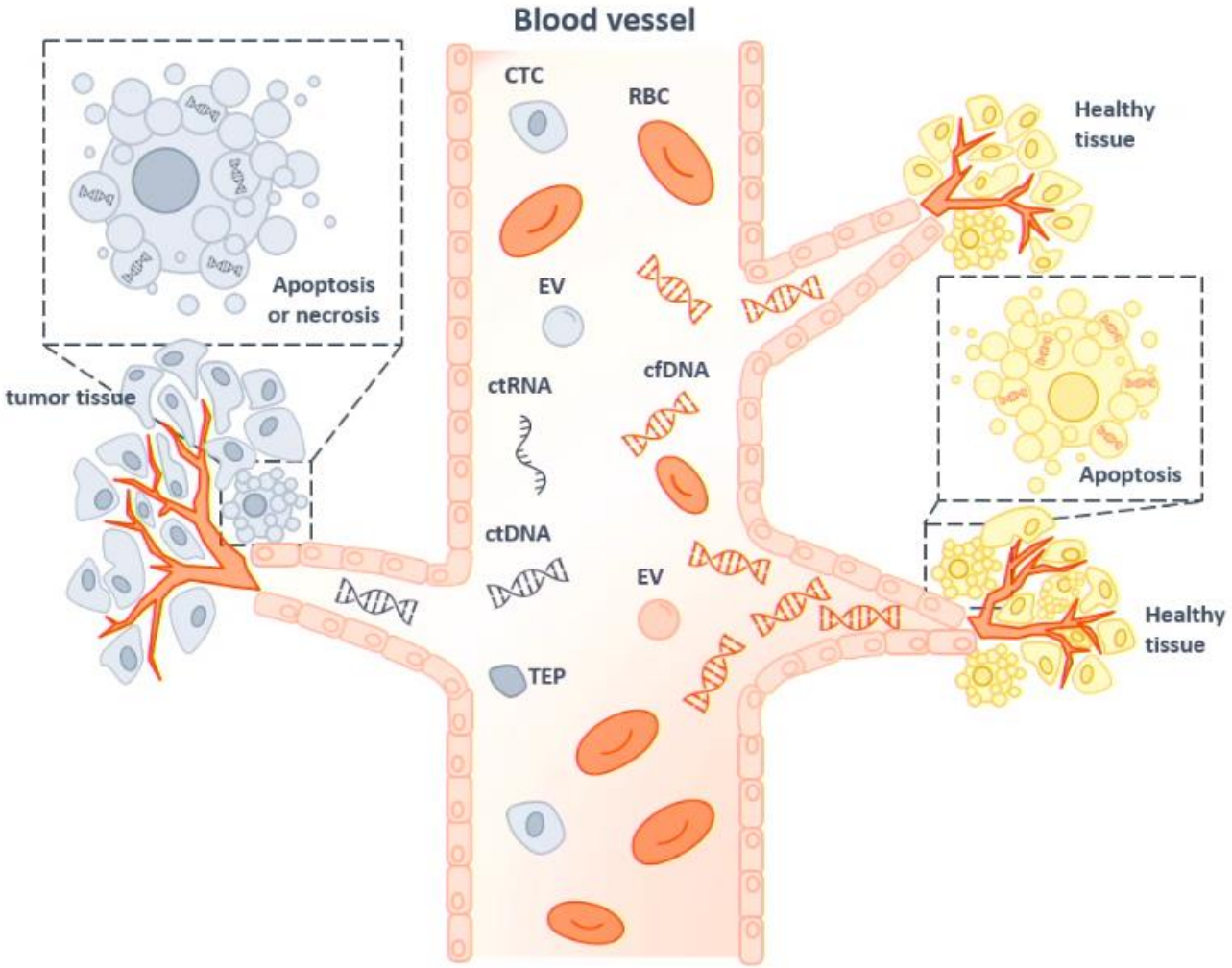
2017-present

- Nivolumab dMMR/ MSI-H colorectal 2017
- Midostaurin AML 2017
- Neratinib Breast 2017
- Osimertinib Lung 2017
- Brigatinib Lung 2017
- Pembrolizumab All rMSI-H 2017
- Olaparib Breast 2018
- Dacomitinib Lung 2018
- Lorlatinib Lung 2018
- Gilteritinib AML 2018
- Enasidenib Ivosidenib AML 2018
- Talazoparib Breast 2018
- Larotrectinib All NTRK-fusion positive 2018
- Entrectinib Lung 2019
- Alpelisib Breast 2019
- Erdafitinib Bladder 2019
- Entrectinib[2] All NTRK-fusion positive 2019
- Atezolizumab TNBC 2019
- Margetuximab Breast 2020
- Capmatinib Lung 2020
- Pembrolizumab All TMB-high 2020
- Rucaparib Prostate 2020
- Olaparib Prostate 2020
- Pemigatinib CCA 2020
- Amivantamab Lung 2021
- Sotorasib Lung 2021
- Dostarlimab All dMMR 2021
- Infigratinib CCA 2021

AML = acute myeloid leukemia; CCA = cholangiocarcinoma; CLL = chronic lymphocytic leukemia; CML = chronic myeloid leukemia; dMMR = mismatch repair deficient; GIST = gastrointestinal stromal tumor; MF = myelofibrosis; MSI-H = microsatellite instability-high; NTRK = neurotrophic tyrosine receptor kinase; TMB = tumor mutational burden; TNBC = triple-negative breast cancer.
*Selected targeted therapies whose FDA-approved use requires the targeted biomarker to be present.

1. FDA. Hematology/Oncology (Cancer) Approvals & Safety Notifications. Updated January 1, 2021. Accessed October 12, 2021. www.fda.gov/drugs/informationondrugs/approveddrugs/ucm279174.htm; 2. FDA. FDA approves entrectinib for NTRK solid tumors and ROS-1 NSCLC. Updated August 16, 2019. Accessed February 12, 2021. https://www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-entrectinib-ntrk-solid-tumors-and-ros-1-nsclc. 3. Data on file, Foundation Medicine Inc., October 2021.

Blood Provides a Rich Source of Tumor-derived Material



Circulating tumors cells (CTCs) are shed into circulation from primary tumor and metastases[3,4]

Healthy and tumor tissue release **cell-free DNA and RNA** into circulation through apoptosis, necrosis and lysis of circulating cells[3,5,6]

Membrane-encapsulated **extracellular vesicles** (EVs) are released from healthy and tumor cells[3]

Tumor-educated platelets (TEPs) may contain tumor-derived RNA and alternatively spliced transcripts[3]

cfDNA: cell-free DNA; ctDNA: circulating tumor DNA; ctRNA: circulating tumor RNA; CTC: circulating tumor cell; EV: extracellular vesicle; TEP: tumor-educated platelet; RBC: red blood cell. Figure adapted from references 1-3. 1. Dahl et al. (2015) Pathologe 36:572-8; 2. Alix-Panabières, C. & Pantel, K. (2013) Nat Rev Clin Oncol 10:472-84; 3. De Rubis, G., et al. (2019)Trends Pharmacol Sci 40:172-86; 4. Yu, M., et al. (2011) J Cell Biol 192:373-82; 5. Francis, G. & Stein, S. (2015) Int J Mol Sci 16:14122-42; 6. Bettegowda, C., et al. (2014) Sci Transl Med

Tissue and Liquid Biopsies

Solid vs liquid biopsy

Solid biopsy

e.g. surgical biopsy / excision
or fine needle aspirate[1]

+ Considered the 'gold standard' for cancer diagnosis and allows both morphological and molecular assessment[1-3]

- Involves a relatively invasive procedure[1,2]
- May not be feasible for some tumors, especially when not amenable or when highly necrotic[1-4,7,8]
- May not provide sufficient sample for all necessary pathological workup[1,3]
- Requires more surgical infrastructure and has longer turn-around time than liquid biopsy[9,5]
- Is not suitable for longitudinal monitoring[2]
- Single site biopsy may not represent tumor heterogeneity[10]

Liquid biopsy

e.g. blood, urine, saliva
or cerebrospinal fluid[1,2,4]

- Not yet comparable to solid biopsy with respect to evidence for clinical utility and applicability in initial cancer diagnosis and management[2,5,6]

+ Less invasive than solid biopsy[1,2]
+ May be used when tissue biopsies cannot be performed due to inaccessibility[1,4]
+ Provides an option when tissue samples are limited or exhausted[1]
+ Requires less surgical infrastructure and has shorter turn-around time than tissue biopsy[9,5]
+ Is suitable for repeat sampling during longitudinal monitoring[2,5]
+ Can capture the genomic heterogeneity of all cancerous lesions[10]

1. Francis, G. & Stein, S. (2015) Int J Mol Sci 16:14122-42; 2. De Rubis, G., et al. (2019) Trends Pharmacol Sci 40:172-86; 3. Chouaid, C., et al., (2014) Lung Cancer 86:170-3; 4. Bardelli, A., et al. (2017) Cell 31:172–9; 5. Wan, J.C.M., et al., (2017) Nat Rev Cancer 17:223-38; 6. Cottox, A.K. (2019) Sci Transl Med 11:eeay1984; 7. Kato, S., et al. (2017) Cancer Res 77:4238-46; 8. Stevenson, M., et al. (2014) Cancer Invest 32:291–8; 9. Temilola , D.O., et al. (2019) Cells , 8, 862; doi:10.3390/cells8080862; 10. Scherer, F. (2020) in Recent Results

Overview of the Liquid Biopsy

- Circulating Tumor Cell assays
  - Launched in 2003
  - Relatively slow adoption
  - Utilized in diseases with high tumor cell shedding rates: prostate cancer
  - Difficult to perform molecular tests on captured cells
- ctDNA (circulating tumor DNA extracted from blood)
  - Popularly known as “The Liquid Biopsy”
    - NGS-based for mutation detection and characterization
    - Non-NGS based for “molecular monitoring”
    - Also in widespread development for early cancer detection
- Other “liquid” samples used for Molecular Studies
  - Bone marrow for hematologic malignancies
  - Urine for bladder, renal and prostate cancers
  - CSF for brain and spinal cord tumors

The Liquid Biopsy (1)

- Detection of ctDNA uses NGS techniques
- Two main types of assays:
  - Hybrid-capture based
  - Non-hybrid capture based
- Two major commercial Liquid Biopsy assays in the USA
  - Guardant 360 (1 companion diagnostic FDA-approved indications)
  - Foundation One Liquid CDx (24 companion diagnostic FDA-approved indications)
- Can serve as a surrogate indicator of overall “disease burden”
  - Too expensive to be used as a frequently ordered monitoring test
  - “Tumor Fraction” emerging as a prognosis guide
- Primary role is to identify genomic alterations that can indicate potential benefit of targeted therapies
- Can provide information on biomarkers associated with immune checkpoint inhibitor response
  - bTMB
  - MSI
  - Others

The Liquid Biopsy (2)

- Different primary tumor types have different frequencies of yielding informative liquid biopsy results
  - Some tumors shed DNA from intact and apoptotic cells more easily than others
    - Breast, prostate and lung cancers more readily shed ctDNA into peripheral blood
    - Pancreas, ovary less readily shed ct DNA into peripheral blood
    - CNS tumors (GBM) rarely if ever yield informative ctDNA in peripheral blood
  - In many settings, tumor stage is more predictive of successful liquid biopsies than tumor type
- The use of other biomarkers to “predict” obtaining an informative liquid biopsy is emerging:
  - PSA of 5 ng/dL as a “requirement” to order a liquid biopsy on a prostate cancer patient
  - Other serum ELISA-based biomarkers in other tumor types guiding liquid biosy selection: CEA, CA 19-9, others
- Clonal hematopoiesis genes (“CHiP”) must be differentiated in liquid biopsies
- A non-informative liquid biopsy is costly and loses time for the patient
- When deciding between a liquid biopsy and a metastatic site tissue biopsy for sequencing:

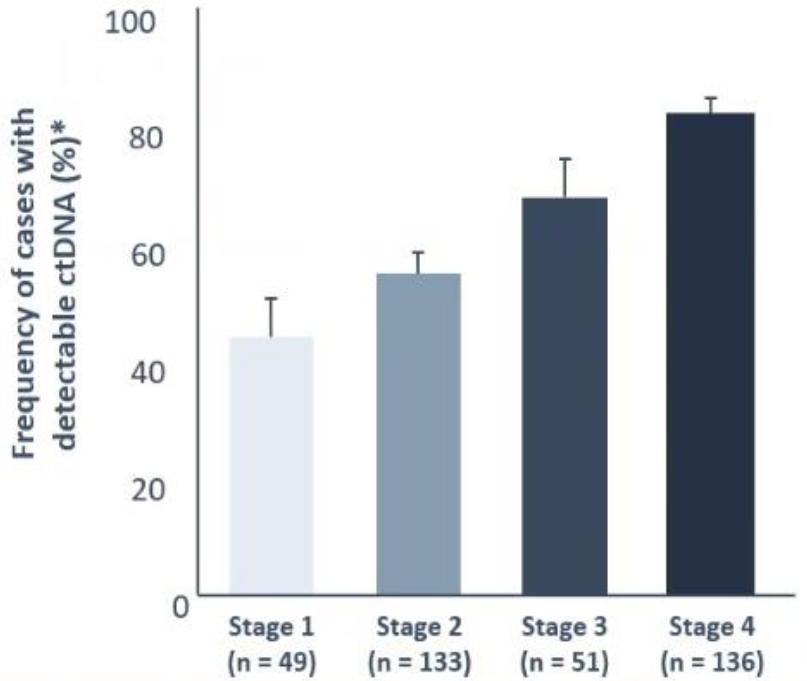
“It is not tissue first. It is not liquid first. It is the patient first”

Analysis of ctDNA Poses Multiple Challenges

ctDNA

- constitutes a **highly variable fraction** of the total plasma cfDNA **from < 0.1% to > 90%**[1,2]
  - if ctDNA fraction is low, detection of **alterations is more challenging**[2,3]
  - need to be able **to detect mutations down to ≤ 0.1%** MAF (particularly for detection of MRD)[3,4]
- is more **fragmented at 134 - 144 bp**, compared with ~166 bp fragments of 'normal' plasma cfDNA[5]
- has a very short **half-life of less than one hour** in circulation[2,6]

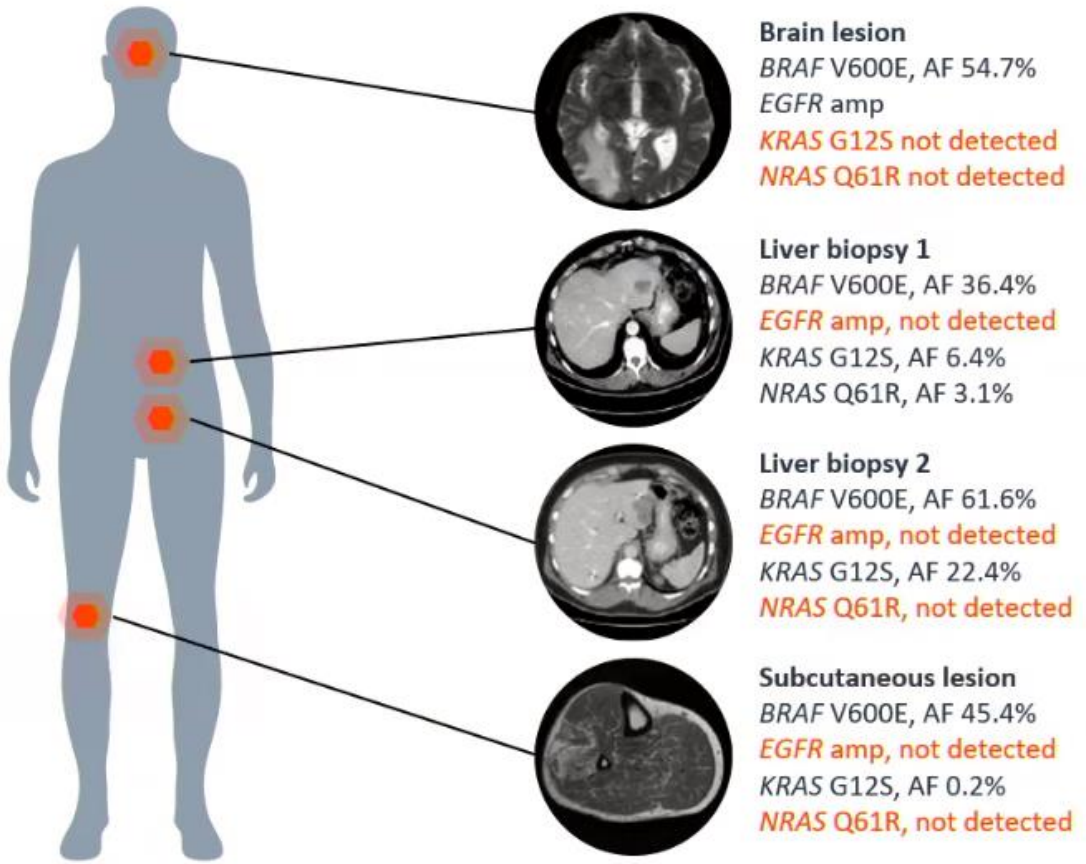
Amount of shedded, or detectable, ctDNA is variable depending on factors such as tumor stage, histology, vascularity and treatment[1,5-8]



Somatic cfDNA alterations were detected in **85%** (18,503 / 21,807) of patients across various cancer types[9]

*Figure adapted from reference 5. cfDNA: cell-free DNA; ctDNA: circulating tumor DNA; MAF: mutant allele frequency; MRD: minimal residual disease. 1. Hinrichsen, T., et al. (2016) J Lab Med 40:313-22; 2. Corcoran, R.B. and Chabner, B.A. (2018) N Engl J Med 379:1754-65; 3. Johansson, G., et al. (2019) Biomol Detect Quantif 17:100078; 4. Jennings, L. et al. (2017) J Mol Diagn, 19:341-65 5. Wan, J.C.M., et al., (2017) Nat Rev Cancer 17:223-38; 6. Mattox, A. K., et al (2019) Sci Transl Med 11:eaay1984; 7. Bettegowda, C., et al. (2011) Sci Transl Med 6:224ra24; 8. Diaz, L.A. and Bardelli, A. (2014) J Clin Oncol 32:579-86; 9. Zill, O.A., et al. (2018) Clin Cancer Res 24:3528-38.

Tumor Heterogeneity: ctDNA can Capture Multiple Mechanisms of Acquired Resistance



Brain lesion
BRAF V600E, AF 54.7%
EGFR amp
KRAS G12S not detected
NRAS Q61R not detected

Liver biopsy 1
BRAF V600E, AF 36.4%
EGFR amp, not detected
KRAS G12S, AF 6.4%
NRAS Q61R, AF 3.1%

Liver biopsy 2
BRAF V600E, AF 61.6%
EGFR amp, not detected
KRAS G12S, AF 22.4%
NRAS Q61R, not detected

Subcutaneous lesion
BRAF V600E, AF 45.4%
EGFR amp, not detected
KRAS G12S, AF 0.2%
NRAS Q61R, not detected

Multiple solid tumor biopsies show diverging resistance mechanisms in different metastases in a patient with advanced *BRAF* V600E CRC

Liquid biopsy captured all 4 resistance mechanisms

cfDNA

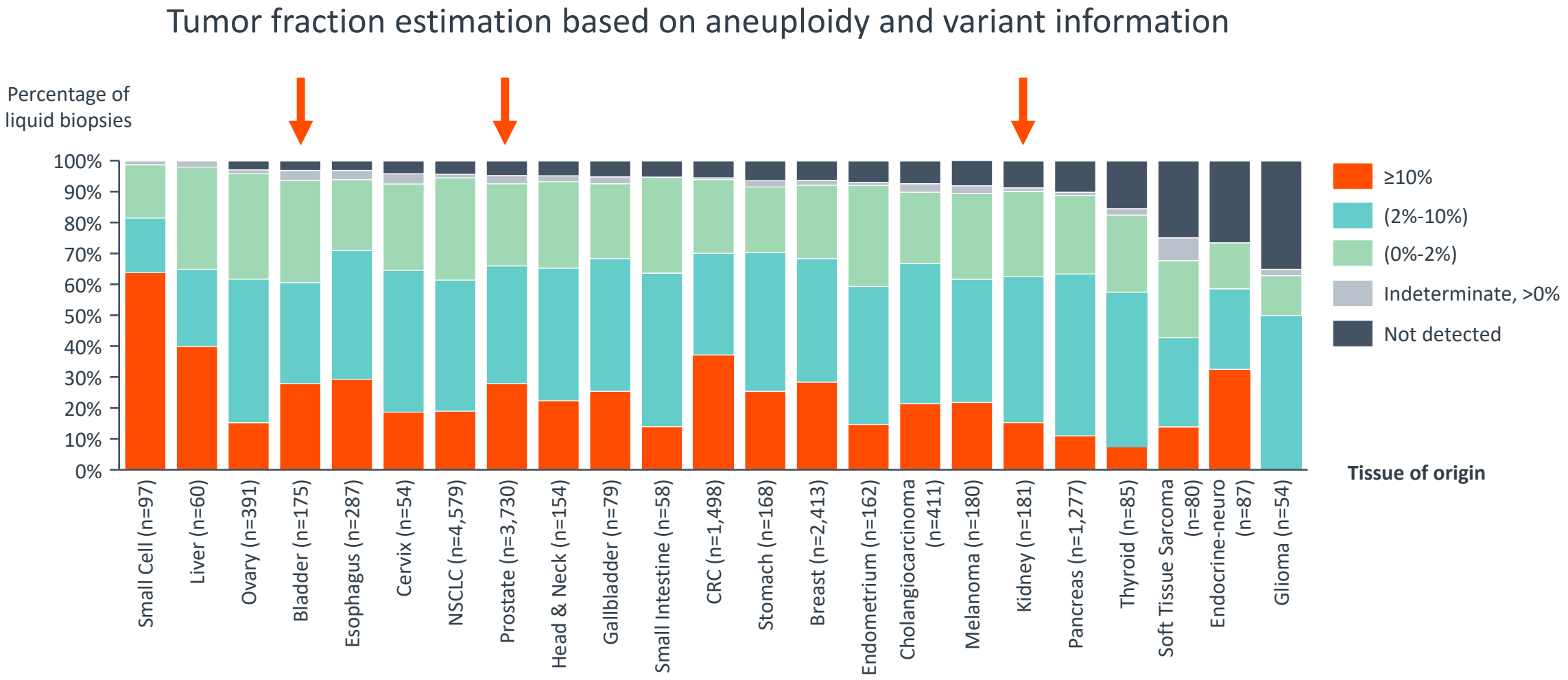
BRAF V600E, AF 24%
EGFR amp
KRAS G12S, AF 2.1%
NRAS Q61R AF 0.6%

Complementary FDA-Approved CGP Tests Across Solid Tumors

	Liquid biopsy NGS[1,2]	Tissue NGS[1,4]
FDA-Approved	FDA-approved CDx for 8 targeted therapies	FDA-approved CDx for 28 targeted therapies and 2 group claims
Target Tumor Types	All solid tumors	
Number of Genes Analyzed	324 (DNA) [2]	324 (DNA)
Genomic Signatures/Biomarkers	bTMB, MSI-H, tumor fraction[3]	TMB, MSI, LOH[5]
Specimen	Peripheral whole blood	FFPE tissue
Variant Types Identified	Point mutations, insertions/deletions, copy number alterations (amplifications and select losses), and rearrangements	Point mutations, insertions/deletions, copy number alterations, and rearrangements
Turnaround Time	Typically ≤10 days from receipt of specimen	Typically ≤12 days from receipt of specimen

bTMB = blood tumor mutational burden; ctDNA = circulating tumor DNA; FFPE = formalin-fixed paraffin-embedded; IHC = immunohistochemistry; LOH = loss of heterozygosity; MSI-H = microsatellite instability-high; TMB = tumor mutational burden.
1. FoundationOne® Liquid CDx and FoundationOne® CDx are FDA-approved in vitro diagnostic tests by Foundation Medicine; **2. FoundationOne® Liquid CDx is FDA-approved to report substitutions and indels in 311 genes, including rearrangements in *ALK* and *BRCA1/2* and copy number alterations in *BRCA1/2* and *ERBB2* (HER2). Comprehensive results across all 324 genes are reported as a laboratory professional service which is not reviewed or approved by the FDA; 3. bTMB, MSI-H status, and tumor fraction are reported as a laboratory professional service which is not reviewed or approved by the FDA;** 4. FoundationOne® CDx is a qualitative next-generation sequencing-based in vitro diagnostic test for advanced cancer patients with solid tumors and is for prescription use only. The test analyzes 324 genes as well as genomic signatures including MSI and TMB and is a companion diagnostic to identify patients who may benefit from treatment with specific therapies in accordance with the approved therapeutic product labeling. Additional genomic findings may be reported and are not prescriptive or conclusive for labeled use of any specific therapeutic product. Use of the test does not guarantee a patient will be matched to a treatment. A negative result does not rule out the presence of an alteration. Some patients may require a biopsy. For the complete label, including companion diagnostic indications and important risk information, please visit www.F1CDxLabel.com; 5. LOH reported in ovarian only; 6. Data on file, Foundation Medicine, Inc., October 2021.

ctDNA Is Detectable but Variable Across Tumor Types



CRC = colorectal cancer; NSCLC = non-small cell lung cancer.

1. Adapted from Tejpar S, et al. Abstract presented at: ESMO 2021. Abstract 457P; 2. Data on file, Foundation Medicine, Inc., October 2021.

Sources of Variability in ctDNA Studies

Clinical Variables	Tumor type, histology, stage of disease
	Definitive therapy type (e.g., surgery, radiation, chemoradiation)
	Therapeutic setting (neoadjuvant, adjuvant)
	Current treatment regimens (dosing/timing) and prior regimens
	Therapeutic class (e.g., targeted, IO, cytotoxic, hormonal, etc.)
ctDNA Collection and Methodology	Sample collection timepoints
	Whole blood collection (i.e., tube type, storage)
	Plasma sample processing (i.e., centrifugation)
Captured Endpoints	Endpoints for clinical and radiographic associations, including methodology and definitions of endpo
	Timing of radiographic surveillance
	Statistical plan (e.g., interim analysis timing, etc.)
Diagnostic Assay and Analysis	Performance parameters (e.g., reference range/interval, LOB, LOD, accuracy, repeatability, reproducibility, clinical cut-off for molecular residual disease)
	Biomarker features assessed (e.g., sequence mutations, structural alterations, methylation, fragmentation, etc.)
	Tumor informed or plasma only platform
	Algorithm design for ctDNA detection and status reporting
	Algorithm design for ctDNA quantification

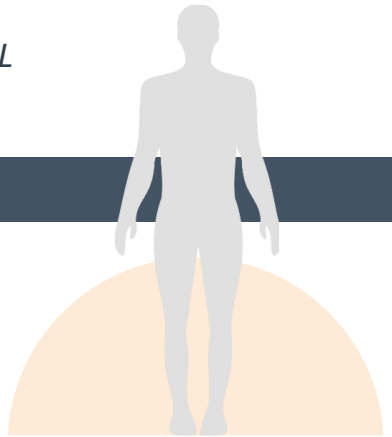
Differences in ctDNA results can be:
biological, logistical, technical, or **algorithmic**

*Table adapted from reference 1. ctDNA: circulating tumor DNA; IO: Immuno-oncology; LOB: Limit of blank; LOD: limit of detection; 1. Friends of Cancer Research. 2021. Assessing the use of ctDNA as an early endpoint in early-stage disease [White Paper].

ctDNA Genomics Across Multiple Tumor Types

All solid tumors: *NTRK1/2/3*, MSI-H/dMMR, TMB-H*

Breast[1]	NSCLC[2]	Prostate[3]	Ovarian[4]	**Colorectal[5,6]**	Melanoma[7]	Thyroid[8]
BRCA1/2 *PIK3CA*[a] *ERBB2* (HER2)	*EGFR*[a] *ALK*[a] *ROS1* *BRAF* *MET*[a] *RET* *ERBB2*[b] *KRAS*	*BRCA1/2*[a] *ATM*[a] *BARD1* *BRIP1* *PALB2* *FANCA* *RAD51B/C/D* *CHEK1/2* *CDK12* *FANCL* *RAD54L*	*BRCA1/2*[a]	*KRAS* *NRAS* *BRAF* *ERBB2*	*BRAF* *KIT* *NRAS* *ALK* *ROS1*	*BRAF* *RET ALK*



Bladder[9]	Gastric[10]/GEJ[11]	Endometrial[12]	GIST[13]	Pancreatic[14]	Bone[15]
FGFR2 *FGFR3*	*ERBB2* (HER2)	*ERBB2* (HER2) *POLE*	*KIT* *SDH* *BRAF* *NF1* *FGFR* *PDGFRA*	*BRCA1/2* *ROS1* *BRAF* *KRAS* *ERBB2* *PALB2*	*IDH1*

dMMR = mismatch repair deficient; GEJ = gastroesophageal junction; GIST = gastrointestinal stromal tumor; MSI-H = microsatellite instability-high; NCCN = National Comprehensive Cancer Network; NSCLC = non-small cell lung cancer; TMB-H = tumor mutational burden high.

Additional genes recommended for germline testing in various tumor types:[1,2,3,4,6,7,16,17,18] *APC*, *ATM*, *BAP1*, *BRCA1/2*, *CDH1*, *CDK4*, *CDKN2A*, *CHEK2*, *EGFR*, *MITF*, *MLH1*, *MSH2/6*, *MUTYH*, *NF1*, *PALB2*, *PMS2*, *POLD1*, *POLE*, *PTEN*, *RET*, *RNF43*, *SMAD4*, *STK11*, and *TP53*.

*See the specific NCCN Guidelines® for detailed testing recommendations; testing is not recommended for some of these biomarkers in certain guidelines.

[a]FoundationOne® Liquid CDx Companion Diagnostic. FoundationOne® Liquid CDx Technical Information. Foundation Medicine; 2021. Accessed August 27, 2021. https://www.F1LCDxLabel.com; [b]Indicates NCCN "emerging" biomarker.

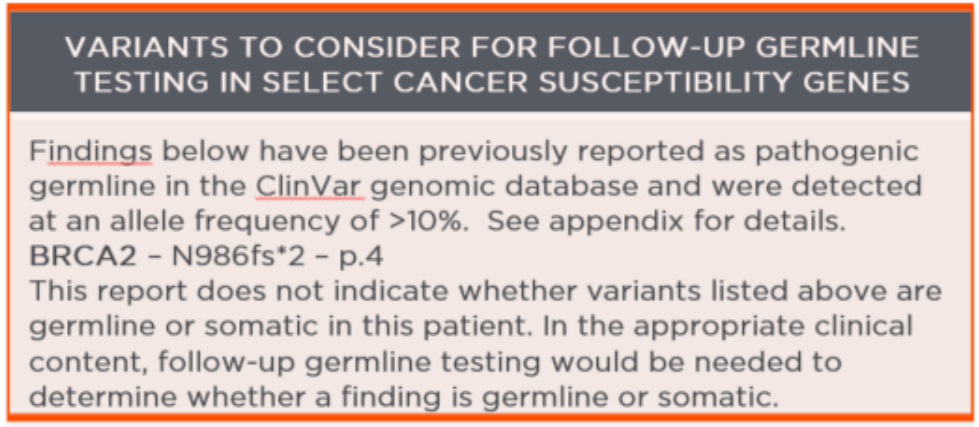
Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for: 1. Breast Cancer V.7.2021; 2. Non-Small Cell Lung Cancer V.5.2021; 3. Prostate Cancer V1.2022; 4. Ovarian Cancer Including Fallopian Tube Cancer and Peritoneal Cancer V.3.2021; 5. Colon Cancer V.3.2021; 6. Rectal Cancer V.2.2021; 7. Melanoma: Cutaneous V2.2021; 8. Thyroid Carcinoma V2.2021; 9. Bladder Cancer V4.2021; 10. Gastric Cancer V4.2021; 11. Esophageal and Esophagogastric Junction Cancers V.4.2021; 12. Uterine Neoplasms V4.2021; 13. Gastrointestinal Stromal Tumors (GISTs) V1.2021; 14. Pancreatic Adenocarcinoma V2.2021; 15. Bone Cancer V1.2022; 16. Genetic/Familial High-Risk Assessment: Colorectal V1.2021; 17. Genetic/Familial High-Risk Assessment: Breast, Ovarian, and Pancreatic V1.2022; 18. American Society of Breast Surgeons. Consensus Guideline on Genetic Testing for Hereditary Breast Cancer. Accessed August 27, 2021. https://www.breastsurgeons.org/docs/statements/Consensus-Guideline-on-Genetic-Testing-for-Hereditary-Breast-Cancer.pdf; 19. Data on file, Foundation Medicine, Inc., October 2021.

Liquid Biopsies and Germline Testing

- The liquid biopsy is not a “formal” germline test
- The patient has not consented for a germline test
- Genetic counseling program has not been activated before the test was obtained
- However, the liquid biopsy is highly informative of the germline status as:
  - Both the ctDNA and the wbcDNA will harbor the germline mutation
  - The variant allele frequency should be at or near 50%
  - The Liquid Biopsy report will alert the ordering physician that formal germline testing on another blood sample is recommended

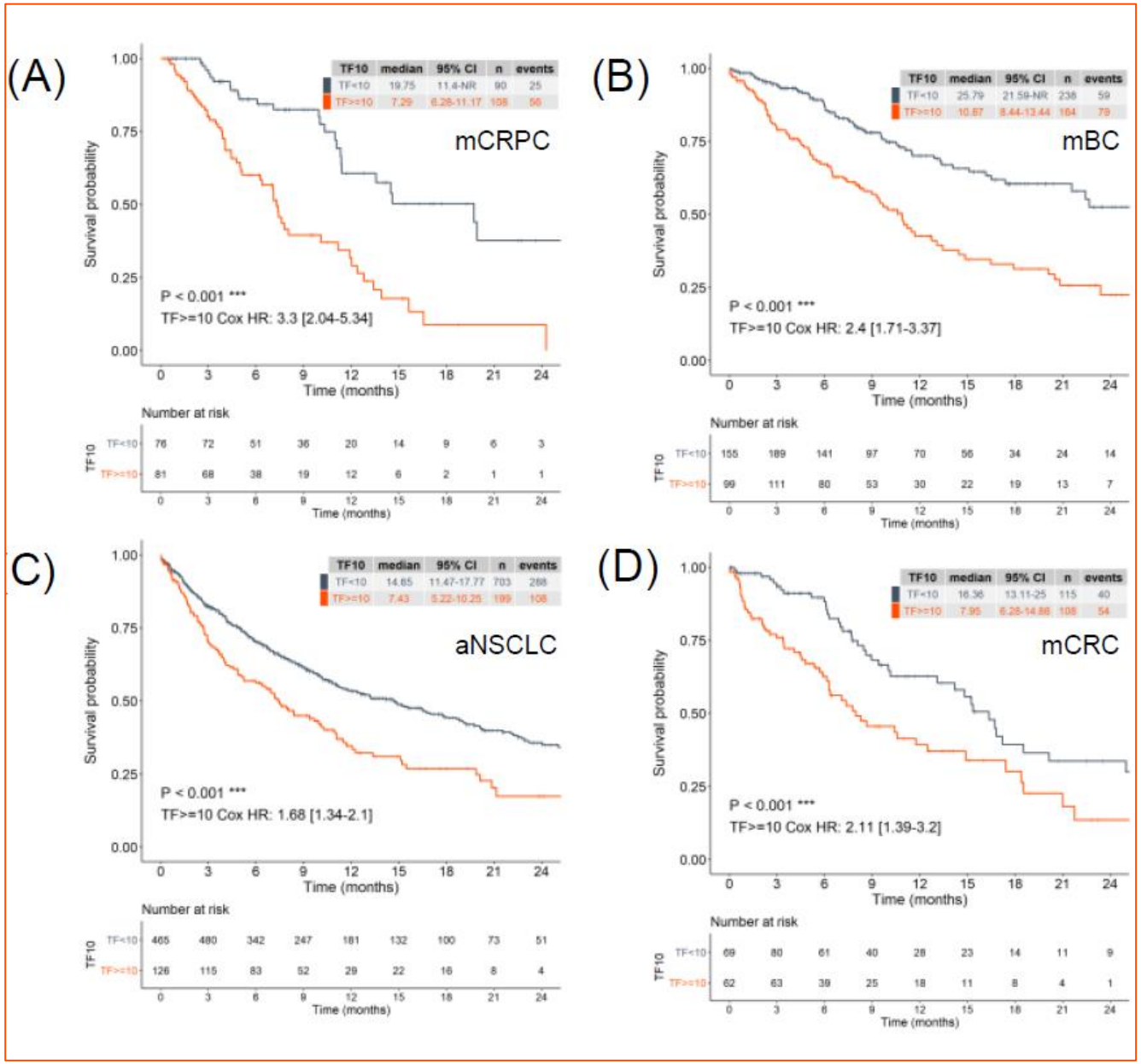
Genes selected per ESMO[1]:

ATM	*PALB2*
BAP1	*PMS2*
BRCA1	*POLE*
BRCA2	*RAD51C*
BRIP1	*RAD51D*
CHEK2	*RET*
FH	*SDHA*
FLCN	*SDHB*
MLH1	*SDHC*
MSH2	*SDHD*
MSH6	*TSC2*
MUTYH	*VHL*



Elevated tumor fraction (TF) is prognostic for worse overall survival in the four tumor histologies studied

Tumor fraction is measured by an emerging method of calculation based on an aneuploidy approach that determines the relative “quantity” of extracted DNA obtained from tumor cells divided by the quantity of all that of all the DNA extracted from the blood sample. A cut-off of 10% TF was used for a “low” vs “high” designation.



1. Zacchery et al Ann Oncol 2022 in press

Case Study: Liquid Biopsy in Prostate Cancer

68 y/o M with advanced prostate cancer

- Presents to oncology clinic after 2 years of abiraterone treatment, PSA rising
- Prefers to avoid chemotherapy, interested in oral options
- Has a bone biopsy from 2 years prior
- What do you do?

- F1LCDx is sent and shows a BRCA2 mutation
- Report indicates the possibility of a germline mutation
- Starts olaparib and is referred for germline counselling and possible testing

68 y/o M with advanced prostate cancer

Liquid Biopsy Findings

Genomic Signatures

Blood Tumor Mutational Burden - 5 Muts/Mb
Microsatellite status - MSI-High Not Detected
Tumor Fraction - Elevated Tumor Fraction Not Detected

Gene Alterations

For a complete list of the genes assayed, please refer to the Appendix.

BRCA2 **N2460fs*7**
MET **exon 14 splice site (D1010N)**
TP53 **L130fs*27**

† See About the Test in appendix for details.

Report Highlights

- Targeted therapies with **NCCN categories of evidence** in this tumor type: **Olaparib** (p. 8), **Rucaparib** (p. 10)
- Variants that may inform **nontargeted treatment approaches** (e.g., chemotherapy) in this tumor type: ***BRCA2*** **N2460fs*7** (p. 5)
- Evidence-matched **clinical trial options** based on this patient's genomic findings: (p. 12)
- Variants with **prognostic implications** for this tumor type that may impact treatment decisions: ***BRCA2*** **N2460fs*7** (p. 5)
- Variants in select cancer susceptibility genes to consider for possible **follow-up germline testing** in the appropriate clinical context: ***BRCA2*** **N2460fs*7** (p. 5)

GENOMIC SIGNATURES	THERAPY AND CLINICAL TRIAL IMPLICATIONS
Blood Tumor Mutational Burden - 5 Muts/Mb	**No therapies or clinical trials. See Genomic Signatures section**
Microsatellite status - MSI-High Not Detected	**MSI-High not detected. No evidence of microsatellite instability in this sample (see Appendix section).**
Tumor Fraction - Elevated Tumor Fraction Not Detected	**Tumor fraction is considered elevated when ctDNA levels are high enough that aneuploidy can be detected. The fact that elevated tumor fraction was not detected in this specimen indicates the possibility of lower levels of ctDNA but does not compromise confidence in any reported alterations. However, in the setting of a negative liquid biopsy result, orthogonal testing of a tissue specimen should be considered if clinically indicated (see Genomic Signatures section).**

GENE ALTERATIONS		VAF %
BRCA2 -	N2460fs*7	51.6%

10 Trials see p. 12

THERAPIES WITH CLINICAL RELEVANCE (IN PATIENT'S TUMOR TYPE)		THERAPIES WITH CLINICAL RELEVANCE (IN OTHER TUMOR TYPE)	
Olaparib	1	Rucaparib	2A
		Niraparib	
		Talazoparib	

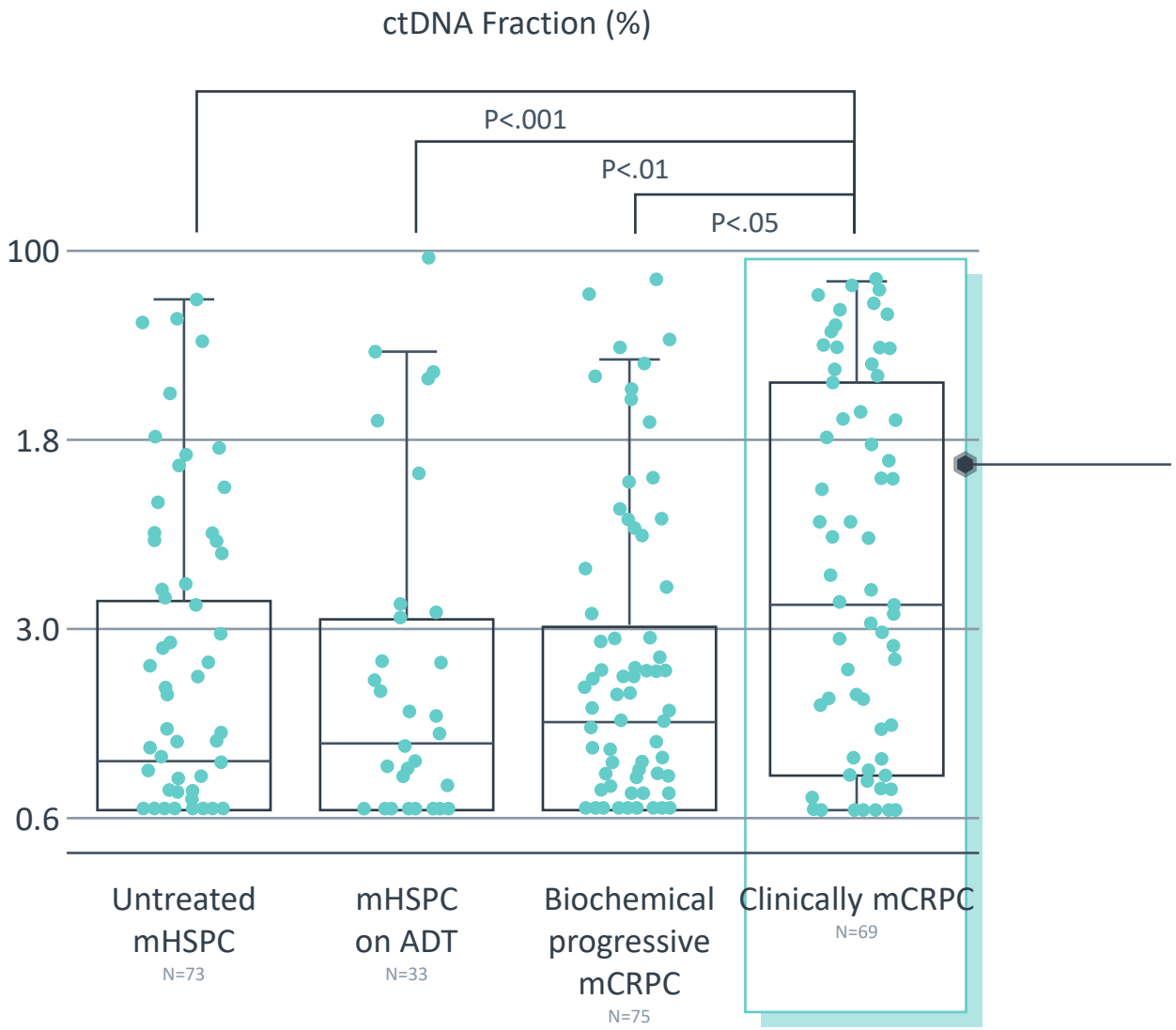
NCCN category

VARIANTS TO CONSIDER FOR FOLLOW-UP GERMLINE TESTING IN SELECT CANCER SUSCEPTIBILITY GENES

Findings below have been previously reported as pathogenic germline in the ClinVar genomic database and were detected at an allele frequency of >30%. See appendix for details.

BRCA2 - N2460fs*7 ..... p. 5

This report does not indicate whether variants listed above are germline or somatic in this patient. In the appropriate clinical context, follow-up germline testing would be needed to determine whether a finding is germline or somatic.



Considerations: When to Draw for Liquid Biopsy?

ctDNA levels highest in patients with mCRPC experiencing clinical progression

Biochemical progression = serial PSA rise on 2 separate occasions after achieving a nadir PSA value

Clinical progression = first appearance of new radiological disease

ADT = androgen deprivation therapy; ctDNA = circulating tumor DNA; mCRPC = metastatic castration-resistant prostate cancer; mHSPC = metastatic hormone-sensitive prostate cancer; PSA = prostate-specific antigen.

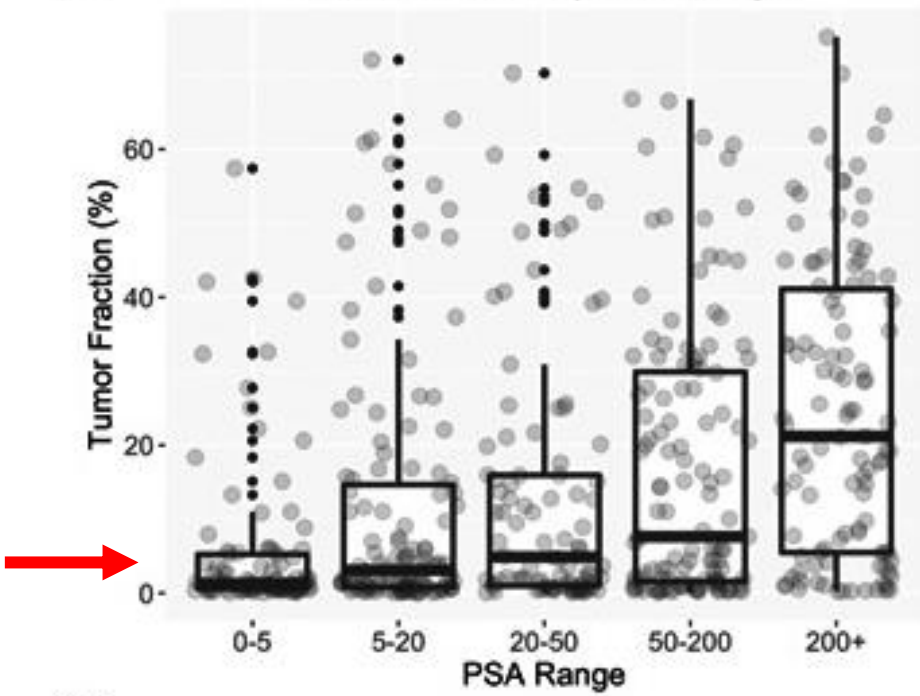
1. Kohli M, et al. EBioMedicine. 2020;54:102728;
2. Data on file, Foundation Medicine, Inc., October 2021.

Determining a Recommended Cut-off for PSA Level Guiding Decision to Perform a Liquid Biopsy in Prostate Cancer

"Our proposed threshold for clinical utility of liquid biopsy assessment is a PSA of >5 ng/ml, at which level 78% of patients would be expected to have a circulating TF of at least 1%, and 23% would have a TF of at least 30%. Conversely, at PSA concentrations of <5 ng/ml in the metastatic prostate cancer setting, a tumor biopsy would be expected to yield more robust CGP results than a liquid biopsy. Liquid and tissue CGP are fundamentally two complementary diagnostics and must be used in parallel to optimize diagnostic yields and to aid treatment decisions for cancer patients."

Antonarakis ES et al. Prostate. 2022 May;82(7):867-875.

(A)



(B)

PSA Range	Patients	Prop TF 1%+	Prop TF 10%+	Prop TF 30%+
PSA 0-5	n=97	0.567	0.155	0.062
PSA 5-20	n=122	0.705	0.311	0.131
PSA 20-50	n=95	0.726	0.400	0.147
PSA 50-200	n=124	0.782	0.476	0.250
PSA >200	n=107	0.897	0.664	0.374

BRCA2 Loss vs *BRCA 2* Point Mutation in mCRPC

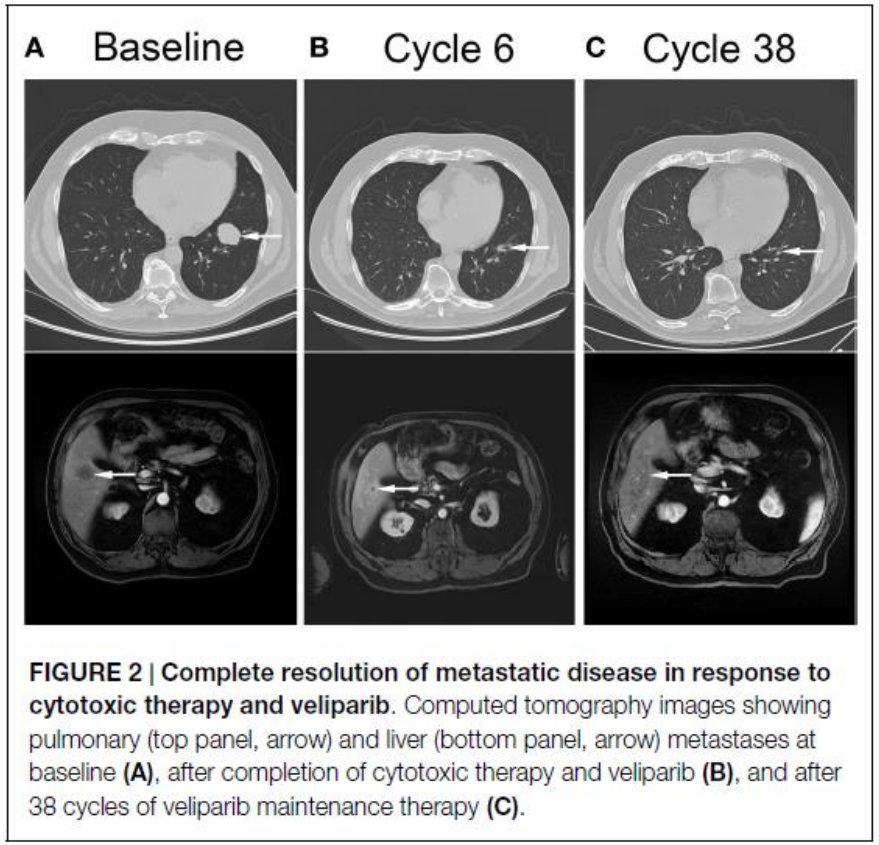


FIGURE 2 | Complete resolution of metastatic disease in response to cytotoxic therapy and veliparib. Computed tomography images showing pulmonary (top panel, arrow) and liver (bottom panel, arrow) metastases at baseline **(A)**, after completion of cytotoxic therapy and veliparib **(B)**, and after 38 cycles of veliparib maintenance therapy **(C)**.

VanderWeele DJ et al. Sustained Complete Response to Cytotoxic Therapy and the PARP Inhibitor Veliparib in Metastatic Castration-Resistant Prostate Cancer - A Case Report. Front Oncol. 2015 Jul 22;5:169

Figure 1: TTD (left) and OS (right) from start of PARPi in *BRCA*del patients vs *BRCA*other patients

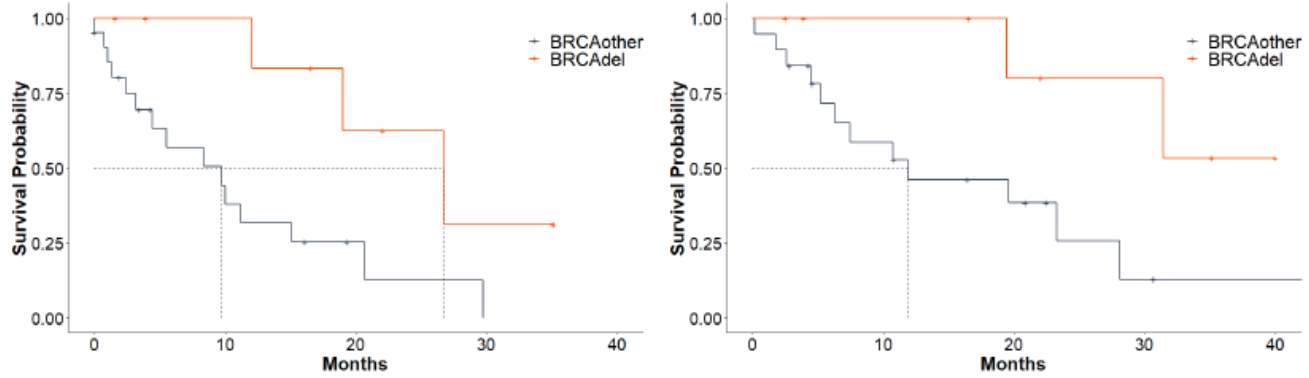
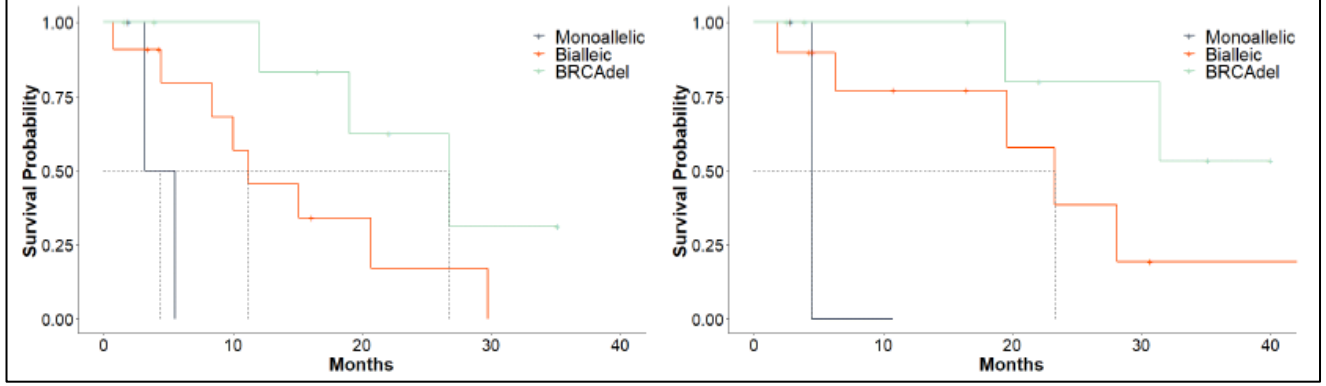


Figure 2: TTD and OS from start of PARPi in *BRCA*alt patients stratified by alteration allelic status

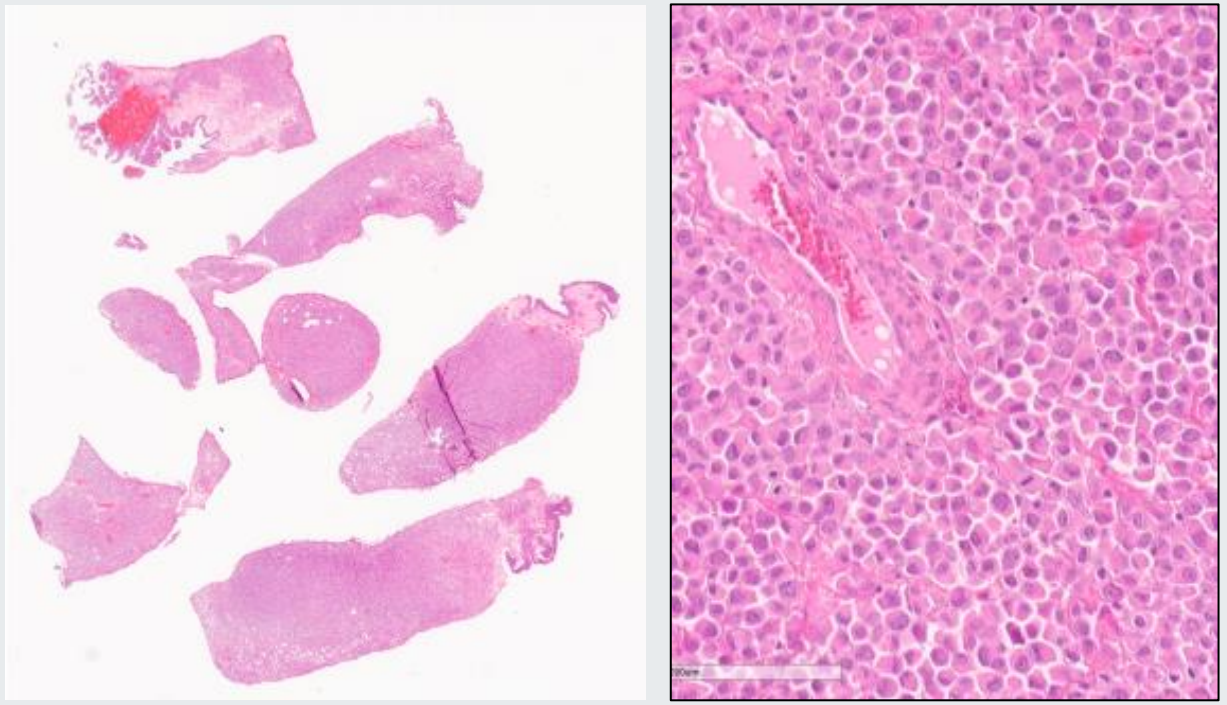


Association of *BRCA* alteration (alt) type with real-world (RW) outcomes to PARP inhibitors (PARPi) in patients (pts) with metastatic castrate resistant prostate cancer (mCRPC)

Emmanuel S. Antonarakis[1], Russell W. Madison[2], Jeremy Snider[3], Tamara Snow[3], Ethan S. Sokol[2], Jon H. Chung[2], Margaret McCusker[3], Gaurav Singal[2], Brian M. Alexander[2], Emily Castellanos[3], Jeffrey M. Venstrom[2], Alexa B. Schrock[2], Jeffrey S. Ross[2,4]
[1]Sidney Kimmel Comprehensive Cancer Center, Baltimore, MD [2]Foundation Medicine Inc. Cambridge, MA [3]Flatiron Health, Inc. New York, NY [4]Upstate Medical University Albany, NY

Case Study: Liquid Biopsy in Urothelial Bladder Cancer: *CDH1* Mutation

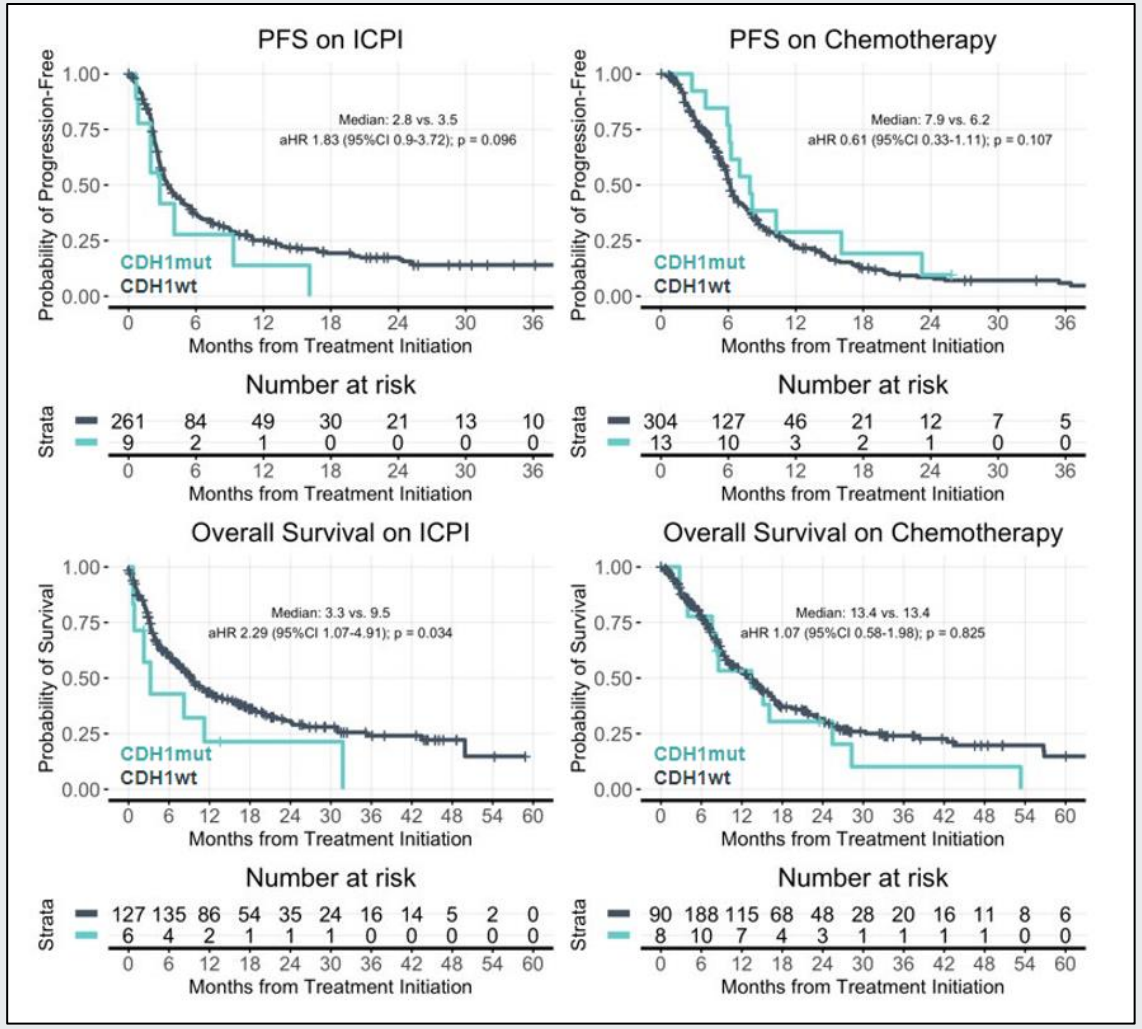
- TURBT specimen showing diffuse infiltration by urothelial carcinoma in a 61-year-old man
- The entire trigone, anterior and left side of the bladder wall was involved with deep smooth muscle invasion
- The bladder appeared to be fixed to the pubic bone on initial imaging studies
- Additional MRI of the pelvis showed diffuse bladder wall thickening with infiltration into pelvic fat and invasion into the right seminal vesicle
- The pathology report was a high grade urothelial carcinoma plasmacytoid type



“Plasmacytoid Urothelial Carcinoma”

Case Study: Liquid Biopsy in Urothelial Bladder Cancer: *CDH1* Mutation

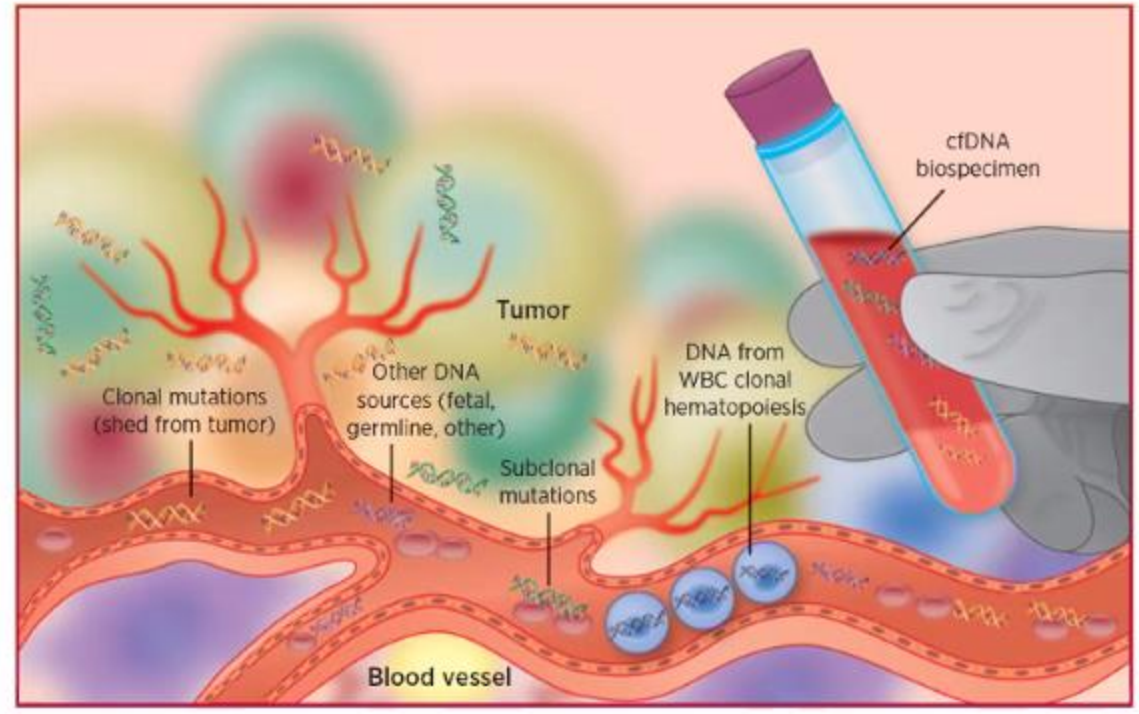
- Comprehensive genomic profiling revealed a MS-stable tumor with low tumor mutational burden of 5 mutations/Mb. A w532* *CDH1* mutation was identified. Additional alterations included both a short variant (Q35*) and short deletion (exon10-11) of *RB1*
- *BRD4* and *NOTCH3* amplifications were identified along with short variant mutations in TERT promoter (-124C>T) and TP53 (E285K)
- Liquid biopsies reveal similar CGP results as tissue samples when tumor fraction is >10% and ctDNA levels are high
- CDH1 mutated UBC (plasmacytoid and non-plasmacytoid types) appear to be associated with resistance to immunotherapy and sensitivity to chemotherapy



Clonal Hematopoiesis (CH)

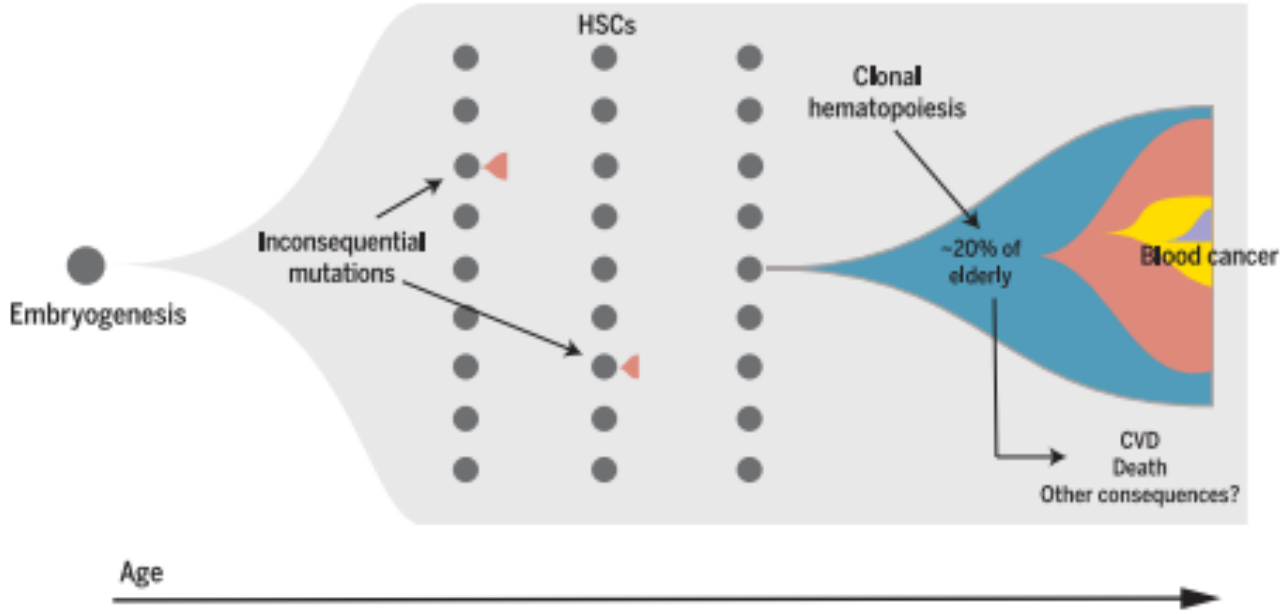
CH adds a layer of complexity when interpreting liquid biopsy results

- Genomic findings from cell-free DNA (cfDNA) may originate from non-tumor somatic alterations, including CHIP[1]
- CH is an age-related condition in which peripheral blood cells accumulate mutations in driver genes known to be associated with hematological malignancies[2]
- Genes with alterations that may be derived from CHIP include, but are not limited to: *ASXL1*, *ATM*, *CBL*, *CHEK2*, *DNMT3A*, *JAK2*, *KMT2D* (*MLL2*), *MPL*, *MYD88*, *SF3B1*, *TET2*, *TP53*, and *U2AF1*[2]



cfDNA biospecimen contains multiple sources of DNA[1]

1. Bauml J, et al. 2018;24(18):4352-4354, 2. Severson EA, et al. *Blood* vol. 131,22 (2018): 2501-2505.



Somatic mutations, clonal hematopoiesis, and aging. Somatic mutations are acquired by all cells throughout life. Most are inconsequential, but rare mutations will lead to clonal expansion of hematopoietic stem cells (HSCs). If additional mutations are acquired, blood cancers may result. Emerging data also associate the presence of such clones with increased risk of cardiovascular disease (CVD) and death. Clonal hematopoiesis provides a glimpse into the process of mutation and selection that likely occurs in all somatic tissues.

- Mutations in genes involved in epigenetic regulation (DNMT3A, TET2, ASXL1) account for the majority of mutation-driven clonal hematopoiesis in humans
- Between 10 and 20% of those older than age 70 harboring a clone of appreciable size
- Clonal hematopoiesis of indeterminate potential (CHIP) is a clinical entity defined by the presence of a cancer associated clonal mutation in at least 4% of nucleated blood cells of individuals without frank neoplasia
- CHIP is associated with an increased risk of developing blood cancers, confirming that it is a bona fide premalignant state
- Factors that influence the likelihood of progression to malignancy include the size of the clone, the number of mutations, and the specific gene or genes that are mutated

Jaiswal and Ebert, Science 366, 586 (2019)

Tissue CGP and Liquid CGP: Complementary FDA-Approved Options for Different Clinical Scenarios

Tissue First

- When quality tissue is available
- For patients with low-shed metastatic sites (CNS disease or local spread)
- For patients on therapy with stabilized disease
- For early-stage cancer

Tissue not immediately available (archival specimen)

Low-quality tissue specimen (eg, bone biopsy, FNA)

If no actionable alterations are detected (reflex)

Specimens with low ctDNA

Liquid First

- When tissue specimen is exhausted from prior testing
- Patient preference to avoid repeat invasive biopsy
- Patients progressing on therapy
- No easily available biopsy site for adequate CGP testing

CGP = comprehensive genomic profiling; CNS = central nervous system; ctDNA = circulating tumor DNA; FNA = fine needle aspiration.

1. Rolfo C, et al. *J Thorac Oncol.* 2021. doi: 10.1016/j.jtho.2021.06.017; 2. Data on file, Founation Medicine, Inc., October 2021.

Conclusions

- Blood based genomic testing is emerging rapidly as a clinically useful approach both to determining underlying driver mutations that can be targeted and biomarkers of immunotherapy response
- When deciding whether to order a blood or urine based or tissue based molecular test, which test to order first is best based on the status of the patient
- Blood based molecular monitoring for solid tumor relapse and progression shows substantial promise to allow earlier treatment adjustments with potential to improve clinical outcomes for these patients
- When used appropriately at the right time, liquid biopsies have potential to add significant additional precision in the modern care of the urologic cancer patient