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Supplemental information

The Liquid Biopsy Consortium: Challenges and opportunities for early cancer detection and monitoring

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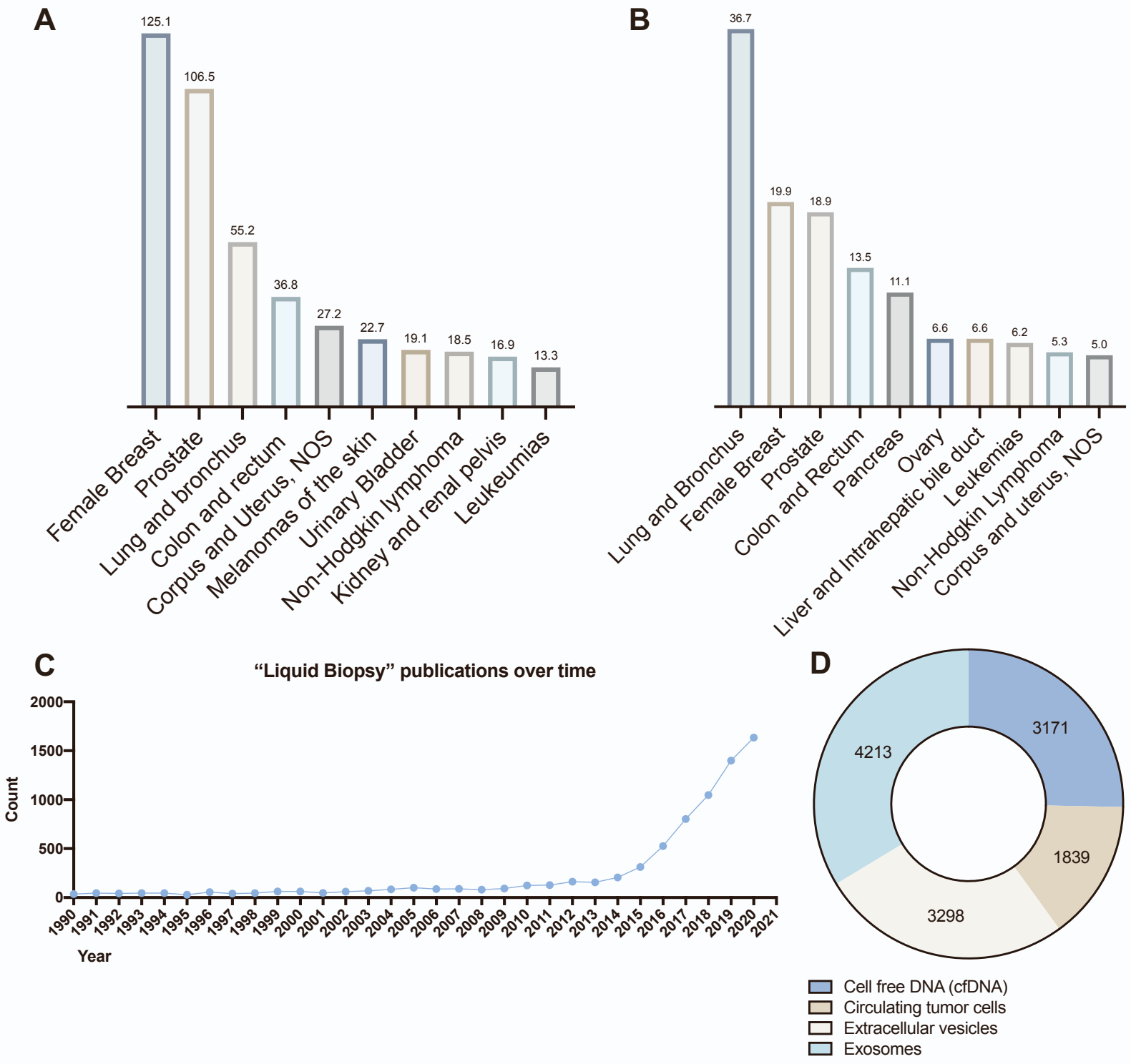

Supplemental Figure 1. Cancer by numbers. **A.** The incidence and **B.** the mortality of top cancers occurring across all races/ethnicities, male and female in the United States. The data are represented as rate per 100,000 people. Data Source- U.S. Cancer Statistics Working Group. U.S. Cancer Statistics Data Visualizations Tool, based on 2019 submission data (1999-2017): U.S. Department of Health and Human Services, Centers for Disease Control and Prevention and National Cancer Institute; www.cdc.gov/cancer/dataviz, released in June 2020. **C.** "Liquid biopsy" publications, with an increasing interest over time. **D.** Individual terms of liquid biopsy registered publications in 2020.

Supplementary Table 1. An overview of studies investigating the clinical utility of multiple liquid biopsy analytes in combination and individually.

Analyte Isolated	Disease	Biofluid	Isolation Method	Platform for biomarker detection	Biomarker	Sensitivity & Specificity	Clinical utility	Advantages	Disadvantages	Reference
EV RNA/DNA + cfDNA (ExoNA)	Non-small cell lung cancer (NSCLC)	Plasma	Exolution™ plus	qPCR	EGFR variants: L858R, T790M	Sensitivity: 90 % (L858R), 83% (T790M) Specificity: 100%	•Diagnosis •Monitoring •Surveillance	ExoNA vs cfDNA is associated with higher sensitivity of rare mutation detection cfDNA released from dying cells EVs are released as an active metabolic process from living cells	Low amounts of analyte in early stage disease. Effect of sampling noise	1
EV RNA/DNA + cfDNA (ExoNA)	Glioma	Plasma	Exolution™ plus	ddPCR	TERT C228T, TERT C250T	Sensitivity, 72% Specificity, 90%	•Diagnosis •Monitoring •Surveillance •	-	-	1,2
EV RNA/DNA + cfDNA (ExoNA)	Melanoma	Plasma	Exolution™ plus	qPCR	BRAF V600E/K	Sensitivity, 50% Specificity, 73%	•Diagnosis •Prediction of worsening prognosis in <2 years	Minimizes false negatives secondary to tissue heterogeneity	-	3
EV DNA vs ctDNA vs (EV DNA + cfDNA)	Melanoma	Plasma	Peptide-based affinity isolation	qPCR		Sensitivity, 56% Specificity, 87%	•Diagnosis •Prognostication (OS, PFS) •Treatment monitoring	Two-fold higher recovery of mutant signal vs ultracentrifugation. Higher sensitivity in low concentration samples	Low diagnostic accuracy Need for clinical studies with more wild-type cohorts	4
EV DNA vs cfDNA	Colon cancer	Serum, Plasma	Invitrogen Total Exosome Isolation MagMAX™ Cell Free DNA Isolation	Next Generation Sequencing	APC, TP53, KRAS, PIK3CA	Sensitivity: EV DNA (61.90 %) cfDNA (66.67 %)	•Diagnosis •Prognostication	High sensitivity in late stage (III-IV) disease. Correlation of circulating mutant DNA levels	Low mutation detection in early stage (I-II) disease Limited coverage of small fragments of cfDNA of	5

								to clinical features (tumor size	varying sizes	
EV DNA vs cfDNA	Pancreatic adenocarcinoma	Plasma	Serial ultracentrifugation QIAmp Circulating Nucleic Acid Kit	ddPCR	KRAS	Localized: (EV DNA 66.7%, cfDNA 45.5%,) Locally advanced: (EV DNA 80%, cfDNA 30.8%) Metastatic: (EV DNA 85%, cfDNA 57.9%)	•Early diagnosis •Disease monitoring •Determination of survival benefit following resection	Higher sensitivity of KRAS detection in early-stage disease using EV DNA Association with disease free survival	Need for larger studies High background in healthy controls- biological role of background oncogenic mutations unclear	6
ctDNA in EV depleted plasma	Small cell lung cancer (SCLC)	Plasma	Sequential Centrifugation (Fractionated Method) DNA Blood Mini Kit	Whole Genome Sequencing PCR	EGFR mutations	Whole Plasma: 10/22 EV depleted Plasma: 14/22	•Diagnosis •Prognostication •Monitoring	Improved method of mutant ctDNA detection Preferential enrichment of smaller size fragments	Limited understanding of the evolving ctDNA content in different plasma fractions in serially collected samples	7
EVs vs Tumor educated platelets (TEPs)	Melanoma	Blood	Centrifugation ExoRNeasy Plasma Isolation	ARMS RT-qPCR	BRAF V600E	EV Fraction, 10/12 Platelets, 0/12	•Diagnosis •Disease Monitoring	Highly sensitive detection of rare events using EV fraction	Higher wild-type background with the use of platelets Mutant RNA transfer to platelets below the limit of detection	7,8
Urine EVs vs Platelet EVs	Prostate	Urine & Blood	Differential Centrifugation	Ultra-Performance Liquid Chromatography-Tandem Mass Spectrometry	Metabolomics	--	•Disease monitoring	Amplified detection of cancer-related changes Increased detection of unique metabolites in urine EVs and platelet EVs	Availability of technology for quantification	9
Plasma EVs/Serum EVs/Whole blood	Healthy	Blood	Differential Centrifugation	Small RNA deep sequencing	miRNA	--	•Diagnosis •Disease Monitoring	Enrichment of miRNAs in EVs	Larger validation studies required	9,10

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