DATA SUPPLEMENT

Retrospective Comparison Between Breast Cancer Tissue- and Blood-Based Next-Generation Sequencing Results in Detection of *PIK3CA*, *AKT1*, and *PTEN* Alterations

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TABLE S1. List of Alterations Defined in the CAPItello-291 Protocol

Gene	**Alteration**
AKT1	SVs E17K
PIK3CA	SVs R88Q E545A Q546E M1043V H1047Y G1049R N345K E545D Q546K M1043I H1047R C420R E545Q

	Q546R H1047L E542K E545K E545G Q546P
PTEN	SVs C124R G129E R130Q C136R S170R R173C C124S G129V R130G C136Y G129R

	R130L R130P Any nonsense, frameshift, or splice site alteration (missense mutations in the start codon and SV deletions spanning from upstream of the start codon are included in this category)
	Homozygous copy number deletion
	Predicted pathogenic rearrangement in *PTEN*

AKT1, Akt serine/threonine kinase 1; *PIK3CA*, phosphatidylinositol-3-kinase catalytic subunit alpha; *PTEN*, phosphatase and tensin homolog; SV, short variant.

TABLE S2. Patient Characteristics in the Liquid Biopsy Cohort With ctDNA TF ≥1%

Characteristic	**Liquid biopsy cohort with ctDNA TF ≥1% (*N* = 3,344)**
Age (years), median (interquartile range)	63 (55-72)
Sex, n (%)	
Male	38 (1.1)
Female	3,306 (98.9)
Tumor type, n (%)	
Breast/breast carcinoma (not otherwise specified)	2,664 (79.7)
Breast invasive ductal carcinoma	533 (15.9)
Breast invasive lobular carcinoma	141 (4.2)
Breast metaplastic carcinoma	4 (0.1)
Other breast[a]	2 (0.1)
Tumor stage, n (%)	
I	67 (2.0)
II	117 (3.5)
III	129 (3.9)
IV	2,358 (70.5)
Unknown	673 (20.1)

Please note that information on hormone receptor and human epidermal growth factor receptor 2 status (including hormone receptor-positive and triple negative breast cancers) was not routinely available. [a]Other breast: breast mucinous carcinoma, breast inflammatory carcinoma. ctDNA, circulating tumor DNA; TF, tumor fraction.

TABLE S3. Characteristics in Patients With Paired Tissue and Liquid Biopsy CGP Data

Characteristic	**Paired cohort (*N* = 289)**	
	Tissue biopsy	**Liquid biopsy**
Age (years), median (interquartile range)	61 (52-71)	61 (52-71)
Sex, n (%)		
Male	7 (2.4)	7 (2.4)
Female	282 (97.6)	282 (97.6)
Tumor type, n (%)		
Breast/breast carcinoma (not otherwise specified)	214 (74.0)	227 (78.5)
Breast invasive ductal carcinoma	61 (21.1)	50 (17.3)
Breast invasive lobular carcinoma	13 (4.5)	12 (4.2)
Breast metaplastic carcinoma	1 (0.3)	0
Other breast[a]	0	0
Tumor stage, n (%)		
I	10 (3.5)	8 (2.8)
II	16 (5.5)	19 (6.6)
III	18 (6.2)	22 (7.6)
IV	188 (65.1)	181 (62.6)
Unknown	57 (19.7)	59 (20.4)

Please note that characteristics might differ for each patient at tissue versus liquid biopsy collection, given these occurred at different timepoints. Additionally, information on hormone receptor and human epidermal growth factor receptor 2 status (including hormone receptor-positive and triple negative breast cancers) was not routinely available. [a]Other breast: breast mucinous carcinoma, breast inflammatory carcinoma. CGP, comprehensive genomic profiling.

FIG S1. Study analysis sets. *AKT1*, Akt serine/threonine kinase 1; CGP, comprehensive genomic profiling; ctDNA, circulating tumor DNA; *ESR1*, estrogen receptor 1; *PIK3CA*, phosphatidylinositol-3-kinase catalytic subunit alpha; *PTEN*, phosphatase and tensin homolog; TF, tumor fraction.

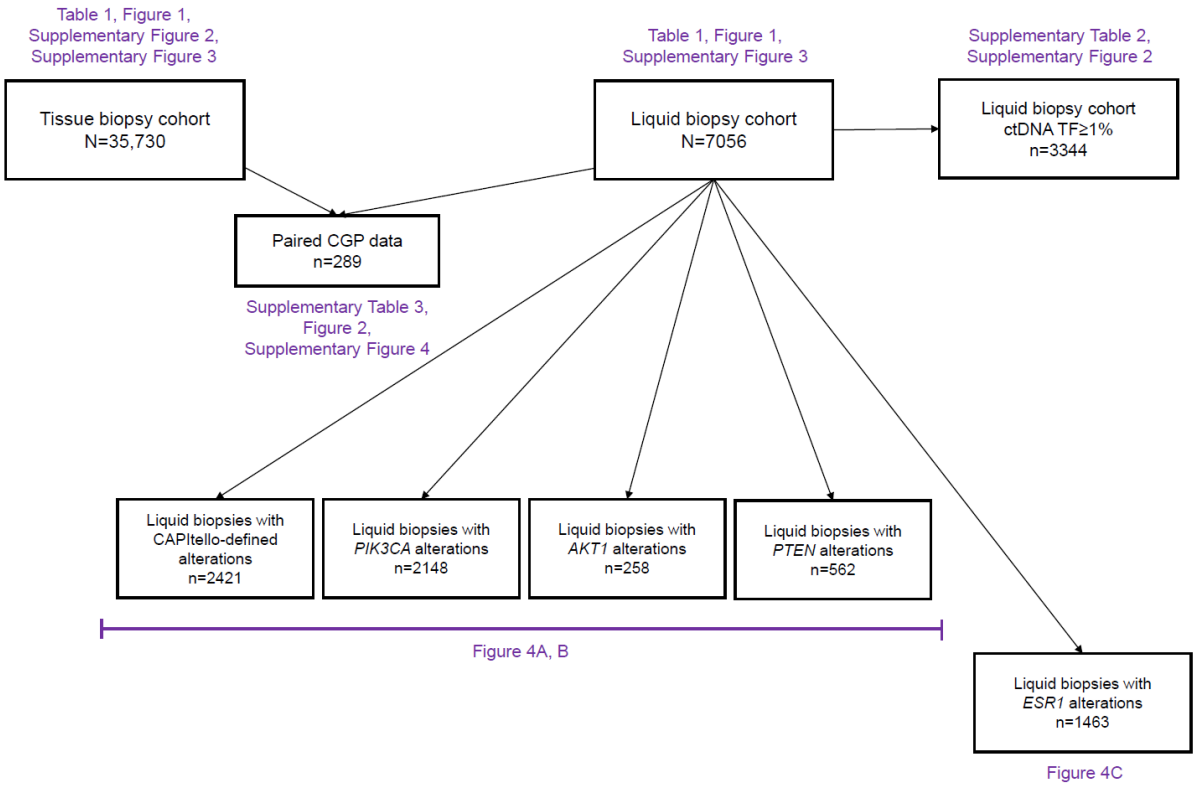


FIG S2. Prevalence of (A) all pathogenic alterations and (B) pathogenic short variants in the tissue and liquid biopsy ctDNA TF ≥1% cohorts. *P < .05; ** P < .01; *** P < .001. Please note that information on hormone receptor and human epidermal growth factor receptor 2 status (including hormone receptor-positive and triple negative breast cancers) was not routinely available. *AKT1*, Akt serine/threonine kinase 1; *AKT2*, Akt serine/threonine kinase 2; *AKT3*, Akt serine/threonine kinase 3; ctDNA, circulating tumor DNA; *PIK3CA*, phosphatidylinositol-3-kinase catalytic subunit alpha; *PTEN*, phosphatase and tensin homolog; TF, tumor fraction.

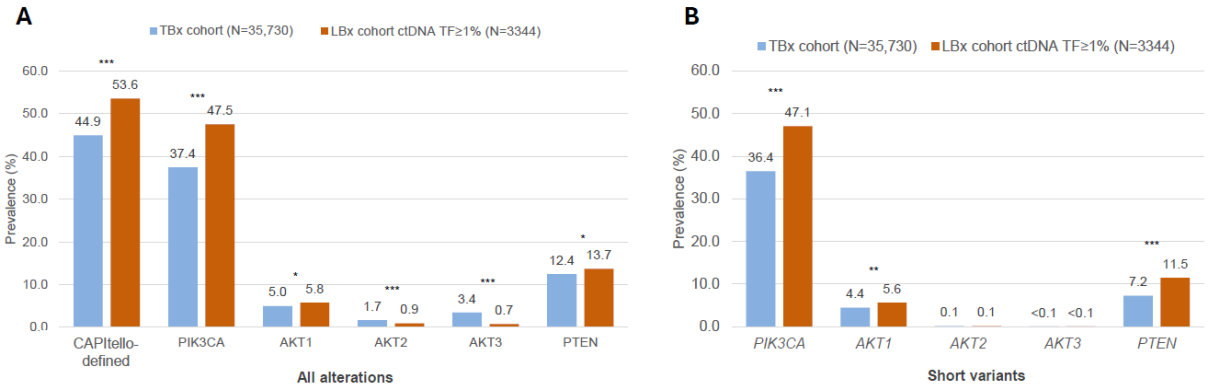


FIG S3. Breakdown of *PIK3CA*, *AKT1*, and *PTEN* pathogenic alterations[a] in breast cancer samples with CAPItello-defined alterations. Please note that information on hormone receptor and human epidermal growth factor receptor 2 status (including hormone receptor-positive and triple negative breast cancers) was not routinely available. [a]*PIK3CA*, *PTEN*, and *AKT1* alterations defined in the CAPItello-291 trial protocol (Table S1). *AKT1*, Akt serine/threonine kinase 1; *PIK3CA*, phosphatidylinositol-3-kinase catalytic subunit alpha; *PTEN*, phosphatase and tensin homolog. **FIG S4.** Negative predictive agreement in detection of alterations between tissue and liquid biopsies according to (A) ctDNA TFs and (B) collection intervals between paired biopsies. Please note that information on hormone receptor and human epidermal growth factor receptor 2 status (including hormone receptor-positive and triple-negative breast cancers) was not routinely available. Additionally, one sample may be counted in more than one category, e.g. both in ‘shared positive’ and ‘tissue-only positive’ for different alterations detected within this sample. Therefore, the total *N* shown here may exceed the number of samples (*n* = 289). NPA = shared negative / (shared negative + liquid-only positive). *AKT1*, Akt serine/threonine kinase 1; *AKT2*, Akt serine/threonine kinase 2; *AKT3*, Akt serine/threonine kinase 3; CNV, copy number variant; ctDNA, circulating tumor DNA; NPA, negative percent agreement; *PIK3CA*, phosphatidylinositol-3-kinase catalytic subunit alpha; *PTEN*, phosphatase and tensin homolog; SV, short variant; TF, tumor fraction.

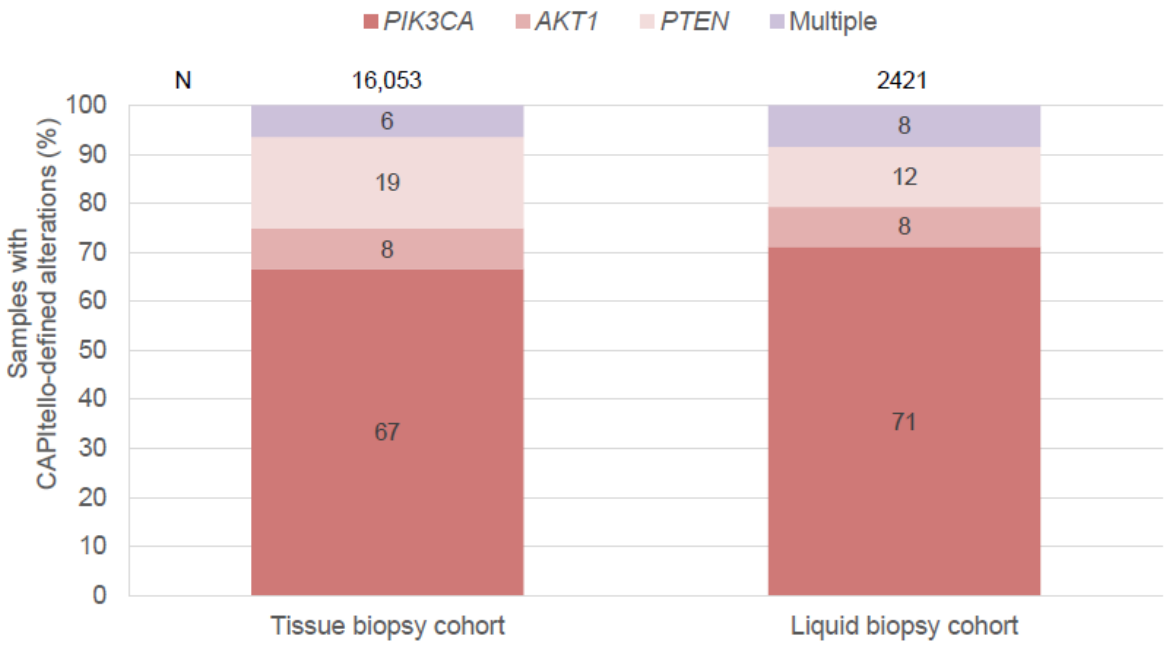
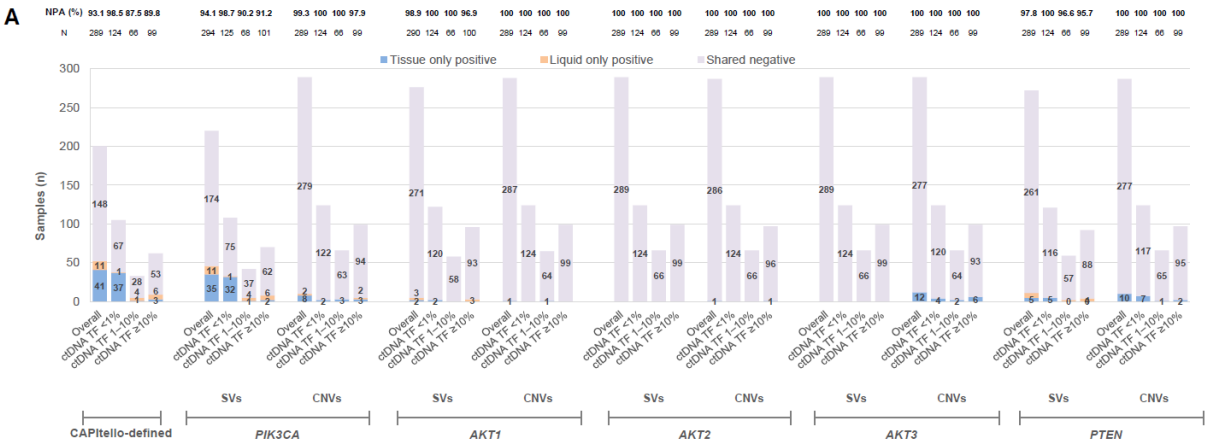
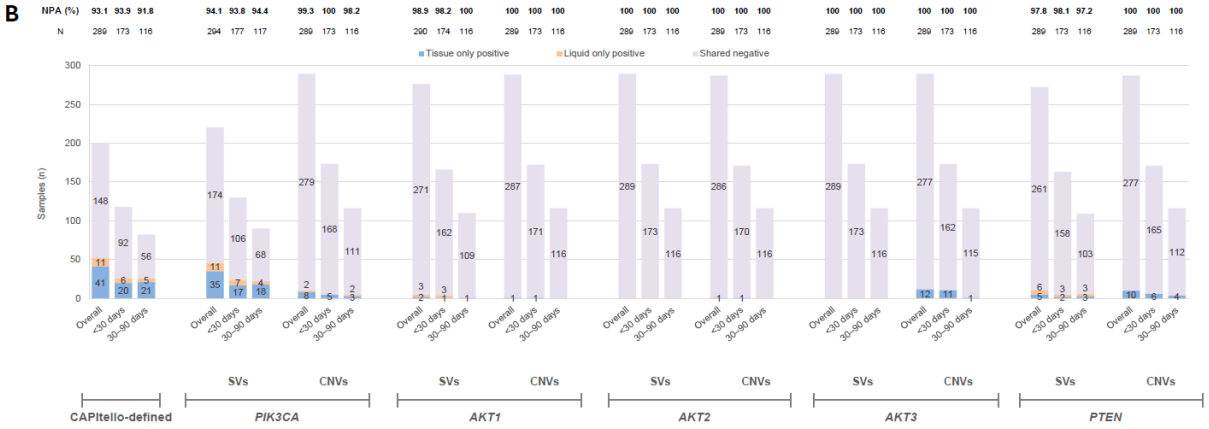


FIG S4. Negative predictive agreement in detection of alterations between tissue and liquid biopsies according to (A) ctDNA TFs and (B) collection intervals between paired biopsies. Please note that information on hormone receptor and human epidermal growth factor receptor 2 status (including hormone receptor-positive and triple-negative breast cancers) was not routinely available. Additionally, one sample may be counted in more than one category, e.g. both in 'shared positive' and 'tissue-only positive' for different alterations detected within this sample. Therefore, the total *N* shown here may exceed the number of samples (*n* = 289). NPA = shared negative / (shared negative + liquid-only positive). *AKT1*, Akt serine/threonine kinase 1; *AKT2*, Akt serine/threonine kinase 2; *AKT3*, Akt serine/threonine kinase 3; CNV, copy number variant; ctDNA, circulating tumor DNA; NPA, negative percent agreement; *PIK3CA*, phosphatidylinositol-3-kinase catalytic subunit alpha; *PTEN*, phosphatase and tensin homolog; SV, short variant; TF, tumor fraction.


A
NPA (%) 93.1 98.5 87.5 89.8 | 94.1 98.7 90.2 91.2 | 99.3 100 100 97.9 | 98.9 100 100 96.9 | 100 100 100 100 | 100 100 100 100 | 100 100 100 100 | 100 100 100 100 | 97.8 100 96.6 95.7 | 100 100 100 100
N 289 124 66 99 | 294 125 68 101 | 289 124 66 99 | 290 124 66 100 | 289 124 66 99 | 289 124 66 99 | 289 124 66 99 | 289 124 66 99 | 289 124 66 99 | 289 124 66 99
Tissue only positive
Liquid only positive
Shared negative
Samples (n)
Overall
ctDNA TF <1%
ctDNA TF 1–10%
ctDNA TF ≥10%
SVs
CNVs
CAPItello-defined
PIK3CA
AKT1
AKT2
AKT3
PTEN


B
NPA (%) 93.1 93.9 91.8 | 94.1 93.8 94.4 | 99.3 100 98.2 | 98.9 98.2 100 | 100 100 100 | 100 100 100 | 100 100 100 | 100 100 100 | 97.8 98.1 97.2 | 100 100 100
N 289 173 116 | 294 177 117 | 289 173 116 | 290 174 116 | 289 173 116 | 289 173 116 | 289 173 116 | 289 173 116 | 289 173 116 | 289 173 116
Tissue only positive
Liquid only positive
Shared negative
Samples (n)
Overall
<30 days
30–90 days
SVs
CNVs
CAPItello-defined
PIK3CA
AKT1
AKT2
AKT3
PTEN