bjclub breast Journal club

L'IMPORTANZA DELLA RICERCA IN ONCOLOGIA

20 - 21 APRILE
2023 ROMA

THE HIVE HOTEL

Via Torino, 6

THE OXFORD DEBATE EDITION

Pro: Linda Cucciniello
Contro: Giuseppe Buono
Provoker: Mario Giuliano

Biopsia liquida: siamo pronti per un uso clinico

Luca Malorni

SOS Ricerca Traslazionale
SOC Oncologia
Ospedale di Prato
Azienda USL Toscana Centro

BioItaLee- Study Design

BioItaLEE
BIOLOGICAL ITALIAN STUDY WITH RIBOCICLIB IN 1L POSTMENOPAUSAL WOMEN WITH ABC

N=287

- Postmenopausal women with HR+, HER2– aBC (locoregionally recurrent not amenable to surgery or metastatic)
- No prior systemic hormonal therapy or chemotherapy for aBC
- TFI >12 months*
- Patients willing to undergo blood and tumor sample collection at baseline and at a scheduled timeframe

Enrollement from 02 February to 28 November 2018
Across 47 Italian centers

First-line ribociclib
(600 mg PO; 3 weeks on/1 week off)
+ letrozole (2.5 mg/d PO)

Baseline (D0)	Day 15 Cycle 1 (D15)	Day 1 Cycle 2 (C2D1)	First Imaging (~ 3 months) (FI)	Disease progression Or end of treatment (EOT)

Liquid biopsy for:
- ctDNA analysis
- Serum thymidine kinase activity

Baseline tumor Tissue sample (fresh or archival) (preferred from a metastatic site)

Buccal swab (pharmacogenomics)

ESMO BREAST CANCER VIRTUAL MEETING

23-24 MAY 2020

BioItaLEE – Comparative Biomarker Analysis of Liquid Biopsies and Paired Tissue Samples of Patients Treated With Ribociclib and Letrozole as First-line Therapy for Advanced Breast Cancer (ABC)

11P

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Introduction

- The addition of ribociclib (RIB) to endocrine therapy (ET) significantly improves the efficacy outcomes, including overall survival (OS), compared to ET alone in patients (pts) with hormone receptor-positive (HR+), human epidermal growth factor receptor 2-negative (HER2–) advanced breast cancer (ABC).[1-3]
- Currently, data on cyclin dependent kinase 4/6 inhibitors (CDK4/6i) predictive biomarkers are limited and inconclusive.[4, 7] ctDNA analysis is emerging as an attractive non-invasive approach to characterize tumor biology and its evolution overtime. Further studies are necessary to fully investigate the clinical utility and feasibility of liquid biopsy in this setting.[5, 6]

Study Objectives

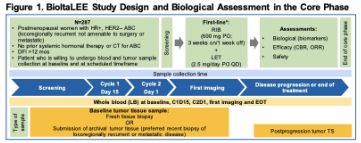
- The primary objective of the BioItaLEE phase 3b, multicenter (47 Italian centers), single-arm trial (NCT03439046) is to study ctDNA alterations, their evolution during treatment and their association with clinical outcome in postmenopausal endocrine sensitive and *de-novo* women with HR+, HER2– ABC receiving RIB and letrozole (LET) as first-line therapy. We recently presented the results of baseline ctDNA mutational status and its correlation with clinicopathological characteristics and response to treatment based on first imaging evaluation.[8] Here, we report a comparative single-nucleotide variant (SNV) analysis of baseline (pretreatment) ctDNA assessment by 2 next-generation sequencing (NGS) methods and tumor tissue DNA (tDNA) collected in the trial.

Methods

Biological Assessment in the Core Phase

- Biological samples such as pretreatment liquid biopsies (LBs) and tumor samples (TS) from metastatic site biopsy or primary tissue were collected prospectively in the trial (**Figure 1**).

Figure 1. BioItaLEE Study Design and Biological Assessment in the Core Phase



*Treatment until disease progression, death, unacceptable toxicity, physician's and/or subject's decision, protocol deviation, study termination by sponsor or lost to follow-up.
ABC, advanced breast cancer; CBR, clinical benefit rate; C1D15, cycle 1 day 15; C2D1, cycle 2 day 1; CT, chemotherapy; DFI, disease free interval; EOT, end of treatment; HER2–, human epidermal growth factor receptor 2-negative; HR+, hormone receptor-positive; LB, liquid biopsy; LET, letrozole; mo, month; ORR, overall response rate; PO, per oral; qd, once daily; RIB, ribociclib; TS, tissue sample.

Next-Generation Sequencing (NGS) Analysis

- tDNA was extracted from tumor samples that had passed preanalytic screening (≥ 100 cells in H&E slides). The quantity and quality of tDNA were estimated via reverse transcription-polymerase chain reaction (RT-PCR) using the Quantiller Trio DNA Quantification Kit (ThermoFisher Scientific). Samples with concentration ≥ 0.1 ng/µL and degradation index ≤ 10 were considered as suitable for NGS.
- Baseline ctDNA and tDNA were assessed by SNV analysis using the same 533-amplicon custom AmpliSeq HD panel.
- Baseline ctDNA was additionally analyzed by the Oncomine Pan-Cancer Cell-Free Assay (ThermoFisher Scientific) (**Table 1**).

Table 1. Molecular Analysis Performed by NGS Testing

NGS Testing	BioItaLEE AmpliSeq HD Custom Panel		Oncomine Pan-Cancer Cell-Free Assay (ThermoFisher Scientific)
Sample tested	Baseline LB (ctDNA)	Baseline TS	Baseline LB (ctDNA)
Mean coverage and LOD	23000 X 0.1% (for 10 ng of cfDNA input)	12000 X 0.1% (for 5 ng of tDNA)	32000 X 0.1% (for 10 ng of cfDNA input)
No. of genes tested for SNV	39		44
Genes analyzed	***AKT1, APC,*** ATM, CDH1, CCND1, CCNE1, CDK4, CDK6, CDH1, CDKN2A, ***EGFR, ERBB2, ERBB3,*** ERBB4, ***ESR1, FGFR1,*** GATA3, ***HRAS, KIT,*** KMT2C, ***KRAS, MAP2K1,*** MAP2K4, MAP3K1, ***MET,*** MLH1, NF1, NOTCH1, ***NRAS, PDGFRA, PIK3CA,*** PIK3R1, RB1, RUNX1, ***PTEN, RET,*** SRC, TBX3, ***TP53***	***AKT1,*** ALK, ***APC,*** AR, ARAF, BRAF, CHEK2, CTNNB1, DDR2, ***EGFR, ERBB2, ERBB3,*** ***ESR1,*** FBXW7, FGFR1, FGFR2, FGFR3, FGFR4, FLT3, GNA11, GNAQ, GNAS, HRAS, IDH1, IDH2, ***KIT, KRAS, MAP2K1,*** MAP2K2, ***MET,*** MTOR, ***NRAS,*** NTRK1, NTRK3, ***PDGFRA, PIK3CA, PTEN,*** RAF1, ***RET,*** ROS1, SF3B1, *SMAD4, SMO,* ***TP53***

- Eighteen genes highlighted in bold are the common ones in the 2 NGS panels used for baseline ctDNA testing. However, not all the amplicons assessed within the same gene by the 2 methods are overlapping.

Comparison Analysis

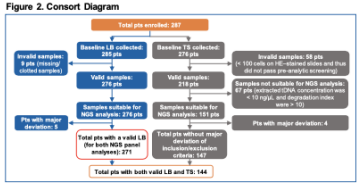
- The results of the SNV analysis, not considering variant of unknown significance, obtained for LB samples using the 533-amplicon custom AmpliSeq HD panel were compared to those obtained for TS. Moreover, the results of the SNV analysis obtained for LB samples using the 533-amplicon custom AmpliSeq HD panel were compared to those obtained for LB samples using the Oncomine Pan-Cancer Cell-Free Assay (considering overlapping amplicons only).
- The Cohen's kappa statistic (agreement/disagreement index) (based on categories from study by Altman DG: Practical statistics for medical research. 1st edition. Oxford: Chapman and Hall 1991; 1-611) was computed, together with its 95% confidence interval (CI), in order to determine a global assessment of agreement between the results.
- The McNemar's test was performed to test the hypothesis that the proportion of pts with an SNV was equivalent between the 2 NGS analyses; *P*-values are reported.
- The distributions of *PIK3CA* variant allele frequency in "concordant positive" (LB+/TS+), "concordant negative" (LB-/TS-) and "discordant" (LB+/TS-, LB-/TS+) were evaluated, and the statistical difference was estimated by Wilcoxon-Mann-Whitney test.
- A comparison between the gene-level mutation frequencies observed in the BioItaLEE cohort (baseline LB [n = 271] and TS [n = 144])with the frequencies observed in TCGA cohort (breast invasive carcinoma [TCGA, provisional], ER+/HER2– subset [n = 590]), was performed. Of all the mutations called, only the subset overlapping with the regions covered by custom panel were included in the analyses. For both datasets, only mutations reported in the COSMIC database were included. The differences in observed frequencies were tested by Fisher's exact test, and *P*-values were corrected using the Benjamini Hochberg method.

Results

Patient characteristics and disposition

- From February to December 2018, 287 postmenopausal women were enrolled.
- Baseline LB and TS were collected from 285 and 276 pts, respectively. Matched LB and TS samples were available for 144 evaluable pts (**Figure 2**).

Figure 2. Consort Diagram



HE, hematoxylin eosin; LB, liquid biopsy; NGS, next-generation sequencing; pts, patients; tDNA, tumor deoxyribonucleic acid; TS, tissue samples.

- Baseline TS were collected from a metastatic site (33.3%) and primary tumors (66.7%). In more than half of the cases (54.2%, n = 78), TS were collected from a recent sampling (within 60 days from the start of study treatment), while 27.8% (n = 40) of the samples were taken from semi-recent (between 6 months and D61 from C1D1) and 18% (n = 26) from archival tissues (> 6 months from C1D1). Notably, 51% of enrolled pts had *de novo* metastatic disease at study inclusion.
- Patient demographics and baseline characteristics of patient population (n = 144) are listed in **Table 2**.

Altered Genes by Patient (Biomarker Analysis Set)

- At least 1 SNV was found in 72.9% (n = 105) and 44.4% (n = 64) of TS and LB, respectively. Of note, 34.0% (n = 49) of TS and 20.7% (n = 24) of LB exhibited > 1 alteration.
- The SNVs found in > 2% of TS samples analysis vs corresponding data observed on LB are included in **Table 3**.
- The following oncoplot represents the pattern, co-occurrence, and type of genomic alterations found both in baseline TS and in LB for genes (n = 18) having alteration frequency ≥ 1% in TS or LB (**Figure 3**).

Genomic Concordance Analysis Between Baseline Tissue and Liquid Biopsy Samples (n = 144)

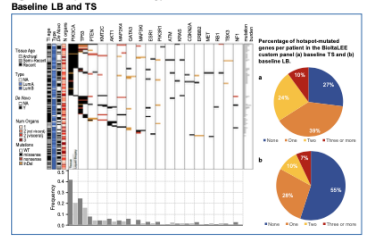
- SNV gene alterations found in baseline TS and LB were compared and their agreement was evaluated.
- The concordance for altered genes found in at least 2% of pts is detailed in **Table 3**.
- The overall concordance in terms of single gene alteration by pts between LB and TS was moderate (K = 0.51, CI: 0.44-0.58, P < 0.0001) mostly due to negative findings in LB. For *PIK3CA*, 18.8% (n = 27) of pts had concordant-positive status (LB+/TS+), 58.3% (n = 84) had concordant-negative status (LB-/TS-), and 23% (n = 33) had discordant status (21.5% [n = 31] had with LB-/TS+, and only 1.4% [n = 2] with LB+/TS-).
- The calculation of an overall concordance in terms of SNVs observed in pts' samples (K "adjusted") confirmed a moderate concordance (K "adjusted" = 0.48; CI: 0.39, 0.56).
- Assessment of agreement in patient subgroups showed that the concordance remained moderate independent of the timing of tissue sampling (archival, semi-recent, and recent) and location of the metastatic sites. In addition, agreement between TS and LB results seems to be better (K = 0.64, good) in pts with > 3 organs affected by metastases and worst in pts with Ki67 ≤ 20% (K = 0.37, fair); this suggests to explore more deeply the potential impact of tumor burden and aggressiveness on ctDNA release.

Table 2. Demographic and Baseline Characteristics of pts With Valid Baseline LB and TS

Demographic Variable		Biomarker Analysis Set (N = 144)
Median age, years (range)		67.0 (47-86)
Age category, years, n (%)	< 65	55 (38.2)
	≥ 65	89 (61.8)
ECOG performance status, n (%)	0	105 (72.9)
	1	37 (25.7)
	2	2 (1.4)
Metastatic disease status, n (%)	*De novo*	74 (51.4)
	Recurrent	70 (48.6)
Estrogen/progesterone positive status, n (%)	Both ER+ and PgR+	125 (86.8)
	ER+ only	15 (10.4)
	PgR+ only	2 (1.4)
	Not assessable	2 (1.4)
Ki67, n (%)	< 20%	46 (31.9)
	≥ 20%	91 (63.2)
	Missing	7 (4.9)
Metastatic sites, n (%)	Bone	103 (71.5)
	Bone only	26 (18.1)
	Visceral	60 (41.7)
	Liver	15 (10.4)
	Lung	45 (31.3)
	Liver and lung	51 (35.4)
	Other visceral	12 (8.3)
	CNS	0
	Lymph nodes	96 (66.7)
	Skin	4 (2.8)
	Breast	11 (7.6)
	Other	12 (8.3)
Number of organs of interest involved by metastases by patient, n (%)	One organ	46 (31.9)
	Bone	30 (20.8)
	Liver and lung	4 (2.8)
	Neither bone nor liver or lung	12 (8.3)
	Two organs	69 (47.9)
	Liver and lung included (visceral)	23 (16.0)
	Liver and lung not included (nonvisceral)	46 (31.9)
	Three organs	28 (19.4)
	No organ of interest involved	1 (0.7)
Tumor type	Luminal A	33 (22.9)
	Luminal B	104 (72.2)
	Unknown	7 (4.9)

ER (PgR and Ki67 data are not centrally assessed but are obtained from local pathology reports.
CNS, central nervous system; ECOG, Eastern Cooperative Oncology Group; ER, estrogen receptor; PgR, progesterone receptor.

Figure 3. Oncoplot: Pattern and Type of Genomic Alterations in Pts With Valid Baseline LB and TS



Luminal A disease was defined as pts with Ki67 < 20%, ER+, PgR ≥ 20%, HER2– status or Ki67 < 20%, ER– , PgR ≥ 20%, HER2– status. Luminal B was defined as Ki67 ≥ 20% or PgR < 20%.
LB, liquid biopsies; Lum A, luminal A; Lum B, luminal B; N, number; NA, not applicable; TS, tissue samples; WT, wild type.

Table 3. Concordance Analysis in Single Gene Alteration by pts Between LBs and TS (n = 144)

Cohen's kappa Values	< 0.20	0.21-0.40	0.41-0.60	0.61-0.80	0.81-1.0
Quality	Poor	Fair	Moderate	Good	Very good

Gene	TS, % (n)	LB, % (n)	K Cohen's (95% CI)	McNemar P-Value	LB+ /TS+ % (n)	LB+ /TS- % (n)	LB- /TS+ % (n)	LB- /TS- % (n)
PIK3CA	40.3 (58)	20.1 (29)	**0.48 (0.34, 0.62)**	< 0.0001	18.8 (27)	1.4 (2)	21.5 (31)	58.3 (84)
TP53	24.3 (35)	16.0 (23)	**0.44 (0.27-0.62)**	0.0186	11.1 (16)	4.9 (7)	13.2 (19)	70.8 (102)
PTEN	7.6 (11)	4.2 (6)	**0.56 (0.28, 0.85)**	0.1250	3.5 (5)	0.7 (1)	4.2 (6)	91.7 (132)
KMT2C	4.2 (6)	4.2 (6)	**0.83 (0.59, 1.00)**	1.0000	3.5 (5)	0.7 (1)	0.7 (1)	95.1 (137)
MAP2K4	4.2 (6)	3.5 (5)	**0.72 (0.41, 1.00)**	1.0000	2.8 (4)	0.7 (1)	1.4 (2)	95.1 (137)
ATM	0.7 (1)	2.1 (4)	0.49 (-0.11-1.00)	0.5000	0.7 (1)	1.4 (2)	0	97.9 (141)
AKT1	5.6 (8)	2.8 (4)	0.48 (0.13, 0.83)	0.2188	2.1 (3)	0.7 (1)	3.5 (5)	93.8 (135)
MAP3K1	4.9 (7)	1.4 (2)	0.43 (0.03, 0.83)	0.0625	1.4 (2)	0	3.5 (5)	95.1 (137)
ESR1	2.8 (4)	1.4 (2)	-0.02 (-0.04, 0.00)	0.6875	0	1.4 (2)	2.8 (4)	95.8 (138)
GATA3	5.6 (8)	0.7 (1)	0.21 (-0.14, 0.56)	0.0156	0.7 (1)	0	4.9 (7)	94.4 (136)
ERBB2	2.1 (3)	0.7 (1)	0.49 (-0.11, 1.00)	0.5000	0.7 (1)	0	1.4 (2)	97.9 (141)
PIK3R1	2.8 (4)	0.7 (1)	-0.01 (-0.03, 0.01)	0.3750	0	0.7 (1)	2.8 (4)	96.5 (139)
Overall			**0.51 (0.44, 0.58)**	**< 0.0001**				
Overall "adjusted"			**0.48 (0.39, 0.56)**	**< 0.0001**				

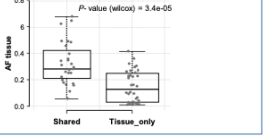
Concordance Analysis Between Custom and Oncomine Pan-cancer NGS Panels

- To verify the consistency of SNV gene alterations found in LB using the custom panel, LB were additionally tested with oncomine pan-cancer cell-free assay.
- The overall concordance between custom and oncomine pan-cancer panels was good (K = 0.73; CI: 0.64, 0.81). For *PIK3CA*, the concordance was very good (K = 0.83; CI: 0.71, 0.94) confirming the validity of ctDNA results obtained.

Correlation of LB/TS Concordance and Variant Allele Frequency in *PIK3CA* Gene

- For PIK3CA, the most frequently altered gene in the patient population (40.3% in TS, 20.1% in LB), an analysis of variant allele frequency by concordance status between LB and TS results was done. Discordant cases showed significantly lower allele frequencies (AFs) (Wilcoxon *P* < 1e 4) (**Figure 4**).

Figure 4. Correlation Between Variant Allele Frequency and Concordance in LB vs TS for *PIK3CA* Gene



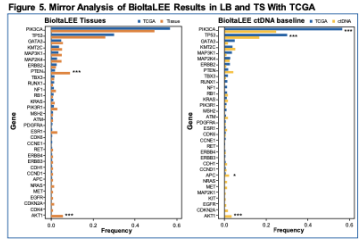
Mirror Analysis With TCGA

- The mutation frequencies in BioItaLEE TS cohort were overall consistent with TCGA. However, a significantly increased mutation rate in *PTEN* and *AKT* as compared to TCGA was detected (*P* < 0.001), in line with genomic studies of metastatic breast cancer cohorts.[10,11] *PIK3CA* and TP53 mutation rate was lower in LB cohort than in TCGA (*P* < 0.001), mainly due to low/undetectable ctDNA content in a significant proportion of pts. Nevertheless, APC (*P* < 0.05) and AKT1 (*P* < 0.001) were mutated at higher rate than in TCGA (**Figure 5**).
- To be noted that in 144 pts with both a valid LB and TS, 22 distinct *PIK3CA* variants with different AFs were observed, suggesting both clonal and subclonal alterations.

Altered Genes in the Patient Population Without a Valid Matched Tumor Sample

- Of the 127 pts with a valid LB but without a matched TS, 47.2% (n = 60) had at least an SNV, 15% (n = 19) had 2 alterations and 4% (n = 5) had 3 or more alterations. Significantly, 24.4% (n = 31) had a *PIK3CA* alteration.

Figure 5. Mirror Analysis of BioItaLEE Results in LB and TS With TCGA



Statistical analysis: two-sided Fisher's exact test, Benjamini-Hochberg adjusted *P*-value. *= *P* < 0.05; **= *P* < 0.01; *** *P* < 0.001.

Conclusions

- In our study, gene mutations were more frequently found in TS rather than LB, supporting the strategy of querying the tissue to complement ctDNA in case of negative results.
- The ultra-deep NGS approach used for TS in this study enabled agreement between TS and LB results. LB+ findings with TS- results were rare.
- Overall, mutation prevalence in TS (in 66.7% of the cases represented by primary tumor samples) was comparable with that found in the TCGA primary BC database. The differences in mutation prevalence of some genes may be due to the different timing of the tumor sampling in our study (60.5% of all biopsies were sampled within 6 months from study enrollment), and the presence of a high proportion of pts with *de novo* metastatic disease in our cohort (51% of all the TS are from biopsies of pts with *de novo* metastatic disease).
- Discordance in *PIK3CA* status between TS and LB is associated with lower AFs in TS, likely due to subclonal events, which may lead to undetectable mutation in ctDNA.

References

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Disclosures

Giampaolo Bianchini: Personal fees for advisory/consultancy from Roche, Novartis, Eisai, AstraZeneca, Pfizer, Lilly, Genomic Health, Amgen, MSD, Sanofi, Daiichi Sankyo, Chugai. **Michelino De Laurentiis**: Personal fees for speaker's honoraria, advisory board honoraria from Pfizer, AstraZeneca, Novartis, Roche, Celgene, Eisai, Eli Lilly, Amgen, MSD, Daiichi Sankyo. **Grazia Arpino**: Personal fees for advisory board from Pfizer, AstraZeneca, Novartis, Roche, Celgene, Eisai, Lilly, Daiichi Sankyo. **Alberto Zambelli**: Advisory/consultant fees from Novartis, Roche, Astra Zeneca, Pfizer, Lilly, Bruger and Gonsior, Health. **Fabio Puglisi**: Grants from AstraZeneca, Eisai, Roche. Personal fees from Novartis, MSD, Eli Lilly, Roche, Pfizer, Pierre Fabre, Eisai. **Lucia Del Mastro**: Personal fees from Roche, Pfizer, Ipsen, Eli Lilly, Novartis, Takeda, MSD, Genomic Health, Seattle genetics. Non-financial support from Celgene. **Marco Angelo Colleoni**: Advisory board personal fees from Pfizer, AstraZeneca, Novartis, Puma Biotechnology, Celltrion, Eisai, GBI Pharma, Pierre Fabre. **Filippo Montemurro**: Travel grants, speaker's honoraria, advisory board honoraria from Roche, Novartis, Pfizer, Lilly, Celgene, Eisai, Daiichi Sankyo, Amgen, Seattle Genetics, Astellas, Merck Sharp & Dohme, Pierre Fabre. **Giulia Bianchi**: Personal fees for advisory board and honoraria from Novartis, speaker honoraria from Eli Lilly. **Ida Paris, Giacomo Allegrini, Laura Amaducci, Marina Elena Cazzaniga, Michele Orditura**: No COI to disclose. **Claudio Zamagni**: Grant, personal fees and nonfinancial support for advisory, travel accommodation, research funding from Roche, Novartis and Pfizer; advisory grant from Eisai; grant, personal fees and nonfinancial support for advisory, research funding from AstraZeneca; grant for advisory, travel accommodation from Pharmadille, Celgene; grant for advisory from Lilly; grant and personal fees for advisory from Daiichi Sankyo; personal fees for advisory, travel accommodation from Amgen, Merck Sharp & Dohme, Ipsen, Bayer, Servier, Sanofi; travel accommodation, research funding from PharmaKern, Italfarmaco, Menarini; grant for research funding from Takeda, Teva, Medivation, Abbvie, Array BioPharma, Nerviano, Synthon, Seattle Genetics. **Sara Bianchetti and Daniela Castelletti**: Employees of Novartis. **Matteo Benelli**: Personal fees from Novartis. **Maurizio Callari**: No COI to disclose. **Luca Malorni**: Grants and personal fees from Novartis, Pfizer.

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ctDNA/tissue concordance in the BIOITALee trial

Pretreatment liquid biopsies (LBs) and tumor samples (TS) were collected prospectively in the trial:

Liquid biopsies (N=285)

Tumor Samples (N= 276)

Matched LB/TS (valid for NGS) (N=144)

Tumor Samples main characteristics:
Primary tumor (66.7%)
52% was recent (taken <60d from enrollment)

NGS Analysis: Baseline ctDNA and tDNA were assessed by SNV analysis using the same 533-amplicon custom AmpliSeq HD panel

NGS Testing	BioItaLEE AmpliSeq HD Custom Panel	
Sample tested	Baseline LB (ctDNA)	Baseline TS
Mean coverage and LOD	23000 X 0.1% (for 10 ng of cfDNA input)	12000 X 0.1% (for 5 ng of tDNA)
No. of genes tested for SNV	39	
Genes analyzed	***AKT1, APC,*** *ATM, CDH1, CCND1, CCNE1, CDK4, CDK6, CDH1, CDKN2A,* ***EGFR, ERBB2, ERBB3,*** *ERBB4,* ***ESR1, FGFR1,*** *GATA3,* ***HRAS, KIT,*** *KMT2C,* ***KRAS, MAP2K1,*** *MAP2K4 , MAP3K1,* ***MET,*** *MLH1, NF1, NOTCH1,* ***NRAS, PDGFRA, PIK3CA,*** *PIK3R1, RB1, RUNX1,* ***PTEN, RET,*** *SRC, TBX3,* ***TP53***	

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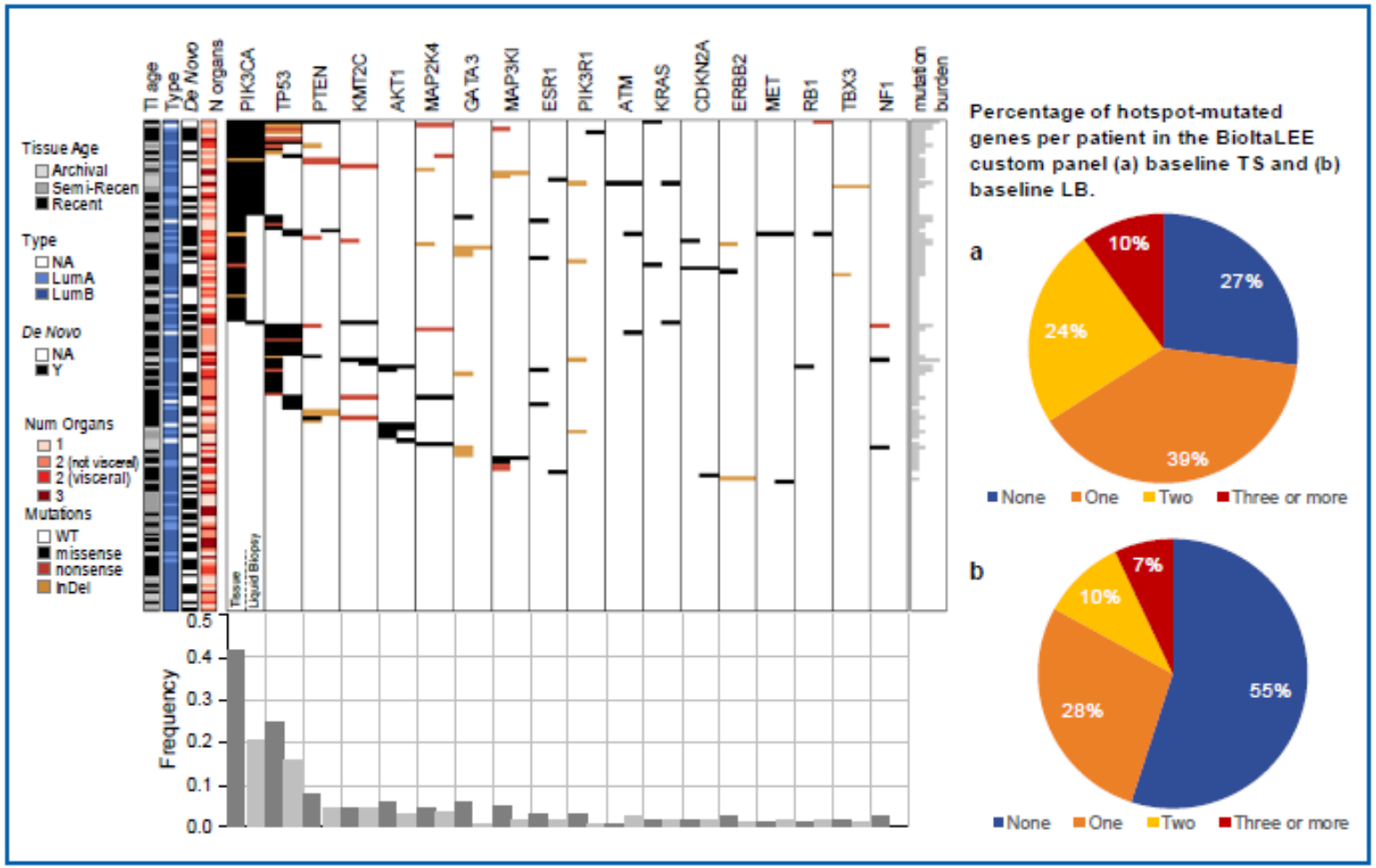
ctDNA/tissue concordance in the BIOITALee trial

At least 1 SNV was found in:

- **72.9%** (n = 105) of tissue samples
- **44.4%** (n = 64) liquid biopsy



Luminal A disease was defined as pts with Ki67 < 20%, ER+, PgR ≥ 20%, HER2– status or Ki67 < 20%, ER–, PgR ≥ 20%, HER2– status. Luminal B was defined as Ki67 ≥ 20% or PgR < 20%.

LB, liquid biopsies; Lum A, luminal A; Lum B, luminal B; N, number; NA, not applicable; TS, tissue samples; WT, wild type.

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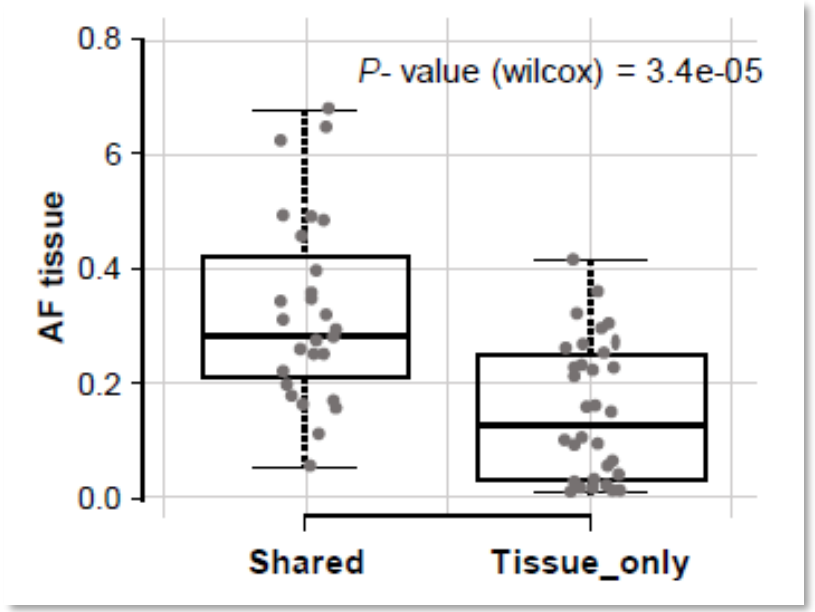
BIOITALee ctDNA vs tissue: Key findings

Concordance Between LBs and TS (n = 144) was moderate

Cohen's kappa Values	< 0.20	0.21-0.40	0.41-0.60	0.61-0.80	0.81-1.0
Quality	Poor	Fair	Moderate	Good	Very good

Gene	TS, % (n)	LB, % (n)	K Cohen's (95% CI)	McNemar P-Value	LB+ /TS+ % (n)	LB+ /TS- % (n)	LB- /TS+ % (n)	LB- /TS- % (n)
PIK3CA	40.3 (58)	20.1 (29)	0.48 (0.34, 0.62)	< 0.0001	18.8 (27)	1.4 (2)	21.5 (31)	58.3 (84)
TP53	24.3 (35)	16.0 (23)	0.44 (0.27-0.62)	0.0186	11.1 (16)	4.9 (7)	13.2 (19)	70.8 (102)
PTEN	7.6 (11)	4.2 (6)	0.56 (0.28, 0.85)	0.1250	3.5 (5)	0.7 (1)	4.2 (6)	91.7 (132)
KMT2C	4.2 (6)	4.2 (6)	0.83 (0.59, 1.00)	1.0000	3.5 (5)	0.7 (1)	0.7 (1)	95.1 (137)
MAP2K4	4.2 (6)	3.5 (5)	0.72 (0.41, 1.00)	1.0000	2.8 (4)	0.7 (1)	1.4 (2)	95.1 (137)
Overall			**0.51 (0.44, 0.58)**	**< 0.0001**				
Overall "adjusted"			**0.48 (0.39, 0.56)**	**< 0.0001**				

- **PIK3CA SNV: 20.1% in LB vs 40.3% in TS**
- **LB-/TS+ discrepancy was more common**
- **PIK3CA Allele frequency was lower in cases LB-/TS+ suggesting subclonal event**



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TAKE HOME messages

- **A negative liquid biopsy may be due to the presence of a subclonal mutation**

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San Antonio Breast Cancer Symposium®, December 7–10, 2021

Circulating tumor DNA (ctDNA) dynamics in patients with hormone receptor positive (HR+)/HER2 negative (HER2-) advanced breast cancer (aBC) treated in first line with ribociclib and letrozole in the BioItaLEE trial

Giampaolo Bianchini

Department of Medical Oncology, Ospedale San Raffaele, Milano, Italy

SAN ANTONIO BREAST CANCER SYMPOSIUM®

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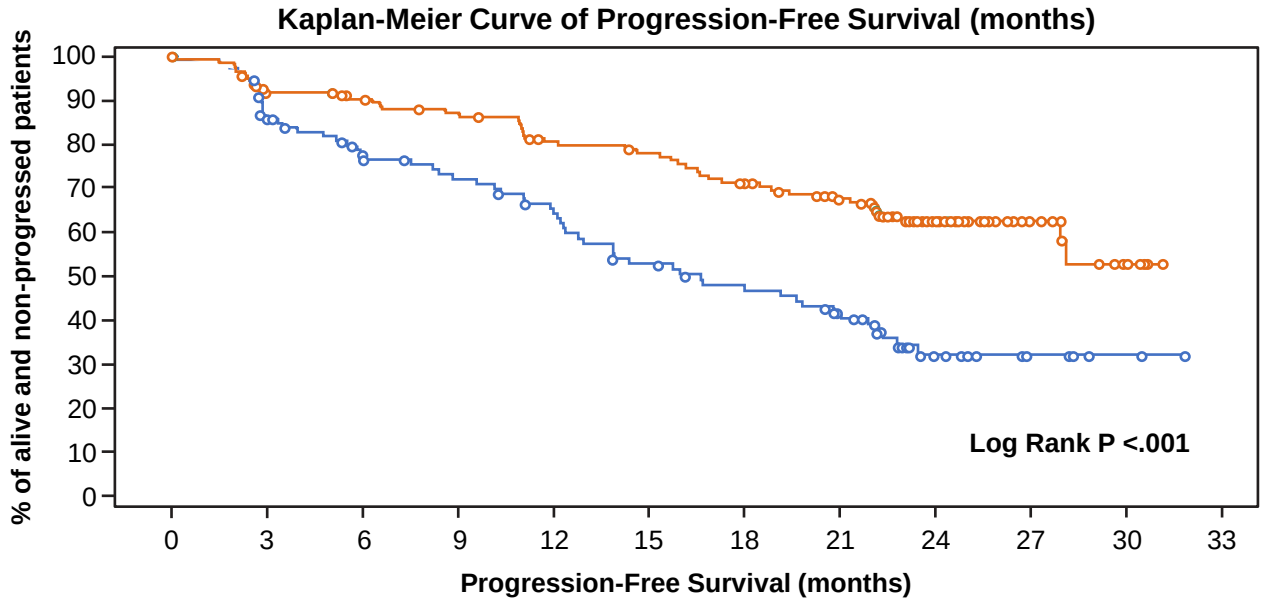
ctDNA dynamics in the BIOITALee trial- key findings BASELINE

Target mutation at baseline	mPFS	HR (95% CI)	P value
Wild type (n=150)	NE	0.41 (0.27,0.61)	<0.0001
Mutated (n=113)	16.59		



Patients at risk

Wild type	150	119	114	107	96	92	82	72	44	20	5	0
Mutated	113	87	73	65	56	45	38	30	13	5	2	0

At baseline, target mutations were detected in 113 patients (43%), whereas 150 patients (57%) were wild type

Mean (SD) pre-treatment VAF at baseline was 11.3% (14.4)

Absence of a target mutation at baseline was associated with good prognosis

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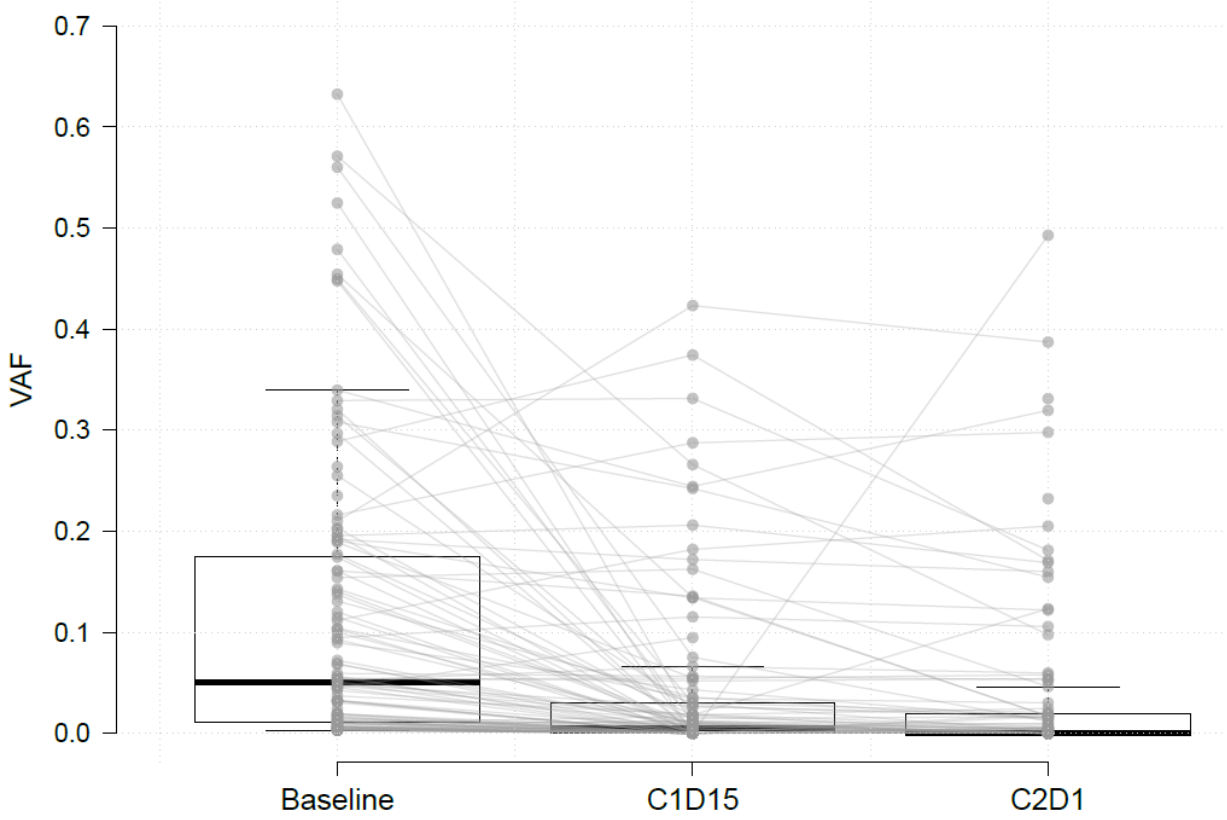
TAKE HOME messages

- A negative liquid biopsy may be due to the presence of a subclonal mutation
- A negative liquid biopsy (using a large panel) is associated with better outcome at baseline

ctDNA dynamics in the BIOITALee trial- key findings
Early changes during C1



Timepoint	mean (SD) VAF Change
D15 (n=104)	– 64.3% (55.9)
C2D1 (n=105)	– 68.6% (52.2)

Early VAF clearance was observed in 47.1% (n=49) of patients at D15 and 52.4% (n= 55) of patients at C2D1

A significant VAF reduction was observed upon ribociclib + letrozole treatment at D15 and C2D1 (but no rebound at C2D1)

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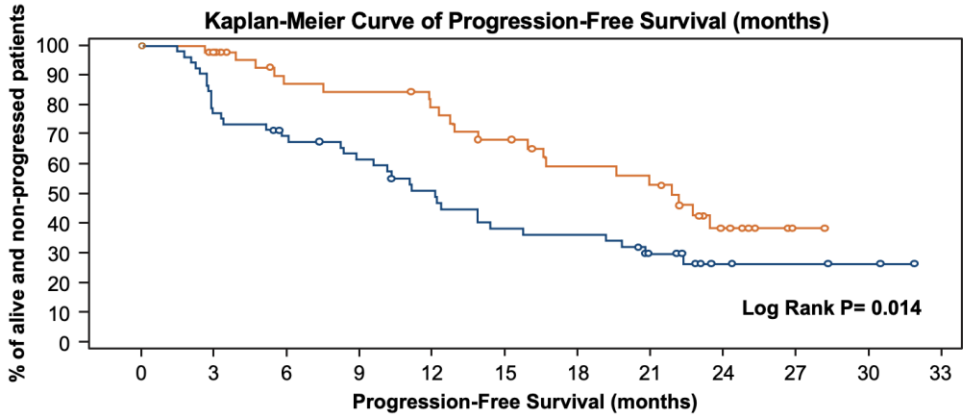
ctDNA dynamics in the BIOITALee trial- key findings
Early changes during C1

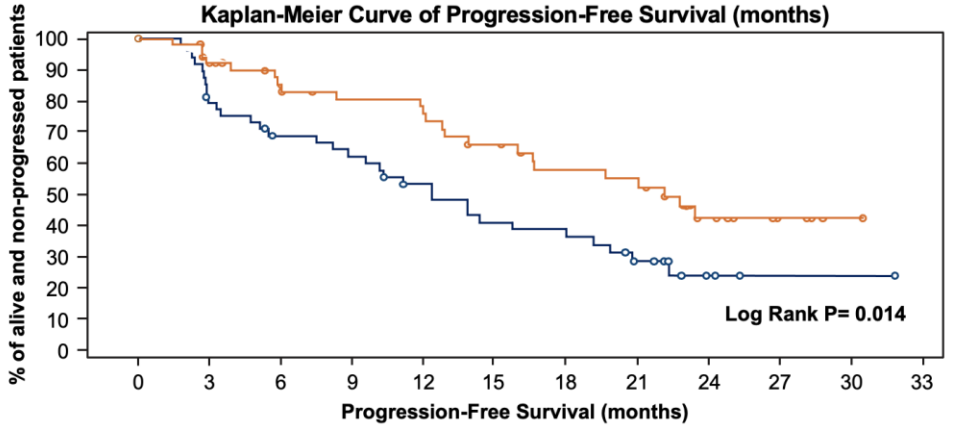
VAF clearance	mPFS	HR (95% CI)	P value
VAF cleared at D15 (n=49)	21.85	0.51 (0.28,0.91)	0.0228
VAF not cleared at D15 (n=55)	12.09		



Patients at risk

VAF cleared at D15	49	41	33	32	29	24	19	17	8	1	0	
VAF not cleared at D15	55	41	35	30	24	18	17	11	4	3	2	0

VAF clearance	mPFS	HR (95% CI)	P value
VAF cleared at C2D1	22.11	0.44 (0.25,0.78)	0.0052
VAF not cleared at C2D1	12.32		



Patients at risk

VAF cleared at C2D1	55	45	38	33	31	26	21	19	10	4	1	0
VAF not cleared at C2D1	50	38	31	28	22	17	15	9	3	1	1	0

Early VAF clearance was associated with improved PFS

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TAKE HOME messages

- A negative liquid biopsy may be due to the presence of a subclonal mutation
- A negative liquid biopsy (using a large panel) is associated with better outcome at baseline
- On-treatment change (from positive to negative) is associated with better outcome

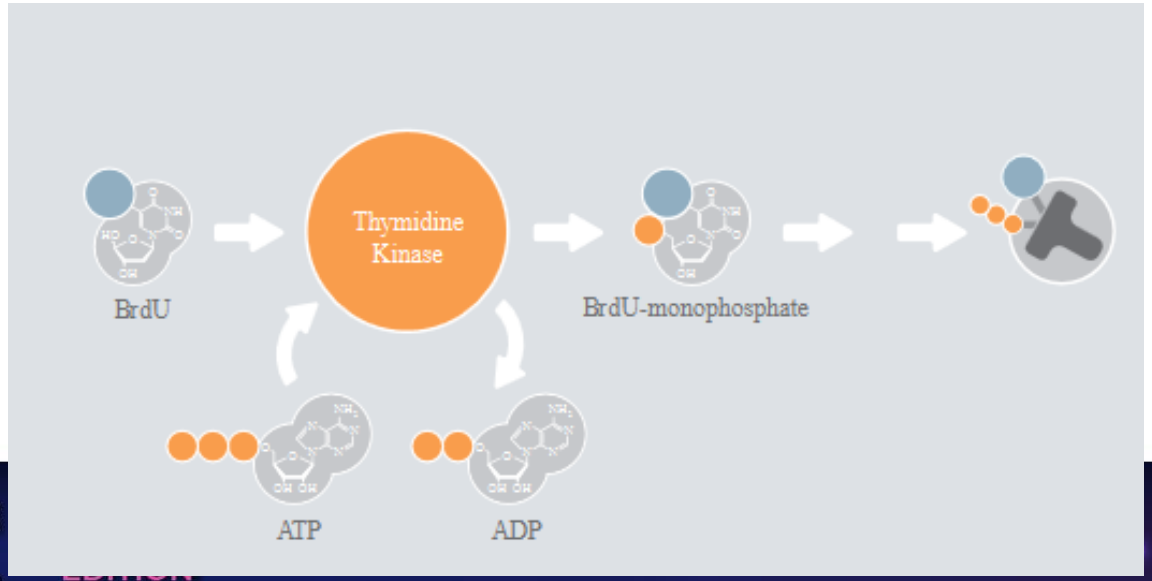
WHAT ELSE CAN WE DO?

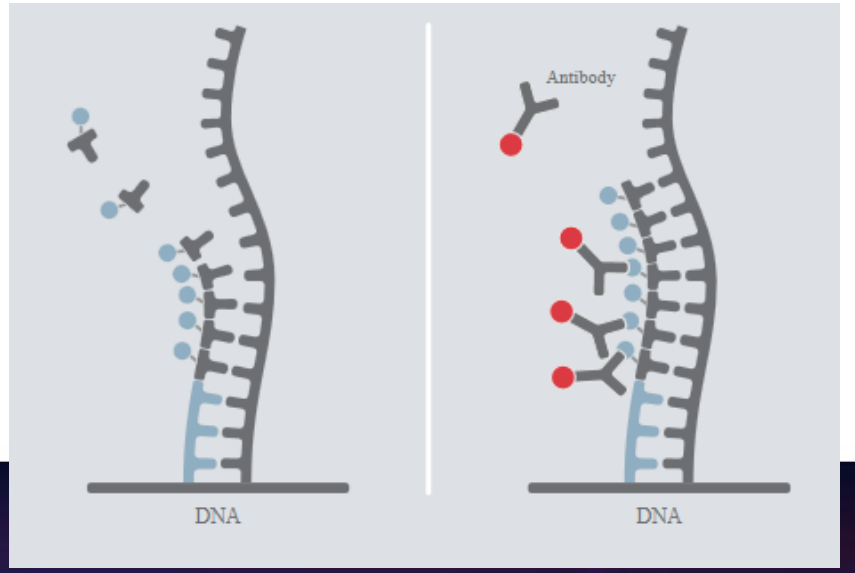
THYMIDINE KINASE ACTIVITY

- TK1 is a cell cycle dependent enzyme playing a critical role in cell proliferation
- TK1 activity rapidly increases after the G1-S transition and then declines
- Cancer cells can secrete pathological levels of TK1 detectable in blood

"Liquid Ki67"

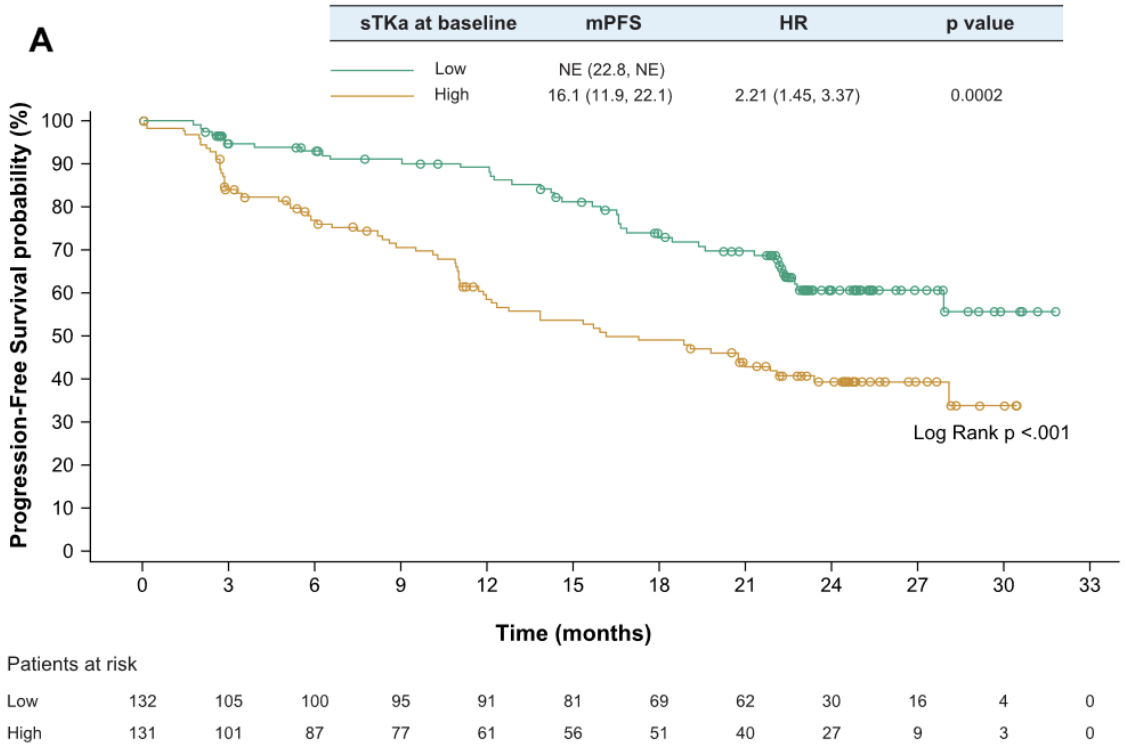
The ELISA based DiviTum™ assay (Biovica International, Uppsala, Sweden) determines the enzymatic activity of TK1 in blood serum/plasma or cell cultures.





Serum Thymidine Kinase 1 (TKa) in the BIOITALee trial- key findings BASELINE

Baseline (median cut-off)



From 263 available samples at baseline, median sTKa was 74.8 Du/L (19–9412)

11,8% of patients had sTKa levels below LOD (20Du/L)

Low sTka at baseline was associated with good prognosis

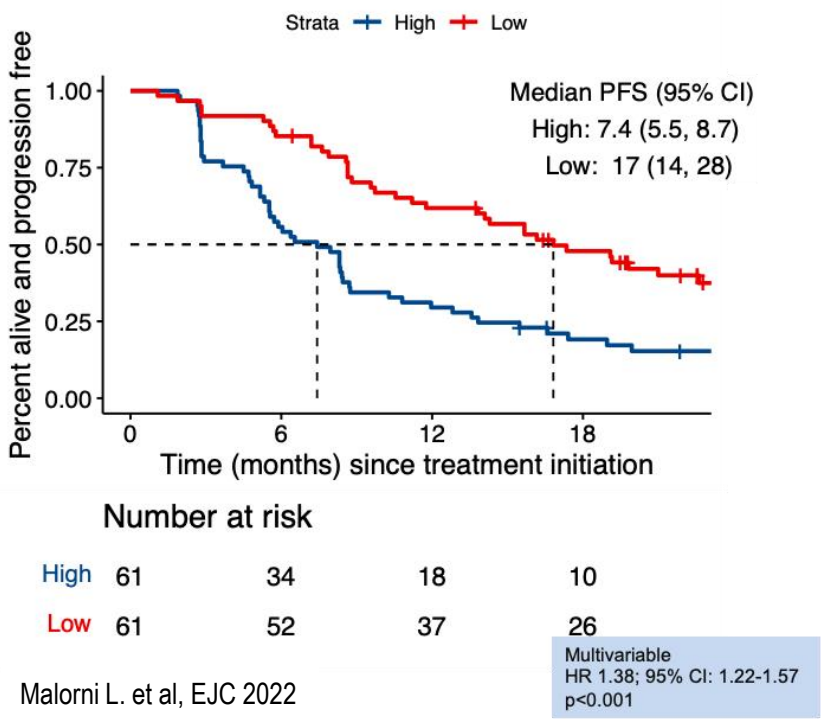
Malorni L. et al, EJC 2023

TKa DATA IN PATIENTS TREATED WITH ET+CDK4/6i: BASELINE

- First/second line tx with PALBO+FUL
- endocrine resistant MBC

PYTHIA

Baseline; median cut-off; 87 Du/L (<20- 14510)



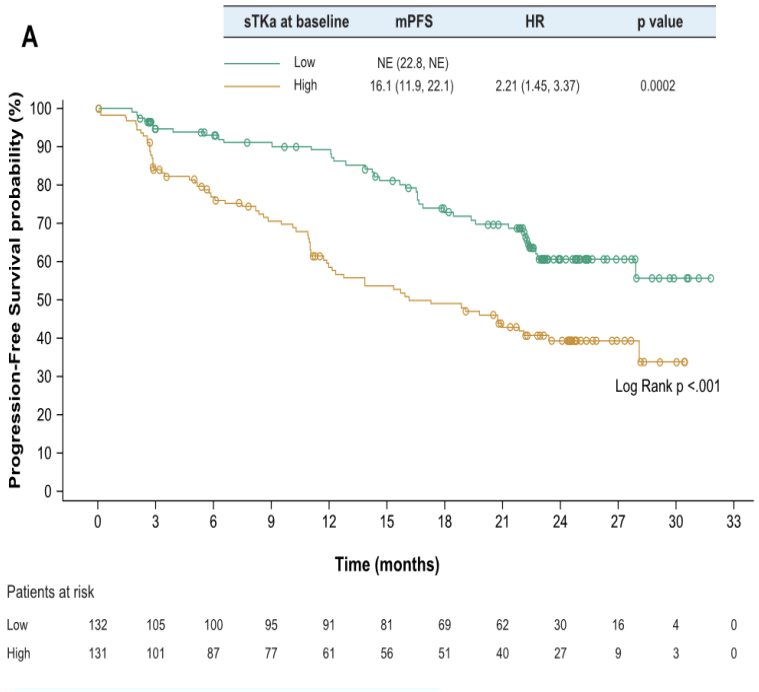
Multivariable
HR 1.38; 95% CI: 1.22-1.57
p<0.001

Malorni L. et al, EJC 2022

- First line tx with RIBO+LET
- endocrine sensitive MBC

BioItaLee

Baseline; median cut-off, 74.8 Du/L (19–9412)

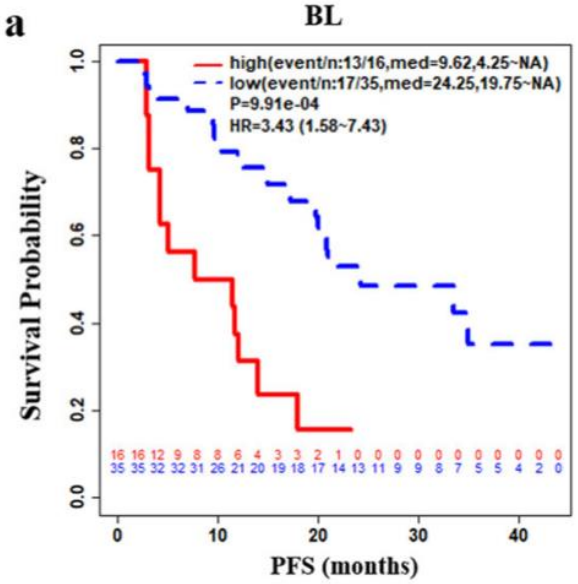


Malorni L. et al, EJC 2023

- First/second line tx with PALBO+HT
- endocrine sensitive/resistant MBC

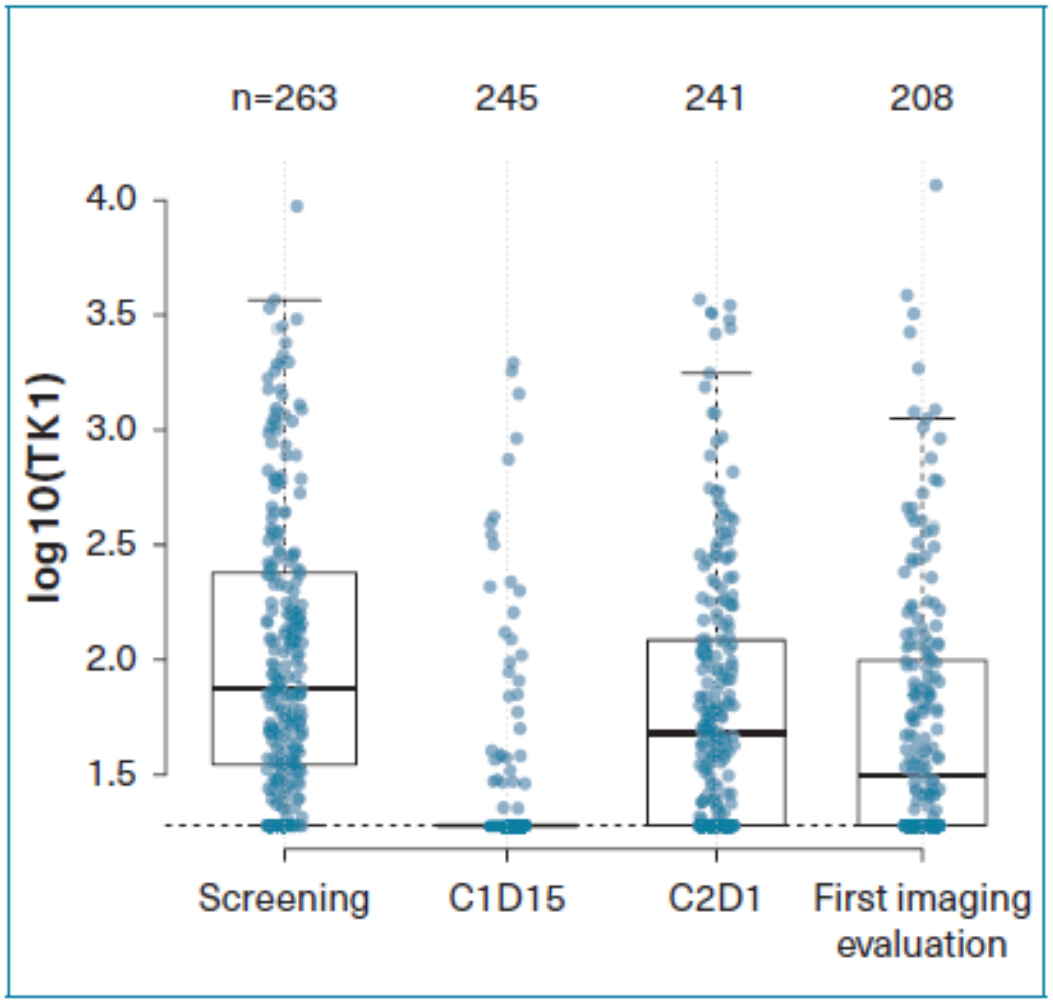
WashU palbo dosing trial

Baseline; median cut-off, 97.9 Du/L (42.4–490.3)



Krishnamurthy, J. npjBC 2022

Serum Thymidine Kinase 1 in the BIOITALee trial- key findings
Early changes during C1



Matched samples.

93.2% of patients had baseline and D15;
88.2% had baseline, D15 and C2D1
74.9% had all time points (baseline, D15, C2D1 and FI)

Early sTKa clearance (levels below LOD) was observed in 84.9% of patients at D15 and 28.6% of patients at C2D1

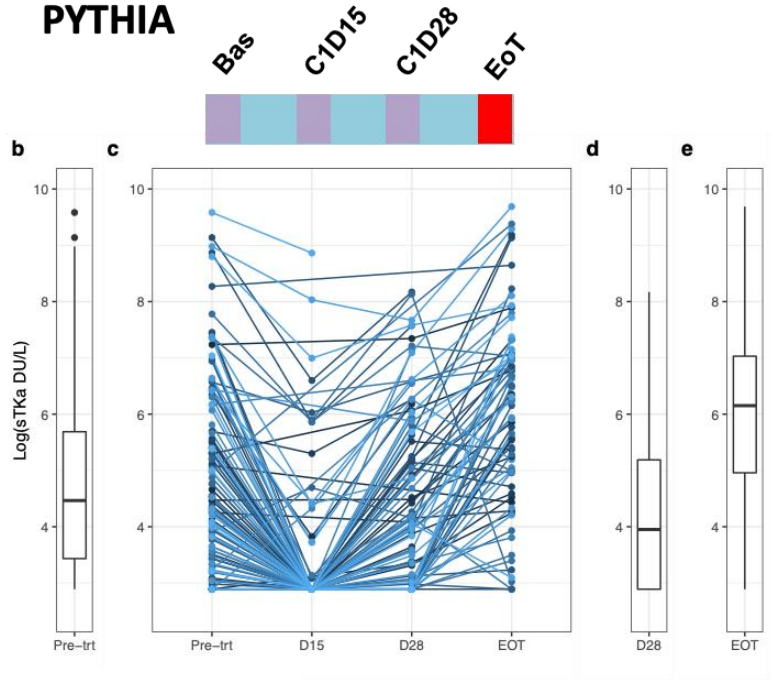
- **A significant reduction in sTKa was observed upon ribociclib + letrozole treatment at D15 and C2D1**
- **A rebound at C2D1 was seen in 68.5% of patients**

Malorni L. et al, EJC 2023

TKa DATA IN PATIENTS TREATED WITH ET+CDK4/6i: changes during treatment

- <LLOD D15 83%
- rebound at D28 54%

PYTHIA



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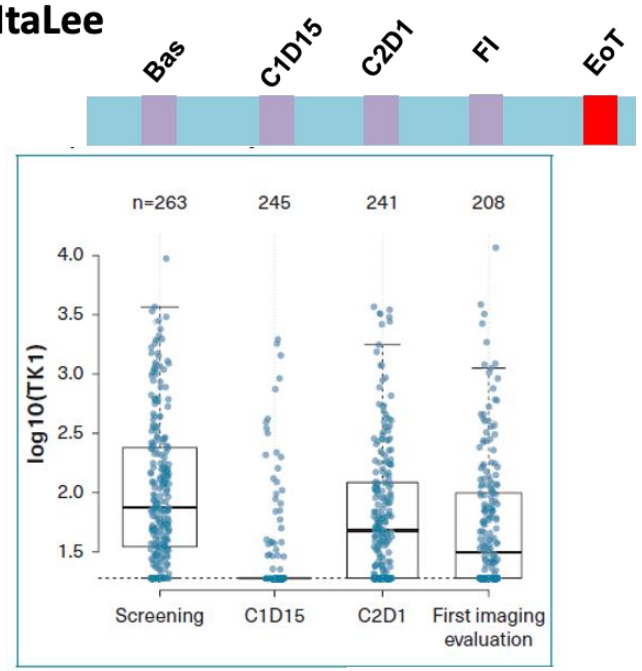
- <LLOD D15 85%
- rebound at D28 68%

BioItaLee



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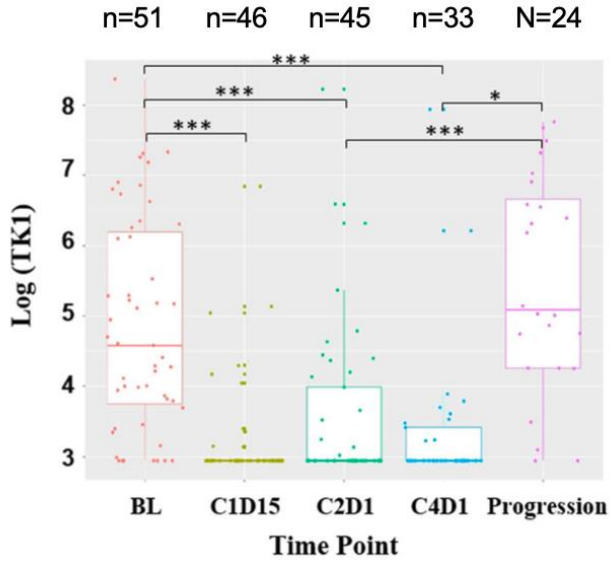
- <LLOD D15 78%
- rebound D28 36%

WashU palbo dosing trial



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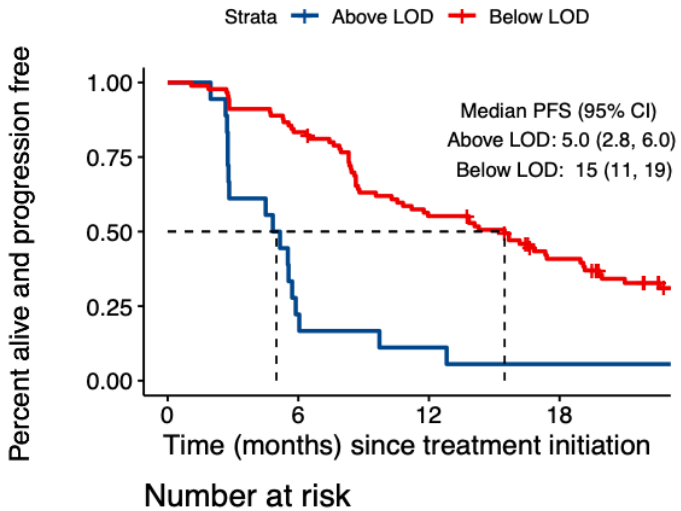
TKa DATA IN PATIENTS TREATED WITH ET+CDK4/6i: CYCLE 1 DAY 15

PYTHIA

C1D15; LoD cut-off, median <20 Du/L (<20- 7060)



Multivariable
HR 1.46; 95% CI: 1.21-1.76
p<0.001

Malorni L. et al, EJC 2022

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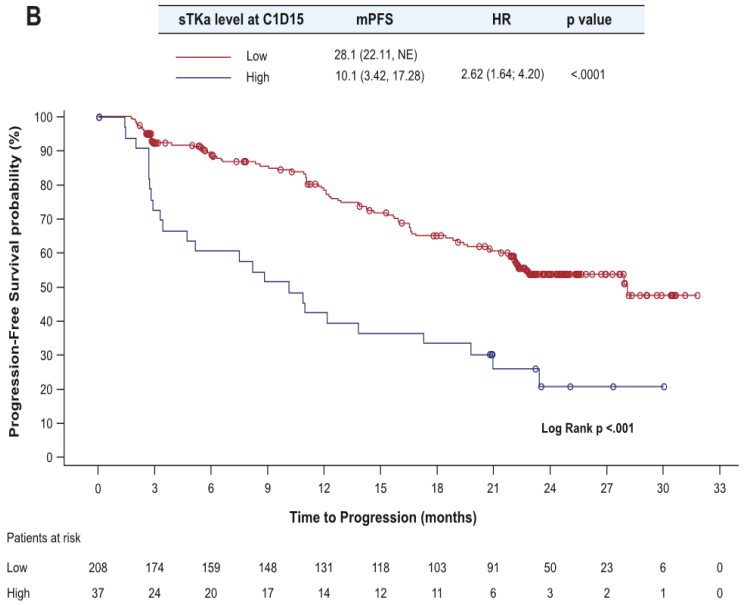
C1D15; LoD cut-off, median 19 Du/L (19–1953)



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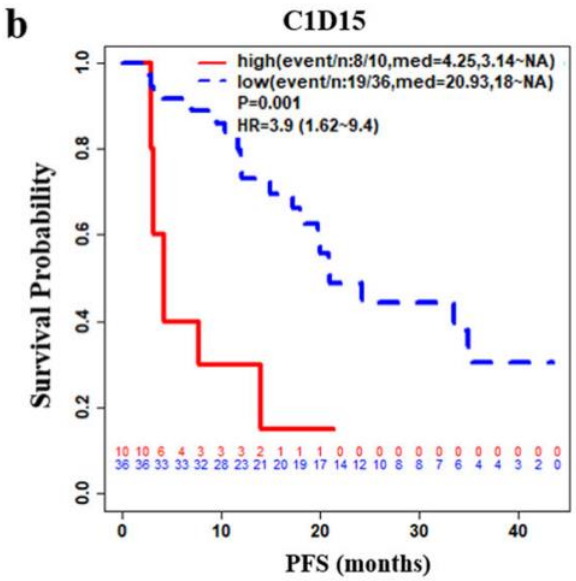
WashU palbo dosing trial

C1D15; LoD cut-off, median <20 Du/L (<20-<20)



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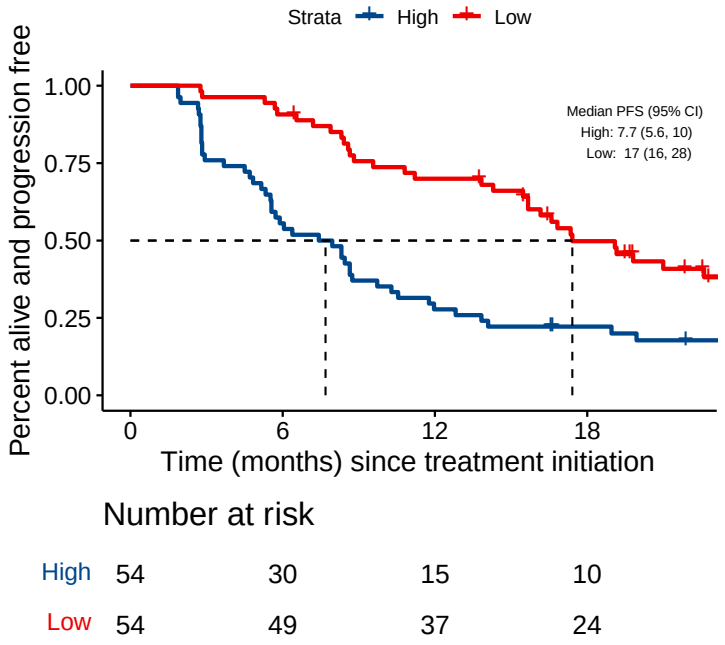
TKa DATA IN PATIENTS TREATED WITH ET+CDK4/6i: CYCLE 2 DAY 1

PYTHIA

C2D1; median cut-off, 52 Du/L (<20, 3533)



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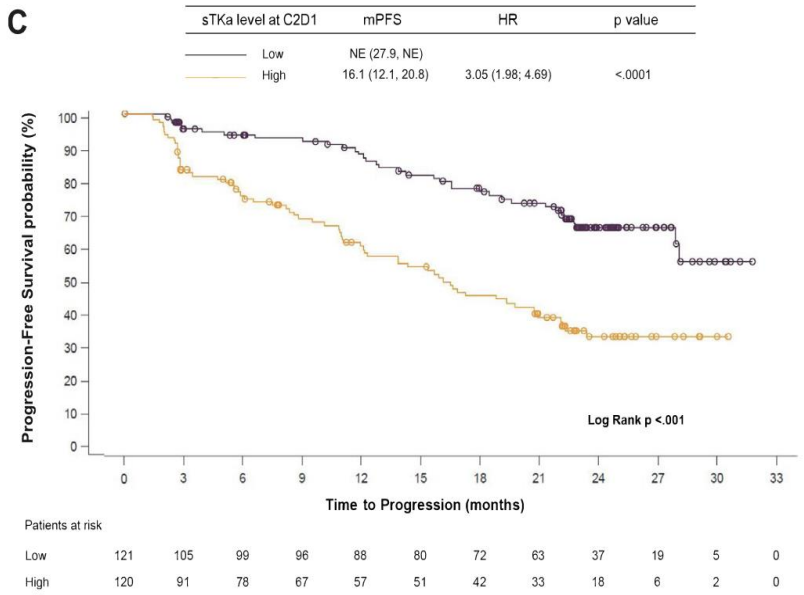
C2D1; median cut-off, 48.1 Du/L (19–3689)



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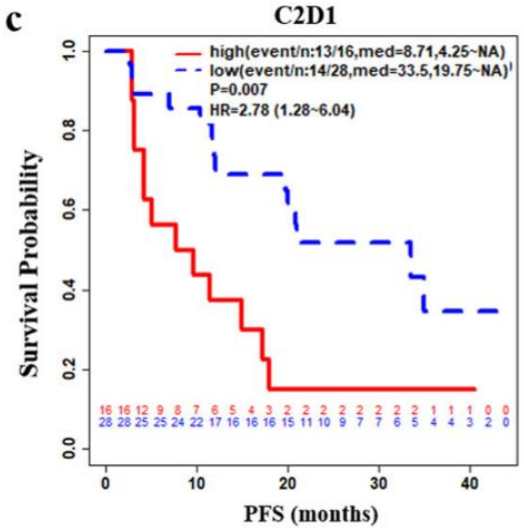
WashU palbo dosing trial

C2D1; median cut-off, <20 (<20~54.1)



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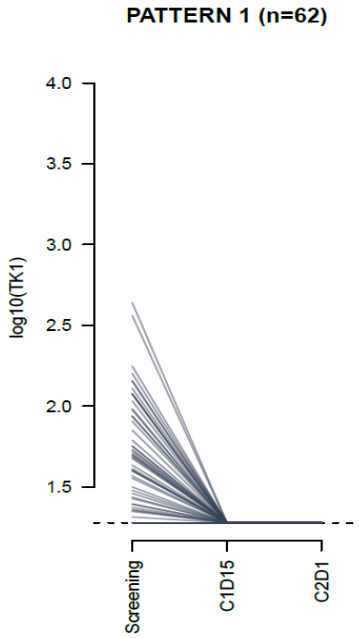
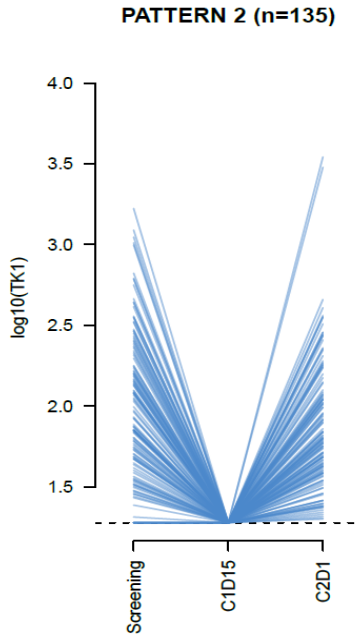
Serum Thymidine Kinase 1 (TKa) in the BIOITALee trial- key findings
Dynamic patterns

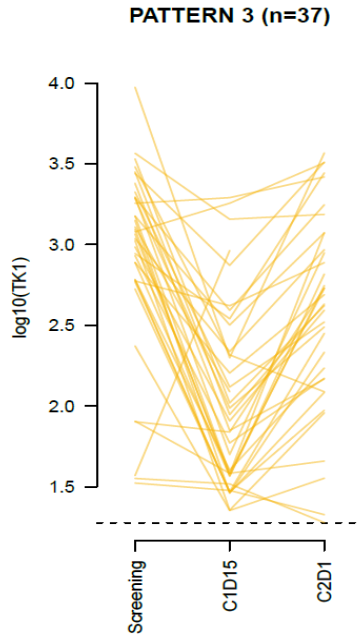
Pattern 1
TKa <LOD at D15 and C2D1



Pattern 2:
TKa <LOD at D15 and >LOD at C2D1



Pattern 3:
TKa >LOD at D15



Malorni L. et al, EJC 2023

Serum Thymidine Kinase 1 (TKa) in the BIOITALee trial- key findings
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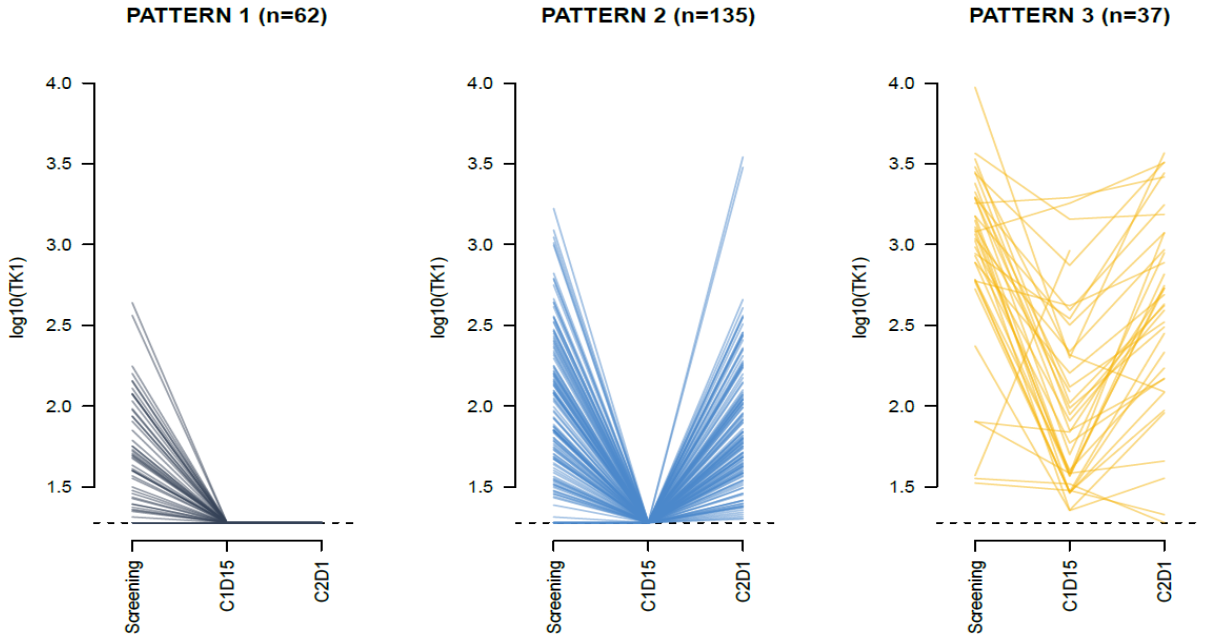
Pattern 1
TKa <LOD at D15 and C2D1

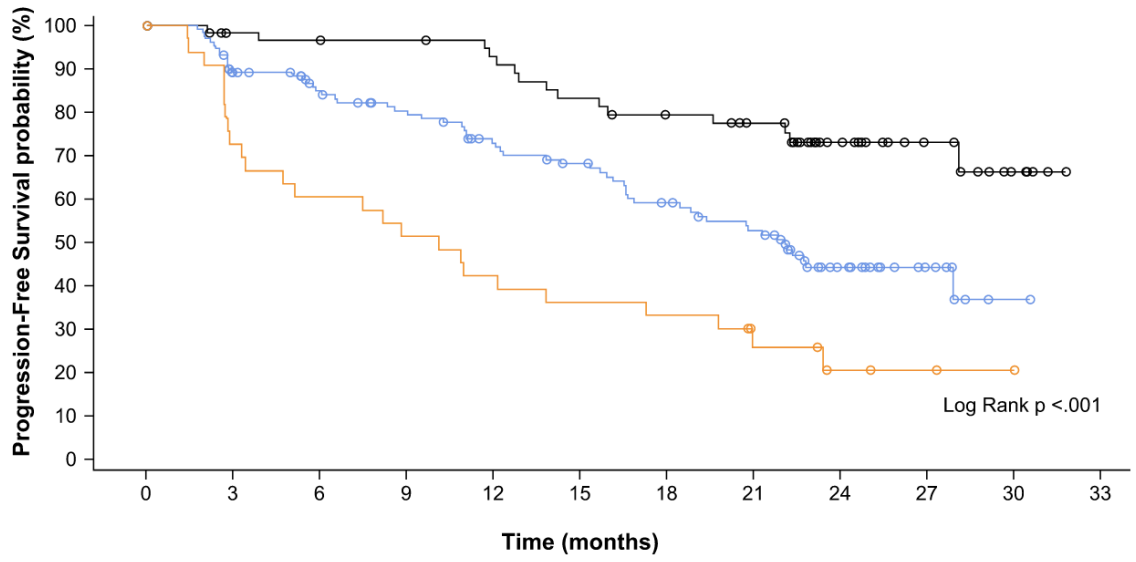
Pattern 2:
TKa <LOD at D15 and >LOD at C2D1

Pattern 3:
TKa >LOD at D15



B

sTKa pattern	mPFS	HR	p value
Pattern 1	NE (28.1, NE)		
Pattern 2	22.1 (16.8, NE)	2.89 (1.57, 5.31)	0.0006
Pattern 3	10.1 (3.1, 17.3)	5.65 (2.84, 11.23)	<0.0001



Patients at risk

Pattern 1	62	54	53	52	49	44	40	36	22	12	5	0
Pattern 2	135	111	97	88	76	69	58	50	27	11	1	0
Pattern 3	37	24	20	17	14	12	11	6	3	2	1	0

Malorni L. et al, EJC 2023

TAKE HOME messages

- A negative liquid biopsy may be due to the presence of a subclonal mutation
- A negative liquid biopsy (using a large panel) is associated with better outcome at baseline
- On-treatment change (from positive to negative) is associated with better outcome
- TKa patterns are prognostic and strongly predictive

2022 ASCO® ANNUAL MEETING

Circulating tumor DNA and serum thymidine kinase 1 activity matched dynamics in patients with hormone receptor–positive, human epidermal growth factor receptor 2–negative advanced breast cancer treated in first-line with ribociclib and letrozole in the BioItaLEE trial

Grazia Arpino[1], Giampaolo Bianchini[2], Luca Malorni[3], Alberto Zambelli[4], Fabio Puglisi[5], Lucia Del Mastro[6], Marco Colleoni[7], Filippo Montemurro[8], Giulia Valeria Bianchi[9], Ida Paris[10], Giacomo Allegrini[11], Stefano Tamberi[12], Marina Elena Cazzaniga[13], Michele Orditura[14], Claudio Zamagni[15], Donatella Grasso[16], Matteo Benelli[17], Maurizio Callari[18], Antonina Benfante[16], Michelino De Laurentiis[19]

[1]Department of Medical Clinics and Surgery, Università Federico II, Napoli, Italy; [2]Department of Medical Oncology, Ospedale San Raffaele, Milano, Italy; [3]Department of Oncology and Translational Research Unit "Sandro Pitigliani", Ospedale di Prato, Azienda USL Toscana Centro, Prato, Italy; [4]U.S.C. Oncologia, Presidio Ospedaliero Papa Giovanni XXIII, Bergamo, Italy; [5]S.O.C. Oncologia Medica e Prevenzione Oncologica, IRCCS, Centro di Riferimento Oncologico, Aviano, Italy; [6]U.O.S.D. Breast Unit, IRCCS Ospedale Policlinico San Martino, Genoa, Italy; [7]Senologia Medica, IEO, Istituto Europeo di Oncologia, IRCCS, Milano, Italy; [8]Istituto di Candiolo, FPO, IRCCS, Candiolo, Torino, Italy; [9]SC Oncologia Medica 1, Fondazione IRCCS Istituto Nazionale Tumori Milano, Milan, Italy; [10]Department of Woman and Child Sciences, Fondazione Policlinico Universitario Agostino Gemelli, IRCCS, Rome, Italy; [11]U.O.C. Oncologia Medica, Presidio Ospedaliero Livorno, Livorno, Italy; [12]U.O. Oncologia, P.O. Ospedale degli Infermi – AUSL, Ravenna, Italy; [13]Phase 1 Research Unit & Oncology Unit, Azienda Socio Sanitaria Territoriale Monza & Milano Bicocca School of Medicine and Surgery, Monza, Italy; [14]U.O.C. Oncologia Medica e Ematologia, A.O.U. Università Degli Studi L. Vanvitelli, Napoli, Italy; [15]IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy; [16]Oncology, Novartis Farma SpA, Origgio, Italy; [17]Department of Oncology and Bioinformatics Unit, Ospedale di Prato, Azienda USL Toscana Centro, Prato, Italy; [18]CRUK Cambridge Institute, University of Cambridge Li Ka Shing Centre, Cambridge, United Kingdom; [19]IRCCS Istituto Nazionale Tumori Fondazione G Pascale, Napoli, Italy

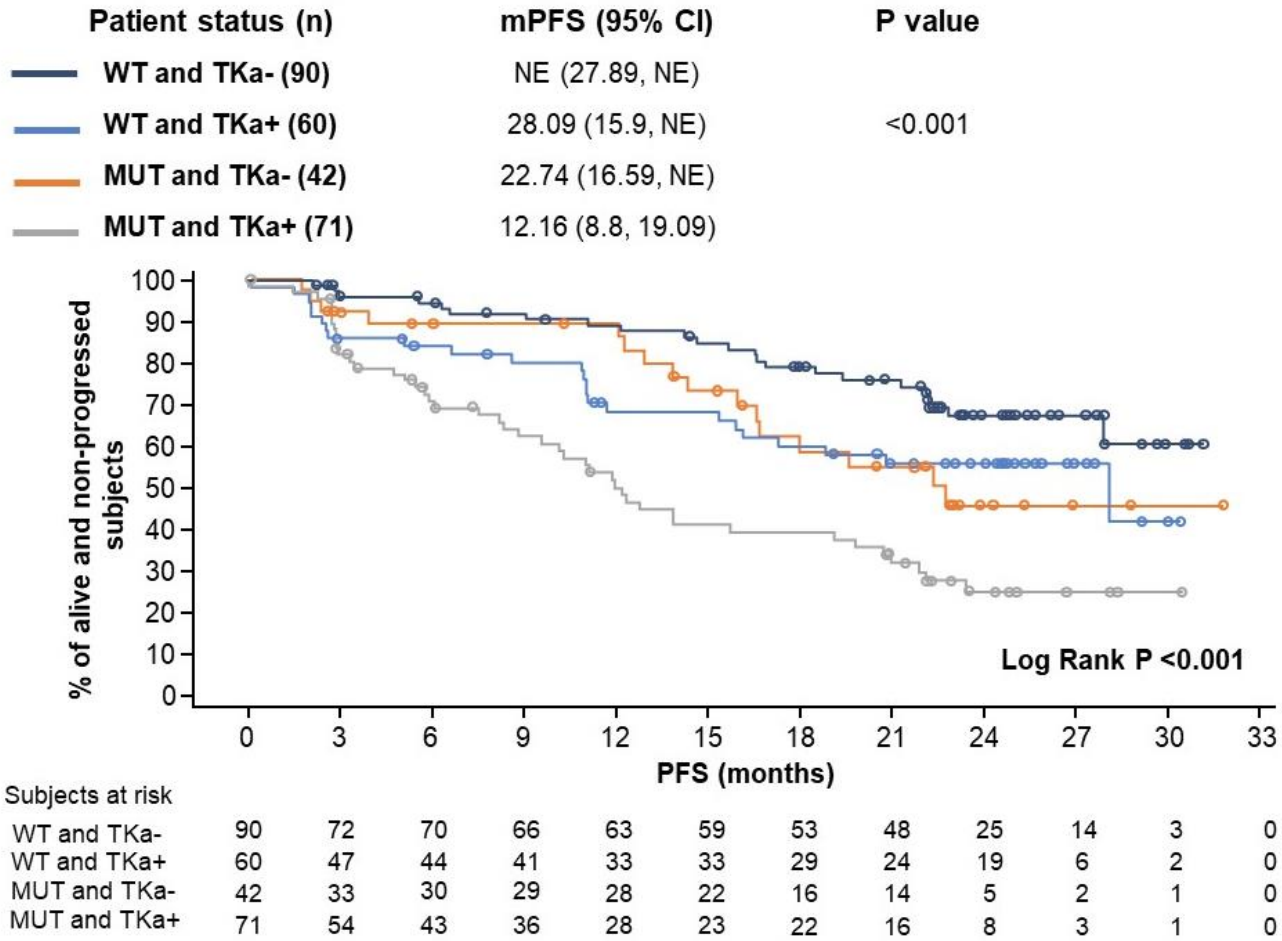
2022 ASCO® ANNUAL MEETING #ASCO22

PRESENTED BY: Grazia Arpino, MD, PhD

Arpino et al, ASCO 2022

TKa and ctDNA in the BIOITALee trial- key findings
BASELINE

84% of the patients had ctDNA and TKa available data



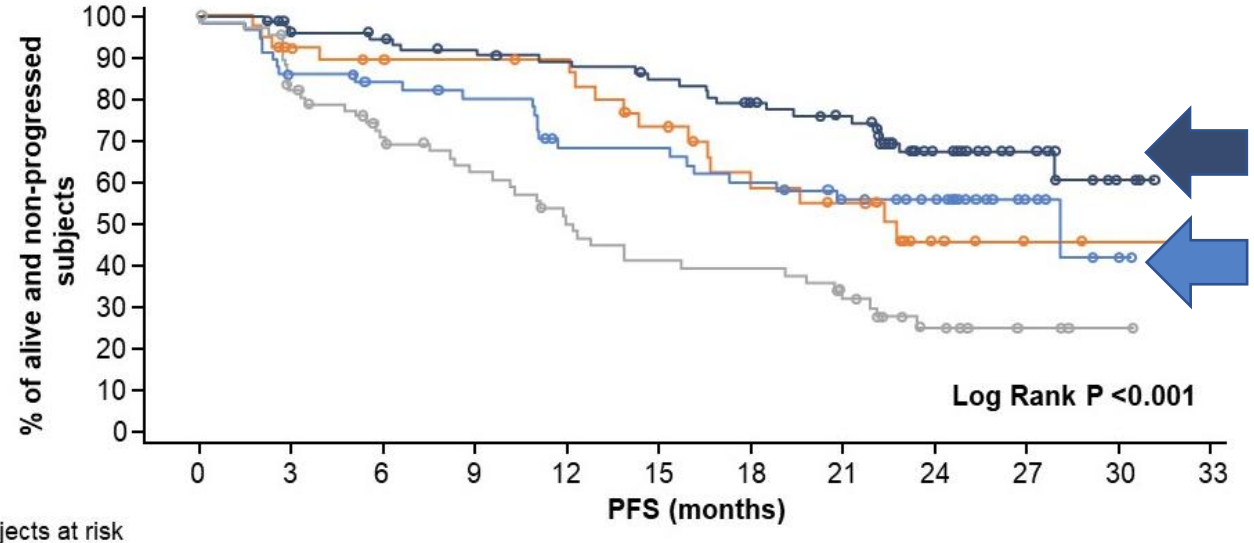
Patient status	HR (95% CI)	P value
Target mutation at baseline		
WT vs. MUT	0.46 (0.30, 0.69)	0.0002
TKa at baseline		
TKa- vs. TKa+	0.53 (0.34, 0.81)	0.0036

Cox model evaluating PFS by presence or absence of ctDNA and TKa status at baseline adjusted for main clinical variables

Baseline ctDNA and TKa are independently informative

TKa and ctDNA in the BIOITALee trial- key findings
BASELINE

Patient status (n)	mPFS (95% CI)	P value
WT and TKa- (90)	NE (27.89, NE)	
WT and TKa+ (60)	28.09 (15.9, NE)	<0.001
MUT and TKa- (42)	22.74 (16.59, NE)	
MUT and TKa+ (71)	12.16 (8.8, 19.09)	



Subjects at risk

WT and TKa-	90	72	70	66	63	59	53	48	25	14	3	0
WT and TKa+	60	47	44	41	33	33	29	24	19	6	2	0
MUT and TKa-	42	33	30	29	28	22	16	14	5	2	1	0
MUT and TKa+	71	54	43	36	28	23	22	16	8	3	1	0

84% of the patients had ctDNA and TKa available data

- **among ctDNA WT, TKa- had the best outcome**

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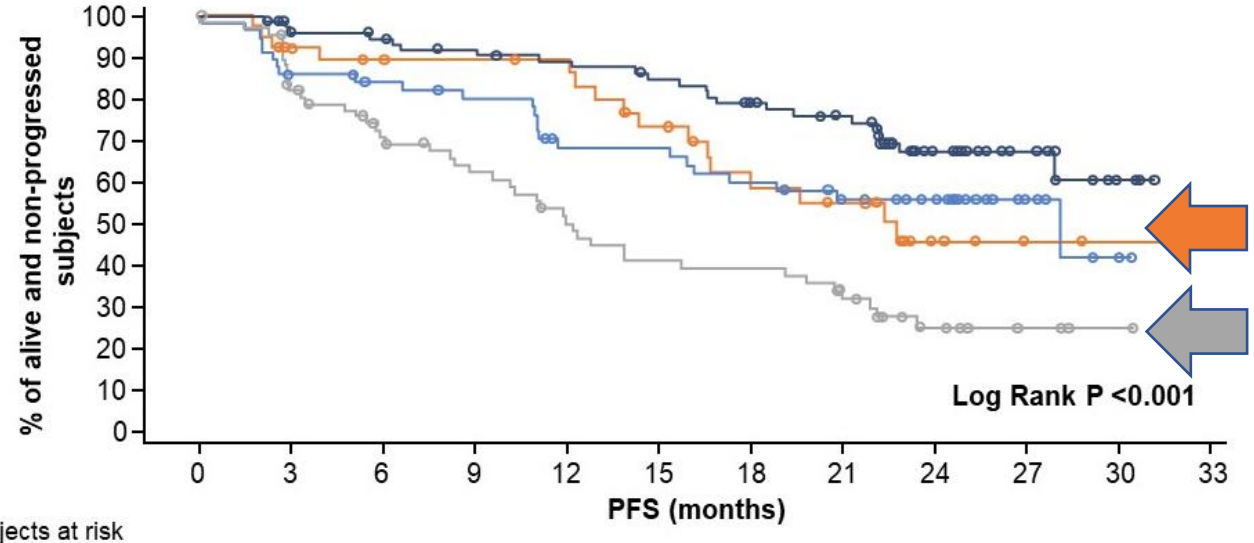

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84% of the patients had ctDNA and TKa available data

- **among ctDNA WT, TKa- had the best outcome**
- **among ctDNA MUT, TKa+ had the worst outcome**

Patient status	HR (95% CI)	P value
Target mutation at baseline		
WT vs. MUT	0.46 (0.30, 0.69)	0.0002
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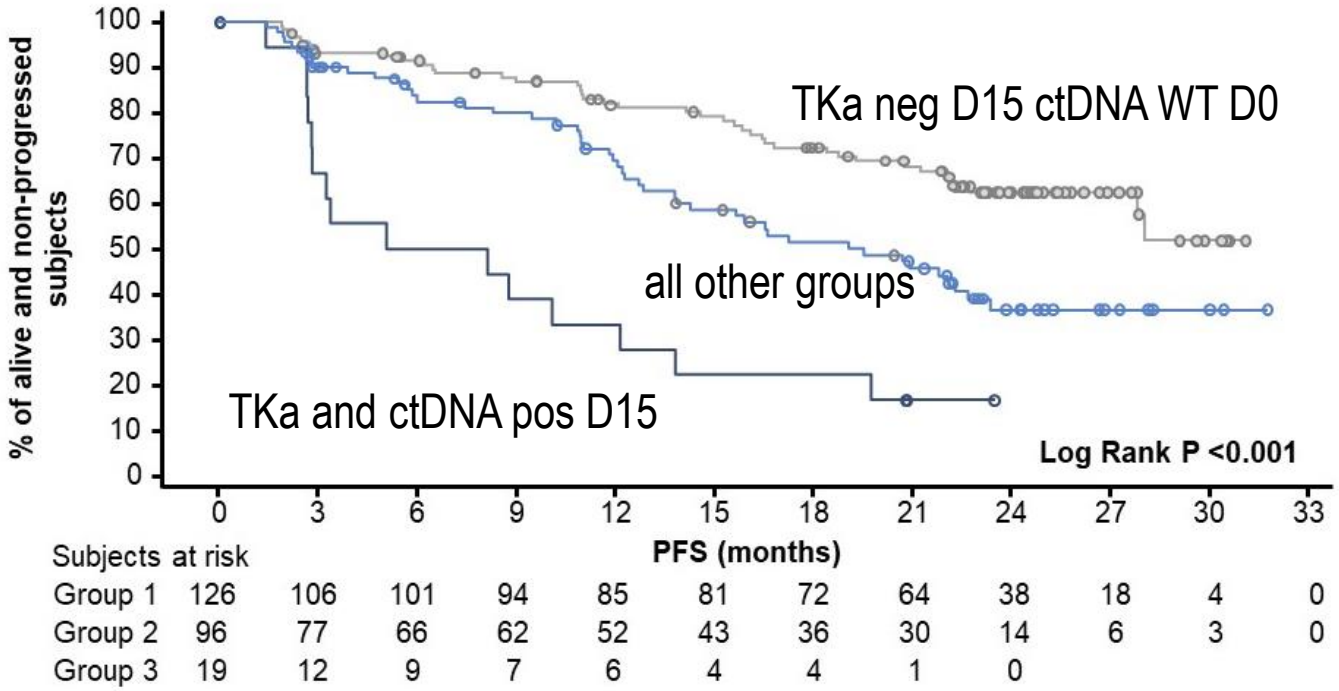
Baseline ctDNA and TKa are independently informative

TKa and ctDNA in the BIOITALee trial- key findings
Change at Day 15

Patient groups (n)	mPFS (95% CI)	P value
Group 1 (126)	NE (27.89, NE)	
Group 2 (96)	19.58 (13.83, 23.39)	<0.0001
Group 3 (19)	6.65 (2.83, 12.16)	



Patient status	HR (95% CI)	P value
Study groups		
Group 1 vs. Group 3	0.17 (0.09, 0.32)	<0.0001
Group 2 vs. Group 3	0.37 (0.20, 0.67)	0.0010

Cox model evaluating PFS by three study groups adjusted for main clinical variables

TKa and ctDNA in the BIOITALee trial- key findings
Change at Day 15

Patient groups (n)	mPFS (95% CI)	P value
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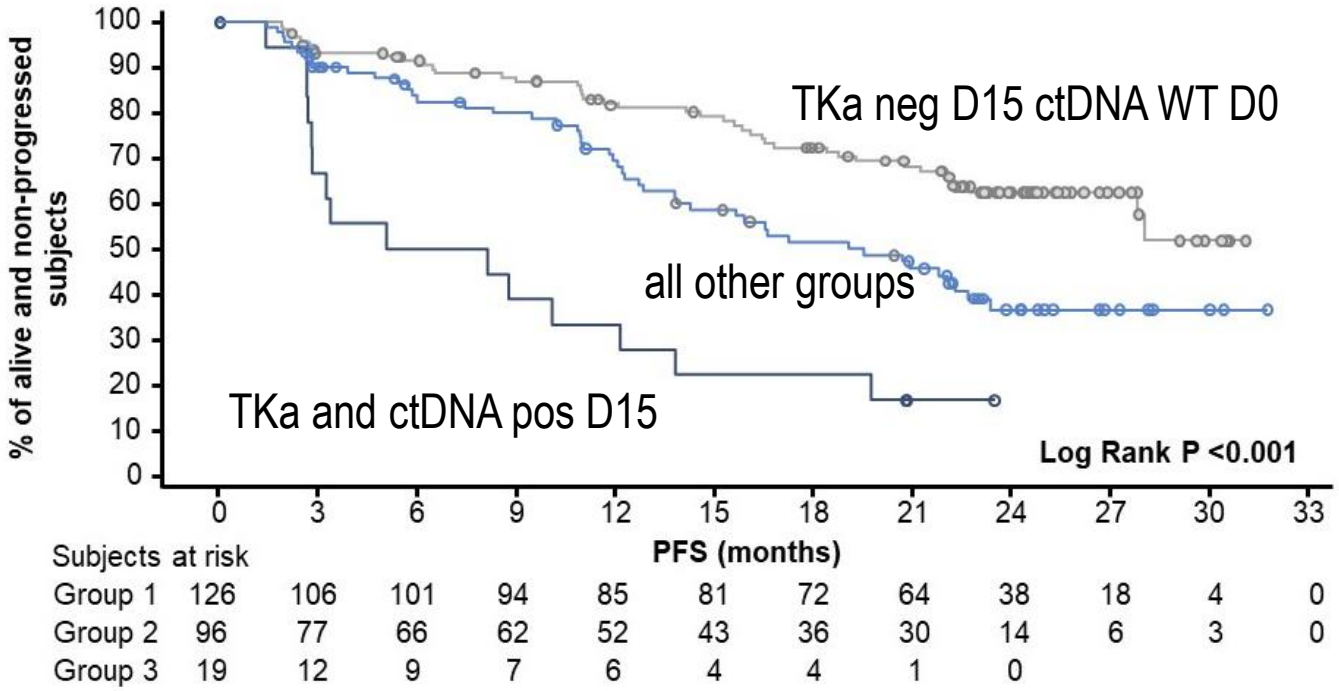
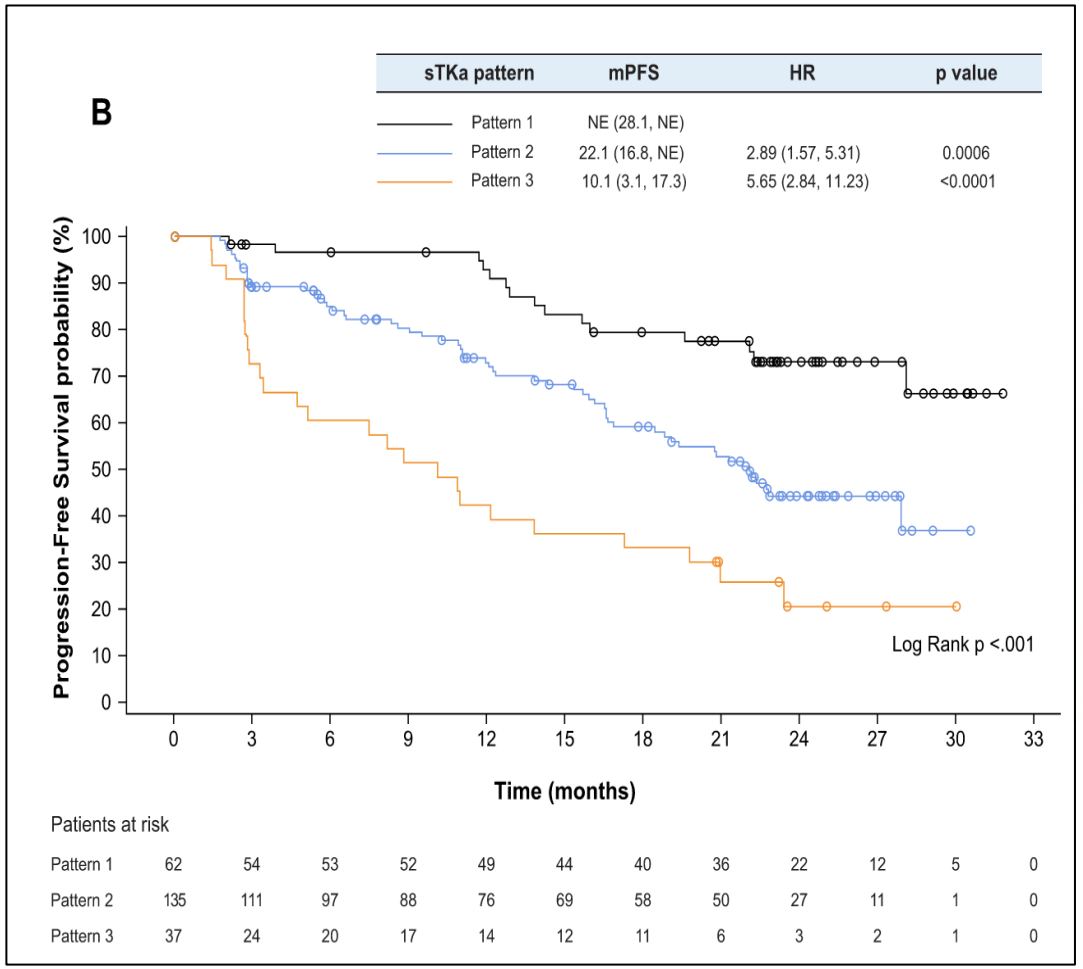


Subjects at risk												
Group 1	126	106	101	94	85	81	72	64	38	18	4	0
Group 2	96	77	66	62	52	43	36	30	14	6	3	0
Group 3	19	12	9	7	6	4	4	1	0			

TKa patterns across first cycle



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TAKE HOME messages

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- A negative liquid biopsy (using a large panel) is associated with better outcome at baseline
- On-treatment change (from positive to negative) is associated with better outcome
- TKa patterns are prognostic and strongly predictive
- TKa and ctDNA give independent information (is TKa sufficient?)

GRAZIE!

All the patients and their families;
All the participating centers.

The BIOItaLee TEAM:

Michelino De Laurentiis
Giampaolo Bianchini
Grazia Arpino

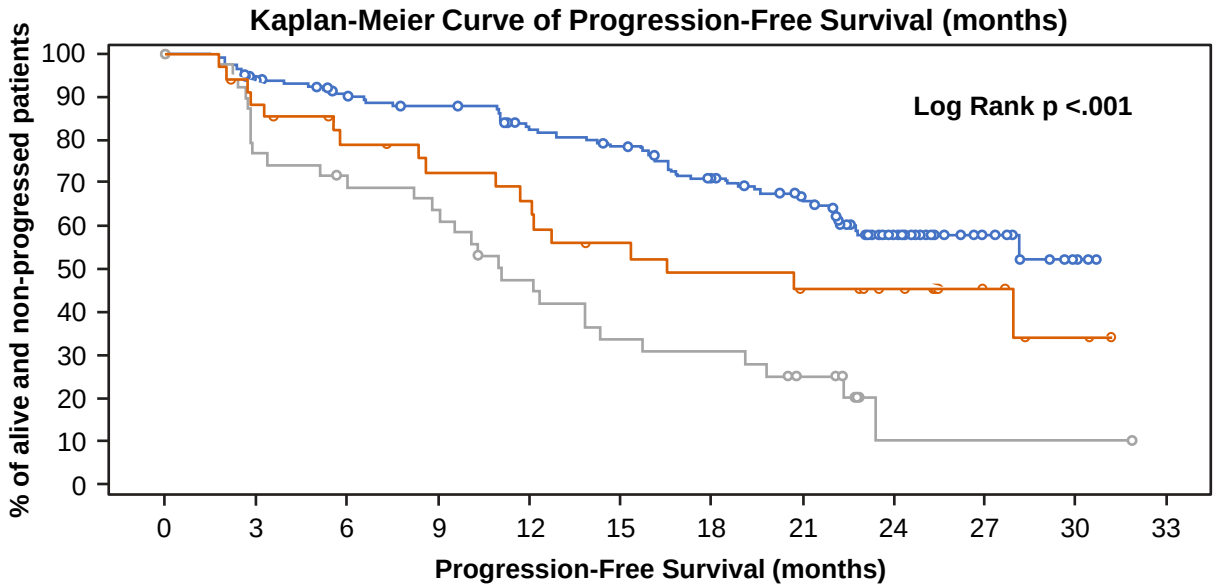
Matteo Benelli
Maurizio Callari

Donatella Grasso
Matteo Suter

Nicola Fenderico
Daniela Castelletti

ctDNA dynamics in the BIOITALee trial- key findings C1D15

VAF status	mPFS	HR (95% CI)	P value
Not mutated	NE	0.32 (0.20,0.51)	<0.0001
Mutated - Low VAF	16.53	0.56 (0.30,1.04)	0.065
Mutated - High VAF	11.07		



Subjects at risk

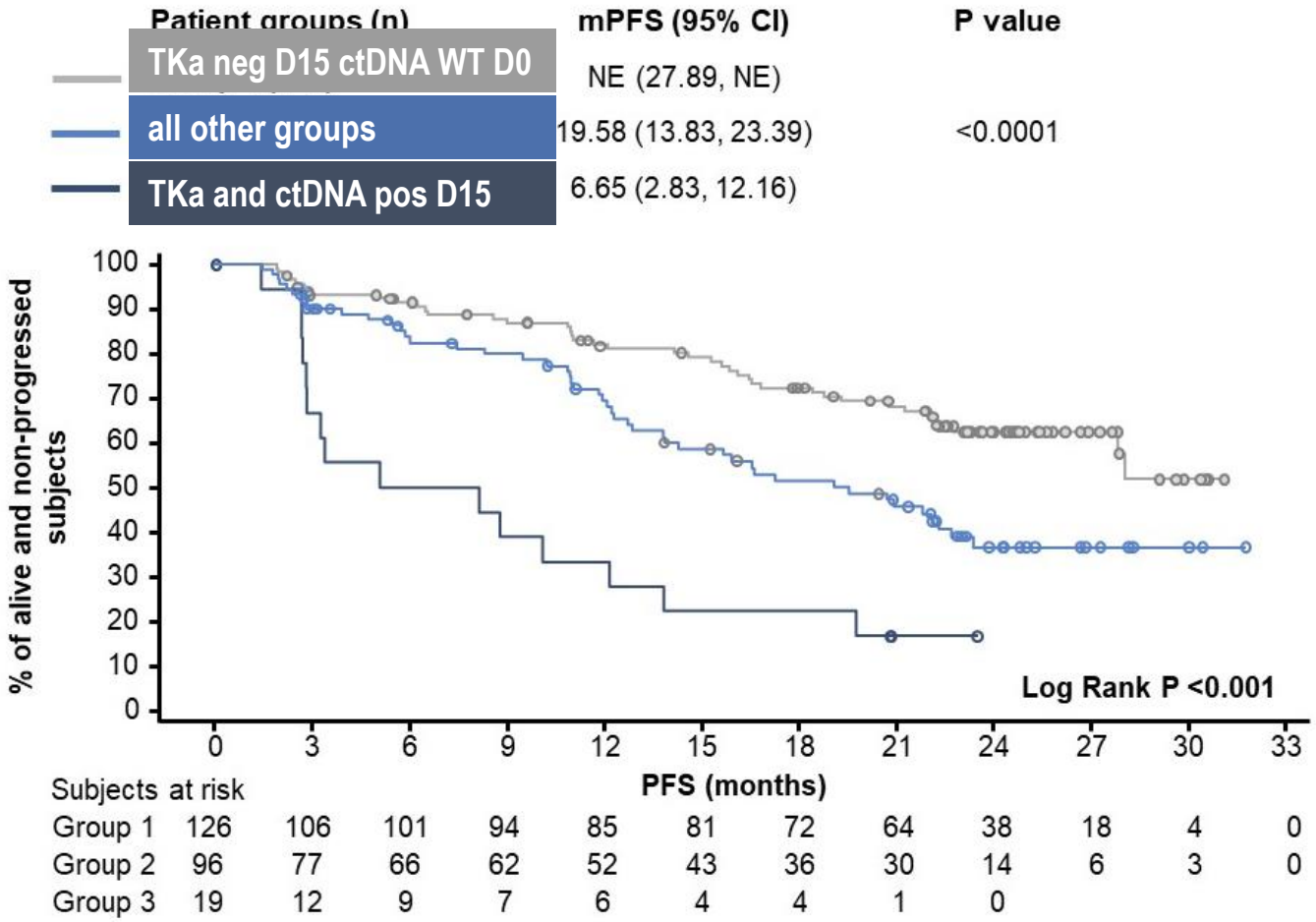
Not Mutated	159	131	120	114	103	97	84	73	41	17	4	0
Mutated - Low VAF	39	30	25	22	20	16	14	12	9	5	2	0
Mutated - High VAF	40	30	27	24	17	12	11	7	1	1	1	0

Patients without target mutation at D15 (n=159, 66.8%) had an extremely favorable outcome

In patients with detectable target mutation at D15, VAF below median (n=39, 16.4%) showed a trend for a better prognosis vs VAF above median (n=40, 16.8%)

Bianchini G. et al SABCS 2021

TKa and ctDNA in the BIOITALee trial- key findings
Change at Day 15



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