


# Genomics to Select Patient for Breast Cancer treatment – why is this study important, in 6 points

Filippo Montemurro

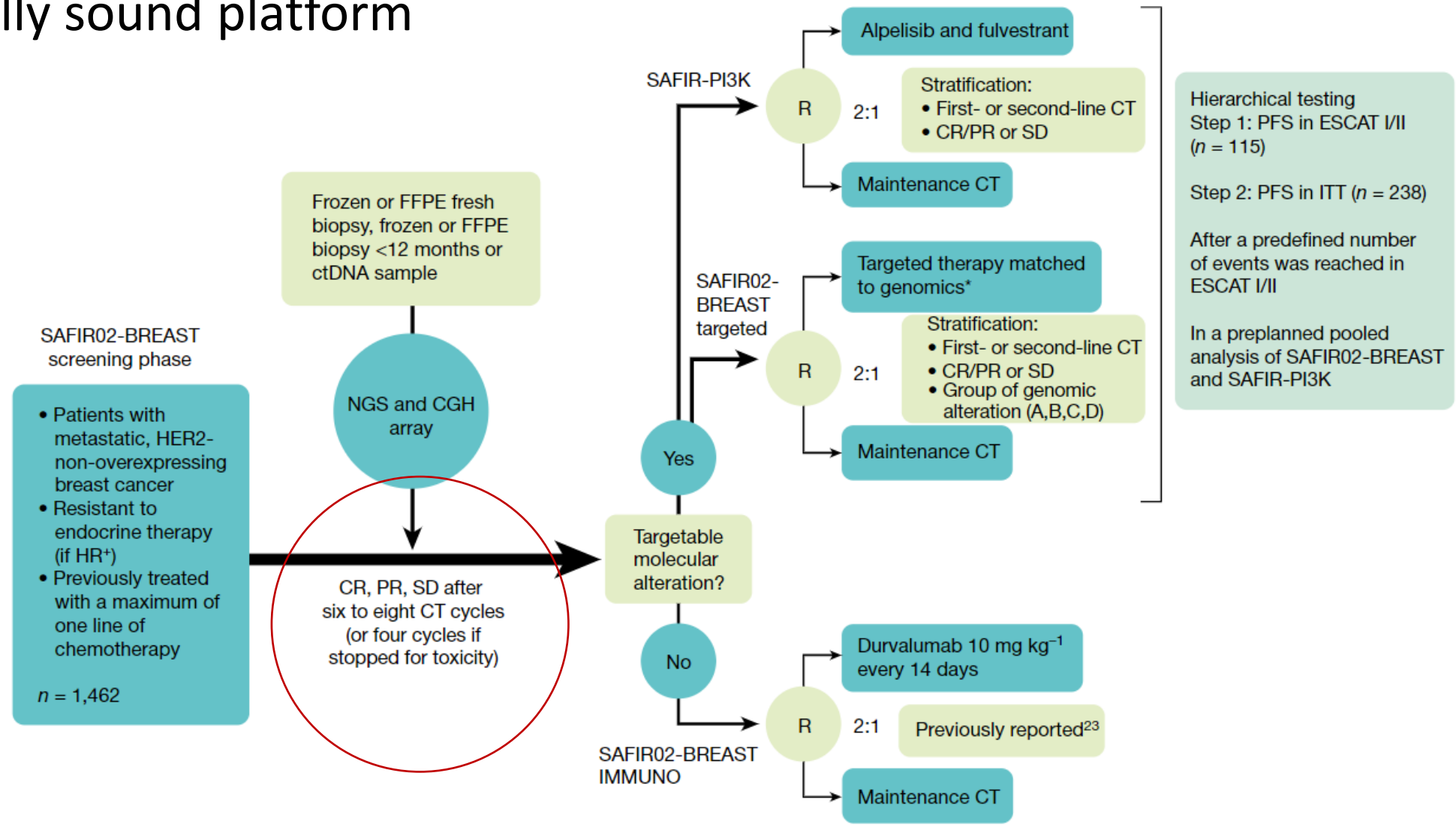
Breast Unit

Istituto di Candiolo, FPO-IRCCS

*Roma, 20-21 Aprile 2023*

# Study design

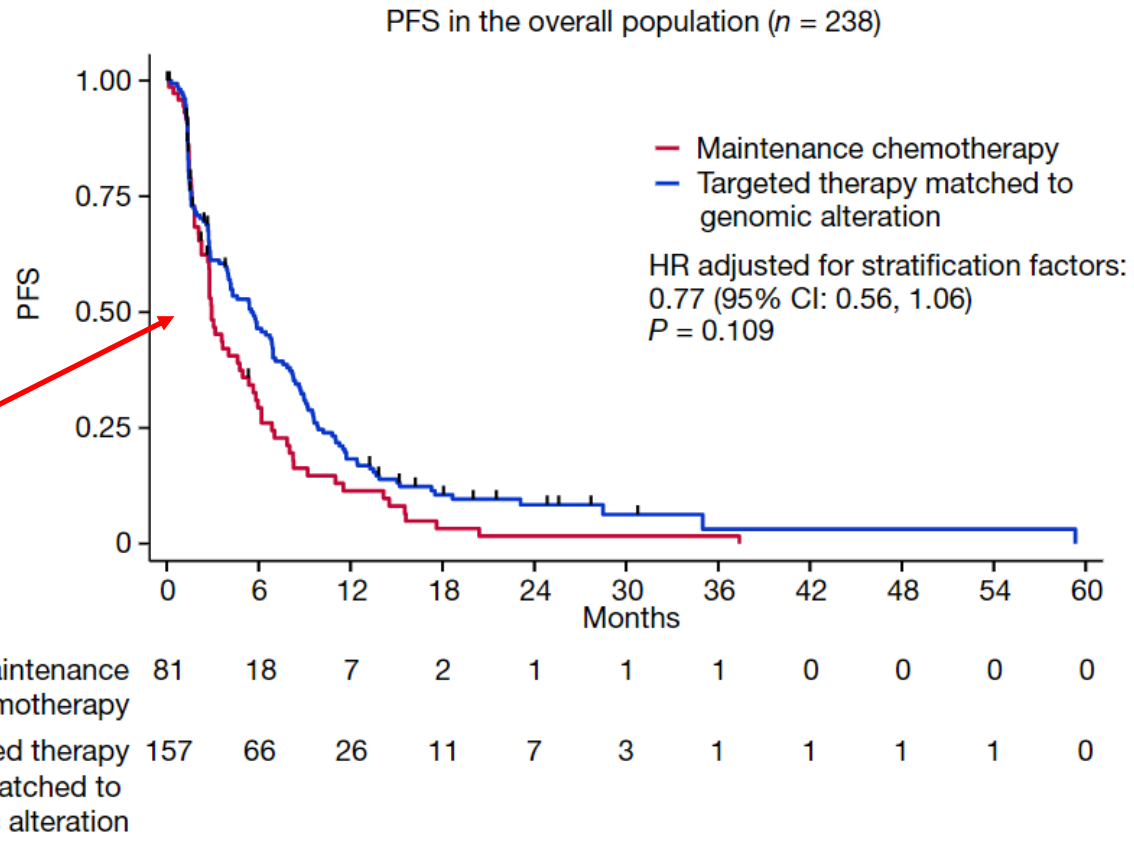
## 1) Ethically sound platform




*olaparib, capivasertib, vistusertib, AZD8931, vandetanib, bicalutamide, AZD4547, selumetinib

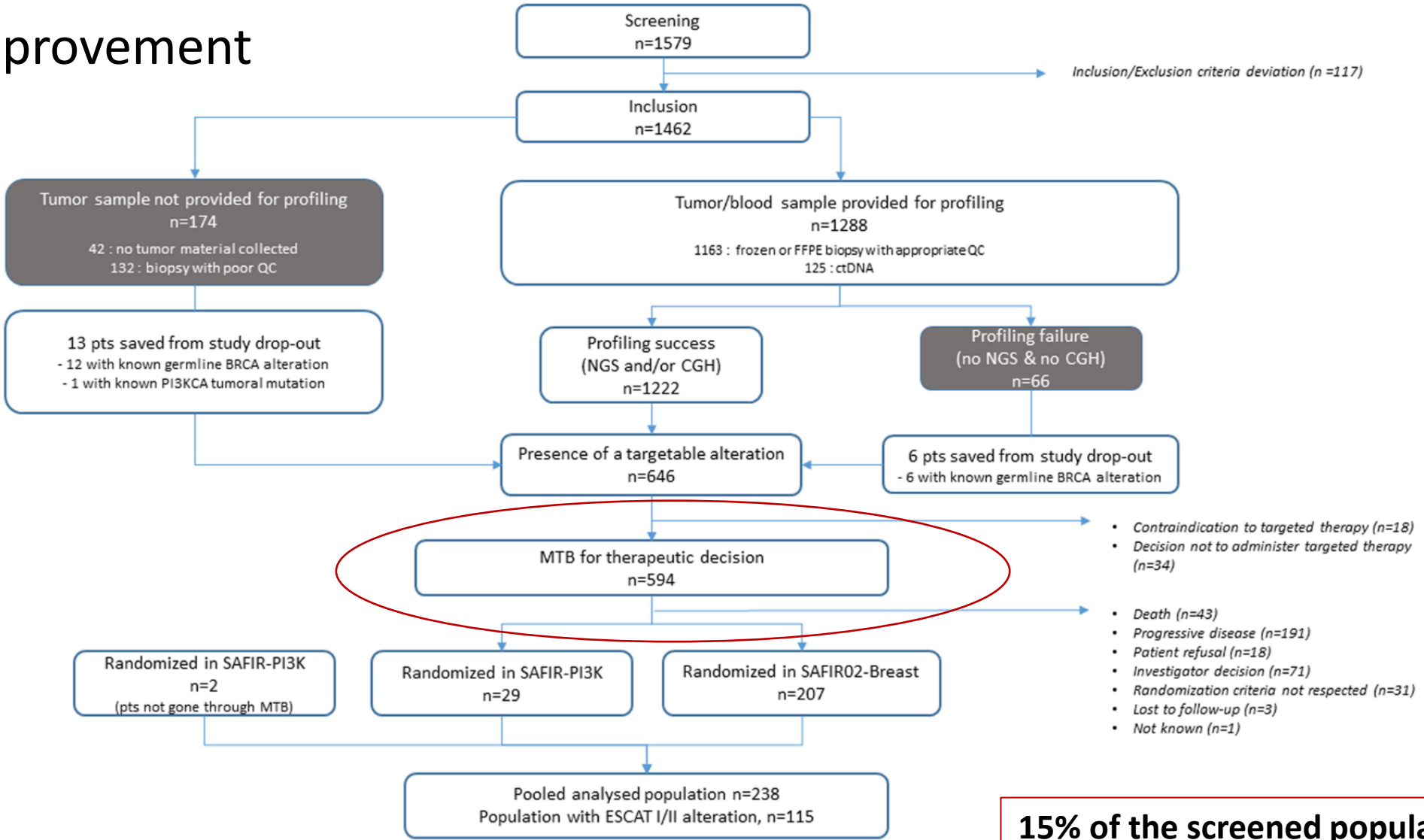
# Post-randomization median PFS in patients receiving maintenance therapy is short

Median PFS
With maintenance conventional therapy
2.9 months



# Consort diagram

2) Pointing at areas for improvement




**Extended Data Fig. 1 | CONSORT diagram of the trial.**

**15% of the screened population was randomized in this trial**

# 3) Foundamental role of the Molecular Tumor Board

**SOMATIC VARIANTS REPORT**

Sample Name: AGN00340
Test: DNA and RNA NGS sequencing

---

Biospecimen: DNA and RNA from FFPE Quantity: 1 vial
Organ: unknown Histology: unknown Cellularity: unknown

---

Technology: Probe capture library (Custom Cancer CUP Panel - 627 genes)
Illumina sequencing

Variants called on DNA:
- Single Nucleotide Variants (SNV): 5 variants in this report
- Small Insertion/Deletions (INDEL): 0 variants in this report
- Tumor Mutation Burden (TMB): NOT analyzed in this report
- Microsatellite Instability (MSI): NOT analyzed in this report
- Copy Number Alterations (CNA): NOT analyzed in this report

Variants called on RNA: Transcripts Fusions: 0 variants in this report

Variants Filtering: SNV/INDEL Virtual panel of 58 clinical relevant genes out of 627 sequenced
Fusion Virtual panel of 10 clinical relevant genes out of 627 sequenced

matched germline N/A population frequency <1%
base coverage ≥50X alternative base depth ≥5X
impact on protein-sequence

Biological databases: Ensembl, gnomAD, COSMIC, ONCOKB.
Automatic filtering is performed without considering the tumor type reported in databases

Selected SNV - INDEL

| Gene | Transcript | Nucleotide change | Amino acid change | VAF | MAF | COSMIC | oncoKB |
|---|---|---|---|---|---|---|---|
| TP53 | ENST00000269305 | c.725G>T | p.C242F | 28.39% | | COSV52661189<br>COSV52677418<br>COSV52689710 | LO |
| BRCA2 | ENST00000380152 | c.10024G>A | p.E3342K | 60.53% | 0.0008% | COSV66337145 | UNK |
| ERBB2 | ENST00000269571 | c.1157C>A | p.A386D | 60.90% | 0.38% | COSV99507790 | |
| CSF1R | ENST00000286301 | c.1237G>A | p.G413S | 35.99% | 0.94% | | |
| BRCA1 | ENST00000471181 | c.5469+1G>C | p.X1823_splice | 57.13% | | | |

**Amino acid change: prediction may be incorrect due to complex variants**
**COSMIC: all the variants of the same nucleotide position are reported**
**oncoKB: O: Oncogenic; LO: Likely Oncogenic; PO: Predicted Oncogenic; N: Neutral; LN: Likely Neutral; UNK: unknown**

→ Type of genetic material sequenced

→ Source

→ Technology

→ What is analyzed and what is not analyzed

→ Filtering

→ Performance of the test (coverage, depth)

→ Biological Databases

Test output:

- Mutated gene
- Transcript Code
- Nucleotide Change
- Amino Acid Change
- VAF
- MAF
- COSMIC matching
- OncoKB matching

*Candiolo CUP Project*

# Commercially available tests provide results interpretation and potential druggability

| | | | | | |
|---|---|---|---|---|---|
| Date of Birth | | Medical Facility | Istituto di Candiolo - FPO IRCCS | | |
| Sex | | Ordering Physician | Dott. Montemurro, Filippo | Specimen Received | |
| FMI Case # | | Additional Recipient | | Specimen Site | Breast |
| Medical Record # | | Medical Facility ID # | | Date of Collection | |
| Specimen ID | | Pathologist | | Specimen Type | Slide |

**ABOUT THE TEST:**

FoundationOne™ is a next-generation sequencing (NGS) based assay that identifies genomic alterations within hundreds of cancer-related genes.

All Report Updates

**Corrected Report 10/05/2018**

This Corrected Report has been issued to update Date of Birth from "21 January 1970" to "26 January 1970".

**PATIENT RESULTS**

- 15 genomic findings
- 9 therapies associated with potential clinical benefit
- 0 therapies associated with lack of response
- 53 clinical trials

**TUMOR TYPE: BREAST INVASIVE DUCTAL CARCINOMA (IDC)**

**Genomic Alterations Identified†**

*CCND1* amplification
*RET* E511K
*AKT2* amplification
*MYC* amplification
*CCND3* amplification
*CCNE1* amplification
*FGF19* amplification
*FGF3* amplification
*FGF4* amplification
*LYN* amplification
*MCL1* amplification
*TP53* R273H
*VEGFA* amplification

**Additional Findings†**

*Microsatellite status* MS-Stable
*Tumor Mutational Burden* TMB-Intermediate; 8 Muts/Mb

**Additional Disease-relevant Genes with No Reportable Alterations Identified†**

*ERBB2*

| Genomic Findings Detected | FDA-Approved Therapies (in patient's tumor type) | FDA-Approved Therapies (in another tumor type) | Potential Clinical Trials |
|---|---|---|---|
| ***CCND1*** amplification | Abemaciclib<br>Palbociclib<br>Ribociclib | None | Yes, see clinical trials section |
| ***RET*** E511K | None | Cabozantinib<br>Lenvatinib<br>Ponatinib<br>Sorafenib<br>Sunitinib<br>Vandetanib | Yes, see clinical trials section |
| ***AKT2*** amplification | None | None | Yes, see clinical trials section |
| ***MYC*** amplification | None | None | Yes, see clinical trials section |
| ***CCND3*** amplification | None | None | None |
| ***CCNE1*** amplification | None | None | None |
| ***FGF19*** amplification | None | None | None |
| ***FGF3*** amplification | None | None | None |
| ***FGF4*** amplification | None | None | None |
| ***LYN*** amplification | None | None | None |
| ***MCL1*** amplification | None | None | None |
| ***Microsatellite status*** MS-Stable | None | None | None |
| ***TP53*** R273H | None | None | None |
| ***Tumor Mutational Burden*** TMB-Intermediate; 8 Muts/Mb | None | None | None |
| ***VEGFA*** amplification | None | None | None |

# Cosmic Interpretation of the RET p.E511K mutation found in this patient

## Samples

This section displays a table of mutated samples, with tissue, histology and zygosity information. Publication information is also included, where available, with links to PUBMED.

Show 10 entries

Export: CSV TSV Search:

| Sample name | Gene name | Transcript | Primary Tissue | Tissue Subtype 1 | Primary Histology | Histology Subtype 1 | Pubmed ID | Zygosity | Somatic Status | Sample Type | LOH | Resistant Mutation | Drugs |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2687137 | RET | ENST00000355710.7 | Breast | NS | Carcinoma | NS | 27284958 | Unknown | Previously Reported | Tumour Sample | Unknown | - | |
| P-0002565-T01-IM3 | RET | ENST00000355710.7 | Large intestine | Rectum | Carcinoma | Adenocarcinoma | 28481359 | Heterozygous | Confirmed Somatic | Tumour Sample | Unknown | - | |
| TCGA-AA-3947-01 | RET | ENST00000355710.7 | Large intestine | Colon | Carcinoma | Adenocarcinoma | - | Unknown | Confirmed Somatic | NS | Unknown | - | |
| PD42111a | RET | ENST00000355710.7 | Skin | Trunk | Malignant melanoma | Superficial spreading | 33024263 | Unknown | Previously Reported | Tumour Sample | Unknown | - | |

Showing 1 to 4 of 4 entries

First Previous 1 Next Last

# Pubmed hit for RET p.E511K mutation

Original Article

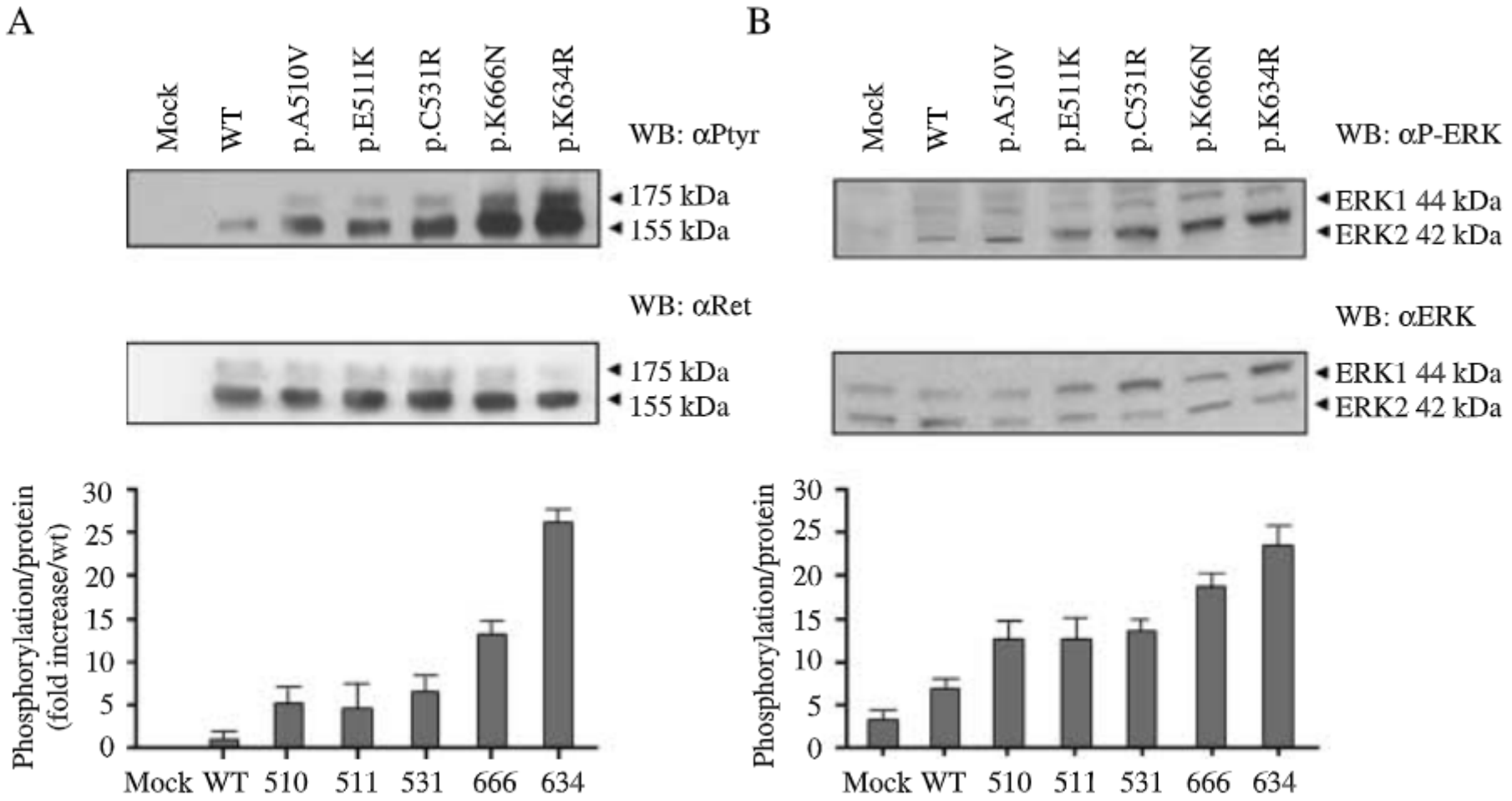
## Nonamplification *ERBB2* Genomic Alterations in 5605 Cases of Recurrent and Metastatic Breast Cancer: An Emerging Opportunity for Anti-HER2 Targeted Therapies

Jeffrey S. Ross, MD[1,2]; Laurie M. Gay, PhD[1]; Kai Wang, MD[1,3]; Siraj M. Ali, MD, PhD[1]; Saranya Chumsri, MD[4]; Julia A. Elvin, MD, PhD[1]; Ron Bose, MD, PhD[5]; Jo-Anne Vergilio, MD[1]; James Suh, MD[1]; Roman Yelensky, PhD[1]; Doron Lipson, PhD[1]; Juliann Chmielecki, PhD[1]; Stanley Waintraub, MD[6]; Brian Leyland-Jones, MD, PhD[7]; Vincent A. Miller, MD[1]; and Philip J. Stephens, PhD[1]

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 090 | 43 | Lymph Node | Metastatic breast carcinoma | PASS | 40 | 529 | Breast carcinoma (NOS) | , BRIP1:NM_032043:c.1240C>T_p.Q414*(0.16,728), ARID1A:NM_006015:c.2989-8_3101del121_p.K997fs*12(0.09,509), ER |
| 091 | 57 | Lymph Node | Metastatic adenocarcinoma | QUALIFIED | 20 | 439 | Breast carcinoma (NOS) | ERBB2:NM_004448:c.2339_2340insGGGCTCCCC_p.P780_Y781insGSP(0.09,454), TBX3:NM_016569:c.1788_1788delT_p.F5 |
| 092 | 35 | Breast | Invasive ductal carcinoma, grade 3 | PASS | 50 | 617 | Breast invasive ductal carcinoma (IDC) | PIK3CA:NM_006218:c.3140A>G_p.H1047R(0.34,776), ERBB2:NM_004448:c.2033G>A_p.R678Q(0.02,8132), CTCF:NM_0065 |
| 093 | 43 | Lymph Node | Metastatic carcinoma consistent wi | PASS | 76 | 390 | Breast carcinoma (NOS) | TP53:NM_000546:c.581T>G_p.L194R(0.82,469), ERBB2:NM_004448:c.2305G>T_p.D769Y(0.86,412), |
| 094 | 67 | Liver | History of breast cancer | QUALIFIED | 30 | 233 | Breast carcinoma (NOS) | ERBB2:NM_004448:c.2329G>C_p.V777L(0.16,238), PIK3CA:NM_006218:c.3140A>G_p.H1047R(0.15,255), CDH1:NM_00436 |
| 095 | 73 | Liver | Positive for malignant cells, adeno | PASS | 40 | 445 | Breast carcinoma (NOS) | AXIN1:NM_003502:c.1577C>T_p.A526V(0.28,430), TP53:NM_000546:c.811G>A_p.E271K(0.25,460), ERBB2:NM_004448:c.2 |
| 096 | 86 | Breast | Invasive ductal carcinoma | PASS | 30 | 487 | Breast invasive ductal carcinoma (IDC) | TP53:NM_000546:c.493C>T_p.Q165*(0.36,421), ERBB2:NM_004448:c.2327G>T_p.G776V(0.7,1372), PIK3CA:NM_006218:c. |
| 097 | 47 | Pleura | Metastatic breast cancer | PASS | 60 | 425 | Breast carcinoma (NOS) | RUNX1:NM_001754:c.601C>T_p.R201*(0.3,488), KRAS:NM_004985:c.35G>A_p.G12D(0.02,465), FBXW7:NM_033632:c.337 |
| 098 | 61 | Chest Wall | Metastatic breast carcinoma | PASS | 60 | 285 | Breast carcinoma (NOS) | TP53:NM_000546:c.524G>A_p.R175H(0.62,267), PIK3CA:NM_006218:c.3140A>G_p.H1047R(0.35,246), ERBB2:NM_004448 |
| 099 | 70 | Liver | Metastatic adenocarcinoma, consis | PASS | 60 | 343 | Breast carcinoma (NOS) | ERBB2:NM_004448:c.2329G>T_p.V777L(0.55,586), **RET:NM_020975:c.1531G>A_p.E511K**(0.46,217), |
| 100 | 73 | Peritoneum | Carcinoma, consistent with metast | PASS | 42 | 458 | Breast invasive lobular carcinoma (ILC) | TP53:NM_000546:c.811G>A_p.E271K(0.02,448), TP53:NM_000546:c.427G>A_p.V143M(0.02,402), TP53:NM_000546:c.112 |
| 101 | 63 | Skin | Metastatic adenocarcinoma consist | PASS | 50 | 590 | Breast carcinoma (NOS) | ERBB3:NM_001982:c.889G>T_p.D297Y(0.01,676), ERBB3:NM_001982:c.994G>A_p.E332K(0.21,703), ERBB2:NM_004448:c.2 |
| 102 | 40 | Breast | Inifiltrating ductal carcinoma | PASS | 30 | 398 | Breast invasive ductal carcinoma (IDC) | ERBB2:NM_004448:c.2329G>T_p.V777L(0.1,723), TP53:NM_000546:c.871_874delAAGA_p.K292fs*52(0.21,409) |
| 103 | 49 | Breast | Inflammatory mammary carcinoma | PASS | 20 | 555 | Breast invasive lobular carcinoma (ILC) | ERBB2:NM_004448:c.2264T>C_p.L755S(0.23,512), ARID1A:NM_006015:c.3826C>T_p.R1276*(0.1,571), MAP2K4:NM_0030 |
| 104 | 64 | Liver | Liver metastatic primary tumor bre | PASS | 44 | 670 | Breast carcinoma (NOS) | TP53:NM_000546:c.843C>A_p.D281E(0.4,716), KEAP1:NM_012289:c.73G>A_p.E25K(0.07,553), PIK3CA:NM_006218:c.1624 |
| 105 | 49 | Lymph Node | Adenocarcinoma consistent with b | PASS | 40 | 370 | Breast carcinoma (NOS) | TP53:NM_000546:c.859G>T_p.E287*(0.21,343), ERBB2:NM_004448:c.1899-1G>C_p.splice site 1899-1G>C(0.07,1015) |
| 106 | 53 | Peritoneal Fluid | Positive for malignant cells | PASS | 13 | 492 | Breast carcinoma (NOS) | ERBB2:NM_004448:c.929C>T_p.S310F(0.08,451), CDH1:NM_004360:c.2329_2332delGACG_p.D777fs*5(0.15,386) |

*Ross et al, Cancer 122;2654, 2016*

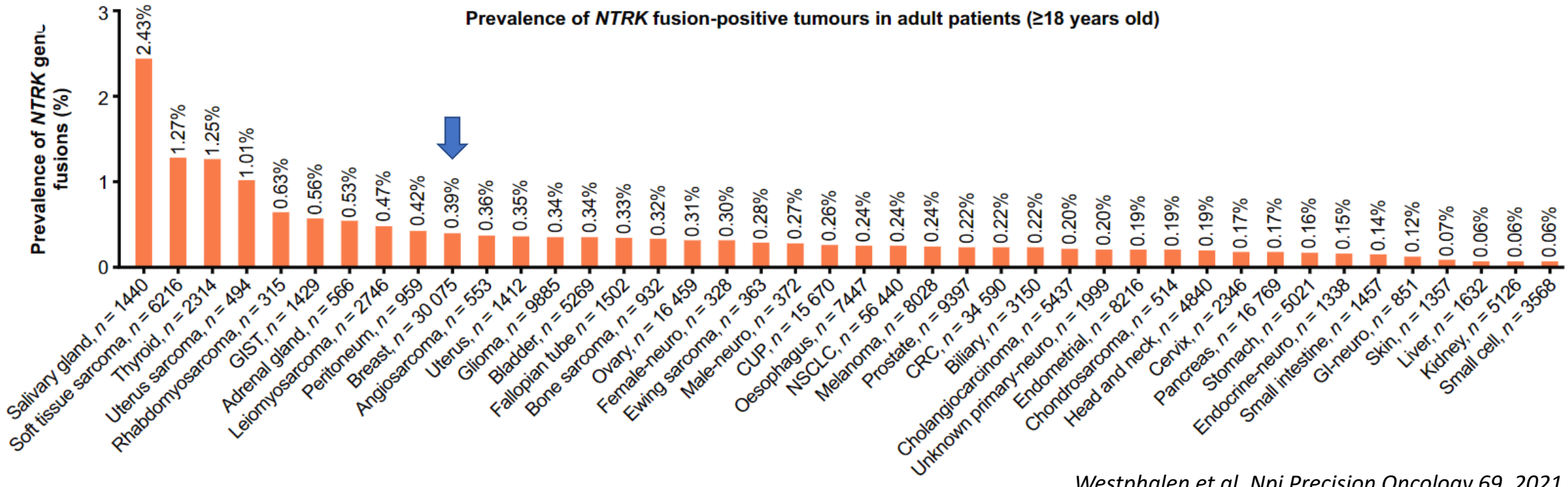
# Is RET p.E511K oncogenic?




Pubmed search string ”Ret” and ("p.E511K" or "c.1531G>A")”

*Muzza et al, Eur j Endocrinol 162; 771, 2010*

# Positive Predictive Value in rare mutations: The case of NTRK fusion



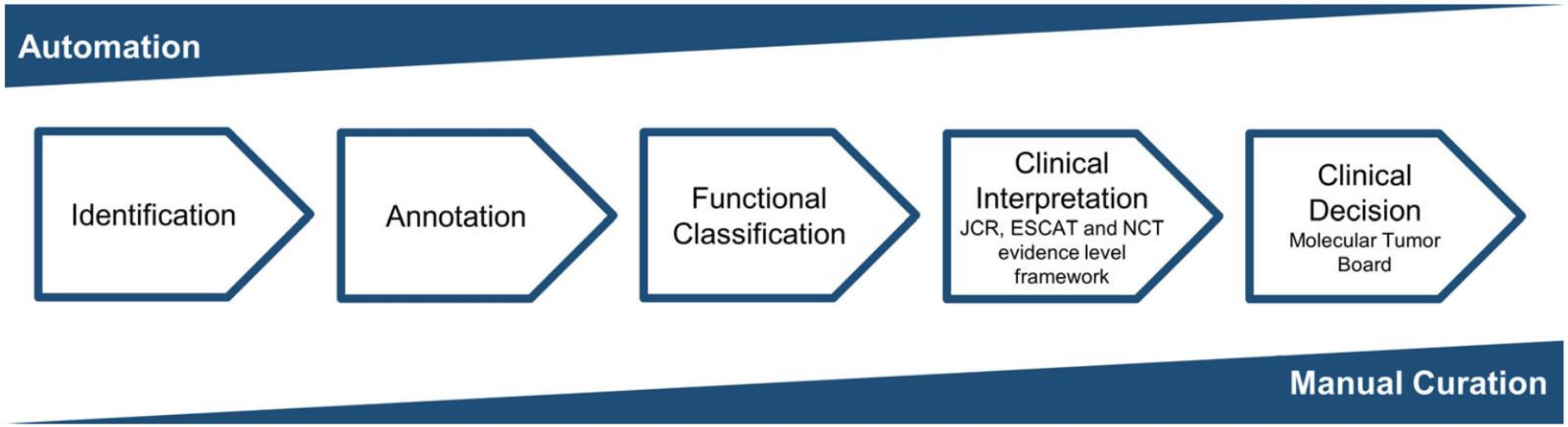

*Westphalen et al, Npj Precision Oncology 69, 2021*

SN=0.99
SP=0.99
P =0.0039

PPV = (0.0039*0.99)/(0.0039*0.99)+(1-0.0039)*(1-0.99) = PPV about 28%

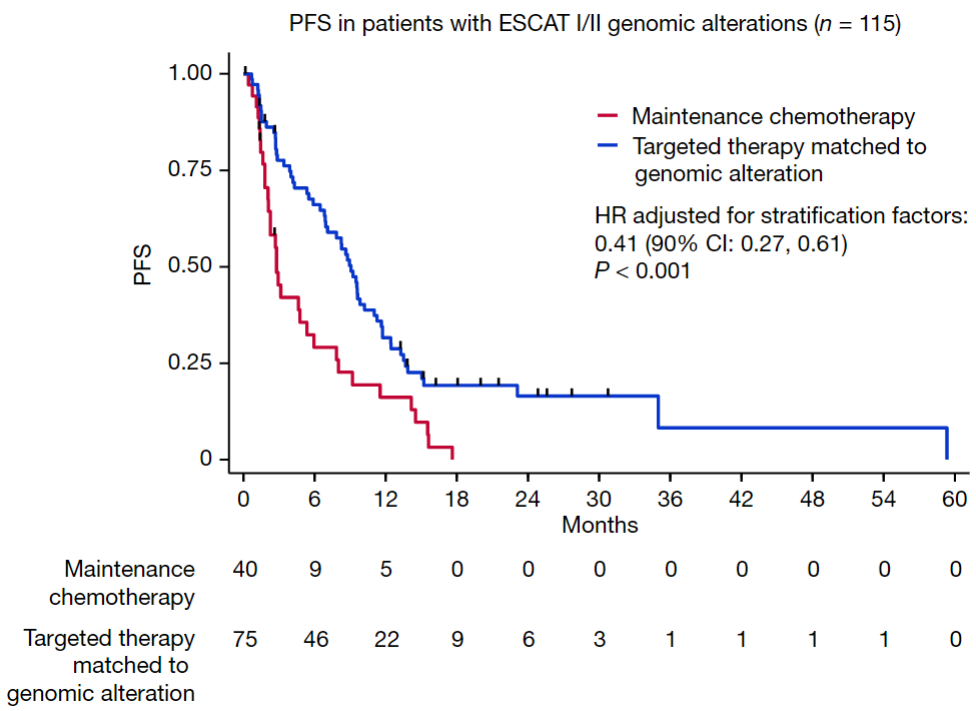
https://www.medcalc.org/calc/diagnostic_test.php

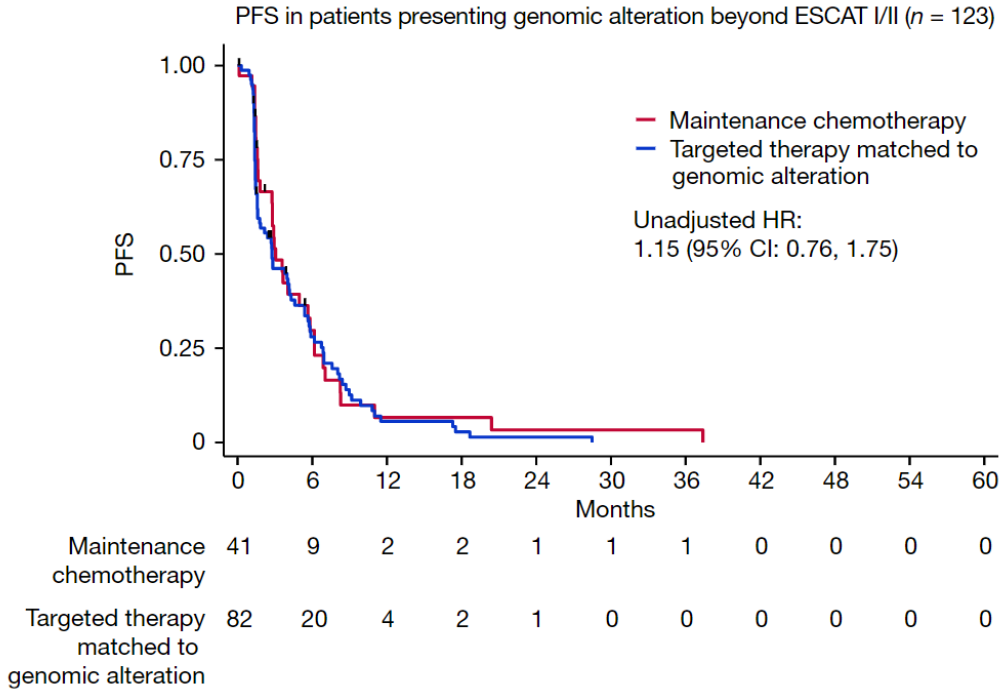
# Technology is outstanding, yet, manual curation plays a crucial role in order to contestualize results and provide real opportunities




*Horak et al, Genes Chromosomes Cancer 2021*

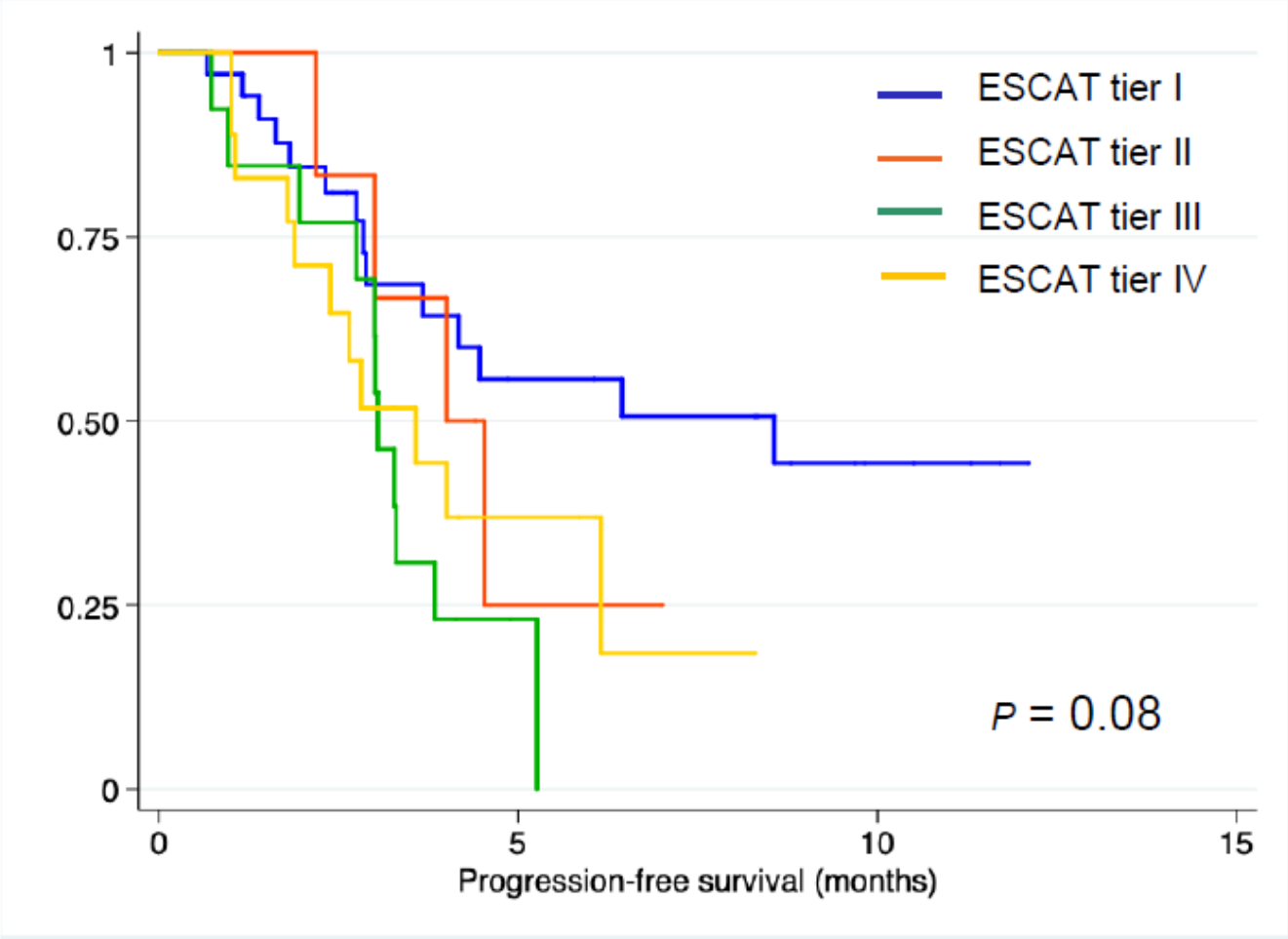
# 4) No signal of benefit beyond ESCAT II matches



# Similar results in a previous study in patients with different cancers

## Genomic alterations & Matched therapy




| ESCAT | Patients (n=73) | Median (95% CI) |
|---|---|---|
| Tier I | 34 | 8.6 (2.9 – NA) |
| Tier II | 6 | 4 (2.2 – NA) |
| Tier III | 14 | 3 (1.9 – 3.8) |
| Tier IV | 19 | 3.6 (1.8 – NA) |

Romano et al, ESMO 2020

# Possible divergence in different actionability scales

**TABLE 2** Examples of divergent variant classifications based on JCR, ESCAT, and NCT classifications

| Molecular biomarker | Drug | Entity | JCR | ESCAT | NCT |
|---|---|---|---|---|---|
| NTRK-Gene Fusions | Larotrectinib | Solid tumors | Tier I-A | I-C | m1A-Z<br>*m1C-Z, m2A-Z* |
| BRAF V600E | Dabrafenib + Trametinib | NSCLC | Tier I-A | I-B | m1A-Z |
| BRAF V600K | Dabrafenib + Trametinib | NSCLC | Tier I-A | III-A | m2A-Z |
| BRAF V600K | Dabrafenib + Trametinib | Melanoma | Tier I-A | I-A | m1A-Z |
| BRAF V600E | Vemurafenib | NSCLC | Tier I-B | I-C | m1A |
| BRAF V600K | Vemurafenib | NSCLC | Tier II-C | II-B<br>*III-A* | m1A<br>*m1C* |
| TMB (≥10 mutations/MB) | Pembrolizumab | Solid tumors | Tier I-A | I-C<br>*I-B* | m1B-Z |
| HRD ≥42 | Olaparib + Bevacizumab | Ovarian Cancer | Tier I-A | I-A | m1A-Z |

*Note*: In italics—alternative classification.

*Horak et al, Genes Chromosomes Cancer 2021;1, 11*

# Feeding the LOA systems is a perpetual process

| Study | Setting | Drug(s) | N pts | ORR | mPFS | Authors' considerations |
|---|---|---|---|---|---|---|
| NCI-MATCH (2020) | MMRd non-colorectal cancer | Nivolumab | 42* | 36% | 6.3m | Activity is promising |
| NCI-MATCH (2020) | FGFR-altered tumors | AZD4547 | 48 | 8% | 3.4m | Promising in FGFR fusions (ORR 22%) |
| NCI-MATCH (2020) | BRAFV600E mutations | Dabrafenib Trametinib | 29 | 39% | 11.4m | Additional investigations warr. |
| NCI-MATCH (2022) | PIK3CAmut, non breast and squamous LC | Copanlisib | 25 | 16% | 3.4m | Primary end-point met |
| NCI-MATCH (2022) | PIK3CAmut, non breast and squamous LC | Taselisib | 61 | 0 | 3.1m | Very limited activity |
| NCI-MATCH | ALK or ROS rearranged, non NSCLS and non lymphoma | Crizotinib | 9** | 50% (ALK) 25% (ROS) | 4.3m | May have a terapeutic role |

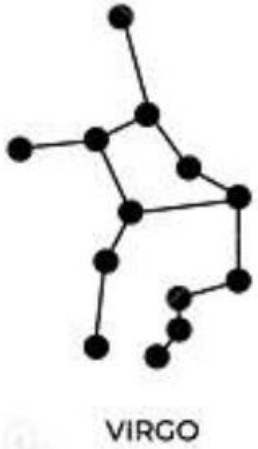
*2% of the non-colorectal screened population showed MMRd by IHC (PPV considering SN and SP =0.99; 0.67)

**0.1 and 0.4% of the screened population show ALK or ROS rearrangementm, respectively (same test performance: PPV and 0.04 and 0.15)

# 5) Tumors of who benefited in Safiro 2 carry well established constellations of druggable alterations

DNA fragility


PIK3CA/AKT/PTEN




Shoudl we go for large gene panels or establish more focused and rapidly adaptable multi-platform tests?

# 6) And last

- (only) one patient with an ESCAT III/IV alteration and treated with targeted therapy had PFS > 12 months.

# Conclusions

1) The Safiro 2 trial has a smart design and confirms that molecular screening can be accomplished in the clinical practice
2) Only 15% of screened patients were randomized. Each step of the consort diagram points at areas for improvement
3) There are two foundamentals elements in the **governance** of this type of approach
    1) A molecular tumor board
    2) An actionability scale
4) There is **no** benefit in ESCAT>2 genomic/drug match
5) While patients who benefited from the genomic approach have tumors bearing two main druggable alterations, exceptional responders may exist, that could reveal newer potentially useful matches. Yet this approach must remain a subject for research and not be adopted in the clinical practice