**Genomic alterations in breast cancer: Level of evidence for actionability according to ESMO Scale for Clinical Actionability of molecular Targets (ESCAT)**

R. Condorelli1,2, F. Mosele1, B. Verret1, T. Bachelot3, P. L. Bedard4, J. Cortes5, D. M. Hyman6, D. Juric7, I. Krop8, I. Bieche9, C. Saura10, C. Sotiriou11, F. Cardoso12, S. Loibl13, F. Andre1, N. C. Turner14

Affiliations:

1 Department of Medical Oncology, INSERM U981, Université Paris Sud, Gustave Roussy, Villejuif, France. 2 Institute of Oncology and Breast Unit of Southern Switzerland, Bellinzona, Switzerland. 3  Department of Medical Oncology, Cancer Research Center of Lyon Inserm, Lyon, France. 4 Department of Medicine, Division of Medical Oncology & Hematology, Princess Margaret Cancer Centre, Toronto, Canada. 5 Ramon y Cajal University Hospital, Madrid & Vall d’Hebron Institute of Oncology (VHIO), Barcelona, Spain. 6 Memorial Sloan Kettering Cancer Center, New York, United States. 7Massachusetts General Hospital (MGH), Boston, United States. 8 Dana-Farber Cancer Institute, Boston, United States. 9 Department of Genetics, Curie Institute, Paris, France 10 Department of Medical Oncology, Vall d’Hebron University Hospital, Vall d’Hebron Institute of Oncology, Barcelona, Spain. 11 J.C. Heuson Breast Cancer Translational Research Laboratory, Université Libre de Bruxelles, Institut Jules Bordet, Brussels, Belgium. 12 Breast Unit, Champalimaud Clinical Center, Champalimaud Foundation, Lisbon, Portugal. 13 German Breast Group, Neu-Isenburg, Germany. 14 Royal Marsden Hospital and Institute of Cancer Research, London, UK.

**Abstract:**

Better knowledge of the tumor genomic landscapes has helped to develop more effective targeted drugs. However, there is no tool to interpret targetability of genomic alterations assessed by next generation sequencing in the context of clinical practice. Our aim is to rank the level of evidence of individual recurrent genomic alterations observed in breast cancer based on the ESMO Scale for Clinical Actionability of molecular Targets (ESCAT) in order to help the clinicians to prioritize treatment. Analyses of databases suggested that there are around 40 recurrent driver alterations in breast cancer. *ERBB2* amplification, germline *BRCA1/2* mutations, *PIK3CA* mutations were classified tier of evidence IA based on large randomized trials showing antitumor activity of targeted therapies in patients presenting the alterations. *NTRK* fusions and *MSI* were ranked IC. *ESR1* mutations and *PTEN* loss were ranked tier IIA, and *ERBB2* mutations and *AKT1* mutations tier IIB. Somatic *BRCA 1/2* mutations, *MDM2* amplifications and *ERBB 3* mutations were ranked tier III. Seventeen genes were ranked tier IV based on preclinical evidence. Finally, *FGFR1* and *CCND1* were ranked tier X alterations because previous studies have shown lack of actionability.

**Introduction**

The development of next-generation sequencing (NGS) technologies allows, today, to profile the mutational landscape of tumors with reasonable times and costs. This translated, in the clinical setting, into the development of molecular screening programs mainly for patients with advanced disease resistant to standard therapies, with the aim of improving outcomes by means of new targeted therapies (1–3). Implementation of clinical genomics assessments in the clinical trial setting, while being a great opportunity to understand the disease’s biology, raises the challenges of data interpretation and their practical applicability. As illustration, nowadays there is no guidance to select genomic alterations in patients with metastatic breast cancer (mBC) and, unfortunately, matching an actionable event with a targeted therapy does not always translate into the expected clinical benefit.

Trying to overcome this lack of information, from recent years, efforts have been made to create a comprehensive classification scheme to guide target prioritization by analyzing level of evidence (LOE) for targetability of each recurrent driver described in solid tumors. These classifications evaluate whether targeting a genomic alteration leads to an antitumor effect, whatever the magnitude of clinical benefit is. Several LOE scales have been reported. In 2014, André and colleagues developed a scale to classify genomic alterations according to their likelihood of being therapeutic targets. In this scale, the LOE combines two levels of assessments: the first (from I to IV) defines the robustness of the data source; the second (from A to C) defines the relevance of the information according to the disease under consideration (4). Another scale was described in 2014 by Van Allen and colleagues with 5 LOE for linking a given alteration to a clinical action, distinguishing and classifying predictive, prognostic and diagnostic markers (5). A different example of classification was presented in 2015 by Dienstmann and colleagues who conceived a “Clinical Targetability Index” resulting from the assessment of genomic alterations at multiple levels (gene-, variant-, tumor type- and drug level) (6). In 2016, Sukhai and colleagues proposed a classification for somatic variants in advanced cancers, organized in five categories according to: known or predicted pathogenicity of the variant, primary site and tumor histology in which the variant is found, recurrence of the variant and evidence of its clinical actionability (7). For the same purpose, in 2017, Chakravarty and colleagues created “OncoKB”, a base stratifying cancer somatic alterations according to their prognostic and predictive significance on the basis of US Food and Drug Administration labeling, National Comprehensive Cancer Network guidelines, experts recommendations, scientific literature (8).

In this paper focused on breast cancer, we will use a ranking launched recently by ESMO: the ESMO Scale for Clinical Actionability of molecular Targets (ESCAT) (Table1) (9). This scale provides a framework to assign DNA alterations into tiers that reflect their clinical utility for selecting patients for treatment with targeted therapies. The scale uses the strength of evidence from clinical studies as the basis to assign tiers to a target.

**Methods**

First, we identified the recurrent drivers described in breast cancer (BC), reviewing the genomic datasets available end of 2017. In addition, we added recent FDA tissue-agnostic drug approvals. For early BC we reviewed The Cancer Genome Atlas (TCGA) (10) and the dataset of the International Cancer Genome Consortium (ICGC) (11); for mBC we reviewed the analysis of Lefebvre and colleagues (12), which performed whole-exome sequencing (WES) on 216 tumor-blood pairs from mBC patients included in molecular screening trials. This work of review resulted in a list of 40 genes, described as recurrent drivers in BC, which are listed in table 2. To assess the efficacy of drugs matched to genomic alterations, we searched for clinical trials in clinicaltrials.gov, pubmed and major scientific meetings (ASCO, ESMO, SABCS, AACR). When all trials were consistent, only one or two was reported in the paper as illustration. When trials where inconsistent, all trials were reported. The first authors (RC, FM) did the literature review and proposed a ranking to the panel which sent their suggestions. All panel members contributed to the ranking.

We graduated the targetability for the most studied genes, according to the ESCAT scale (9). This scale classifies the molecular targets into different tiers (I-V and X) based on the strength of evidence from clinical studies. One limitation of this classification is that only focus on DNA alterations.

**Tier I**

Tier I, according to ESCAT classification, is when the alteration-drug match is associated with improved outcome in clinical trials so the access to the treatment should be considered standard of care. Tier I includes tier IA, IB and IC.

*Tier IA* is based on prospective, randomized clinical trials that show the alteration-drug match in a specific tumor type results in a clinically meaningful improvement of a survival end point. *ERBB2* amplification, germline *BRCA1/2* mutations and *PIK3CA* mutations are classified at this l tier.

*Tier IB* is based on prospective, single arm clinical trials that show the alteration-drug match in a specific tumor type results in clinically meaningful benefit as defined by ESMO MCBS 1.1.

*Tier IC* is based on clinical trials across multiple tumor types or basket clinical trials that show clinical benefit (ESMO MCBS 1.1) for the alteration-drug match, with similar benefit observed across the different tumor types. *NTRK* translocations and microsatellite instability (MSI) are examples of this tier.

*ERBB2* amplification (IA)

*ERBB2* amplification reaches tier IA on the basis of randomized, prospective trials that result in overall survival (OS) and progression free survival (PFS) improvement in early BC and mBC patients harboring the *ERBB2* amplification. The pooled analysis of NSABP-B31 and NCCTG N9831, two phase III trials comparing chemotherapy to trastuzumab + chemo in patients with HER2 amplified BC, showed a 12% absolute benefit on 3 year DFS in patients treated with trastuzumab (13). At the opposite, the NSABP B-47 trial testing trastuzumab in Her2-low BC did not detect a benefit in patients treated with trastuzumab (5 year DFS: 89.6% *vs* 89.2%; HR 0.98; 95% CI [0.77-1.26]; p=0.90) (14).

Since trastuzumab could mediate its efficacy through ADCC and not by oncogene de-addiciton, we looked at studies that evaluated tyrosine kinase inhibitors. A randomized trial compared lapatinib and paclitaxel *versus* placebo and paclitaxel as first-line therapy for mBC. Overall 580 patients were enrolled, with 15% of them harboring an *ERBB2* amplified BC. The analysis in the HER2 positive subgroup reported a significantly longer time to progression (TTP) in the lapatinib arm, in comparison with the placebo arm (median 36.4 *vs* 25.1 weeks, respectively; HR 0.53; p=0.005). On the contrary, in the HER2 negative subgroup there was no difference in terms of TTP in the treatment arm with lapatinib compared with the treatment arm with placebo (median 25.1 *versus* 24.0 weeks respectively; HR 1.05; p=0.6617) (15) .

Gene expression arrays suggest that tumors driven by *ERBB2* amplifications are frequently Estrogen Receptor (ER) negative (16).

Germline *BRCA 1/2* mutations (IA)

In the randomized, controlled, phase III OlympiAD trial, patients with germline *BRCA 1/2* mutations mBC were randomized to receive olaparib or standard therapy with single-agent chemotherapy of the physician’s choice. The results showed a median PFS significantly longer in the olaparib group than in the standard therapy group (7.0 *vs.* 4.2 months; HR 0.58; p <0.001). Moreover, the response rate was 59.9% in the olaparib group and 28.8% in the standard-therapy group (17).

In the randomized, controlled, phase 3 EMBRACA, patients with germline *BRCA 1/2* mutations and mBC were randomized to receive talazoparib 1 mg/day or standard therapy with single-agent chemotherapy of the physician’s choice. The median PFS was 8.6 months for patients in the talazoparib arm vs 5.6 months for those in the physician’s-choice arm (HR 0.54; p <0.0001) (18).

*PIK3CA* mutations (IA)

Approximately 40% of hormone receptor positive (HR+) mBC present mutations that activate the α isoform PI3K, called *PIK3CA* mutations. The data supporting the targetability of *PIK3CA* mutations in mBC comes from the phase III randomized trial SOLAR-1. Patients were randomized to receive alpelisib 300 mg/day + fulvestrant 500 mg *vs* fulvestrant + placebo. They were assigned to two cohorts: *PIK3CA* mutant (n=341) *vs* *PIK3CA* non-mutant (n=231). The median PFS in the *PIK3CA* mutant cohort was 11 months for patients in the alpelisib arm vs 5.7 months in placebo arm (HR:0.65, p=0.00065). Alpelisib did not improve PFS in the not mutant cohort (19). While *PIK3CA* mutations predict efficacy of alpha-selective PI3K inhibitors, it does not predict sensitivity to everolimus (20).

Microsatellite instability (MSI) (IC)

The incidence of MSI in breast cancer is around 1% (21). Mismatch repair-deficient tumors are more responsive to PD-1 blockade (pembrolizumab) (22).The study that led to its approval in this setting is a trial of 149 patients (2 breast cancer) with MSI solid tumors, enrolled from five uncontrolled, open-label, multi-cohort, multi-center, single-arm trials. The overall response rate (ORR) was 39.6% (95% CI [31.7-47.9]). Both patients with BC had partial response (PR). The identification of MSI status was determined using polymerase chain reaction (PCR) tests or immunohistochemistry (IHC) (23). While methods of MSI detection using NGS are being developed; it is important to acknowledge that the current gold standard for detection is PCR and IHC.

*NTRK* translocations (IC)

Tropomyosin receptor kinase (Trk) family is composed by 3 transmembrane proteins (TrkA, TrkB and TrkC) that are encoded by the *NTRK1*, *NTRK2* and *NTRK3* genes, respectively. Fusion genes, resulting from chromosomal alterations involving *NTRK* genes, lead to transcription of chimeric Trk proteins with constitutively activated or overexpressed kinase function conferring oncogenic potential (24).

Larotrectinib, a selective small-molecule pan-Trk inhibitor, demonstrated efficacy in the LOXO-101 trial. A total of 55 patients were enrolled and treated with larotrectinib, including one patient with breast cancer (2%). The ORR was 75% (95% CI [61–85]). At 1 year, 71% of the responses were ongoing and 55% of the patients remained progression-free. The median duration of response and the median PFS had not been reached (25).

**Tier II**

Tier II is defined by the existence of antitumor activity associated with the matched alteration-drug, but without information about the magnitude of benefit because lack of prospective outcome data. Tier II includes tier IIA and IIB. Patients who present tier II alterations should have access to drugs in the context of prospective trials, or more optimally in the context of access programs.

*Tier IIA* is based on retrospective studies of prospective trials showing that patients presenting the alteration in the tumor of interest derive a benefit from the matched drug. *PTEN* loss and *ESR1* mutations are classified at this tier.

*Tier IIB* is based on prospective clinical trial(s) showing objective responses in patients presenting the alteration, but without conclusive data on outcome. *AKT1* mutations and *ERBB2* mutations are classified at this tier.

Table 3 reports the list of genomic alterations that, according to ESCAT scale of targetability, reach the tier I and II.

*ESR1* mutations (IIA)

It is known that resistance to hormonal therapies develops through a variety of mechanisms, including acquisition of *ESR1* mutations resulting in ligand-independent constitutive ER activity. These mutations have a high prevalence in mBC previously treated with aromatase inhibitors (AIs). Fribbens and collegues (26) developed a prospective-retrospective analysis of two phase III randomized trials (SoFEA and PALOMA 3) where they evaluated the impact of *ESR1* mutations on sensitivity to standard therapies. In SoFEA trial, for patients with *ESR1* mutant ctDNA, the median PFS was 2.6 months (95% CI [2.4-6.2]) for patients given exemestane and 5.7 months (95% CI [3.0-8.5]) for those given fulvestrant (HR 0.52; 95% CI [0.30-0.92]; p=0.02). At the opposite, patients with wild-type *ESR1* had a median PFS of 8.0 months (95% CI [3.0-11.5]) when given exemestane and a median PFS of 5.4 months (95% CI [3.7-8.1]) when given fulvestrant (HR 1.07; 95% CI [0.68-1.67]; p=0.77). The interaction test between treatment allocation and *ESR1* mutation status was p=0.07. In PALOMA 3 trial, *ESR1* mutation was not predictive of efficacy of palbociclib (interaction test; p=0.74).

Some could argue that *ESR1* mutations do not meet the criteria of targetable alterations since they define a resistant genotype, rather than predicting sensitivity to targeted agents. Nevertheless, considering the incidence and the relevance of the alteration, the panel has preferred to keep it into the ranking.

*PTEN* loss (IIA)

Loss of *PTEN* function, through mutational inactivation, homozygous deletion or down-regulation of expression, results in activation of AKT-mTOR signaling. This is a common finding in triple negative BC (TNBC). AKT inhibitors could block the cell signaling mediated by *PTEN* loss.

In a phase II randomized trial Schmid and colleagues (27) evaluated capivasertib (AZD5363), a small molecule AKT inhibitor. Overall 140 patients with untreated triple negative mBC were randomized to receive paclitaxel plus capivasertib 400 mg orally twice daily (n=70) or paclitaxel plus placebo (=70). In the overall population median PFS was 5.9 months in the capivasertib arm vs. 4.2 months in the placebo arm (HR:0.74 95% CI [0.50-1.08]; p=0.06). A retrospective analysis showed a significant improvement of median PFS in the subgroup of patients presenting *PIK3CA/AKT1/PTEN* alterations (n=28); in this group the median PFS was 9.3 months in the capivasertib arm vs. 3.7 months in the placebo arm (HR 0.30; 95% CI [0.11-0.79]; p=0.01) while in the *PIK3CA/AKT1/PTEN* no altered patients the median PFS was 5.3 months in the capivasertib arm vs. 4.4 in the placebo arm (HR 1.13; 95% CI [0.70-1.82]; p=0.61).

*AKT1* mutations (IIB)

Preliminary evidence about the targetability of *AKT1* mutations comes from a phase 1 multi-histology basket study, in which the drug AZD5363, a pan-AKT kinase inhibitor, was tested in a cohort of 58 patients with solid cancers. 20 BC patients enrolled harbored hot spot *AKT1 E17K*–mutant tumor. The response rate was 19% and median PFS was 5.5 months (95% CI [2.9-6.9 months]) (28). In a phase I of AZD5363 that included patients with *PIK3CA* mutation, without enrichment on *AKT1* mutations (n=54), the partial response was 5.6% of which 1 response was in the ER+ breast cancer cohort (29).

*ERBB2* mutations (IIB)

In the multi-histology, phase 2, basket trial SUMMIT, the activity of neratinib, an irreversible pan-HER tyrosine kinase inhibitor, was tested in patients affected by solid tumors with somatic *ERBB2/3* mutations. Breast cancer was the second most frequent tumor type, with 25 patients representing the 17.7% of the whole cohort In the *ERBB2*-mutant BC cohort, a single-agent activity for neratinib was observed, with an ORR of 24%, clinical benefit rate of 40% and median PFS of 3.5 months (30).

In another single-arm phase 2 trial, focused only on mBC, 22 patients were found to present a somatic *ERBB2* mutation without amplification. Among them, 16 patients received neratinib 240 mg daily. The clinical benefit rate was 31% (90% CI [13%-55%]), including one complete response (CR), one PR, and three stable disease (SD) ≥24 weeks. Median PFS was 16 weeks (90% CI [8–31]) (31). On the negative control arm (*ERBB3*-mutant, *ERBB2*-wildtype), there was no response to the drug. Therefore we assigned *ERBB2* mutations to tier IIB.

**Tier III**

Tier III, according to ESCAT classification, is defined by a matched drug-alteration that led to clinical benefit in another tumor type or by alterations on genes that belong to the same families of tier I genes. Tiers III includes tier IIIA and tier IIIB.

*Tier IIIA* targets include alterations that define a patient population with proven benefit from a targeting agent in a specific tumor type that is not the tumor of interest. These clinical scenarios are an ideal setting for prospective studies. Somatic *BRCA1/2* mutations and *MDM2* amplification are classified at this tier.

A tier *IIIB* alteration has a similar predicted functional impact as a tier I abnormality in the same gene or pathway, but does not have supportive clinical data. *ERBB3* mutations are classified at this tier.

The table 4 reports the gene alterations ranked III by the panel.

Somatic *BRCA 1/2* mutations (IIIA)

Over 5% of all cancers present a bi-allelic pathogenic alteration in homologous recombination (HR)-related genes, with 25% and 10% of ovarian and breast cancers, respectively, having this pathway altered (32).

Somatic *BRCA 1/2* mutations are ranked tier IIIA because of very little data in BC, while a substantial evidence of efficacy of PARP-inhibitors exists in metastatic ovarian cancer. In December 2016, FDA approved the PARP-inhibitor rucaparib as monotherapy for patients with advanced ovarian cancer and deleterious germline and/or somatic *BRCA* mutations. Approval was based on efficacy data from a pooled analysis of two single-arm, multi-center trials (ARIEL2 and Study 10) reporting an ORR of 54%, a median duration of response of 9.2 months and a median PFS of 10.0 months for treatment with rucaparib (33). Data still need to be generated in metastatic breast cancer.

*MDM2* amplifications (IIIA)

The murine double minute 2 (*MDM2*) is involved in the negative regulation of p53 and itself serves as an oncogene. *MDM2* is amplified or overexpressed in 40% to 60% of liposarcomas. Six patients with a *MDM2*-amplified liposarcoma were enrolled in a phase 1 trial testing a MDM2 inhibitor. There was a partial response in three patients and stable disease in two patients (15.7 months and 4.7 months) (34).

*ERBB3* mutations (IIIB)

On the MOSCATO 01 trial, overall 12 patients (less than 2%) with different diseases (one invasive lobular BC) harbored an *ERBB3* mutation and 7 of them received HER3 partners' inhibitors (trastuzumab and/or lapatinib or afatinib); 4 patients received other molecularly targeted agents (mTOR, PI3K or NOTCH inhibitors). Authors reported two partial responses. Out of 6 patients with SD, the breast cancer patient had 504 days on capecitabine + lapatinib association, and the lung squamous cell carcinoma patient had 420 days on afatinib (35). On the contrary, no drug’s activity was observed in the *ERRB3*-mutant cohort in the SUMMIT trial (n=16) (30).

While there is no consistent clinical data confirming the targetability of *ERBB3* mutations, its similarity to Her2 and preliminary evidence observed in MOSCATO-01 suggest this target is tier IIIB.

**Tier IV**

Lastly tier IV is defined by pre-clinical evidence of actionability for an alteration-drug match, without clinical data. This tier is divided in tier IVA and IVB. These targets are hypothetical and are best considered as qualifying evidence for future clinical testing. They may also be appropriate for enrichment of patients in early phase clinical trials. This category contains a very large number of putative markers proposal based on laboratory evidence, but space limitations preclude discuss them. They are mentioned in table 4.

*Tier IVA* evidence that the alteration influences drug sensitivity in preclinical *in vitro* or *in vivo* cancer models.

At tier *IVB* the actionability is based *in silico* bioinformatic predictions.

**Tier X**

Tier X is defined by an alteration for which clinical data indicate that it’s not actionable. These alterations should not be taken into account for clinical decisions. *CCND1* and *FGFR1* amplifications belong to this class. It must be acknowledge that this tier is defined based on data obtained with currently available therapies and using currently available technologies. Some of these alterations could move to other tiers when better drugs or technologies will be available.

*CCND1* amplification (X)

In the PALOMA-1/TRIO-18 study, patients with *CCND1* amplification did not derive more benefit than the population without amplification (36). The phase 3 PALOMA-2 (37) and MONALEESA-2 (38) trials (palbociclib + letrozole and ribociclib + letrozole respectively) also reported no differential benefit for the CDK4/6 inhibitors in *CCND1* amplified tumors.

*FGFR1* amplification (X)

*FGFR1* is amplified in around 15% of luminal breast cancers In a phase I study with patients with advanced solid tumors harboring alterations in fibroblast growth factor receptors (FGFRs), among the 43 patients with BC treated with BGJ398, 10 had a best response of SD and no response was observed. The authors concluded that the lack of objective responses and the limited disease control in BC patients challenge the idea that *FGFR* amplifications could be a driver alteration in this disease. It is still unclear whether alternative or additional biomarkers would better predict responders, or whether FGFR signaling is less essential for tumor growth in BC (39).

The safety and preliminary efficacy of lucitanib (an oral inhibitor of FGFR1 and FGFR2) have been investigated in an open-label phase I/IIa study in patients with advanced solid tumors (40) and others FGFR-inhibitors as dovitinib and AZD4547 are currently investigated in clinical trials (41,42).

**Conclusions**

Our work aimed at ranking genomic alterations observed in breast cancer in order to help the oncologist to prioritize drugs when receiving results of multigene sequencing. We found six gene alterations that met criteria for being ranked Level I, and four alterations ranked Level II, suggesting that multigene sequencing could provide benefit to patients pending optimal drug access.

More work has to be done to keep up-to-date this classification, and it is planned to update this ranking on an annual basis. It is imperative that the scientific community shares all results, even and especially the negative ones, in order to improve knowledge in precision and personalized cancer medicine.

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