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STRATEGIES TO PREDICT TREATMENT RESPONSE AND SELECT THERAPIES IN METASTATIC BREAST CANCER PATIENTS USING A NEXT GENERATION SEQUENCING (NGS) MULTI-GENE PANEL

INTRODUCTION

The standard of care for many patients with advanced breast cancer (BC)is gradually evolving from empirical treatment based on clinical-pathological characteristics to the use of targeted approaches based on the molecular profile of the tumor.

In the last decade, an increasing number of molecularly targeted drugs have been developed for the treatment of metastatic BC. These drugs target specific molecular abnormalities that confer to cancer cells a survival advantage [1]. Interestingly, the ability to perform multi-gene testing for a range of molecular alterations may provide an opportunity to clarify the mechanisms of treatment response, to find the strategies to overcome treatment resistance and thus, to identify patients who are more likely to develop relapse and who may be candidates for matched targeted therapies [2-3].

The main aim of this study is to find prognostic and predictive molecular biomarkers for the management of metastatic BC patients in clinical practice.

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MATERIALS AND METHODS

The amplicon-sequencing analyses took advantage of the Ion AmpliSeq™ technology (Thermo Fisher, Waltham, MA, USA). A custom panel was designed with the help of the Designer online tool (www.ampliseq.com), which was employed to generate optimized primers encompassing the coding DNA sequences (with 100bp of exon padding and the UTRs regions) of 25 genes in the Human Reference Genome (hg19); these genes were selected searching and screening scientific literature for treatments resistance in BC and are reported in Table 1. Primer pairs were divided into two pools to optimize multiplex PCR conditions and the coverage, that assessed to 89.02%. The customized Ion AmpliSeq panel was employed on samples from 7 primary BC samples and matched metastatic sites (3 skin, 3 lymph node and 1 lung metastases). They were all processed using the Ion AmpliSeq Library Kit 2.0, starting from 15 nanograms of FFPE extracted DNA/pool. Samples were barcoded with the Ion Express Kit to optimize matched patients pooling on the same 318 Chip v2 sequencing chip. The template-positive Ion Sphere Particles were sequenced on a Personal Genome Machine (Thermo Fisher, Waltham, MA, USA)

TABLE 1. NGS targeted panel of 25 genes involved in pathways of resistance to endocrine and targeted treatments

EGFR	ERBB2	ERBB3	UGF1R	FGFR1
MET	AKT1	PDK1	MTOR	PIKECA
PIK3R1	PTEN	INPP4B	BRAF	KRAS
MEK1	MEK2	ERK1	ERK2	CCDN1
CCDN2	CCDN3	CDK4	CDK6	ESR1

RESULTS

The mutation profiles of paired primary and secondary tumors of the seven patients enrolled in this study are presented in Table 2. Ten different genes (PTEN, PIK3CA, mTOR, ERBB2, ERBB3, MET, INPP4B, MAP2K1, CDK6, KRAS) in 6 different patients showed possible damaging variants as shown in Table 2.

- Four patients (number 1, 3, 5 and 6) showed no additional or different mutations in secondary tumors if compared to primary samples.
- In patient number 2, the metastatic site presented new mutations if compared to the primary tumor.
- Finally in patient number 4 and 7 we did not detect in metastases some of the mutations found in the primary tumor.

	PRIMARY TUMOR IHC	PRIMARY TUMOR MUTATIONS	TREATMENTS	METASTATIC SITES	TYPE OF METASTASIS BIOPSY	METASTASIS CHARACTERISITICS	METASTASIS MUTATIONS	SUBSEQUENT TREATMENTS	RESPONSE
Patient 1	ER 50%, PR 70%, HER2 negative	x	5-FU, Epirubicin, Cyclophosphamide, Taxotere, Anastrozole	SKIN	SKIN NODULE EXCISION	ER 100%, PR 20%, HER2 negative	x	Exemestane, Taxolo, Eribulina, Vinorelbine + Capecitabine, Faslodex, Palbociclib + Letrozole, Carboplatin + Gemcitabine	**PR** with Exemestane, **PD** with all the other agents
Patient 2	ER 60%, PR 2%, HER2 positive	x	5-FU, Epirubicin, Cyclophosphamide, Tamoxifen, Taxotere, Trastuzumab, Letrozole	LUNG	SINGLE LUNG NODULE RESECTION	ER 95%, PR 95%, HER2 positive	**ERBB3, ERBB2, KRAS**	Trastuzumab and Letrozole in maintenance	**CR**
Patient 3	ER 90%, PR 90%, HER2 negative	**PTEN**	Taxotere, Cyclophosphamide, Tamoxifen	SKIN	SKIN PUNCH	ER 90%, PR 60%, HER2 negative	**PTEN**	Taxotere, Exemestane, Faslodex, Anastrozole, Vinorelbine + Capecitabine	**PR** with Exemestane and Faslodex, **PD** with all the other agents
Patient 4	ER 95%, PR 95%, HER2 negative	**PIK3CA (exon10), PIK3CA (exon21), mTOR**	Tamoxifen	BONE, SKIN	SKIN PUNCH	ER 90%, PR 50%, HER2 negative	**PIK3CA (exon10), mTOR**	Single skin metastasis resection and Tamoxifen, Letrozole, Capecitabine, Myocet + Cyclophosphamide, Fulvestrant, Taxolo, Vinorelbine	**SD** with Letrozole and Capecitabine, **PD** with all the other agents
Patient 5	ER 90%, PR 80%, HER2 negative	**PIK3CA**	Tamoxifen, Taxol + Neratinib, Trastuzumab, Letrozole	BONE, LIVER, LYMPH NODES	LYMPH NODE	ER 10%, PR 1%, HER2 negative	**PIK3CA**	Myocet, Zometa, Examestane, Eribulin, Carboplatin + Taxol, Capecitabine, Vinorelbine	**SD** with Myocet, **PD** with all the other agents
Patient 6	ER 100%, PR 10%, HER2 positive	**ERBB2**	Taxotere + Cyclophosphamide, Anastrozole	LYMPH NODES	LYMPH NODE	ER 90%, PR 1%, HER2 positive	**ERBB2**	Taxotere + Trastuzumab + Pertuzumab, Exemestane	**PR** with Taxotere + Trastuzumab + Pertuzumab and Examestane
Patient 7	ER 90%, PR 40%, HER2 negative	**ERBB2, MET, INPP4B, MAP2K1, CDK6**	Epirubicine + Taxole, Zometa, Letrozole, Fulvestrant + Trastuzumab	BONE, LYMPH NODES	LYMPH NODE	ER 0%, PR 0%, HER2 negative	x	Capecitabina, Anastrazole + Dehydroepiandrosterone (DHEA), Exemestane, Taxole + Trastuzumab	**PR** with Capecitabine, **PD** with Anastrozole + DHEA, Examestane, **PR** with Taxole + Trastuzumab

TABLE2

PD = progressive disease
RC = complete response
PR = partial response
SD = stable disease

DISCUSSION

In 5 patients (71,4%) the mutational status of primary tumor could explain treatment resistance and thus predict relapse, in one patient the mutational status of the new subclones could be relevant for guiding differently the subsequent treatment choices.

In 2 patients (28,5%) we were not able to detect in metastases some of the mutations found in the primary tumor. This could be explained by considering the clonal evolution of metastases.

These preliminary data suggest that the multi-gene panel analysis of primary and secondary tumors may help clinicians:

- in discriminating BC patients HR+ and/or HER2+ with mutations predicting an increased risk of adjuvant treatment resistance and thus relapse
- in guiding treatment selection strategies in the metastatic setting.

The study is still open and we are currently recruiting other patients.

REFERENCES

1. Breast Cancer Res. 2015 Apr 23; 17:60
2. Clin Cancer Res. 2012 Nov 15; 18(22):6373-83
3. Nature. 2011 Apr 7; 472(7341):90-4