Intratumor and circulating clonal heterogeneity shape the basis of precision breast cancer therapy

“The recent evidence of spatiotemporal genomes and tumor evolution has led to the development of breakthrough next-generation sequencing (NGS) technologies. Intratumor heterogeneity (ITH) and circulating clonal diversity represent two of the most possible explanations of primary and secondary resistance. In this editorial, we discuss how extensive biobanking for each individual patient with subsequent genome sequencing can open novel horizons for precision medicine in breast cancer.

Although the ENCODE project [1,2] has revolutionized biomedical research highlighting the necessity for a long-term basic research efforts, at the same time it has shaped innovative horizons in improving NGS technologies and developing breakthrough methods for understanding cancer evolution and resistance to current therapies [3,4].

Progress in personalized prevention & treatment
Progress in basic, translational and clinical research and implementation of new discoveries into routine clinical practice is faster in breast cancer than in any other cancer type. Advances in single gene testing have led to the development of a personalized approach in the prevention setting and targeted therapy. Breast cancer is still a major health problem for women taking into consideration that 1.67 million new cases are diagnosed each year worldwide [5].

1Centre for Biosystems & Genomic Network Medicine, University of Ioannina, Ioannina, Greece
2Department of Surgery, ‘G Hatzikosta’ General Hospital, Ioannina, Greece
3Department of Surgery, METAXA Cancer Memorial Hospital, Botsisi 51, TK 18535, Piraeus, Greece.
4Department of Plastic Surgery, Ioannina University School of Medicine, Ioannina, Greece
5First Department of Propaedeutic Surgery, Hippocration Hospital, Athens Medical School, National & Kapodistrian University of Athens, Athens 11525, Greece
6Department of Surgery, Ioannina University Hospital, Ioannina, Greece
7Biomedical Research Foundation of the Academy of Athens (BRFAA), Athens, Greece
*Author for correspondence: Tel.: +30 265 100 7423; Fax: +30 265 100 7094; droukos@uoi.gr
20 years after the discovery of germline mutations BRCA1 and BRCA2, which are associated with life-time high risk of breast and ovarian cancer, effective primary prevention, has developed as a result of personalized therapies based on both family history and BRCA1/2 testing [6]. Moreover, more aggressive risk-reducing surgery including bilateral mastectomy and bilateral salpingo-oophorectomy has been suggested for BRCA1 than BRCA2 mutation carriers [6].

In the multimodal treatment setting, established clinical models on the basis of both traditional and genetic criteria provide better selection of patients improving oncological and quality-of-life outcomes. For example, age, tumor size (T), node status (N), histological grade (G) and molecular features including estrogen receptor and progesterone receptor (ER/PR) status as well as HER2 status represent the modern algorithm for decision-making in adjuvant treatment of early breast cancer (M0).

Trastuzumab (Herceptin®, Genentech, Inc., CA, USA) added to chemotherapy for HER2-positive patients has improved overall survival (OS) rates in the adjuvant [7] and metastatic setting (M1). More recently, trastuzumab–emtansine conjugate (T-DM1, Kadcyla®, Genentech, Inc., CA, USA) prolongs mean OS [8]. Moreover, Palbociclib has recently received regulatory fast-track approval opening a new therapeutic horizon for postmenopausal women with ER+/HER2-negative metastatic breast cancer [9].

**From interpatient to intrapatient heterogeneity**

Tumor heterogeneity has long been considered as genetic variation for most solid malignancies. In recent years, the development of high-throughput technologies has enhanced the ability of many studies to access the impact of tumor heterogeneity in the clinic. Most of these studies have suggested different genetic characteristics not only among patients with the same cancer type (interpatient heterogeneity) [10], but also between primary and metastatic tumor(s) in the same individual patient [3]. However, a few other studies have shown high similarities between primary and metastatic tumor, suggesting the pre-existence of a small cell population within the primary tumor responsible for metastasis (reviewed also in [3]).

Next-generation sequencing systems

This relative uncertainty of individual patient’s tumor heterogeneity could be overcome in recent years by the implementation of NGS systems. NGS systems have revolutionized biomedical research because of clinical validity and continuing lowering cost of these technologies.

Numerous genomic studies using tumor-normal pairs for each individual patient including whole-exome sequencing (WES) [11] and whole-genome sequencing (WGS) [12,13] have dramatically been increased over the last 6 years. Even in this NGS era, multiple challenges are emerging, regarding their potential for improving clinical treatment and patients’ outcomes. Two NGS strategies including a conventional and a breakthrough approach have been developed providing promise for clinical implications.

Following single biopsy-based modern oncology, most NGS studies available are based on the same concept. In the largest WES study reported by Lawrence et al. [11] on 4742 tumor-normal pairs, 892 breast cancers were analyzed. In this study, the SETBD1 gene has been discovered for
Intratumor & circulating heterogeneity in breast cancer

EDITORIAL

Intratumor & circulating clonal heterogeneity

Exploiting recent evidence on dynamic clonal evolution [15,16] we could explain therapeutic resistance-based metastatic relapse. Preliminary data suggest that dynamic evolution depends on the kind of mutation. On one hand most data support dynamic evolution of point mutations only, while on the other large structural genome changes are pre-existing and remain stable during tumor growth and progression [18,19]. However, these findings require validation by large studies.

Clinical evidence that almost 25% of women with HER2-positive breast cancer treated with guidelines-based recommendation therapy develop relapse, clonal evolution identification in response to neoadjuvant treatment. In 13 patients, targetable mutations were resulted from clonal evolution following neoadjuvant treatment.

Noninvasive methods with major potential clinical implications have recently been developed such as the circulating tumor DNA followed by NGS analysis (ctDNA-NGS). The aim from a clinical perspective is to use this method as a biomarker to predict therapeutic resistance and relapse before it clinically occurs. Murtaza et al. [17] have performed a serial ctDNA-NGS analysis in 19 plasma samples obtained from six patients with breast, ovarian and lung cancer. Quantification of allele fractions in plasma identified the emergence of mutations associated with acquired therapeutic resistance. This method not only can potentially predict resistance-based metastatic relapse, but also opens new avenue for drug-development design. In breast cancer, one patient that was treated with chemotherapy, was found to have an activating mutation in PIK3CA. These data establish a proof-of-principle that ctDNA exome sequencing can be used as biomarker to predict acquired resistance but additional larger studies are required.

Future perspective & conclusion

The unprecedented potential of advancing NGS systems and methods to explore spatiotemporal genomes and tumor evolution, raises for the first time rational hope for deep understanding of mechanisms underlying therapeutic resistance and metastasis in response to therapy.

Indeed, once the vast majority of women with nonmetastatic breast cancer receive systemic chemotherapy and targeted therapy and a substantial proportion among them develop relapse, it is crucial to understand the reasons why.

Exploiting recent evidence on dynamic clonal evolution [15,16] we could explain therapeutic resistance-based metastatic relapse. Preliminary data suggest that dynamic evolution depends on the kind of mutation. On one hand most data support dynamic evolution of point mutations only, while on the other large structural genome changes are pre-existing and remain stable during tumor growth and progression [18,19]. However, these findings require validation by large studies.

Clinical evidence that almost 25% of women with HER2-positive breast cancer treated with guidelines-based recommendation therapy develop relapse, clonal evolution identification
following systemic therapy could reduce this resistance-based treatment failure. The potential of ITH and subclonal emergence identification in the neoadjuvant treatment and postsurgical setting [16] along with repeated ctDNA-NGS can reveal the emergence of resistant genome-wide alterations responsible for clinical relapse several months before it clinically occurs.

In summary, rapid developments in technological genome systems and computational network methods open new avenues in understanding etiopathogenesis on the basis of large-scale translational research studies. This understanding of molecular mechanisms landscape underlying therapeutic resistance shapes new horizons in robust biomarkers and drug-development strategy to reach precision cancer medicine.

Financial & competing interests disclosure
The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

References

10.2217/fon-2016-0307 Future Oncol. (Epub ahead of print)