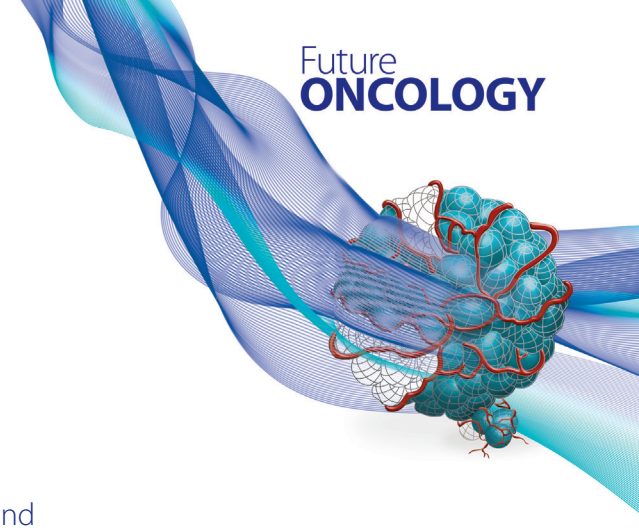
Future ONCOLOGY



EDITORIAL

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

"...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."

Demosthenes E Ziogas[1,2], John Spiliotis[3], Efstathios G Lykoudis[4], Georgios C Zografos[5] & Dimitrios H Roukos*[1,6,7]

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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.

Approximately half a century following the war against cancer and the discovery of DNA double helix, millions of patients still die from the disease. The initial enthusiasm on personalized medicine after the completion of the first human genome sequence draft at the beginning of this millennium, was followed by skepticism on the basis of the complexity of noncoding genome functionality and nonlinear transcription. 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].

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[1]Centre for Biosystems & Genomic Network Medicine, University of Ioannina, Ioannina, Greece
[2]Department of Surgery, 'G Hatzikosta' General Hospital, Ioannina, Greece
[3]Department of Surgery, METAXA Cancer Memorial Hospital, Botosi 51, TK 18535, Piraeus, Greece.
[4]Department of Plastic Surgery, Ioannina University School of Medicine, Ioannina, Greece
[5]First Department of Propaedeutic Surgery, Hippocration Hospital, Athens Medical School, National & Kapodistrian University of Athens, Athens 11525, Greece
[6]Department of Surgery, Ioannina University Hospital, Ioannina, Greece
[7]Biomedical 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

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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–emtasine 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].

Limitations

Despite these advances, much more work is required to reach a personalized cancer medicine model for the clinic. Among women with significant family history who account for 25% of all breast cancers, only 7% are caused by the known *BRCA1/2* mutations, while for the remaining 18% no other high-penetrance gene has yet been identified and thus no precise risk estimation can be made. For sporadic breast cancer that accounts for 70–80% of all breast cancers, no personalized risk prediction is feasible. Therefore, a generalized strategy including mammography and ultrasound has been established as an evidence-based approach for early-stage breast cancer diagnosis and OS benefit recommended by guidelines.

In the treatment setting, anti-estrogen for ER$^+$ has long been considered as standard treatment. For HER2-positive women trastuzumab has been recommended in the adjuvant setting, while trastuzumab–emtansine has been suggested in trastuzumab-resistant metastatic disease. More recently, palbociclib plus letrozole for ER$^+$/HER2-negative postmenopausal women has become a new standard therapy, improving treatment efficacy and survival. However, despite advances and standardization of systemic chemotherapy and targeted therapy, intrinsic and acquired resistance remains an unresolved problem.

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

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the first time with strict statistically significant criteria ($p < 0.01$) [11]. Three other new genes, *CBFB*, *RUNX1* and *GATA3* involved in triple negative breast cancer were reported for the first time by Banerji *et al.* [14]. However, because of the relatively small number of patients enrolled in this study (n = 103), confirmation by larger studies is required to reach the statistical level of accuracy which was recommended by Lawrence *et al.* [11] in the discovery of new cancer driver genes.

At least four WGS breast cancer studies have been reported. In one of these studies, Ellis *et al.* [12] identified five genes (*RUNX1*, *CBFB*, *MYH9*, *MLL3* and *SF3B1*) in 46 patients' samples with ER^+ breast cancer. These genes have previously been linked with hematopoietic disorders, but are reported for the first time in breast cancer. However, this conventional single biopsy-based WES/WGS strategy has strong limitations in understanding primary and secondary resistance because of more recent evidence on dynamics genomic clones evolution [15] and ITH [16]. Based on this new knowledge, innovative methods and NGS applications have been developed.

Intratumor & circulating clonal heterogeneity

Exploiting these new data from basic and translational research, landmark studies open new predictive and therapeutic horizons for breast cancer. Developing new methods, dynamics of genomics clones' evolution [16], ITH and circulating genomic clones diversity can now be explored. These techniques and methods provide the potential not only to explain, but also to overcome therapeutic resistance and prevent metastasis.

Identification of ITH, which represents genetic characteristics of different cell subpopulations within the primary tumor, could provide important clinical implications in overcoming resistance to current therapeutics. Multiregional NGS analysis has been suggested as the optimal method to reveal ITH. In the largest ITH study available, Yates *et al.* [16] have performed WGS and targeted sequencing of the primary tumor in 50 patients with breast cancer including 303 solid tumor samples. Sequencing data were compared in 18 patients who had undergone neoadjuvant treatment followed by surgical resection. This intelligent method could reveal not only ITH but also clonal evolution 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-existed and remain stable during tumor growth and progression [18,19]. However, these finding 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

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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.

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