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Journal Club

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Breast cancer gene expression profiling has revolutionized our view on breast cancer biology and given insights into the extensive heterogeneity of the disease. However, despite these novel transcriptomic insights, a large number of questions have not yet been answered. As one means to generate a few of these answers, a novel technology has recently been introduced: next generation sequencing analyses allow for the rapid analysis of the (breast cancer) genome and therefore provide a basis to get further insights into the disease biology. In this journal club, Thomas Karn from the University of Frankfurt discusses a recent paper that has used several genomic and transcriptomic assays in parallel to derive 'comprehensive molecular portraits of human breast tumours' and may deservedly been described as one landmark publication in this area of research.

Since the introduction of tamoxifen endocrine therapy has become one major approach to systemically treat hormone receptor positive breast cancer. Although the standard of care nowadays is a 5-year-schedule of antihormonal therapy through either tamoxifen and/or aromatase inhibitors there is still debate whether extending treatment to 10 years might be beneficial for certain subgroups of patients, such as those with a priori node negative disease. In this journal club, Christian Jackisch from the Klinikum Offenbach discusses the results from the ATLAS trial that have recently been presented at the San Antonio Breast Cancer Symposium (SABCS) 2012 and have been published in *The Lancet* at the very same day.

Cornelia Liedtke, Lübeck

Combination of Genomic and Transcriptomic Assays Gives New Insights in Breast Cancer Biology

Koboldt DC, Fulton RS, McLellan MD, et al.: Comprehensive molecular portraits of human breast tumours. Nature 2012;490:61–70.

We analysed primary breast cancers by genomic DNA copy number arrays, DNA methylation, exome sequencing, messenger RNA arrays, microRNA sequencing and reverse-phase protein arrays. Our ability to integrate information across platforms provided key insights into previously defined gene expression subtypes and demonstrated the existence of four main breast cancer classes when combining data from five platforms, each of which shows significant molecular heterogeneity. Somatic mutations in only three genes (TP53, PIK3CA and GATA3) occurred at > 10% incidence across all breast cancers; however, there were numerous subtype-associated and novel gene mutations including the enrichment of specific mutations in GATA3, PIK3CA and MAP3K1 with the luminal A subtype. We identified two novel protein-expression-defined subgroups, possibly produced by stromal/microenvironmental elements, and integrated analyses identified specific signalling pathways dominant in each molecular subtype including a HER2/phosphorylated HER2/EGFR/phosphorylated EGFR signature within the HER2-enriched expression subtype. Comparison of basal-like breast tumours with high-grade serous ovarian tumours showed many molecular commonalities, indicating a related aetiology and similar therapeutic opportunities. The biological finding of the four main breast cancer subtypes caused by different subsets of genetic and epigenetic abnormalities raises the hypothesis that much of the clinically observable plasticity and heterogeneity occurs within, and not across, these major biological subtypes of breast cancer.

Commentary Thomas Karn, Frankfurt/M.

One decade ago the advent of gene expression profiling through microarrays has allowed global analysis of changes in the cancer transcriptome. These techniques improved understanding of breast cancer biology and led to both new prognostic information [1, 2] and a tumor classification according to molecular breast cancer subtyping [3]. During the last year we again faced a similar 'revolution' through novel techniques from the molecular biology laboratory. While just 2 genome sequences of breast cancers were published in the years 2009 [4] and 2010 [5], the sample sizes of those studies literally exploded during 2011 and 2012. Last year the total number of published cancer genomes surpassed 2,500 cases and still counting. The number of breast cancer genomes has also reached more than 800 samples with the publication from The Cancer Genome Atlas (TCGA) Network commented on

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here [6]. This dramatic increase in sequencing throughput has been achieved through next generation sequencing (NGS) technologies reducing costs by 10,000 to 100,000 fold compared to the classical Sanger method. Genome sequencing projects that previously required decades of work can now be accomplished within days. All NGS methods rely on highly parallel sequencing of very short stretches of clonally amplified, immobilized fragments of DNA. Subsequent analysis is performed by digitally counting the short sequence reads after aligning them to a reference genome sequence. In addition to the detection of somatic mutations and germline variants this quantitative analysis also allows to determine DNA copy number variations. Moreover, sequencing of RNA through NGS even allows transcriptome expression profiling (RNA-seq) [7].

In the TCGA study, however, not only genome sequencing was performed. In addition, a series of 'omics' methods were applied to the more than 500 primary tumors by the 357 authors of the paper: Exome sequencing, single nucleotide polymorphism (SNP) and comparative genomic hybridization (CGH) arrays, DNA methylation analysis as well as both transcriptome, proteome, and microRNA expression analysis. Many of the 'comprehensive' insights of the study were enabled through integrative analysis across platforms. The results suggest breast cancer as a highly heterogeneous disease not only by gene expression but mutational and DNA copy number profiles as well. The most frequently mutated genes were TP53 (37% overall) and PIK3CA (36% overall), somewhat preferentially in the basal-like and the luminal A subtypes, respectively. From the more than 30,000 mutations detected among 507 patients, statistical methods identified 35 significantly mutated genes, nearly all of them previously implicated in breast cancer. One of the most intriguing findings of the paper was apparent from the analysis of pathways containing mutated genes. These and results from integrating other platforms suggest that basal-like tumors more closely resemble ovarian cancers than other breast cancer subtypes. This raises the exciting possibility that therapies for ovarian cancer might benefit patients with basal-like breast cancer, and vice versa.

When putting this study into context with the many other whole genome sequencing papers appearing recently, what can we learn from these results? Two main points can be made for all cancer genome studies: Firstly, the number of genes with frequent alterations in cancers is rather low [8]. Only 3 genes (PIK3A, TP53, GATA3) were mutated in at least 10% of the patients [9–11]. Secondly, the observed heterogeneity is high [12]. This holds true both for comparing different tumors and when studying the intratumoral heterogeneity. Regarding the inter-tumor heterogeneity it appears that there are virtually no two tumors with a similar mutational pattern. Nevertheless, further analysis of the genetic changes seem to suggest that different mutational events may be grouped to common oncogenic pathways somewhat reducing this complexity. On the other hand, a large degree of intratumoral heterogeneity has been detected through 'ultra deep sequencing': This highly redundant sequencing of the genome allows to digitally count the relative proportion of specifically mutated DNA molecules and thereby establish the frequency of different genetically distinct subclones within the tumor. Such analyses have already been extensively performed for hematological and other cancers [13] but also data on breast cancer are available [10, 14]. These data demonstrate waves of subclonal evolution within the cancer adding further complexity to the disease [15]. Taken together these results from the recent large sequencing projects corroborate data from the earlier studies on genome sequencing [16, 17]. Further informative studies will depend on the advised selection of defined sample material according to a specific clinical question.

There are also different exciting possibilities for integrating NGS in clinical practice. One approach will be targeted resequencing of mutations with therapeutic importance. Moreover, pilot studies have already shown that it is possible to analyze the complete genome of patients' tumors in a cost-effective and clinically relevant time frame [18]. Since data suggest that each breast cancer has at least one DNA rearrangement, such an approach could be used for the development of highly sensitive PCR assays for each individual tumor allowing personal monitoring of disease through specific detection of tumor DNA in peripheral blood.

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Optimal Treatment Duration for Endocrine Sensitive Breast Cancer in the Adjuvant Setting – Still a Matter of Debate

Davies C, Pan H, Godwin J, et al.: Long-term effects of continuing adjuvant tamoxifen to 10 years versus stopping at 5 years after diagnosis of oestrogen receptor-positive breast cancer: ATLAS, a randomised trial. Lancet 2012; doi: 10.1016/S0140-6736(12)61963-1. [Epub ahead of print]

Background: For women with oestrogen receptor (ER)-positive early breast cancer, treatment with tamoxifen for 5 years substantially reduces the breast cancer mortality rate throughout the first 15 years after diagnosis. We aimed to assess the further effects of continuing tamoxifen to 10 years instead of stopping at 5 years. **Methods:** In the worldwide Adjuvant Tamoxifen: Longer Against Shorter (ATLAS) trial, 12 894 women with early breast cancer who had completed 5 years of treatment with tamoxifen were randomly allocated to continue tamoxifen to 10 years or stop at 5 years (open control). Allocation (1:1) was by central computer, using minimisation. After entry (between 1996 and 2005), yearly follow-up forms recorded any recurrence, second cancer, hospital admission, or death. We report effects on breast cancer outcomes among the 6846 women with ER-positive disease, and side-effects among all women (with positive, negative, or unknown ER status). Long-term follow-up still continues. This study is registered, number ISRCTN19652633. **Findings:** Among women with ER-positive disease, allocation to continue tamoxifen reduced the risk of breast cancer recurrence (617 recurrences in 3428 women allocated to continue vs 711 in 3418 controls, p = 0·002), reduced breast cancer mortality (331 deaths vs 397 deaths, p = 0·01), and reduced overall mortality (639 deaths vs 722 deaths, p = 0·01). The reductions in adverse breast cancer outcomes appeared to be less extreme before than after year 10 (recurrence rate ratio [RR] 0·90 [95% CI 0·79–1·02] during years 5–9 and 0·75 [0·62–0·90] in later years; breast cancer mortality RR 0·97 [0·79–1·18] during years 5–9 and 0·71 [0·58–0·88] in later years). The cumulative risk of recurrence during years 5–14 was 21·4% for women allocated to continue versus 25·1% for controls; breast cancer mortality during years 5–14 was 12·2% for women allocated to continue versus 15·0% for controls (absolute mortality reduction 2·8%). Treatment allocation seemed to have no effect on breast cancer outcome among 1248 women with ER-negative disease, and an intermediate effect among 4800 women with unknown ER status. Among all 12 894 women, mortality without recurrence from causes other than breast cancer was little affected (691 deaths without recurrence in 6454 women allocated to continue versus 679 deaths in 6440 controls; RR 0·99 [0·89–1·10]; p = 0·84). For the incidence (hospitalisation or death) rates of specific diseases, RRs were as follows: pulmonary embolus 1·87 (95% CI 1·13–3·07, p = 0·01 [including 0·2% mortality in both treatment groups]), stroke 1·06 (0·83–1·36), ischaemic heart disease 0·76 (0·60–0·95, p = 0·02), and endometrial cancer 1·74 (1·30–2·34, p = 0·0002). The cumulative risk of endometrial cancer during years 5–14 was 3·1% (mortality 0·4%) for women allocated to continue versus 1·6% (mortality 0·2%) for controls (absolute mortality increase 0·2%). **Interpretation:** For women with ER-positive disease, continuing tamoxifen to 10 years rather than stopping at 5 years produces a further reduction in recurrence and mortality, particularly after year 10. These results, taken together with results from previous trials of 5 years of tamoxifen treatment versus none, suggest that 10 years of tamoxifen treatment can approximately halve breast cancer mortality during the second decade after diagnosis.

Commentary Christian Jackisch, Offenbach

If one issue might have been settled today, most of us were considering it would be that the use of tamoxifen in the adjuvant setting should be limited to a 5-year period. Endocrine therapy is the most effective and long-lasting treatment option we can offer and we have been using it for a very long time around the world. In addition, this treatment is affordable in nearly every part of the globe. Reviewing the periodically updated Oxford overview data of the Early Breast Cancer Trialist's Colloborative Group (EBCTCG) the most fascinating issue is the carry-over effect of tamoxifen therapy for all age groups, regardless of the menopausal status; beyond the actual treatment duration [1]. The introduction of the third-generation aromatase inhibitors has shed some light on alternatives in the endocrine adjuvant setting, but only for postmenopausal women, resulting in the upfront treatment, the classical sequence (tamoxifen followed by aromatase inhibitors), or the inverse sequence (aromatase inhibitors followed by tamoxifen). The most important information remains that the optimal benefit of these treatments is gained only by compliance to a given endocrine treatment for the intended duration. Switching from tamoxifen to any aromatase inhibitor might face the women with different side effects, jeopardizing compliance, i.e. adherence to treatment over time. Since the publication of the MA.17 trial has demonstrated substantial benefit in postmenopausal women from extension of treatment duration after 5 years of tamoxifen by additional 5 years of letrozole, the discussion of the optimal duration of targeting the steroid receptor in endocrine responsive disease is ongoing. In this context the importance of the recently published data of the ATLAS trial are of major clinical relevance for all age groups, not only the postmenopausal patients.

Clinical oncology still remains a field of controversy. Until recently the duration of endocrine adjuvant treatment with tamoxifen was strictly limited to a 5-year period. Some

randomized controlled trials (RCTs) have suggested that the extension of this treatment might have negative impact on survival [2–5]. The Adjuvant Tamoxifen – Longer Against Shorter (ATLAS) trial is one of two trials asking the question if the extension of treatment duration is of any benefit for the patients, together with the ATTOM trial (Adjuvant Tamoxifen Treatment: Offer More?). This issue was evaluated in nearly 20,000 women with endocrine responsive early breast cancer. The ATLAS trial recruited 13,000 women with operable breast cancer who completed 5 years of tamoxifen. The recent release reported on 6,846 women who were randomly assigned to either receive additional 5 years of tamoxifen or no treatment in the control group [5]. The analysis at 10 years reports a reduced risk of relapse, breast cancer mortality, and all-cause mortality in comparison with the control group. These important findings have to be balanced against the reported toxicity. The most important adverse effects in the ATLAS trial were increased risks for endometrial cancer (RR 1.74) and pulmonary embolism (RR 1.87). In contrast, no increased risk was seen in stroke incidence or ischaemic heart disease. Form a clinical point of view there were no relevant safety concerns against recommending the extended use of tamoxifen in clinical routine as long as the women were under regular medical surveillance, as the protocol suggested. The follow-up of the ATTLAS trial is announced to last until 2015.

The really new point in this debate is that by now pre- and perimenopausal women for whom tamoxifen is indicated have first-time evidence that in this setting the extension of tamoxifen use is of clinical relevance. The MA.17 trial only suggested that women starting on tamoxifen for 5 years and turning postmenopausal during this time might benefit from the extended adjuvant therapy using letrozole [6]. No data were available for those women remaining premenopausal by the end of a 5-year treatment with tamoxifen. In my view the most important information from the ATLAS trial is that premenopausal breast cancer patients with ER-positive disease benefit from the extension of tamoxifen exposure.

Again, this trial demonstrates nicely that the best benefit is obtained in patients adhering to their assigned treatment. At 7 years after the initial diagnosis 84% of patients in the tamoxifen group were on treatment. It is of special interest that recurrence after 10 years versus 5 years of tamoxifen differs by 1.4% and was 3.7% 10 years after study entry in the ATLAS trial. Mortality at 10 years after study entry differs by around 2.8% [6]. This carry-over effect after the end of the extended adjuvant endocrine therapy with tamoxifen improves over time.

The final take-home message in endocrine treatment of early breast cancer remains that we need to allow for a robust follow-up period of these trials to gain the optimal benefit for the women willing to take part in RCTs.

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