What have we gained from the application of biological 'rationales'?

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Abstract

We have pinned our hopes for the future of breast cancer treatment on the application of biological rationales. However, recent conclusions regarding the complexity of the breast cancer genome have given us pause for thought. On the one hand, solid successes for biological rationales such as endocrine therapy, bisphosphonates and HER2 directed therapy cannot be argued with; however, the question of where the next HER2-like 'Achilles Heel' will come from does seem overwhelming when confronted with so many recently described genomic aberrations to investigate. Recent advances in molecular profiling simply seem to add to the current confusion. While these tests place patients in different risk categories, there is little information from the lists of genes in these prognostic signatures that provides any insight into mechanisms that are exploitable with new biological agents. Recent attempts to combine endocrine therapy with signal transduction agents in unselected populations of patients with ER+ breast cancer have been failures, including studies of HER1 inhibitors, rapamycin analogues and farnesyl transferase inhibitors. The way forward must be to have a better idea of the exact endocrine resistance mechanisms in play in any particular tumour so that the pharmacology can be more precisely targeted, i.e. we need more biological rationale for these studies before we will begin to see success. The ideal of mechanistic precision is beginning to play out in the setting of PI3 kinase inhibitors since breast cancers exhibit multiple anomalies in this pathway such as PTEN loss, S6 kinase amplification and alpha catalytic subunit mutation that can now be put in context of therapy with rapamycin analogues, AKT inhibitors, direct inhibitors of the PI3 kinase catalytic subunit and mTOR. The interplay between pharmacological targeting and the breast cancer genome will be most likely addressed in the neoadjuvant setting and we can expect a good deal of progress in the near future from a number of critical trials that are currently underway or in the planning stages.

Cellular versus molecular targets

The application of endocrine therapy for breast cancer marked the first success for biologically rational therapy for this disease [1]. Beatson's rationale was based on reproductive physiology of course, and when the therapeutic benefits of ovarian ablation and later pharmacological agents became evident, the endocrine manipulation of oestrogen receptor in breast cancer cells became an early paradigm for targeted therapy. Other examples of physiologic rationales for breast cancer therapy have included bisphosphonate therapy for the treatment of bone metastasis [2], and most recently anti-angiogenesis therapy [3]. In all these cases the cancer cell is not the target, but the environment of the cancer cell, which can be rendered inhospitable by interfering with the cancer growth-supporting physiology of non-malignant cell types (granulosa cells of the ovary, aromatase expressing adipocytes, osteoclasts and endothelial cells). Other examples of successful targeting of non-malignant cells may emerge. For example, cancer associated fibroblasts are much more supportive of tumour growth than fibroblasts from healthy breasts. An understanding of the cell biological basis for this interesting finding could well lead to new treatment approaches [4].

HER2 targeting is the paradigm to follow but where is the needle in the haystack?

The development of the ERBB2 targeting monoclonal antibody trastuzumab opened a new chapter in the biological rationale textbook in which physiological principles were replaced by insights into the DNA recoding events that take place during the cellular evolution of a breast cancer. Remarkably, the demonstration that the ERBB2 gene was amplified in breast cancer occurred very early on in the 'genomic era' and was based on a search for cancer specific anomalies in the cellular homologues of the

acutely transforming retroviral oncogenes described in birds and mammals [5]. However, ERBB2 gene amplification is now recognised to be only one of a large number of somatic mutations that occur in breast cancer cells. Large scale tumour DNA resequencing projects, focused on a small number of tumours, and the analysis of many genes, suggest that by the time breast cancer enters the terminal phase, approaching tens to hundreds of individual somatic mutations may have accumulated [6]. Similarly, array comparative hybridisation experiments have uncovered multiple gene amplification and deletion events [7,8]. The complexity of these changes has given rise to the concept of a 'cancer genome atlas' in which all the recurring mutations in cancer are documented in publicly available data bases to assist clinical investigation and translational medicine. Understanding the clinical and biological significance of these somatic changes must represent one of the most important challenges facing breast cancer researchers today because when complete, a functionally annotated breast cancer genome atlas will provide predictive/prognostic biomarkers and therapeutic opportunities that will transform our approach to this common disease.

Molecular profiles that predict poor outcome for patients with oestrogen receptor positive breast cancer

Poor prognosis ER+ breast cancer accounts for a large proportion of the 40,000 breast cancer deaths that occur each year in the USA. Unfortunately, the molecular basis for endocrine therapy failure in ER+ disease remains poorly understood [9] and clinical progress limited, particularly since conventional chemotherapy regimens have only a limited impact for this disease subtype [10]. The only example of a definitive molecular mechanism for endocrine therapy resistance concerns the 8–10% of ER+ tumours that harbour ERBB2 gene amplification at 17q12. ER+ ERBB2 amplified (ERBB2+) tumours behave in an aggressive fashion in both the early stage and advanced disease settings and are poorly responsive to endocrine therapy [11,12]. These poor outcomes are partially reversed by an ERBB2 directed antibody, demonstrating that once a molecular target is defined, improvements can be anticipated through the use of precisely targeted pharmacology. Unfortunately, ERBB2 amplification only accounts for a small proportion of relapses on endocrine therapy. Multigene signatures have been developed to prospectively identify patients with poor prognosis ER+ disease. However, these tests (Mammaprint and Oncotype DX) do not provide any molecular insights into the basis for poor outcome ER+ disease. Thus, while Mammaprint and Oncotype DX may improve the targeting of standard chemotherapy they are not helpful with respect to either understanding the molecular mechanisms of endocrine therapy resistance or for defining populations of patients who might benefit from biological therapies.

Endocrine therapy signal transduction inhibitor combination trials – more failure than success

The success of ERBB2 targeting has stimulated interest in studying endocrine therapy in combination with signal transduction inhibitors (STI) that target other proteins in the ERBB2 signal transduction pathway. However, early results have raised the concern that these trials will fail unless there is a precise understanding of the subpopulation of ER+ tumors that might respond favorably to the STI in question. For example, the HER1 inhibitor gefitinib was studied as neoadjuvant treatment in combination with the aromatase inhibitor (AI) anastrozole in patients with ER+ primary tumours. The rationale was based on studies with MCF7 cells that suggested gefitinib could inhibit or delay the development tamoxifen or oestrogen-deprivation therapy resistance [13,14]. The clinical trial results, however, indicate that if anything, the gefitinib anastrozole combination was less effective than the aromatase inhibitor treatment alone [15]. A similar counter-intuitive result was obtained in a randomised Phase 2 study of the farnesyl transferase (FNTA) inhibitor (FNTAI) tipifarnib in combination with letrozole versus letrozole alone (also based on MCF7 cell-based preclinical experiments) [16]. The FNTAI-AI combination was associated with a trend for a shorter progression free interval and lower response rates than AI alone [17]. A third example was the early closure for futility of a Phase 3 trial in the advanced disease setting that examined the combination of the rapamycin analog CCI-779 with letrozole versus single agent letrozole (personal communication). These negative trials, set against the success of the ERBB2/trastuzumab studies, raise a number of concerns regarding drug development approaches for endocrine therapy STI combinations. First, the unsuccessful trials were based on preclinical studies focused on the MCF7 model. We must therefore accept that MCF7 based models can be misleading and do not predict the complexities of targeting HER1, mTOR or FNTA in a clinical setting and should not be

relied upon for proof of principle for other agents either. Secondly, unsuccessful trials did not select for sensitive tumours harbouring somatic mutations in the target pathway, largely because validated selection criteria for HER1, mTOR or FNTA treatment did not, and still do not, exist. Thirdly, several of these trials suggest that the addition of an STI may, in some cases, reduce the efficacy of single agent AI treatment. We do not have an adequate explanation of this observation. However, STI exposure may trigger compensatory events that interfere with the response to oestrogen receptor directed treatment. For example, treatment of cells with HER1 or HER2 directed TKI increases HER3 phosphorylation [18] and treatment with rapamycin increases AKT phosphorylation [19]. One can speculate that the adverse effects of STI activated feedback signalling may be particularly adverse when target pathway inhibition would have been successfully achieved through oestrogen deprivation alone. A review of clinical trials registered with the NCI indicates a number of ongoing studies of STI in combination with endocrine agents in unselected patients with ER+ advanced tumours [12]. These include letrozole in combination with lapatinib (a combined HER1 and ERBB2 inhibitor), anastrozole in combination with sorafenib (targets include B-RAF, VEGFR-2, PDGFβ, FLT-3 and c-KIT) and exemestane in combination with sunitinib (VEGFR and PDGFα and β, FLT-3 and c-KIT). Strong preclinical evidence does not exist for the use of lapatinib in ER+ ERBB2– disease or for sorafenib or sunitinib in unselected ER+ tumours. The activity of multi-targeted STI may be hard to predict and inhibition of a spectrum of tyrosine kinase (TK) and serine threonine kinase (STK) targets could activate multiple feedback loops whose clinical consequences are unpredictable. Furthermore, most of these studies are being conducted in the advanced disease setting, consequently the opportunity for tissue based correlative science to resolve the issue of drug targeting cannot be conducted. A better design, where feasible, would be to conduct STI aromatase inhibitor studies in the neoadjuvant setting so that molecular profiling can be an integral part of the study.

Neoadjuvant endocrine therapy trials – a setting to resolve the genomic complexity of ER+ breast cancer

A randomised neoadjuvant endocrine therapy trial comparing 4 months of tamoxifen with letrozole in postmenopausal women with hormone receptor rich tumours established letrozole as a potential standard of care for women with Stage 2 and 3 breast cancer who require tumour regression to achieve an improvement in surgical outcome [20]. Extensive biomarker investigations, focused on defining populations of patients who benefit most from preoperative aromatase inhibitor treatment, have been conducted by the project principal investigator's laboratory [20, 21]. Strong relationships between oestrogen receptor content and response clearly show a need to focus on the treatment of tumours with high level oestrogen receptor expression [20]. However, even within the most oestrogen receptor rich subset, responses are highly variable, implying other factors strongly influence response. Efforts to identify these factors focused on ERBB2 over-expression and amplification since contemporary evidence singled out this biomarker as a key endocrine therapy resistance factor. We initially demonstrated that ERBB2 over-expression did not reduce the neoadjuvant clinical response rate to letrozole, although tamoxifen performed poorly under these circumstances [20]. However, when the question of letrozole efficacy was examined in terms of the Ki67-based tumour proliferation data, it became clear that the majority of ERBB2 FISH tumours exhibited positive Ki67 staining despite letrozole treatment, implying that almost all ER+ ERBB2+ tumours are capable of some degree of oestrogen independent cell cycling and therefore must be partially or completely resistant to oestrogen deprivation [11]. Recent data from large aromatase inhibitor adjuvant trials confirms that ER+ ERBB2+ breast cancers have an elevated relapse rate [22]. This suggests that Ki67 proliferation data provides a more reliable intermediate endpoint for detecting molecular resistance events early in the course of endocrine treatment possibly because clinical regressions may only be transient in some cases while Ki67 analysis reveals microscopic tumour subpopulations that are already exhibiting letrozole resistant re-growth. Recently Dowsett and colleagues have reported that high Ki67 levels in 2 week biopsies are associated with an elevated risk for relapse and death from breast cancer [23]. Based on these findings we are currently conducting a screen for endocrine therapy resistance genes based on Ki67 data as the most reliable short term surrogate endpoint for the effectiveness of aromatase inhibitors in longer term studies. In order to have an adequate sample size we have activated the American College of Surgeons Oncology Group (ACOSOG) Z1031 trial (Fig. 1). This study will accrue 375 patients with ER rich stage 2 and 3 tumours (Allred 6–8) to a comparison of letrozole, anastrozole and exemestane. Frozen and

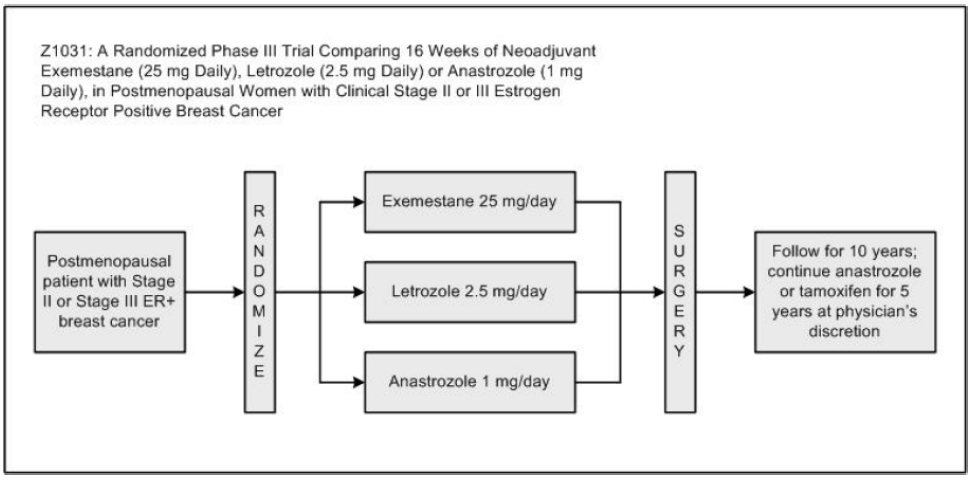

Fig. 1. The schema for the ACOSOG Z1031 trial, a randomised comparison between 4 months of preoperative exemestane, letrozole and anastrozole. Frozen tissue cores (2) and formalin fixed cores (2) at baseline and from the surgical specimen are a mandatory aspect of the protocol. Accrual is proceeding on schedule.

formalin-fixed tumour samples at baseline and surgery. The study is accruing on schedule.

The PIK3 kinase pathway and endocrine therapy resistance

The central hypothesis in the endocrine therapy resistance field focuses on the concept that constitutive activation of peptide growth factor pathways causes oestrogen independent tumour growth. Exactly how this event occurs mechanistically remains unclear. However, one line of evidence indicates that ER becomes phosphorylated and consequently exhibits oestrogen independent transcriptional activities – referred to as 'ligand-independent growth'. In addition, growth factor signalling may cause ER down-regulation and 'ER independent growth'. ERBB2 gene transfection studies suggest that over-expression may produce resistance through both mechanisms, which can be reversed with an agent that targets ERBB2. ERBB2 functions through a complex signal transduction cascade with two major arms, the PI3 kinase cascade and the RAS mediated signal transduction cascade. Theoretically, endocrine therapy resistance could also result from additional somatic mutations that cause constitutive activation of these pathways. Interestingly, while somatic mutations in the RAS pathway appear to be uncommon in breast cancer, the PIK3 kinase pathway has been shown to commonly exhibit mutations in multiple components, including PIK3CA gain of function mutations and PTEN loss of function mutations (Fig. 2). In the absence of HER2 amplification, investigators are beginning to ask if these 'down-stream' mutations affect endocrine therapy outcomes. In the only well powered study reported to date, PIK3CA gain of function mutations do appear to increase the event rate in an ER+ ERBB2– population [24]. DNA mutation analysis with PTEN is more difficult than for PIK3CA because loss of function mutations do not occur in hotspots. However, published studies using IHC do indicate PTEN deficient tumours also exhibit resistance to adjuvant tamoxifen therapy [25]. Interestingly, PTEN loss and PIK3CA mutation may be mutually exclusive events while ER+ tumours have a predominance of PIK3CA mutation and ER- disease tends to exhibit PTEN loss [26]. To date there have been no reports on the outcome of ER+ breast cancer that takes into account all the potential mutations in the PIK3 kinase signalling network. However, it is increasingly clear that once all classes of mutation have been identified, the opportunities for pharmacological targeting are immense. Multiple classes of PI3 kinase inhibitors are now in clinical development, including direct inhibitors of the PI3 kinase catalytic subunits, TOR itself, as well as AKT [27]. Matching these inhibitors to the precise constallation of molecular defects will be a critical exercise.

Conclusion

Doubtless we will see more remarkable progress in the treatment of breast cancer by the application of biological rationales, but we must not shy away from the complexity of the problem or be over reliant on simplistic pre-clinical models. In the future, breast

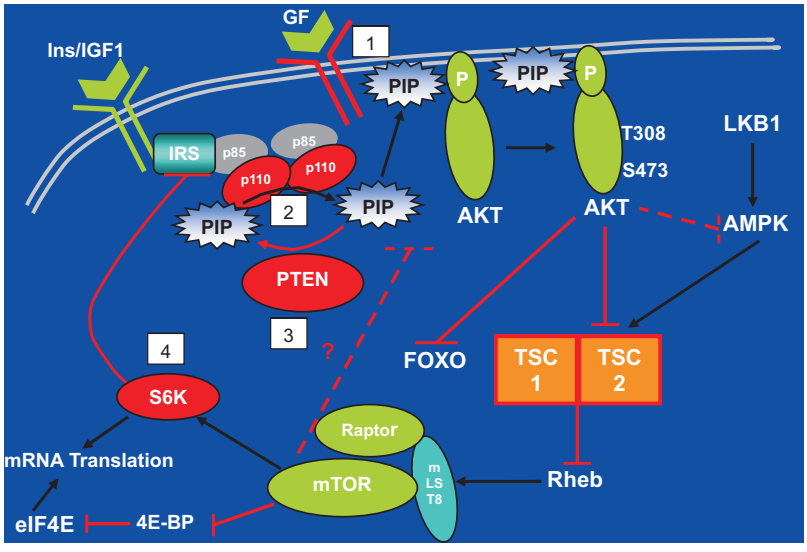

Fig. 2. The PI3 kinase pathway contains multiple somatic mutations that may affect endocrine therapy sensitivity (indicated in dark grey), including ERBB2 amplification (1), p110 gain of function mutation – PIK3CA (2), PTEN loss of function (3) and S6 kinase gene amplification (4).

cancer treatment is likely to become more complex for the physician, but not necessarily for the patient, since we may be able to avoid therapies in patients who do not benefit from them, as well as apply new approaches to patients who are not cured with conventional therapy. However, the ability to match the correct pharmacology to individual tumour genetic profiles will not only require vast progress in our biological classification of this disease but a global collaboration so we can conduct clinical trials in small patient subsets in an efficient manner.

Conflict of interest statement

Novartis: Laboratory Grant support, Speaker Fees and Consulting Fees Genentech: Consulting Fees GSK: Clinical Trial Grant support, Consulting Fees and Speaking Fees Astra Zeneca: Clinical Trial Support, Consulting Fees, Speaking Fees

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