E1. Tailored systemic treatment for breast cancer: dream or reality?[1]

Martine J. Piccart, M.-A. Colozza, C. Sotiriou, F. Cardoso

Jules Bordet Institute, Brussels, Belgium

Introduction

Nowadays, when considering adjuvant systemic therapy for breast cancer, the two most important tasks for medical oncologists are the identification of those patients who need treatment and the selection of the most appropriate treatment for the individual patient. This lecture will emphasise the current limitations of the prognostic and predictive factors used in the clinic and the sub-optimal nature of clinical trials developed so far; it will advocate the need for innovative clinical trials that allow for a rapid transition from empirical to molecular-based oncology.

Identification of patients who need adjuvant treatment

Three important initiatives have been undertaken to analyse, digest, and interpret the growing amount of data generated by randomised clinical trials: the Oxford Overview, the National Institutes of Health (NIH) Consensus Conference and the St-Gallen Consensus Conference. In addition, since 1996, the American Society of Clinical Oncology has regularly published evidence-based, clinical practice guidelines for the use of prognostic and predictive factors. These panels [1–8] concluded that the only molecular markers recommended for broad clinical use are the hormone receptors for endocrine therapy and HER-2 status for treatment with trastuzumab, a rather disappointing conclusion in view of the huge diversity in molecular pathways dissected by basic scientists and relevant to breast cancer biology. As a result, the management of small invasive tumours (<1 cm) without axillary nodal involvement remains highly controversial. However, there is hope for substantial progress in the coming years.

Using DNA microarray technology, the Amsterdam group was able to establish an expression signature based on 70 genes for the prediction of early distant metastases in 78 untreated women with node-negative disease, 34 of whom relapsed within 8 years of follow-up, while 44 remained relapse-free. The researchers went on to compare this new prognostic tool with the St-Gallen criteria as a way to assist the clinician in treatment decision-making. Both methods performed well in identifying those patients with an early relapse who need treatment. However, the St-Gallen criteria classified more than 66% of the relapse-free patients in an average-high risk group requiring therapy, while the gene prognosis signature did so in only 27%, which implies the potential for a marked reduction in overtreatment. The same group published the results of a confirmatory study performed on a larger set of 295 node-negative and node-positive breast cancer patients, followed for a median of 7 years: the gene prognosis signature clearly separated a subgroup with an excellent 10 year survival rate from one with a high mortality rate [9]. Although very impressive and interesting, this work has some weaknesses such as the retrospective nature of the study, the small sample size, the selection of a group of young women (all <52 years of age) and all treated in one hospital. This makes extrapolations to other age groups and other countries difficult. Additionally, microarray analysis is a new and expensive technology that is evolving rapidly, and that needs to be fully standardised and reproducible across different laboratories. Therefore, before this new tool can be successfully used in clinical practice, these results need to be duplicated [10] in an independently run study and then validated in a large, independent, prospective trial. This effort is currently ongoing through a "sister network" of the Breast International Group (BIG) – the TRANS-BIG network – and coordinated by the European Organisation for Research and Treatment of Cancer (EORTC). These ambitious research projects, funded by the European Commission under the Framework VI Programme, includes a validation/standardisation initial phase, followed by a large, prospective, randomised study, the MINDACT trial. In this trial, 5000 node-negative breast cancer patients will be randomised between risk assessment based on common clinical-pathological criteria or based on the 70 gene expression signature. While the clinical outcome is expected to be similar in the two groups, it is postulated that prescription of adjuvant chemotherapy will be reduced by roughly 15–20% in the group managed according to the tumour gene expression profile. Since ongoing research may show that gene expression arrays are of value in selecting

[1] Based on a publication for the Breast Cancer Conference; Current Controversies and New Horizons (Cambridge, MA, USA, Dana-Faber Cancer Care and Harvard Medical School, July 10–12, 2003). Keynote-Lubrano Lecture: *Bringing Molecular Prognosis and Prediction to the Clinic.*

1359-6349/$ – see front matter

optimal treatment at the time of relapse, the plan is to offer this technology to all women participating in the trial, but with a delayed potential use in the "control" group. This project will hopefully confirm that this new prognostic tool outperforms the traditional prognostic factors.

Treatment selection for the individual patient

Among the systemic treatment modalities available to treat early breast cancer, chemotherapy (CT) has the worst reputation in terms of side-effects, and it suffers from poor "tailoring" in view of a lack of evidence-based predictive molecular markers. It is not surprising, therefore, that our current understanding of the "optimal adjuvant CT regimen" for the individual patient is very limited. The large randomised clinical trials, run in patients unselected for their tumour biological characteristics, tell us that, *on average*, treatment A is superior to treatment B [11], a result that will guide treatment for future patients across the board (i.e., all future patients will receive treatment A). However, in a very heterogeneous disease such as breast cancer, this global treatment effect "A is better than B", might be compatible with different scenarios: a) all subpopulations derive a benefit; b) one subpopulation derives a benefit and another a negligible one; and c) one subpopulation derives a large benefit and another has, in fact, a detrimental effect (A < B). Examples supporting such large differences in the magnitude of the treatment effect for subpopulations of patients divided according to oestrogen receptor (ER) or HER2 expression, have been reported.

Anthracyclines, taxanes, or CMF?

The two most active classes of cytotoxic agents available for breast cancer treatment are the anthracyclines and the taxanes. A decade of randomised clinical trials has established the level 1 evidence-based superiority of the former over CMF (cyclophosphamide, methotrexate, 5-fluorouracil), while further gain in survival with the incorporation of taxanes into anthracycline-based regimens is starting to emerge and will most likely be level-1 evidence-based in one to two years. In spite of the huge numbers of women participating in these traditional clinical trials ($\approx$ 50,000), we still have a long way to go before being able to tailor the use of anthracyclines and taxanes to the individual patient. Ongoing efforts in this direction will be summarised, and the design of two innovative trials attempting to prospectively validate the molecular hypothesis of taxanes' or anthracyclines' selective benefit will be discussed. These two studies – the EORTC p53 trial and the TOP trial – are trying to exploit the great potential of the new DNA microarray technology, which offers the hope of identifying molecular signatures of drug sensitivity or drug resistance.

The dose-dense approach

The publication of the Cancer and Leukemia Group B (CALGB) 9741 trial [14] shook the oncology community given its striking results favouring dose-dense CT over conventionally-timed CT. However, collectively, the data available so far suggest that the superiority of a sequential dose-dense approach might be specific to paclitaxel; data on docetaxel are too scarce and suboptimal in nature, and the data on anthracyclines are not convincing [15]. More importantly, there is an obligation to understand which subset of patients derives a large benefit from CT acceleration.

As we progressively leave the era of empirical medicine and enter one of "molecular medicine", it is becoming increasingly more obvious that the "one shoe fits all" theory will find ever fewer supporters. Gene-profiling studies have confirmed that ER-positive and ER-negative breast cancer are essentially two different diseases. Furthermore, a dissection of the ER-negative subset [12,13] reveals the existence of at least two subtypes: a HER-2-overexpressing subset and a basal-like one. Among the latter, a further subgroup can be delineated with overexpression of several key genes involved in cellular proliferation. It is extremely tempting to speculate that this subset could be the one that derives the greatest benefit from a CT dose-densification approach [10].

One of these key genes is the focus of recent attention, namely cyclin E. Cell cycle alterations are common in human cancers. Eukaryotic cells are driven through the cell cycle by successive activation and inactivation of cyclin-dependent kinases (Cdks). The Cdks are regulated by a number of different proteins including cyclins that bind and activate the Cdks to form serine/threonine kinase holoenzyme complexes. The different cyclins have a temporally distinct and highly regulated pattern of expression i.e. they are synthesised and degraded at specific stages of the cell cycle. Cyclin E appears in late G1 after the passage through the 'restriction point'. The level of cyclin E peaks in late G1 and disappears in early S phase. It associates with Cdk2 and activates its kinase activity shortly before entry of the cells into the S phase. The targets for the cyclin E-Cdk2 kinase are largely unknown. Cyclin E is believed to control S phase entry by phosphorylating proteins involved in DNA replication [16]. It may also control centrosome duplication [16], histone gene transcription [17,18] and pre-mRNA splicing [19]. The degradation of cyclin E is regulated by ubiquitin-dependent proteolysis during S phase [20,21]. Two structurally unique families of Cdk inhibitors (CKIs) negatively regulate cyclin–cdk

complexes. The Ink4 inhibitor family (p15, p16, p18, and p19) specifically interacts with D-type cyclin–Cdk4/6 complexes, while KIP/CIP family members (p21, p27, p57) regulate both D-type cyclin–cdk4/6 and E-type cyclin–cdk2 complexes [22–24]. Cyclin E exists in 2 isoforms, that have a high homology with one another, designed cyclin E1 (formerly called cyclin E) and cyclin E2. Like cyclin E1, cyclin E2 associates with cdk2 and activates its kinase activity at the G1/S boundary [25,26]. Cyclin E2 shares 47% overall similarity to cyclin E1 and it remains to be determined if structural features outside of the conserved regions impart unique functions. Using the reverse transcriptase-polymerase chain reaction (RT-PCR) it has been demonstrated that the levels of cyclin E1 and cyclin E2 are elevated in 23% and 38% of primary breast carcinomas, respectively, relative to normal breast controls. Interestingly, the expression level of both cyclins is higher in ER-α negative tumours.

The prognostic and/or predictive value of cyclin E (E1) has been retrospectively evaluated in several studies [27–35]. Most of these studies were adjuvant trials and the number of patients included ranged from 114 to 395. The systemic treatments were different, varying from no treatment to CT and/or hormonal therapy (HT). Immunohistochemistry (IHC) was the most common evaluation method even if the antibodies and the cut-off values used were different. Western blotting analysis was applied in two studies while real-time RT-PCR was used in one. Unfortunately, the results generated from these studies are heterogeneous and therefore no definitive conclusion exists today regarding the prognostic and/or predictive role of cyclin E1 in breast cancer. There is a need for a standardisation of the evaluation methods and the scoring systems. Additionally, these preliminary results need to be validated in the context of large prospective clinical trials. The role of cyclin E1 as a predictive marker to systemic therapy has been evaluated in only one study using quantitative RT-PCR [35]. These results showed that cyclin E1 might have a predictive value for resistance to HT. Data on the potential prognostic and/or predictive role of cyclin E2 in breast cancer are very scarce. Recently, our group assessed the prognostic and predictive value of cyclin E1 and E2 in a series of 326 breast cancer patients using quantitative RT-PCR (Sotiriou C. *et al.*, abstract submitted to ASCO 2004). The expression levels of both cyclin E1 and cyclin E2 were associated with relapse-free and overall survival in the total cohort of patients. Interestingly, cyclin E1 was found to be a predictor of resistance to HT, as previously reported by others [35]. We are currently increasing the number of patients to be evaluated in order to assess the potential predictive role of both cyclin E1 and cyclin E2 with respect to a poor response to conventionally-timed CT.

Should these results be encouraging, cyclin E would become an attractive marker for a molecular hypothesis-driven randomised study of CT dose-densification versus conventionally-timed CT to be conducted as a confirmatory trial of CALGB 9741.

Conclusions

We are clearly at a transition point in oncology, moving from empirical to molecular medicine. These are exciting times for oncologists, but there is a crucial need to break from tradition in the philosophy and the design of our clinical trials if we want "tailored treatment" to become a reality for our patients in a not too distant future.

References

[1] Early Breast Cancer Trialists' Collaborative Group. Tamoxifen for early breast cancer: An overview of the randomized trials. Lancet 1998; 351: 1451–1467.

[2] Early Breast Cancer Trialists' Collaborative Group. Ovarian ablation in early breast cancer. Overview of the randomized trials. Lancet 1996; 348: 1189–1196.

[3] Early Breast Cancer Trialists' Collaborative Group. Polychemotherapy for early breast cancer: An overview of the randomized trials. Lancet 1998; 352: 930–942.

[4] Early Breast Cancer Trialists' Collaborative Group. 2000 analysis overview results. Fifth Meeting of the Early Breast Cancer Trialists Collaborative Group, Oxford, UK, September 21–23, 2000.

[5] NIH Consensus Conference: Treatment of early-stage breast cancer. JAMA 1991; 265: 391–395.

[6] National Institutes of Health Consensus Development Conference. J Natl Cancer Inst Monogr 2001; 30: 1–152.

[7] Gorldhirsch A, Glick JH, Gelber RD, et al. Meeting highlights: International Consensus Panel on the treatment of primary breast cancer. J Natl Cancer Inst 1998; 90: 1601–1608.

[8] Gorldhirsch A, Glick JH, Gelber RD, et al. Meeting highlights: International Consensus Panel on the treatment of primary breast cancer. Seventh International Conference on Adjuvant Therapy for Primary Breast Cancer. J Clin Oncol 2001; 19: 3817–3827.

[9] van de Vijver MJ, He YD, van't Veer L, et al. A gene-expression signature as a predictor of survival in breast cancer. N Engl J Med. 2002; 347(25):1999–2009.

[10] Sotiriou C, Powles TJ, Dowsett M, Jazaeri AA, Feldman AL, Assersohn L, Gadisetti C, Libutti SK and Liu ET. Gene expression profiles derived from fine needle aspiration correlate with response to systemic chemotherapy in breast cancer. Breast Cancer Res 2002; 4:R3.

[11] Cardoso F, Di Leo A and Piccart MJ. Controversies in the adjuvant systemic therapy of endocrine-nonresponsive breast cancer. Cancer Treatment Reviews Volume 28(6): 275–290, 2002.

[12] Sorlie T, Perou CM, Tibshirani R: Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci USA 98: 10869–10874, 2001.

[13] Gruvberger S, Ringner M, Chen Y, et al.: Estrogen receptor status in breast cancer is associated with remarkably distinct gene expression patterns. Cancer Res 61: 5979–5984, 2001.

[14] Citron ML, Berry DA, Cirrincione C, et al.: Randomized trial of dose-dense versus conventionally scheduled and sequential versus concurrent combination chemotherapy as postoperative adjuvant treatment of node-positive primary breast cancer: First Report of

Intergroup Trial C9741/Cancer and Leukemia Group B Trial 974. J Clin Oncol 21:1431–1439, 2003.

[15] Piccart MJ. Mathematics and oncology: a match for life? Journal of Clinical Oncology, 2003, 21(8): 1425–1428.

[16] Even ME. Where the cell cycle and histones meet. Gene development 2000; 14: 2265–2270.

[17] Ma T, Van Tine BA, Wei Yue et al. Cell cycle-regulated phosphorylation of p220 ^{NPAT} by cyclin E/Cdk2 in Cajal bodies promotes histone gene transcription. Gene development 2000; 14: 2298–2313.

[18] Zhao J, Dynlacht, B, Imai T, et al. Expression of NPAT, a novel substrate of cyclin E-CDK2, promotes S-phase entry. Gene development 1998; 12: 456–461.

[19] Seghezzi W, Chua K, Shanahan F et al. Cyclin E associates with components of the pre-mRNA splicing machinery in mammalian cells. Mol Cell Biol. 1998; 18: 4526.

[20] Won KA, Reed. Activation of cyclin E/CDK2 is coupled to site specific autophosphorylation and ubiquitin-dependent degradation of cyclin E. EMBO J 1996; 15: 4182–4193

[21] Clurman BE, Sheaff RJ, Thress K et al. Turnover of cyclin E by the ubiquitin-proteasome pathway is regulated by CDK2 binding and cyclin phosphorylation. Gene Development 1996; 10: 1979–1990.

[22] Sherr CJ, Roberts JM. Inhibitors of mammalian G1 cyclin-dependent kinases. Genes Dev 1995; 9: 1149–1163.

[23] Sherr CJ Roberts JM. CDK inhibitors: positive and negative regulators of G1 phase progression; Genes Development 1999; 13: 1501–1512.

[24] Slingerland J, Pagano M. Regulation of the Cdk inhibitor p27 and its deregulation in cancer. J Cell Physiol 2000; 183: 10–17.

[25] Payton M, Coats S. Molecules in focus Cyclin E, the cycle continues. The Int J Biochem Cell Biol 2002; 34: 315–320.

[26] Payton M, Scully S, Chung G et al. Deregulation of cyclin E2 expression and associated kinase activity in primary breast tumors. Oncogene 2002; 21: 8529–8534.

[27] Nielsen NH, Arnerlov C, Emdin SO and Landberg G. Cyclin E over expression, a negative prognostic factor in breast cancer with strong correlation to oestrogen receptor status. Br J Cancer 1996; 74: 874–880.

[28] Porter PL, Malone KE, Heagerty PJ et al. Expression of cell-cycle regulators p27 and cyclin E alone and in combination, correlate with survival in young breast cancer patients. Nat Med 1997; 3: 222–225.

[29] Donnellan R, Kleinschmidt I, Chetty R. Cyclin E immunoexpression in breast ductal carcinoma: pathologic correlations and prognostic implications. Hum Pathol 2001 32: 89–94.

[30] Kim HK, Park IA, Heo DS et al. Cyclin E over expression as an independent risk factor of visceral relapse in breast cancer. Eur J Surg Oncol 2001; 27: 464–471.

[31] Keiomarsi K, Tucker SL, Buchholz TA et al. Cyclin E and survival in patients with breast cancer. N Engl J Med 2002; 347: 1566–1575.

[32] Kuhling H, Alm P, Olsson H, et al. Expression of cyclins E, A, and B, and prognosis in lymph node-negative breast cancer J Path 2003; 199: 427–431.

[33] Rudolph P, Kuhling H, Alm P et al. Differential prognostic impact of the cyclins E and B in premenopausal women with lymph node-negative breast cancer. Int J Cancer 2003; 105: 674–680.

[34] Han S, Park K, Bae B-N et al. Prognostic implication of cyclin E expression and relationship with cyclin D1 and p27Kip1 expression on tissue microarrays of node-negative breast cancer. J Surg Oncol 2003; 83: 241–247.

[35] Span PN, Tjan-Heijnen VCG, Manders P et al. Cyclin-E is a strong predictor of endocrine therapy failure in human cancer. Oncogene 2003; 22: 4898–4904.