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Review

Molecular Genetic Tools Shape a Roadmap Towards a More Accurate Prognostic Prediction and Personalized Management of Cancer

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ABSTRACT

The continuous flow of molecular genetic information has cautiously started integrating into clinical practice changing the future landscape of the clinical management of cancer. Germline mutation analysis for individuals at familial risk and testing result-based surgical or nonsurgical preventive intervention can protect from hereditary breast-ovarian, colorectal and stomach cancer and reduce mortality. Research is focusing now on the development of effective chemoprevention to replace prophylactic surgery for improving quality of life and to provide novel targeted therapies for hereditary cancer patients. The TNM staging system and conventional clinicopathologic factors have led clinical decisions on adjuvant therapy for decades improving survival in patients with early-stage tumors. However, current staging methods and therapeutic decisions remain suboptimal. Patients with early-stage cancer who are at low risk of recurrence could be spared the toxicity of systemic treatment if clearly distinguished, while others at high risk of distant recurrence could get maximal benefit if therapy matched the molecular genetic profile of either the host or the tumor. With the establishment of validated molecular analysis techniques it is believed that clinical biomarkers will gradually overtake TNM by their capacity to form more accurate prognostic systems and delineate better predictors of response to specific therapies. Areas of cancer research such as germ-cell mutation analysis, tumor gene-signature identification, gene expression profile linked targeted therapy, cancer stem cells, circulating cancer cells and single-nucleotide polymorphism keep on producing promising data which is believed to refine future preventive and early intervention strategies on an individual basis. Today these gene-based strategies are in a transition phase prior to full implementation into clinical practice. At present they wait for the results of large-scale prospective validation studies which compare molecular against "classic" markers. It is anticipated that molecular genetic biomarkers when implemented in clinical practice will considerably improve both biologically guided therapeutic decisions and clinical outcomes.

INTRODUCTION

Cancer affects millions of people worldwide with a trend towards increase in morbidity and mortality. It is estimated that the worldwide annual incidence of cancer will increase from 10.9 to approximately 20 million new cases per year by the year 2020, and that cancer deaths will increase from 6.6 million to more than 10 million.[1] Cancer preventing policies with the potential to influence modifiable risk factors can unequivocally make an impact in reducing cancer incidence.[2] Second to that, transfer of research achievements from basic sciences to medical practice in the fields of individual risk assessment, early detection and treatment has been recognized as a potent way to fight cancer.[3]

During the last decade, with the advent and refinement of high-throughput technology, genomic-wide cancer research has generated considerable data of a potentially high clinical importance[4] (Table 1). However, in the era of evidence-based medicine, prospective validation of molecular biomarkers in large randomized trials is required prior to their establishment into clinical practice.[5]

FAMILIAL CANCER

Cancer is distinguished, according to identified inherited or environment causative factors, into familial and sporadic. Overall, 30% cancers are considered familial and 70% are named sporadic; yet these rates vary considerably among various cancer histotypes.[6] Although indistinguishable inherited genetic factors may possibly contribute to the development of most cancers, persons with a definite cancer family history have a two-fold risk

Table 1 **Research approaches that lead individualized cancer management**

Research Field	Clinical Target
Germline mutation analysis	Personalized risk assessment and guided prevention
Gene expression profiling (multigene assays, gene signatures)	Prognosis optimization, predictive response to specific therapies
Tissue single-gene mutation analysis	Improvement of predictive precision
Circulating tumor cells	Individual prognosis, fine-tuning
Single-nucleotide polymorphism analysis	Host tailored therapy
Cancer stem cells	Cancer eradication therapy

of developing cancer compared to the general population, and those who carry germline mutations in specific genes may have a 80% lifetime risk for developing cancer. However, for the majority of people with a familial cancer history but unidentified germline genetic aberrations, we are still unable to make risk assessments and propose appropriate cancer prevention strategies.[7]

Hereditary breast, ovarian and colorectal cancer. Germline mutations in specific high-penetrance tumor suppressive genes have consistently been associated with a number of hereditary cancer syndromes: *BRCA1* and *BRCA2* in breast and ovarian cancer (HBOC),[7] mismatch-repair genes *MLH1, MSH2, MSH6* and *PMS2 in* hereditary nonpolyposis colorectal cancer (HNPCC), also called Lynch syndrome,[8] adenomatosis polyposis coli gene (*APC)* in familial adenomatous polyposis (FAP) related colorectal cancer, *PTEN* (phosphatase and tensin homolog) in multiple endocrine neoplasias and *CDH1* in hereditary diffuse gastric cancer.[9]

Among these syndromes, HBOC and HNPCC/FAP are of the highest clinical significance because a large body of evidence supports the implementation of genetic analysis in clinical intervention strategies in these clinical situations. Indeed, although HBOC accounts for 5–10% of all breast-ovarian cancers and HNPCC for 2–4% of colorectal cancers,[7,10] the high incidence of these tumor types reflect a substantial number of inherited cancers. It is estimated that in the US among 300,000 new annual breast and ovarian cancer cases, approximately 15,000 to 30,000 are attributed to germline *BRCA* mutations.[11] Similarly among 150,000 colorectal cancer cases approximately 3300 to 6000 are considered to be familial.[12] It is clear that a sensible number of cancer patients could be offered a life-saving early diagnosis, or optimally tumor prevention if genetic screening programs were applied. The development of such strategies could help prevent cancer onset in mutation carriers. However, despite evolving advances in genetic testing and preventive interventions there are still limitations and potential harms to be addressed (Table 2). A synthesis of most recent reviews, meta-analyses, and imposed guidelines summarize a number of visible weaknesses and current limitations of cancer prevention strategies.[13-17]

Prophylactic clinical interventions. Prophylactic surgery is increasingly accepted today as an effective clinical prevention strategy although the negative impact on quality of life can not be disregarded.[13] Bilateral prophylactic salpingo-oophorectomy, for example, appears to offer optimal risk-benefit ratio compared with bilateral mastectomy, and of course with surveillance alone. Therefore, this surgical practice is currently recommended as the best available prevention management for *BRCA* mutation carriers.[18] In other cases a timely and regularly conducted colonoscopy for HNPCC risk carriers and an appropriate surveillance for familial endometrial cancer should be offerd.[19] Benefits of preventive interventions can offset risks if only cancer genetic services are provided by well trained multidisciplinary teams to well-informed individuals. This is a demanding and extremely complicated job given that accurate risk estimates and potential effectiveness must carefully be considered in each individual case.[20]

The impact familial cancer genetics has made on the public, has led to a direct-to-consumer marketing of genetic tests over the internet which has raised concerns by clinicians, experts and the FDA.[21,22] On the other side the Human Fertilization and Embryology Authority (HFEA) in the United Kingdom has decided to allow and include in the preimplantation genetic diagnosis genetic testing for families who are known carriers of cancer susceptibility genes.[23]

SPORADIC CANCER

In contrast to familial cancer, sporadic cancer occurs in people at random. In those cases tumor genesis is attributed to a number of environmental causative factors with a possible contribution of unidentified low-penetrance genes. Risk assessment in individuals without a cancer family history can not be approached.[24]

Treatment. Improvements in early detection and treatment of solid tumors have resulted to a decrease in death rates for the three most common cancers in western countries during the last ten years.[2,11] The most impressive clinical results are reported for breast cancer. Advances in systemic adjuvant therapy including cytotoxic, hormonal, and most recently targeted therapy with the anti-HER2 therapeutic antibody trastuzumab demonstrate survival improvements in specific target groups of patients with early-stage breast cancer.[25,26] However, adjuvant therapy in breast cancer remains suboptimal because treatment decisions are based on traditional prognostic factors and result in over-treatment or inadequate treatment in approximately half of the treated patients. This observation indicates the need to develop more accurate prognostic and predictive molecular markers.[27]

Adjuvant therapy and prognostic/predictive factors. To improve prognostication, models that combine traditional prognostic factors are being developed and validated.[28,29] However despite the progress made, over-treatment and inadequate treatment remains a challenge. At present, tailoring optimal therapy for each individual patient remains wishful thinking and clinical investigators look forward to establishing potent predictive and prognostic molecular biomarkers.[30]

Markers to guide individualized treatment. A large number of candidate biomarkers are being evaluated but only a few will succeed in reaching clinical implementation. These include validated markers for targeted therapy, circulating cancer cells, gene expression profiles (gene signatures) and lately cancer stem cells.

Cancer tissue biomarkers. Hormone receptors in breast cancer. Estrogen receptor (ER) status is a well established prognostic and predictive factor for breast cancer.[31] Tamoxifen, the first ever targeted cancer therapy, has saved thousands of lives of breast cancer patients and now aromatase inhibitors appear to further improve the

Table 2 **Prevention of hereditary breast ovarian cancer and colorectal cancer: Limitations, potential harms and challenges in current clinical practice**

1. Lack of level I evidence for the safety and effectiveness of comprehensive prevention strategies including genetic testing and cancer preventive interventions.
2. Lack of standardized criteria to define populations at cancer risk to apply genetic testing.
3. Inability of accurate risk estimates among individuals with a positive test result because of wide range of risk; BRCA and mismatch-repair genes mutation carriers have a lifetime risk of 50 to 85% for breast cancer and colorectal cancer and 11 to 55% for ovarian cancer and 40 to 60% for endometrial cancer respectively.
4. Difficulties in the interpretation of uninformative or negative test results (familial, nonhereditary cancer).
5. Imposed challenges in selecting clinical preventive interventions over surveillance.

outcome in postmenopausal women with early stage, ER-positive breast cancer.[25,32] However, some of these patients could be further benefited by adding chemotherapy. The critical question in this case is how to identify from among ER-positive cases those who are most likely to gain benefit from chemotherapy, hopefully this will be addressed by gene expression profile-based information.[33]

Human epidermal growth factor Receptor 2 (HER2) in breast cancer. HER2 is recognized as an important prognostic and predictive factor for metastatic and also for early-stage breast cancer. HER2 gene amplification and protein overexpression has been established as a validated marker for targeted therapy.[34] Randomized controlled trials have shown that the HER2 targeted therapeutic antibody trastuzumab, can improve tumor response in metastatic, and disease-free and overall survival in early-stage HER2-positive breast cancer when added to chemotherapy.[26] Moreover, the fact that a substantial proportion of HER2 positive tumors fail to respond to trastuzumab prompted the initiation of search for trastuzumab resistance predictors.[35]

Human epidermal growth factor receptor 1 (EGFR) in non-small-cell-lung cancer. Lung cancer is the leading cancer killer in the economically developed world with less than 15% of affected patients surviving at two years. Conventional chemotherapy appears to have reached a plateau in efficacy and despite a continuous inflow of novel drugs the outcome of lung cancer has improved little over the last decades.[36] Recently, small molecule therapeutics that can intervene with cellular transduction pathways, such as the epidermal growth factor receptor inhibitors gefitinib and erlotinib entered clinical investigation, and the first published results generated some enthusiasm. However, they were pushed into a rather unorthodox clinical development plan in regard to study populations and combinations with cytotoxic chemotherapy and now struggle to find their place in lung cancer therapy.[37] Their negative results in randomized trials can be explained when we consider that these therapeutics targeted only 10-15% of the trial patients, i.e., those who carried target gene amplification or mutations in the EGFR gene.[38] Clinical investigators now consider conducting targeted trials with eligibility restricted to patients predicted to respond to the drug on a molecular basis.[39] Moreover major efforts are being undertaken to identify predictive molecular markers of higher fidelity.[40]

Disseminated tumor cells. The occurrence of tumor metastasis after a successful local therapy is attributed to the existence of disseminated viable cancer cells. For a clinically obvious metastasis to be established, tumor cells must invade the surrounding tissue, enter the circulation, spread throughout the body, arrest in capillary beds at distant organs, invade the host tissue, evade immunological surveillance and start proliferating long before they become clinically evident. Therefore, detection of occult disseminated and circulating cancer cells (CTC) in patients without clinical evidence of metastatic disease was believed and lately was proven to predictive of risk of distant recurrence.[41] The development of high-discrimination assays, capable to detect these rare cells long before clinical manifestation of distant metastases is prognostically important and may lead guide a more individualized therapeutic approach. This search started in the late 1980s, and today both immunohistochemical staining and real-time polymerase chain reaction (RT-PCR)-based approaches are available to detect CTC and DTC.[42] Large-scale clinical trials are in progress assessing the validity and clinical utility of such markers.[41]

Gene expression profiles (gene signatures). The advent and use of new genome-wide high-throughput technology has revolutionized cancer research in molecular classification, and in prognosis and treatment response prediction over the last few years with cDNA microarrays taking the lead and protein microarrays to follow.[43,44]

Solid tumors are clinically and biologically heterogeneous diseases. The observation of different clinical outcomes among patients with the same TNM tumor stage, clinicopathologic factors and treatment can rationally be explained by cancer heterogeneity. Indeed, genomic and proteomic studies confirm the presence of distinct molecular subtypes among the conventional classifications Several microarrays-based studies have shown that gene-expression profiles of human cancers such as breast and lung cancer can provide a better prognostic classification.[45-47] An "explosion" in gene expression research during the last few years has already led to the development of several genetic classifiers. Only a few of these classifiers have reached the level of entering phase III randomized trials.

Intrinsic subtype classifier. This 306-gene expression profile distinguishes breast cancer into two major types, basal and luminal with each subdivided into two or three subtypes with a distinct prognosis. ER-positive tumors can be subdivided into luminal A and luminal B subtypes. It appears that luminal A tumors are adequately treated with endocrine therapy alone, whereas the more aggressive luminal B tumors should benefit if chemotherapy was added to endocrine therapy. Regarding the ER-negative tumors, they can be subdivided into a basal-like subtype which is ER/PR/HER2-negative (triple negative) that are responsive to chemotherapy and a HER2-like subtype (HER2+) responsive to combined trastuzumab-chemotherapy regimens.[48]

70-gene signature (mammaprint). The 70-gene signature developed in the Netherlands Cancer Institute was shown to predict distant metastasis and survival of patients with early-stage breast cancer, significantly better than conventional clinicopathologic factors. The 70-gene signature tested in 295 breast cancer patients was found capable to distinguish patients into a good and a poor prognosis group according to their 10-year survival outcome.[45,46] Currently, the prognostic capability of this signature was confirmed in a multi-center validation European study. The 70-gene signature was found to add independent prognostic information to clinicopathologic risk assessment for patients with early breast cancer.[49]

21-gene signature (oncotype Dx). This 21-gene signature development was based on tissue bank and data of the patients enrolled in the National Surgical Adjuvant Breast and Bowel Project (NSABP)

clinical trials. Paik et al using reverse transcriptase-polymerase chain reaction (RT-PCR) in formalin-fixed, paraffin-embedded tissue calculated a recurrence score (RS) for node-negative, ER-positive breast cancers into categories of high, intermediate and low risk of recurrence. Currently, RS was shown not only to quantify the likelihood of breast cancer recurrence node-negative, ER-positive breast cancers, but also predict chemotherapy efficacy.[50,51]

Gene expression studies have widely been criticized for methodology, diversity of genes used and the reproducibility outside highly-specialized laboratories.[52,53] However, despite criticism expressed on the validity of gene-sets, a recently published study produced encouraging results by confirming that most gene signature models had high rates of concordance in their outcome predictions for the individual samples.[54] Evidence-based medicine imposes that gene signatures can only enter the clinical practice if they prove superior over standard predictors in large-scale trials. This issue is expected to be addressed by two large randomized trials the TAILORx [ClinicalTrials.gov Identifier: NCT00310180] designed by the NCI in the USA and the MINDACT [EORTC Trial 10041, BIG 3-04] in the European Union, that are currently recruiting patients with the aim to test the validity of the 21-gene recurrence score and the 70-gene profile respectively. In general it is foreseen by file experts that genomic research will soon be in the position to provide easily applicable versions of gene-sets that will give clinicians the opportunity to provide cancer patients a truly personalized treatment.[55]

Cancer stem cells. Stem cells are characterized for their capacity for self-renewal, multilineage differentiation, and the proliferative ability to drive continued expansion of the population of malignant cells. These properties enable them to generate functional tissues during development and to regenerate these tissues following injury or degenerative processes. Stem-cell fate is regulated by the combination of extrinsic and intrinsic signals, many of which are poorly understood.[56] An understanding of the molecular mechanisms that govern stem-cell fate and the identification of specific stem-cell markers is of fundamental significance in cell and developmental biology and has important biomedical applications.[57]

In cancer research the cancer-stem-cell model is increasingly attracting attention. This model not only provides a logical explanation of current treatment failures, but also it may lead to the discovery of therapeutic targets that will specifically eradicate cancer stem cells improving treatment efficacy even leading to cure.[57-59] Recent data have indicated that several tumor types, including haematopoietic,[60] brain,[61] breast,[62] prostate[63] and other tumors, are probably maintained by cancer stem cells.

It is increasingly recognized that a tiny subpopulation of cancer stem cells plays a most crucial role in tumor initiation, growth, chemotherapy resistance and metastasis (Fig. 1).[57-59] They have been shown to be intrinsically resistant to chemotherapy[64] and radiotherapy.[65] Moreover data support that it is cancer stem cells that cause early metastasis and recurrence and not nonstem cancer cells.[57-59,66] Cancer stem cells may hold the answer to the mystery of commonly observed chemotherapy failures even in cases where they achieve to shrink bulky tumors to disappearance.[67] It is envisaged that recognition of committed genes and signaling pathways, as for example BMI1 and the SHH, Notch and Wnt/β-catenin pathways, which are characteristically activated in cancer stem cells may lead to identification of markers and potentially to the discovery of novel targeted therapeutics.[68] Yet this research is still in its infancy and a good deal of work has to be done before CSC biomarkers and CSC targeted therapeutics start entering clinical investigation studies.[69]

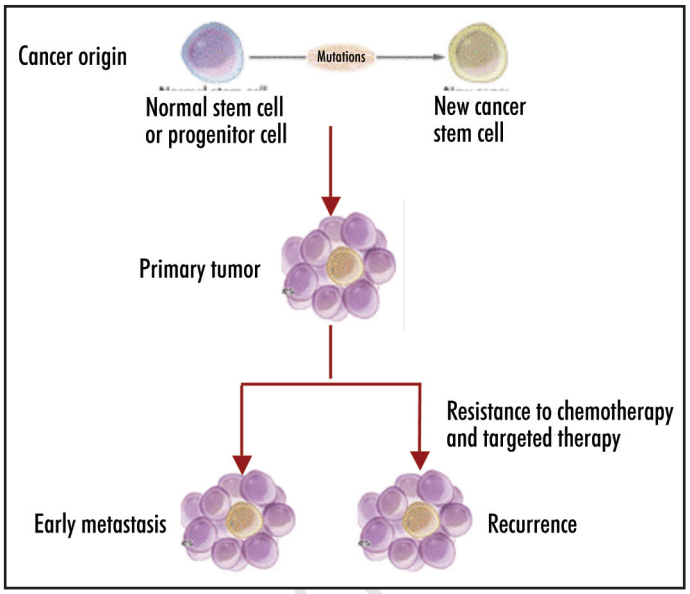

Figure 1. Scenarios of origin, growth and metastasis of cancer stem cells (CSC). Normal tissue arises from normal stem cells and progenitor cells that acquire the ability for self-renewal. First, cancer stem cells arise by means of a mutation in normal stem cells or progenitor cells that are responsible for sustaining a primary tumor. Second, cancer stem cells, by contrast to nonstem cancer cells, have intrinsically the ability to create metastatic lesions, even at early-tumor stages, or to form recurrent tumors at distant sites despite chemotherapy or targeted therapy (resistance).

CONCLUSIONS

Dramatic advances in basic, translational and clinical cancer research that have evolved over the last few years are driving the clinical management of cancer towards more personalized treatment approaches. The first data sets are now mature enough to justify clinical validation studies. In the future these techniques will help guide life-saving clinical management of persons at-risk to develop a hereditary cancer and patients diagnosed with cancer of a particular gene-signature.

However, we have to wait for molecular genetic biomarkers and novel targeted agents to provide clinical evidence of robustness and superiority over established clinicopathologic prognosticators and conventional chemotherapy respectively before they enter routine clinical practice. Data suggest gene-signature molecular classifiers will be the first to be validated in large randomized trials prior to obtaining approval for guiding clinical management. They have already shown to add independent information on tumor behaviour and clinical outcome, to improve prognostic prediction and assist in making therapeutic decisions.

It must be emphasized that despite intense international scientific cooperation, considerable research funding and hard laboratory and clinical work, a conserved, step-by-step process and not a revolution in cancer management can be expected. Eventually a "bench-to-bedside" evidence-based transfer of basic and translational research into clinical practice is believed to progressively build-up and benefit cancer-prone and cancer affected people. Through this progress it is hoped that individualized cancer management will be substantiated; however striking reductions in cancer mortality are rather unlikely to be seen in the next few years.

References

1. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin 2005; 55:74-108.
2. Kamangar F, Dores GM, Anderson WF. Patterns of cancer incidence, mortality, and prevalence across five continents: Defining priorities to reduce cancer disparities in different geographic regions of the world. J Clin Oncol 2006; 24:2137-50.
3. Ludwig JA, Weinstein JN. Biomarkers in cancer staging, prognosis and treatment selection. Nat Rev Cancer 2005; 5:845-56.
4. bd El-Rehim DM, Ball G, Pinder SE, et al. High-throughput protein expression analysis using tissue microarray technology of a large well-characterised series identifies biologically distinct classes of breast cancer confirming recent cDNA expression analyses. Int J Cancer 2005; 116:340-50.
5. Maltoni M, Caraceni A, Brunelli C, et al. Prognostic factors in advanced cancer patients: Evidence-based clinical recommendations-a study by the Steering Committee of the European Association for Palliative Care. J Clin Oncol 2005; 23:6240-8.
6. Lichtenstein P, Holm NV, Verkasalo PK, et al. Environmental and heritable factors in the causation of cancer-analyses of cohorts of twins from Sweden, Denmark, and Finland. N Engl J Med 2000; 343:78-5.
7. Narod SA, Offit K. Prevention and management of hereditary breast cancer. J Clin Oncol 2005; 23:1656-63.
8. Lynch HT, de la CA. Hereditary colorectal cancer. N Engl J Med 2003; 348:919-32.
9. Oliveira C, Seruca R, Carneiro F. Genetics, pathology, and clinics of familial gastric cancer. Int J Surg Pathol 2006; 14:21-33.
10. Hampel H, Frankel WL, Martin E, et al. Screening for the Lynch syndrome (hereditary nonpolyposis colorectal cancer). N Engl J Med 2005; 352:1851-60.
11. Jemal A, Siegel R, Ward E, et al. Cancer statistics, 2006. CA Cancer J Clin 2006; 56:106-30.
12. Boland CR. Decoding hereditary colorectal cancer. N Engl J Med 2006; 354:2815-7.
13. Guillem JG, Wood WC, Moley JF, et al. ASCO/SSO review of current role of risk-reducing surgery in common hereditary cancer syndromes. J Clin Oncol 2006; 24:4642-60.
14. Genetic risk assessment and *BRCA* mutation testing for breast and ovarian cancer susceptibility: Recommendation statement. Ann Intern Med 2005; 143:355-61.
15. Lindor NM, Petersen GM, Hadley DW, et al. Recommendations for the care of individuals with an inherited predisposition to Lynch syndrome: A systematic review. JAMA 2006; 296:1507-17.
16. Nelson HD, Huffman LH, Fu R, Harris EL. Genetic risk assessment and *BRCA* mutation testing for breast and ovarian cancer susceptibility: Systematic evidence review for the U.S. Preventive Services Task Force. Ann Intern Med 2005; 143:362-79.
17. Roukos DH, Fatouros M, Tsianos E, Kappas AM. Does a new model improve decisions about mismatch-repair genetic testing and Lynch syndrome identification? Nat Clin Pract Oncol 2006; 3:656-7.
18. Kauff ND, Satagopan JM, Robson ME, et al. Risk-reducing salpingo-oophorectomy in women with a *BRCA1* or *BRCA2* mutation. N Engl J Med 2002; 346:1609-15.
19. Bradshaw N, Holloway S, Penman I, Dunlop MG, Porteous ME. Colonoscopy surveillance of individuals at risk of familial colorectal cancer. Gut 2003; 52:1748-51.
20. Fukushima Y, Sakurai A. Comprehensive genetics clinic for familial tumors: Proposal for a suitable system in Japan. Int J Clin Oncol 2004; 9:304-7.
21. Wolfberg AJ. Genes on the Web-direct-to-consumer marketing of genetic testing. N Engl J Med 2006; 355:543-5.
22. Roche PA, Annas GJ. DNA testing, banking, and genetic privacy. N Engl J Med 2006; 355:545-6.
23. Braude P. Preimplantation diagnosis for genetic susceptibility. N Engl J Med 2006; 355:541-3.
24. Ponder BA. Cancer genetics. Nature 2001; 411:336-41.
25. Anonymous. Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: An overview of the randomised trials. Lancet 2005; 365:1687-717.
26. Piccart-Gebhart MJ, Procter M, Leyland-Jones B, et al. Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer. N Engl J Med 2005; 353:1659-72.
27. Andre F, Pusztai L. Molecular classification of breast cancer: Implications for selection of adjuvant chemotherapy. Nat Clin Pract Oncol 2006; 3:621-32.
28. Kattan MW, Karpeh MS, Mazumdar M, Brennan MF. Postoperative nomogram for disease-specific survival after an R0 resection for gastric carcinoma. J Clin Oncol 2003; 21:3647-50.
29. Olivotto IA, Bajdik CD, Ravdin PM, et al. Population-based validation of the prognostic model ADJUVANT! for early breast cancer. J Clin Oncol 2005; 23:2716-25.
30. Briasoulis E, Liakakos T, Dova L, et al. Selecting a specific pre or postoperative adjuvant therapy for individual patients with operable gastric cancer. Expert Rev Anticancer Ther 2006; 6:931-9.
31. Goldhirsch A, Glick JH, Gelber RD, Coates AS, Thurlimann B, Senn HJ. Meeting highlights: International expert consensus on the primary therapy of early breast cancer 2005. Ann Oncol 2005; 16:1569-83.
32. Bria E, Ciccarese M, Giannarelli D, et al. Early switch with aromatase inhibitors as adjuvant hormonal therapy for postmenopausal breast cancer: Pooled-analysis of 8794 patients. Cancer Treat Rev 2006; 32:325-32.
33. O'Shaughnessy JA. Molecular signatures predict outcomes of breast cancer. N Engl J Med 2006; 355:615-17.
34. Gschwind A, Fischer OM, Ullrich A. The discovery of receptor tyrosine kinases: Targets for cancer therapy. Nat Rev Cancer 2004; 4:361-70.
35. Nahta R, Yu D, Hung MC, Hortobagyi GN, Esteva FJ. Mechanisms of disease: Understanding resistance to HER2-targeted therapy in human breast cancer. Nat Clin Pract Oncol 2006; 3:269-80.
36. Ioannidis JP, Polycarpou A, Ntais C, Pavlidis N. Randomised trials comparing chemotherapy regimens for advanced nonsmall cell lung cancer: Biases and evolution over time. Eur J Cancer 2003; 39:2278-87.
37. Giaccone G, Rodriguez JA. EGFR inhibitors: What have we learned from the treatment of lung cancer? Nat Clin Pract Oncol 2005; 2:554-61.
38. Paez JG, Janne PA, Lee JC, et al. *EGFR* mutations in lung cancer: Correlation with clinical response to gefitinib therapy. Science 2004; 304:1497-500.
39. Simon R, Maitournam A. Evaluating the efficiency of targeted designs for randomized clinical trials. Clin Cancer Res 2004; 10:6759-63.
40. Balko JM, Potti A, Saunders C, Stromberg A, Haura EB, Black EP. Gene expression patterns that predict sensitivity to epidermal growth factor receptor tyrosine kinase inhibitors in lung cancer cell lines and human lung tumors. BMC Genomics 2006; 7:289.
41. Braun S, Naume B. Circulating and disseminated tumor cells. J Clin Oncol 2005; 23:1623-6.
42. Ring A, Smith IE, Dowsett M. Circulating tumour cells in breast cancer. Lancet Oncol 2004; 5:79-8.
43. Pusztai L, Ayers M, Stec J, Hortobagyi GN. Clinical application of cDNA microarrays in oncology. Oncologist 2003; 8:252-8.
44. Haab BB. Multiplexed protein analysis using antibody microarrays and label-based detection. Methods Mol Med 2005; 114:183-94.
45. van 't Veer LJ, Dai H, van d, V, et al. Gene expression profiling predicts clinical outcome of breast cancer. Nature 2002; 415:530-6.
46. Van de Vijver MJ, He YD, van't Veer LJ, et al. A gene-expression signature as a predictor of survival in breast cancer. N Engl J Med 2002; 347:1999-2009.
47. Potti A, Mukherjee S, Petersen R, et al. A genomic strategy to refine prognosis in early-stage nonsmall-cell lung cancer. N Engl J Med 2006; 355:570-80.
48. Sotiriou C, Neo SY, McShane LM, et al. Breast cancer classification and prognosis based on gene expression profiles from a population-based study. Proc Natl Acad Sci USA 2003; 100:10393-8.
49. Buyse M, Loi S, van't VL, et al. Validation and clinical utility of a 70-gene prognostic signature for women with node-negative breast cancer. J Natl Cancer Inst 2006; 98:1183-92.
50. Paik S, Shak S, Tang G, et al. A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. N Engl J Med 2004; 351:2817-26.
51. Paik S, Tang G, Shak S, et al. Gene expression and benefit of chemotherapy in women with node-negative, estrogen receptor-positive breast cancer. J Clin Oncol 2006; 24:3726-34.
52. Simon R, Radmacher MD, Dobbin K, McShane LM. Pitfalls in the use of DNA microarray data for diagnostic and prognostic classification. J Natl Cancer Inst 2003; 95:14-8.
53. Ransohoff DF. Rules of evidence for cancer molecular-marker discovery and validation. Nat Rev Cancer 2004; 4:309-14.
54. Fan C, Oh DS, Wessels L, et al. Concordance among gene-expression-based predictors for breast cancer. N Engl J Med 2006; 355(6):560-569.
55. Bild AH, Yao G, Chang JT, et al. Oncogenic pathway signatures in human cancers as a guide to targeted therapies. Nature 2006; 439:353-7.
56. Moore KA, Lemischka IR. Stem cells and their niches. Science 2006; 311:1880-5.
57. Clarke MF, Fuller M. Stem cells and cancer: Two faces of eve. Cell 2006; 124:1111-5.
58. Weigelt B, Peterse JL, van 't Veer LJ. Breast cancer metastasis: Markers and models. Nat Rev Cancer. 2005; 5:591-602.
59. Huff CA, Matsui WH, Douglas SB, Jones RJ. Strategies to eliminate cancer stem cells: Clinical implications. Eur J Cancer 2006; 42:1293-7.
60. Krivtsov AV, Twomey D, Feng Z, et al. Transformation from committed progenitor to leukaemia stem cell initiated by MLL-AF9. Nature. 2006; 442:818-22.
61. Singh SK, Clarke ID, Hide T, Dirks PB. Cancer stem cells in nervous system tumors. Oncogene 2004; 23:7267-73.
62. Al-Hajj M, Clarke MF. Self-renewal and solid tumor stem cells. Oncogene 2004; 23:7274-82.
63. Patrawala L, Calhoun T, Schneider-Broussard R, et al. Highly purified CD44+ prostate cancer cells from xenograft human tumors are enriched in tumorigenic and metastatic progenitor cells. Oncogene 2006; 25:1696-7.
64. Jordan CT, Guzman ML, Noble M. Cancer stem cells. N Engl J Med 2006; 355:1253-61.
65. Bao S, Wu Q, McLendon RE, Hao Y, et al. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. Nature 2006, [Epub ahead of print].
66. Glinsky GV. Genomic models of metastatic cancer: Functional analysis of death-from-cancer signature genes reveals aneuploid, anoikis-resistant, metastasis-enabling phenotype with altered cell cycle control and activated Polycomb Group (PcG) protein chromatin silencing pathway. Cell Cycle 2006; 5:1208-16.
67. Mehlen P, Puisieux A. Metastasis: A question of life or death. Nat Rev Cancer 2006; 6:449-58.
68. Galmozzi E, Facchetti F, La Porta CA. Cancer stem cells and therapeutic perspectives. Curr Med Chem 2006; 13:603-7.
69. Clarke MF, Dick JE, Dirks PB, et al. Cancer stem cells-perspectives on current status and future directions: AACR workshop on cancer stem cells. Cancer Res 2006; 66:9339-44.