Novel approaches to cancer therapeutics

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Advancements in our understanding of the biology of cancer have progressed dramatically over the past decade. The application of cutting-edge molecular profiling techniques analysing the cancer genome is elucidating an appreciable amount of information. This data is now being integrated into a catalogue that is providing researchers with a revolutionary roadmap of the molecular mechanisms behind cancer. Recent accomplishments in cancer research are also being introduced into the clinic through the development of innovative diagnostic technologies and targeted therapies. Lessons from the past, along with novel therapeutic approaches being developed today, have stimulated an optimistic promise for tomorrow's fight against cancer.

Introduction

Cancer has the largest burden of disease on the health care system in Australia. [1] Over 100,000 new cases were diagnosed nation-wide in 2005 and the incidence projections from 2006 through 2010 are expected to grow by over 3000 cases annually. [1] Combinations of surgery, radiation and chemotherapy are the most applied treatment modalities in cancer. Unfortunately, surgery and radiation are often palliative interventions for metastasising cancers and the number of systemic treatment options available for cancer is relatively limited. Many current chemotherapeutic treatments for cancer use the 'shotgun' approach, which targets DNA replication with an attempt to exploit the high rates of cell division, a concept that was discovered over 50 years ago and has since changed very little. [2] However, a revolution in the methodology applied to modern molecular medicine is elucidating fundamental characteristics of the genetics behind cancer, providing researchers with a 'molecular handle' to develop novel targeted systemic therapeutic strategies.

The advent of the human genome project sprouted a revolution in '-omics' technologies which accelerated our comprehension of the molecular mechanisms involved in health and disease. Cancer is no exception. The biotechnology industry is now developing novel diagnostic microarray technologies capable of characterising the extensive variability of cancer genetics between patients with considerable accuracy and detail, while the pharmaceutical industry is racing to fill the pipeline with targeted molecular therapeutic agents never before used clinically. [2-4] Together, 'personalised' diagnostic analyses in partnership with a new generation of drugs have the potential to change the way cancer is treated and subsequently improve patient outcomes and survival. Armed with the right tools, tomorrow's clinicians could become well equipped cancer-killing assassins.

Molecular profiling and tools in the making

In 1975, a technique called Southern Blotting was developed which exploited the A-T G-C sequence-specific hybridisation of DNA. This efficiently enabled the specific identification of individual gene sequences. [5] This approach is now being applied in a novel way to gene chips, or microarrays. Microarrays containing thousands of specific gene sequences are capable of identifying specific genomic changes and allow visualisation of entire gene expression profiles of cells under a given set of conditions. [3,6,7] For example, a cell under normal conditions without any stressors or stimulants will express a particular set of "housekeeping" genes for optimising survival through the regulation of mRNA transcription. The relative levels of mRNA

transcripts can be visualised, monitored and catalogued for future reference using microarray-based gene expression profiling. [3,6,7] The expression profiles of cancer cells can then be tested in real time under various conditions for comparison against the reference catalogues. [6] The differences between the two expression profiles can be used to identify changes in gene activation in carcinogenesis.

Tumours are traditionally classified by histology, which enables the crude prediction of characteristics and prognosis of a cancer. Microarray analyses of cancers have demonstrated that tumours with

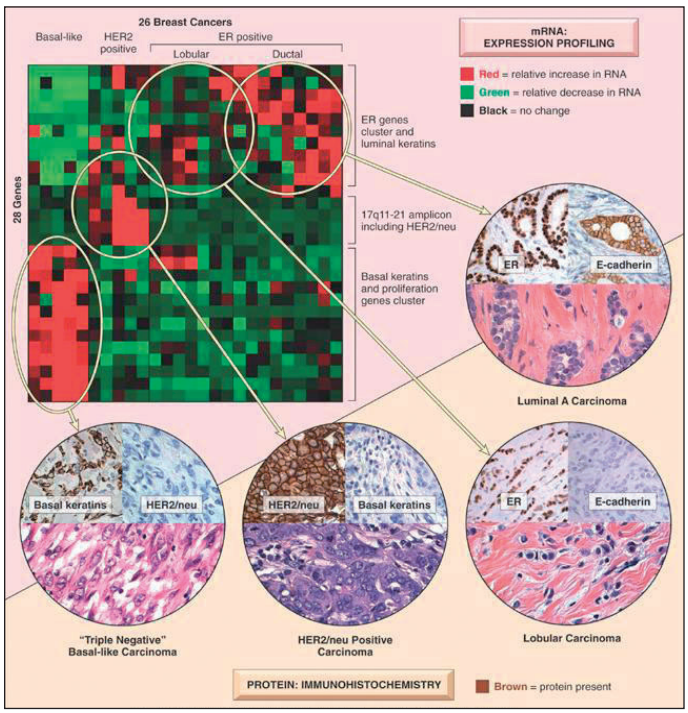

Figure 1. *Gene expression portraits of breast carcinomas. Alterations in mRNA and protein expression identify breast cancer subtypes previously recognized by morphology (e.g., lobular carcinomas) and define new subtypes ("luminal A," "HER2/neu positive," and "basal-like"). [10] (Array data courtesy of Dr. Andrea Richardson, Brigham and Women's Hospital, Boston, MA, as modified from Signoretti S, Di Marcotullio L, Richardson A, Ramaswamy S, Isaac B, Rue M et al. Oncogenic role of the ubiquitin ligase subunit skp2 in human breast cancer. J Clin Invest 2002;110:633. This image was published in Robbins and Cotran Pathological Basis of Disease, 8th Edition, Kumar* et al.*, Copyright, 2009, Saunders, an imprint of Elsevier. Used with permission.)*

histological similarity can develop from distinct genetic mechanisms which influence the progression of disease. [2,3,8,9] Figure 1 demonstrates the conjoining of histological analyses with microarrays, allowing greater accuracy in the characterisation of disparate types of cancer. [2,3,8-10]

Gene expression profiles are also divulging information about the susceptibility of tumours to different targeted chemotherapeutic agents which can help clinicians decide on the most effective treatments. [2,3,8-10] This is the basis of personalised therapeutics.

Following suit with these genomic technologies are proteomics and metabolomics, which utilize analytical methods to monitor the set of proteins and metabolites within a cell. [4,6] Collectively, these techniques have many implications in modern medicine. For example, they allow the analysis of the effects of various drug compounds on different cell types or the precise characterisation of specific tumours or other cells in diseased states. [4,6] The applications of molecular profiling technologies such as these have the potential to increase the rate of success of drug discovery and development. By predicting drug response and toxicity before the compound ever enters clinical trials, failure rates and overall development costs would ultimately be reduced. [3,11]

The first eukaryotic gene expression microarray was developed in 1997 and now, more than a decade later, this technology is eliciting an immense amount of data about the molecular biology of the cell at an ever increasing rate.

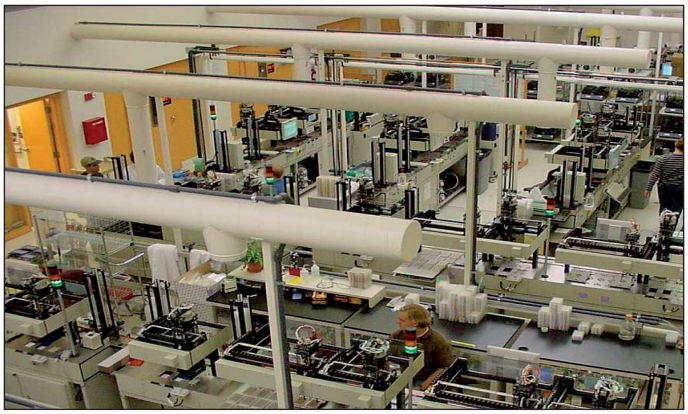

Figure 2. *High-throughput large-scale sequencing centre. Taken from The Cancer Genome Atlas website. [6] Courtesy: The Broad Institute of MIT and Harvard, the National Cancer Institute (NCI) and the National Human Genome Research Institute (NHGRI). [6]*

Building a roadmap of cancer

In 2006, a joint effort between the National Cancer Institute (NCI) and the National Genome Research Institute (NGRI) in the United States spurred a pilot project called The Cancer Genome Atlas (TCGA), aimed at creating a reference data set of the genomic changes that occur in three major types of cancer. [6,7] This project utilised high-throughput technologies (Figure 2) to sequence and catalogue gene mutations, chromosome rearrangements, gene copy numbers, gene expression profiles and epigenetic changes from collected tissue samples of brain, lung and ovarian cancers. [6,7] This has generated an immense amount of data which is being integrated into an online catalogue, publicly available to researchers around the world. [6]

Mapping the genetic changes in cancer with the integrative analysis of multidimensional genomic data such as DNA copy numbers, gene expressions and DNA methylation aberrations is proving to be extremely informative. In 2008, TCGA researchers identified three major signalling pathways (RB, p53 and RTK/RAS/PI3K) which harboured mutations in 75% of cases of glioblastoma multiforme (GBM), the most common and deadliest form of brain cancer. [12] This suggests that these pathways are promising targets for drug development.

The TCGA database has also contributed to a number of other studies focusing on the genetics of cancer and the list is growing rapidly. [6] One study using data from TCGA identified the gene ANXA7 as a tumour suppressor for the epidermal growth factor receptor gene, EGFR. [13] Mutations of ANXA7 have previously been associated with breast and prostate cancer, whereas EGFR is the most common genetic defect in growth factor signalling in GBM and is implicated in several other cancer types. [13,14] Genetic players such as these, with roles in multiple cancer types, are encouraging targets for the pharmaceutical industry.

In October of 2009, TCGA announced its expansion efforts to characterise over 10,000 tumours from 20 different tumour types by the year 2015, creating a comprehensive database describing the foundations of cancers. [6] This endeavour will significantly increase the rate of advancement for the treatment and management of cancer in tomorrows' clinics. The clinical relevance of understanding the molecular origins of carcinogenesis have already been demonstrated in leukaemia, breast and lung cancer. [2,8] Prognostic tests for specific genetic mutations from tumour biopsies are giving direction regarding which drugs patients will most likely respond to. [7,9,11,14]

Retrospective as well as prospective samples are being collected for TCGA to analyse the changes that occur in the progression of drug resistance, a frequent problem in the treatment of cancer. [6] This will provide insight for the direction of drug development and the future management of the disease. Many patients with GBM initially respond to temozolomide treatment, an alkylating agent, but develop therapeutic resistance which invariably leads to death. [15,16] TCGA data from tumours of GBM patients were analysed before and after treatment with temozolomide for the identification of elements responsible for the development of therapeutic resistance. [16] Mutations in the mismatch repair gene, MSH6, arose after treatment and were found to give rise to a hypermutation phenotype that mediated the resistance of GBM against temozolomide. [16] Eventually, the initially effective treatment developed resistance by selecting for cells with genetic aberrations that enabled the cancer to resist the therapy.

The ammunition: Examples of currently used targeted therapy

Standard cytotoxic chemotherapy does not adequately discriminate between cancerous and normal cells, reducing the efficacy of treatment and increasing side effects. In contrast, targeted therapeutic treatment regimens generally permit the use of higher concentrations for longer durations, with fewer harmful side-effects. Effective therapeutic strategies such as these are already showing promise. Table 1 summarizes several examples of targeted therapies currently in use or being tested. Advances in high-throughput technologies and molecular profiling have also sparked new developments in cancer research, identifying the mechanisms involved in acquired resistance.

Imatinib is a synthetic tyrosine kinase inhibitor used in the treatment of chronic myeloid leukaemia (CML). It is specifically designed to inhibit the BCR-ABL fusion protein which is a result of a chromosome translocation, known as the Philadelphia chromosome. [17] The constitutively active tyrosine kinase targeted by imatinib, BCR-ABL, activates signalling pathways involved in the regulation of bone marrow stroma cell adhesion, cell proliferation and apoptosis. [17] Imatinib has also been shown to block the activity of additional tyrosine kinases, including c-Kit receptor and the platelet-derived growth factor receptor (PDGFR), both of which promote tumour growth. [17,18] Imatinib has significantly increased the effectiveness of CML treatment with fewer complications and side-effects compared to the traditional chemotherapy regimen, with an improved five year survival rate. [17]

Approximately 25% of invasive primary breast cancers exhibit amplification of the receptor tyrosine kinase human epidermal growth factor receptor 2 (HER2). [18] Trastuzumab is a monoclonal antibody which inhibits the activity of the HER2 tyrosine kinase. [19] This targeted monoclonal antibody significantly improved outcomes of patients with HER2-positive breast cancer when used in combination with traditional chemotherapy. [20-22] However, over time the

treatment loses efficacy through the development of resistance, likely mediated through IGF-1 and related EGFR signalling pathways. [19] Pre-clinical studies are currently elucidating the mechanisms regulating trastuzumab resistance for the identification of additional candidate targets for drug development. [19] In addition, another novel antibody, lapatinib, appears to reduce HER2, EGFR and IGF-1 signalling and is showing promise in combination therapy. [19]

The addition of rituximab, an anti-CD20 monoclonal antibody, to the treatment of B-cell non-Hodgkin's lymphoma (NHL) is another example where targeted molecular therapy in combination with standard chemotherapy has become first-line treatment. [11,23] The side-effects associated with rituximab are minimal and have proven to be tolerable for long durations. [11,23] The antibody treatment is now being tested as a long-term maintenance therapy for NHL, and preliminary results from phase III clinical trials have shown that progression-free survival is significantly improved on the maintenance regimen. [24]

The activation of the hedgehog signalling pathway has been implicated in several types of cancer, including basal-cell carcinoma of the skin and medulloblastoma of the brain. [25] The hedgehog pathway is responsible for the control of several processes in embryogenesis and is mostly inactive in adult tissues; thus, blocking this pathway may reveal a large degree of selectivity against the cancer with fewer harmful side-effects. [26] A new compound, GDC-O449, has been found to inhibit the hedgehog signalling pathway and has recently reported to generate beneficial responses in two preliminary studies, a phase I clinical trial and a case report of a patient with refractory medulloblastoma. [25,27] Both studies demonstrated compelling evidence that therapy directed at hedgehog signalling is an encouraging new direction in the treatment of basal-cell carcinoma, medulloblastoma and other cancer types. [25-27] Importantly, molecular profiling of tumours in both studies is helping to characterise the mechanisms involved in hedgehog-regulated carcinogenesis and the development of therapeutic resistance to GDC-0449.

Ligand-independent epidermal growth factor receptor (EGFR) activation occurs in a subset of non-small-cell lung (NSCL) cancer, resulting in constitutive activation of the intracellular tyrosine kinase domain. [14,28] Erlotinib and gefitinib are targeted tyrosine kinase inhibitors of the EGFR that give rise to an improved patient outcome, although not a cure, with modest side effects. [14,18] Mutations in the EGFR tyrosine kinase domain have been demonstrated in a number of other metastatic cancers such as pancreatic, colorectal, breast and glioma, where the efficacy of erlotinib is also being tested. [28]

An exciting new development in the area of gene expression is the discovery of so-called microRNAs (miRNAs). These short regulatory RNA molecules function in the control of gene expression. Microarray analyses of tumours are revealing increasing evidence that these microRNAs also play a functional role in cancer as oncogenes and tumour suppressor genes. [29-31] Studies are elucidating which are key players, and a new class of anticancer therapeutics under investigation is aimed at regulating these microRNAs. [29-31] Theoretically, all RNA or protein molecules that are cancer targets can be down-regulated through a process called RNA interference. This requires the synthetic production of sequence-specific siRNAs which specifically target the molecule in question. Although effective delivery of siRNAs is likely to be the most challenging hurdle in the effective use of RNAi therapeutics, novel strategies exploiting lipid complexes, viral capsids and antibodies may prove to be successful in overcoming these obstacles. [29,32]

Tomorrow's promise

The most troublesome attribute of cancer is its ability to develop resistance against traditional and new age classes of drugs. This is primarily done by manipulating the network of interacting signalling pathways that mediate growth, replication, survival and apoptosis. Past lessons have taught us that monotherapeutic approaches will likely continue to fail in this comprehensive cross-talk model of correlated genes interacting with related signalling pathways. [33] Yet a great sense of optimism remains, as current and prospective technological advances continue to expand our understanding of the mechanisms that drive cancer. The progression towards drug resistance is an evolutionary process of selection, familiar to the field of medicine. However, past successful approaches preventing nature's propensity to adapt should be emphasised, the treatment of HIV being one example. The 'cocktail' regimen of antiretroviral therapy uses several medications which target a combination of biological processes essential for HIV replication, and has been successful in preventing mutations in the virus which regulate drug resistance. The further development of novel therapeutic approaches targeting additional specific mechanisms in cancer will hopefully provide tomorrow's clinicians with a 'cocktail' arsenal of ammunition, capable of surmounting cancer's tendency to adapt.

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Table 1. *Examples of some targeted therapeutic drugs currently approved for use or being tested.*

Cancer Type	Drug(s) currently approved or being tested	Drug type	Drug target	Comments
Chronic myeloid leukaemia	Imatinib	Synthetic tyrosine kinase inhibitor	BCR-ABL fusion protein	May also block other tumour-promoting receptors (e.g. c-KIT, PDGFR)
Breast (HER2-positive)	Trastuzumab	Monoclonal antibody	HER2 tyrosine kinase	Resistance possibly mediated through IGF-1 signalling
	Lapatinib	Monoclonal antibody	HER2 & EGFR tyrosine kinases	May also block IGF-1 signalling (implicated in tumour growth and resistance)
B-cell non-Hodgkin's lymphoma	Rituximab	Monoclonal antibody	CD20	Likely mediated through antibody-dependent cell-mediated cytotoxicity, complement and apoptosis
Medulloblastoma	Compound GDC-0449	Synthetic ligand inhibitor	Hedgehog signalling	Hedgehog signalling is implicated in basal-cell carcinoma, medulloblastoma and possibly others
Non-small-cell lung cancer	Erlotinib Gefitinib	Synthetic tyrosine kinase inhibitorSynthetic tyrosine kinase inhibitor	EGFR tyrosine kinase EGFR tyrosine kinase	EGFR mutations are also implicated in other metastatic cancers (e.g. pancreatic, colorectal, breast and glioma)

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Conflict of Interest

None declared.

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Glossary of Terms

Epigenetic processes are changes in the regulation of the expression of gene activity without alteration of genetic structure.

Molecular profiling studies utilise measurement of global mRNA and protein patterns towards identification of individual genes and groups of genes that mediate particular aspects of cellular physiology and pathology. Proteomics is the global analysis of cellular proteins.

Proteomics uses a combination of sophisticated techniques including two-dimensional (2D) gel electrophoresis, image analysis, mass spectrometry, amino acid sequencing, and bio-informatics to resolve comprehensively, to quantify, and to characterize proteins. The application of proteomics provides major opportunities to elucidate disease mechanisms and to identify new diagnostic markers and therapeutic targets.

Metabolomics is the study of the biological metabolic profile of a cellular specimen in a specific environment at an isolated time point. This discipline depicts the physiological states of cells and organisms by focusing on carbohydrates, lipids, and other metabolites. Several analytical techniques are utilized to quantify the metabolic content of specimens such as mass spectrometry and electrophoretic applications.

miRNA or *MicroRNA* is a sequence of single-stranded RNA which is typically 20-25 nucleotides in length and may regulate the expression of other genes. *mi*RNAs are regulatory RNA molecules which are transcribed from DNA, but are not translated into proteins.

RNAi or *RNA Interference* is sequence-specific posttranscriptional gene silencing. It is mediated by 21- and 22-nucleotide small interfering RNAs (siRNAs).

siRNA or *Small Interfering RNA* is 21- and 22-nucleotide double-stranded RNAs. These are the mediators of a sequence-specific messenger RNA degradation process known as RNA interference. *siRNAs* can also be synthetically produced.

Definitions are in part adapted from the National Cancer Institute (NCI) Terminology Browser (http://nciterms.nci.nih.gov/NCIBrowser/Dictionary.do) using the NCI Thesaurus terminology.

References

[1] Australian Institute of Health and Welfare (AIHW) & Australasian Association of Cancer Registries (AACR). Cancer in Australia: an overview, 2008. Canberra (NSW): Australian Institute of Health and Welfare Publishing; 2008. (Cancer series; 42(46)).
[2] Varmus H. The new era in cancer research. Science 2006;312(5777):1162-5.
[3] Stoughton R, Friend S. How molecular profiling could revolutionize drug discovery. Nat Rev Drug Discov 2005;4(4):345-50.
[4] Chin L, Gray JW. Translating insights from the cancer genome into clinical practice. Nature 2008;452(3):553-63.
[5] Southern E. The birth of the southern blot: 1975. Scientist 2003;17(21):14.
[6] The Cancer Genome Atlas (TCGA). National Cancer Institute: National Institue of Health. [Online]. 2009. [cited 2009 Oct 20]; Available from: URL:http://cancergenome.nih.gov/
[7] Collins F, Barker A. Mapping the cancer genome. Sci Am 2007;296(3):50-7.
[8] Dalton WS, Friend S. Cancer biomarkers - an invitation to the table. Science 2006;312(5777):1165-8.
[9] Pao W, Kris MG, Lafrate J, Ladanyi M, Jänne PA, Wistuba II, *et al.* Integration of molecular profiling into the lung cancer clinic. Clin. Cancer Res 2009;15(17):5317-22.
[10] Lester SC. The Breast. In: Kumar V, Abbas AK, Fausto N, Aster JC. Robbins & Cotran Pathologic Basis of Disease. 8th ed. Philadelphia (PA): Saunders; 2009.
[11] Abramson JS, Shipp MA. Advances in the biology and therapy of diffuse large B-cell lymphoma: moving towards a molecularly targeted approach. Blood 2005;106(4):1164-74.
[12] The Cancer Genome Atlas Research Network, (2008). Comprehensive genomic characterization defines human glioblastoma genes and core pathways. Nature 2008;455(7216):1061-8.
[13] Yandav AK, Renfrow JJ, Scholtens DM, Xie H, Duran GE, Bredel C, *et al.* Monosomy of chromosome 10 associated with dysregulation of epidermal growth factor signalling in Glioblastomas. JAMA 009;302(3):276-89.
[14] Gazdar AF. Personalized medicine and inhibition of EGFR signalling in lung cancer. N Engl J Med 2009;361(10):1018-20.
[15] Sarkaria JN, Kitange GJ, James CD, Plummer R, Calvert H, Weller M, *et al.* Mechanisms of chemoresistance to alkylating agents in malignant glioma. Clin. Cancer Res 2009;14(10):2900-8.
[16] Yip S, Miao J, Cahill DP, Lafrate AJ, Aldape K, Nutt CL, *et al.* MSH6 mutations arise in glioblastomas during temozolomide therapy and mediate temozolomide resistance. Clin Cancer Res 2009;15(14):4622-9.
[17] Moen MD, Mckeage K, Plosker GL, Siddiqui MA. Imatinib: a review of its use in chronic myeloid leukaemia. Drugs 2007;67(2):299-320.
[18] Baselga J. Targeting tyrosine kinases in cancer: the second wave. Science 2006;312(5777):1175-8.
[19] Nahta R, Esteva FJ. Trastuzumab: triumphs and tribulations. Oncogene 2007;26(25):3637-43.
[20] Baselga J, Tripathy D, Mendelsohn J, Baughman S, Benz CC, Dantis L, *et al.* Phase II study of weekly intravenous recombinant humanized anti-p185HER2 monoclonal antibody in patients with HER2/neu-overexpressing metastatic breast cancer. J Clin Oncol 1996;14(3):737-44.
[21] Cobleigh MA, Vogel CL, Tripathy D, Robert NJ, Scholl S, Fehrenbacher L, *et al.* Multinational study of the efficacy and safety of humanized anti-HER2 monoclonal antibody in women who have HER-2 overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease. J Clin Oncol 1999;17(9):2639-48.
[22] Vogel CL, Cobleigh MA, Tripathy D, Gutheil JC, Harris LN, Fehrenbacher L, *et al.* Efficacy and safety of trastuzumab as a single agent in first-line treatment of HER2-overexpressing metastatic breast cancer. J Clin Oncol 2002;20(14):719-26.
[23] Bendandi M. Aiming at a curative strategy for follicular lymphoma. CA Cancer J Clin 2008;58(5):305-17.
[24] Bekiesz B. PRIMA trial of rituximab maintenance for follicular lymphoma stopped early due to PFS benefit. Oncology Stat. Elsevier [Online]. 2009 Sept 18. [cited 2009 Oct 23]. Available from: URL: http://www.oncologystat.com/news-and-viewpoints/news/PRIMA_Trial_of_Rituximab_Maintenance_for_Follicular_Lymphoma_Stopped_Early_Due_to_PFS_Benefit.html
[25] Von Hoff D, Lorusso PM, Rudin CM, Reddy JC, Yauch R, Tibes R, *et al.* Inhibition of the hedgehog pathway in advanced basal-cell carcinoma. N Engl J Med 2009;361(12):1164-72.
[26] Dlugosz AA, Talpaz M. Following the hedgehog to new cancer therapies. N Engl J Med 2009;361(12):1202-5.
[27] Rudin CM, Hann CL, Laterra J, Yauch RL, Callahan CA, Fu L, *et al.* Treatment of medulloblastoma with hedgehog pathway inhibitor GDC-0449. N Engl J Med 2009;361(12):1173-8.
[28] Tang PA, Tsao MS, Moore MJ. A review of erlotinib and its clinical use. Expert Opin Pharmacother 2006;7(2):177-93.
[29] Kim DH, Rossi JJ. Strategies for silencing human disease using RNA interference. Nat Rev Genet 2007;8(3):173-84.
[30] Zhang B, Pan X, Cobb GP, Anderson TA. microRNAs as oncogenes and tumor suppressors. Dev Biol 2007;302(1):1-12.
[31] Ji J, Shi J, Budhu A, Yu Z, Forgues M, Roessler S, *et al.* MicroRNA expression, survival, and response to interferon in liver cancer. N Engl J Med 2009;361(15):1437-47.
[32] Bumcrot D, Manoharan M, Koteliansky V, Sah DWY. RNAi therapeutics: a potential new class of pharmaceutical drugs. Nat Chem Biol 2006;2(12):711-20.
[33] Bredel M, Scholtens DM, Harsh GR, Bredel C, Chandler JP, Renfrow JJ, *et al.* A network model of a cooperative genetic landscape in brain tumors. JAMA 2009;302(3):261-75.