Tuesday 19 November

Michel Clavel lecture

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Drug Resistance reversal - are we getting closer?

S.B. Kaye, *Royal Marsden Hospital, Sutton, Surrey, United Kingdom*

Conventional chemotherapy in solid tumours has achieved a modicum of success, but results are limited in most cases by the presence of drug resistance. Data from experimental models indicate that at the level of the tumour cell, factors underlying this resistance are likely to include alterations in membrane drug transport, changes in drug activation or target enzyme activity, changes in repair capacity (either enhanced repair mechanisms or failure to recognise damage to DNA) and/or a shift in the balance of pro-apoptotic and survival signals in favour of survival following exposure to cytotoxics.

There are now two key issues which must be addressed if progress is to be made in the clinic:

(a) *Which of these experimental data have relevance to the clinical situation?*

To answer this, translational research groups are at last beginning to conduct pharmacogenomics studies linked to clinical trial databases, using stored samples of tumour taken prior to therapy for increasingly sophisticated analyses of gene expression profiles. While much is expected of these studies, it is conceivable that at least as much information will be obtained by careful analysis of tumour tissue from patients whose disease has relapsed and demonstrated a degree of drug resistance, as well as from patients pre-treatment. To contribute to this, we have elected to focus on ovarian cancer, and have initiated the systematic collection of tumour cells separated from ascites in patients whose disease has relapsed following platinum-based chemotherapy. Sequential material from patients before and at the onset of drug resistance may prove the most informative of all.

(b) *Can knowledge of the relevant underlying mechanisms lead to successful therapeutic reversal of drug resistance?*

To answer this, a number of avenues are being explored in our Institute, which address some of the mechanisms described above.

(i) As regards membrane transport, modulation of P-glyco-protein function by specific inhibitors has so far proved unsuccessful in the clinic. Alternative approaches to exploit tumour-specific transport could involve the utilisation of specific receptors, e.g. the a folate receptor. This is expressed at high levels in certain tumours, eg ovarian cancer and mesothelioma and overexpression has been linked to drug resistance (to cisplatin). A novel and highly potent thymidylate synthase inhibitor with a high degree of specificity for this receptor is under development in our laboratory(1), with expectations for entry into the clinic in the near future.

(ii) Failure to recognise DNA damage, following exposure to a wide range of cytotoxic agents has been attributed experimentally to mismatch repair deficiency in tumour cells, specifically through inactivation of hMLH1 resulting from gene methylation. Experimentally, resistance which is due to this deficiency can be reversed, using the demethylating agent, decitabine, at non-toxic doses. Successful reversal has been achieved in human tumour xenografts, treated with a range of agents, including cisplatin and doxorubicin(2). A Phase I clinical trial is now underway in our Institute, in which carboplatin and decitabine are given together, and preliminary data indicate that gene demethylation is likely to be achievable at feasible decitabine doses.

(iii) The balance of signals determining whether or not apoptosis is engaged following cytotoxic exposure is highly complex. Already there are clinical data suggesting that the inhibition of certain survival signals, eg the EGFR pathway, with monoclonal antibodies, can reverse drug resistance. Since the degree of cross-talk between several of these pathways is very substantial, it seems likely that novel molecules capable of affecting a number of pathways simultaneously will be particularly effective. An example which is under clinical trial in our Institute is the molecular chaperone (Hsp90) inhibitor, 17 - allylamino-geldanamycin.(3) Molecular pharmacodynamic endpoints are now essential in clinical trials and both gene expression microarray and proteomic profiling have been used to identify molecular signatures of Hsp 90 inhibition.(4) Clinical data including tumour biopsies indicate the potential to reach effective drug concentrations within tumours, and preclinical data indicate the potential for synergy with a range of agents, including paclitaxel.

One of several Hsp 90 client proteins is AKT, a serine/threonine kinase, which is activated by phosphatidylinositol-3-kinase (PI3K) and is involved in promoting cell survival, and potentially in cytotoxic drug resistance. Preliminary data from our study in ascitic tumour cells support the notion that this could be clinically relevant, and highly specific PI3K inhibitors, currently being developed in our Institute, will therefore be assessed in this context.

Clearly these, and other molecularly targeted agents may possess significant antitumour activity in their own right. The extent will depend on the presence of relatively specific oncogenic signalling pathways capable of inhibition, and this is likely to be tumour specific. A recent exciting example is the discovery by Stratton and co-workers of a mutation within the kinase domain of the BRAF oncogene in 66% of samples of malignant melanoma. Since the mutated BRAF proteins have elevated kinase activity, BRAF-inhibitors clearly merit exploration in malignant melanoma, and clinical trials are now underway in our unit. It is quite conceivable that these agents will find a role in combination with chemotherapy as a means of enhancing the apoptotic response.

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Keynote Lectures

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Combining kinase inhibitors with chemotherapy

J. Mendelsohn*, *University of Texas M.D. Anderson Cancer Center, Office of the President, Houston, USA*

EGFR are expressed at high levels in about 1/3 of epithelial cancers, and autocrine activation of EGFR appears to be critical for the growth of many tumors. We hypothesized that blockade of the binding sites for EGF and TGF-alpha on EGFR with an antireceptor monoclonal antibody (mAb) might be an effective anti-cancer therapy. Murine mAb 225 blocked EGFR function, and inhibited tumor cell growth in cultures and in nude mouse xenografts. C225 is the human:murine chimeric version of mAb 225. Pharmaceutical companies have developed a number of soluble, low molecular-weight inhibitors which act intracellularly on the ATP binding site of EGFR, blocking receptor activation. These molecules differ in their specificity for the EGF receptor and their reversibility of binding. The mechanisms of tumor inhibition by anti-EGF receptor agents involve growth inhibition through upregulation of p27Kip1, enhancement of apoptosis, and inhibition of angiogenesis and metastasis. In addition, these agents enhance the cytotoxicity of chemotherapy and radiotherapy. These findings in extensive preclinical studies led to clinical trials of EGF receptor inhibitors, both as monotherapy and in combination with chemotherapy or radiotherapy. Results from Phase I and II trials involving thousands of patients are promising, and data from Phase III trials will be forthcoming soon. Many challenges remain to be addressed. Is EGF receptor signaling different in cancer cells expressing 106 receptors than in normal cells expressing 104? What are the relative advantages of agents with high specificity for the EGF receptor vs. agents that cross-react with other receptors in the family? Why do some but not all patients with an EGF receptor-expressing type of cancer respond to receptor inhibitors? What is the basis for greater response rates in some types of cancers? Are there tissue markers that would identify responsive cancers? Are there specific mechanisms for synergism between EGF receptor inhibitors and chemotherapeutic agents, and between EGF receptor inhibitors combined with agents promoting apoptosis or blocking angiogenesis? These questions suggest the need for further preclinical studies, for carefully targeted clinical trials, and for ways to speed up the sequence of trials required to obtain answers.

*A director and holder of stock options in ImClone.

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Molecular diagnosis of cancer by gene expression profiling

L.M. Staudt, *National Cancer Institute, NIH, Center for Cancer Research, USA*

Cancer patients within the same diagnostic category often vary considerably in their response to therapy and this clinical heterogeneity can be traced to molecular differences in their tumors. Gene expression profiling of diffuse large B-cell lymphoma (DLBCL) has revealed that this single diagnosis actually contains two distinct diseases. One DLBCL subgroup, termed germinal center B-like (GCB) DLBCL, strongly resembles normal germinal center B cells in gene whereas the other DLBCL subgroup, termed activated B-like (ABC) DLBCL, expresses genes that are induced in blood B cells upon mitogenic stimulation. Recurrent oncogenic events in DLBCL are seg-

regated according to the DLBCL subgroup distinction demonstrating that the DLBCL subgroups represent pathogenetically distinct diseases. GCB DLBCL patients have a relatively favorable clinical outcome. However, the DLBCL subgroup distinction did not fully capture the clinical variability of these patients, and therefore clinical data were used to discover individual genes with expression patterns that predicted overall survival. The majority of predictive genes fell into gene expression signatures that reflected the cell of origin, proliferation rate, and the host immune response to the tumor. 17 genes representing these biological features were used to create a multivariate model that divided the patients into quartiles with strikingly distinct 5-year survival rates of 73%, 71%, 34% and 15%. It is critical to improve the cure rates for those DLBCL patients predicted to have a poor response to conventional therapy. Gene expression profiling revealed that ABC DLBCL tumors express genes that are activated by the NF-kB family of transcription factors, and this was not a feature of GCB DLBCLs. ABC DLBCL cell lines had nuclear NF-kB due to constitutive activity of the IkB kinase. Inhibition of the NF-kB pathway was selectively toxic to ABC DLBCL cells, thus defining a new molecular target in the currently refractory subgroup of DLBCLs. Gene expression profiling is also very effective in defining the mechanism of action of oncogenes and tumor suppressors and thus can reveal new targets for therapeutic intervention. Recent results using this approach in the lymphoid malignancies will be presented.

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PLENARY SESSION 1

EGF-receptor targeting – clinical achievements

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Biology of ErbB/HER receptors

Y. Yarden, *Weizmann Institute of Science, Department of Biological Regulation, Rehovot, Israel*

Cancer arises following stochastic accumulation of several independent mutations in oncogenes and tumor suppressor genes, but inhibition of any single target can potentially reverse the oncogenic process. Unlike conventional therapeutic approaches, targeted therapy is based upon detailed mechanistic understanding of the oncogenic process and its molecular players. Currently, immunotherapies targeting cell surface receptors like HER2/ErbB-2 and the epidermal growth factor receptor (EGFR/ErbB-1) show effectiveness in breast and head and neck cancers, respectively. Likewise, highly specific low molecular weight inhibitors of tyrosine kinases effectively block certain malignancies. The mechanisms underlying these pharmacological strategies will be discussed in the context of a signaling network and the biology of ErbB/HER receptors

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Update on tyrosine kinase inhibitors

M. Hidalgo, *The Sydney Kimmel Cancer Center at Johns Hopkins, Drug Development ProgramRm 1M88, Baltimore, MD, USA*

Over the last few years, an increasing number of small molecules inhibitor of the EGFR TK have entered clinical trials. Though similar in their basic mechanism of action these agents differs with regard to their specificity for members of the EGFR family and the nature of their interaction with the ATP binding pocket of the receptor. Selected examples include ZD1839; OSI-774; EKB-569; CI-1033; GW216 and PKI1-16. In phase I studies these compounds were well tolerated and resulted in dose related diarrhea and cutaneous acneiforme rashes as the most significant toxicities. Pharmacological studies demonstrated adequate oral absorption and the achievement of biologically relevant plasma concentrations. Correlative biological studies showed inhibition of the targeted receptor and related pathways. Subsequent Phase II and III studies explored the efficacy of these agents. Key features of these trials include the use of phase II randomized design to explore the activity and toxicity of different doses and the early launching of randomized phase III studies. In non-comparative trials, objective responses and prolonged stables disease were observed in a substantial number of patients accompanied by improvement of symptoms. Preliminary data from the first large randomized clinical trials with Iressa in NSCLC, however, are negative. A major debate in the disease oriented development of this class is whether or not patients should be selected for disease-oriented studies based on the expression of the receptor. The lack of robust and well standardized methods to assess receptor expression as well as suitable tissues for analysis in most patients led to the predominant conduction of studies in non selected patient populations. While the non-selection of patients appeared appropriate, every effort should be made to collect tumor tissues for retrospective biological studies. In conclusion, a significant number of small molecules inhibitors of the EGFR are currently in clinical development. Although phase II studies showed a substantial number of patients with evidence of antitumor response, the impact of these agents in cancer mortality remains to be determined. Additional clinical and translational studies are needed to fully elucidate the real value of this target and its inhibitors in cancer treatment.

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Strategies to optimise anti-EGF receptor therapies

J. Baselga, *Vall Hebron University Hospital, Department of Medical Oncology, Barcelona, Spain*

Anti-epidermal growth factor receptor (EGFR) agents including monoclonal antibodies (Mabs) and tyrosine kinase inhibitors have clear, but modest, single-agent antitumor activity in epithelial tumors. A strategy to augment their activity would be to treat tumors that depend on the EGFR pathway. Early data from anti-EGFR Mabs clinical trials have shown that, unlike with anti-erbB2 therapies, higher levels of EGFR expression by immunohistochemistry (IHC) do not predict higher response rates. Therefore, a comprehensive evaluation of EGFR regulating and dependent genes may be required. We are performing pre and post treatment IHC assays to study tumor expression of EGFR ligands and activated EGFR, ERK, PI3K and Akt. The recently reported ability to profile gene expression using fixed paraffin-embedded tissues (FPET) could enable development of new molecular assays to guide selection of patients for anti-EGFR (and other targeted) therapies. We are collaborating with scientists at Genomic Health, Inc. who have developed new validated assays that can quantitate gene expression of up to 400 cancer-related genes from archival tumor blocks. We are exploring in head and neck carcinoma, colon carcinoma, and breast carcinoma the correlation between molecular profiles and other assessments, such as activation of the ERK's by IHC and clinical response to EGFR inhibitor therapy. Archival tumor tissue from 75 patients, including patients treated with anti-EGFR agents, are being assayed for quantitative expression of the HER kinase system, and for more than 160 other genes important in growth, proliferation, signaling, and apoptosis. The following HER kinase system genes are assayed: EGFR, ErbB2, ErbB3, ErbB4, TGFalpha, Amphiregulin, Betacellulin, HB-EGF, MMP9, Erk1, Erk2, STAT1, STAT3, STAT5A, and STAT5B. Initial data on 19 patients with head and neck cancer showed that EGFR and TGFalpha genes were expressed in the tumor tissue in all patients. Of note, there was a large variation in EGFR expression between patients (up to 100-fold) and a smaller variation in TGFalpha expression between patients (up to 15-fold). We will present and discuss the data we have obtained on the correlation between quantitative gene expression and IHC expression. The results from this study will be used to design larger studies required to confirm the clinical utility of these new FPET tumor assays to guide the selection of patients for EGFR-targeted therapy.

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Epidermal growth factor inhibitors: issues in clinical development

J. Dancey, *National Cancer Institute, National Institutes of Health, Investigational Drug Branch,Cancer Therapy Evalua, Rockville, USA*

EGFR is a member of the receptor tyrosine kinase family that includes HER2, HER3, and HER4. When EGFR-related ligands bind the HER receptors, they trigger a network of signaling pathways that may alter cell proliferation, survival, and motility. In cancer cell, hyperactive signaling through EGFR may occur through overproduction of ligands or receptors, or constitutive receptor activation. Such aberrant signaling activates pathways that stimulate many of the properties associated with cancer cells: proliferation, migration, stromal invasion, angiogenesis, and resistance to apoptotic signals. Because of the frequency of abnormalities in EGFR signaling in human cancer and because of the success of agents such as trastuzumab (Herceptin™) for the treatment of breast cancer and imatinib (Gleevec/Glivec™) for chronic myelogenous leukemia and gastrointestinal stromal tumors, which are directed at specific molecular alterations in cancer cell signaling pathways, EGFR is an attractive target for therapeutics development. Strategies to inhibit EGFR signaling include blocking ligand