Tuesday 19 November

Michel Clavel lecture

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Drug Resistance reversal - are we getting closer?

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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

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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

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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-