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Towards Improved Cancer Diagnosis and Treatment Founded on Current Developments in the Basic Sciences: Options for Intensified European Efforts

An EORTC Research Branch Scientific Advisory Board Consensus Paper

RAPID AND EXCITING advances during the past few years in the areas of molecular genetics, cell and developmental biology and immunology have resulted in an increased demand for improved and intensified interaction between basic research and clinical oncology in Europe. In order to accelerate the transfer of information and innovative concepts derived from "new biology" to the clinic, the Research Branch of the EORTC has appointed a Scientific Advisory Board, which consists of basic scientists and several clinical oncologists. The function of the board is to evaluate continuously developments in the basic sciences and their potential for application in clinical oncology. In a process of further re-evaluation within the EORTC Research Branch, recommendations from the Boards are subsequently condensed into Consensus Papers, the first of which is presented here. In addition, this paper describes the activities of several EORTC Research Groups concerned with the development and evaluation of anticancer agents. While primarily intended for European researchers and clinicians, the information contained in these Consensus Papers should also assist European science funding agencies—and the Commission of the European Com-

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munities (CEC) in particular—in evaluating research projects proposed jointly by high quality European research groups with complementary skills.

ESSENTIALS OF CANCER DIAGNOSIS AND THERAPY

Before describing a number of topical developments in the basic sciences that have a potential for applicability in clinical oncology, it should be stressed that the key requirements for successful cancer therapy remain (i) the earliest possible detection of small numbers of malignant cells (and their non-tumorigenic precursors) in order to avoid excessive phenotypic diversification in the course of tumour progression prior to therapy; and (ii) the development of strategies to improve the selectivity of the therapeutic attack on cancer cells. These two criteria must be used in evaluating the superiority of any new diagnostic or therapeutic approach over existing procedures. It is equally clear that fulfilment of these two criteria requires analysis of both predisposing (heritable) and somatic genetic alterations as well as of characteristic phenotypic changes associated with the stepwise process of malignant conversion and tumour progression in specific types of human cells. Further progress in the clinic, therefore, remains critically dependent on concurrent advances in the basic sciences.

GENETIC SUSCEPTIBILITY TO CANCER

One area of research for which well-planned cooperative efforts between basic scientists and clinicians in Europe are particularly important is that of genetic susceptibility to cancer. This field includes (i) the recognition, mapping and cloning of the genes that predispose to common cancers; (ii) the study of the basis for the variability of expression of specific genes seen in "cancer families"; (iii) the establishment of "marker phenotypes" indicative of the presence of predisposing genes during precancerous stages (analogous to the polyps in familial intestinal polyposis); (iv) the application of knowledge about inherited predisposition to the practical management of individuals at risk (which will require transfer of information to both the medical profession and the public and careful evaluation, because of the ethical issues involved); (v) elucidation of the interactions between DNA-reactive carcinogens and the human genome, including polymorphisms in the enzymatic bioactivation of potential carcinogens, genetic variability with regard to the expression of repair enzymes for specific types of DNA damage, and the performance of corresponding case-control studies; and (vi) to complement these studies, investigation of somatically acquired inactivating or activating alterations (deletions, mutations) in critical genes ("suppressor genes", proto-oncogenes) in specific cell types.

In all such studies, collaboration is essential in collecting the necessary material and in joint analysis of results. A good start has been made with the establishment of CEC Concerted Actions for familial adenomatous polyposis, the molecular cytogenetics of solid tumours (including thyroid cancer), and DNA repair and cancer; with the setting up of a collaborative study of familial breast cancer by the UK Cancer Family Study Group with wide European participation; and with a projected European collaboration for the study of familial ovarian cancer.

MINIMAL RESIDUAL DISEASE IN EPITHELIAL CANCER AND EARLY MICROMETASTASIS

Concerted efforts are also urgently needed to carry out systematic studies to detect small numbers of disseminated cancer cells (early micrometastasis). Such screening analyses can now be performed, and further refined, in particular for micrometastases originating from epithelial tumours by the use of epithelial cell-specific (e.g. anti-cytokeratin) antibodies as well as the polymerase chain reaction. These procedures will allow us, for example, to monitor patients in complete remission and the effectiveness of adjuvant therapy by repeated, long-term testing of bone marrow samples. After primary and regional tumours have been removed, residual disseminated cancer cells are likely to represent readily accessible targets for therapy (e.g. for antibodies and other high molecular weight agents). Investigations into the biological properties of these disseminated cells and their interactions with the microenvironment are of great importance because the cells may not always express fully malignant phenotypes ("dormant" tumour cells). The problem of minimal residual disease, particularly in epithelial cancer, represents a relatively neglected area, which requires novel investigative and therapeutic strategies.

MOLECULES INVOLVED IN SIGNAL TRANSDUCTION AND THE CONTROL OF CELL PROLIFERATION AND DIFFERENTIATION

Recent rapid advances in characterisation of the molecules that are involved in the positive and negative control of proliferation and differentiation in specific cell lineages, and their cellular receptors and intracellular signal transduction pathways, led the EORTC Research Branch to establish a special European Task Force on Cytokines (Chairman: H. Zwierzina, Innsbruck). Cytokines, i.e. soluble molecules that mediate cell–cell communication, are produced by different types of cells (e.g. cells of the immunohaematopoietic system, and endothelial and stromal cells). These molecules often interact with more than one cell type (and a given type of cell can interact with more than one cytokine) and exhibit overlapping activities. They can elicit distinct cell activation programmes and are involved, for example, in viral infection (interferons), interactions between monocytes, T-lymphocytes and B-lymphocytes (interleukins), the control of proliferation and differentiation of haematopoietic cells (colony-stimulating factors), and in the recruitment and control of tumour-associated macrophages (e.g. monocyte chemotactic protein, which belongs to the emerging superfamily of inflammatory cytokines). Of particular relevance in relation to cancer are cytokine-controlled mechanisms in tumour vascularisation and metastasis.

The cytokine network and the molecules involved in intracellular signal transduction represent relevant new foci for therapeutic approaches, including the induction of differentiation in malignant cells concomitant with a cessation of cell proliferation. In view of the emerging complexity and delicately balanced nature of these multicomponent control systems in normal cell populations, however, it is clear that the development of therapeutic strategies (e.g. the use of specific recombinant cytokines) will require more knowledge and a high degree of sophistication.

MOLECULES INVOLVED IN CELL ADHESION AND MOTILITY

The acquisition of invasive properties by tumorigenic cells constitutes an essential step in tumour progression. Recent observations indicate that invasive behaviour of epithelial cells may result from disturbances of intercellular adhesion (owing to the cessation of expression or inhibition of, for example, the cell adhesion molecule E-cadherin) and from the effects of motility factors. Further elucidation of the factors and molecular

mechanisms responsible for the invasiveness of tumorigenic cells, together with the development of procedures for specific interference with these processes (e.g. by inhibitors of proteases or toxic substrates for these enzymes), represents another important area of research for cancer therapy).

Joint European studies on molecular and cellular mechanism associated with tumour progression in malignant melanoma and precursor lesions, together with research into early diagnosis and therapy, fall within the framework of a CEC Concerted Action and the EORTC Melanoma Cooperative Group. Another CEC Concerted Action is focused on cell surface determinants of cancer cells and their role in metastasis and in the host immune response.

MONOCLONAL ANTIBODIES

Monoclonal antibodies (Mabs) have become available that are directed against cell type-specific and differentiation stage-specific molecules, against mutant oncogene or suppressor gene products, and against specific DNA constituents structurally modified by DNA-reactive carcinogens or chemotherapeutic agents. Their use is gaining considerable importance in terms of histopathological diagnosis and the analysis of cellular DNA repair capacity; however, with respect to cancer therapy, use of Mabs has not yet fulfilled initial expectations. In particular, Mabs carrying cytoxic agents—although active in a number of experimental models—cannot yet be applied advantageously in cancer patients. Continued efforts will be needed, for example, to develop suitable ligand and linker molecules as well as enzyme–antibody conjugates. CEC Concerted Actions have been established on immunoconjugates and on drug carrier systems in general, for use in cancer therapy. Recent advances in the production of genetically engineered antibodies (e.g. "humanised" mouse–human chimaeric antibodies, including reshaped antibodies in which only the hypervariable regions are of murine origin; and immunoglobulin molecules structurally optimised by site-specific mutagenesis) are highly encouraging. Recombinant antibody fragments optimised for application in clinical oncology may become available, with better tissue penetration due to their small size, lower antigenicity and suitable pharmacokinetics.

ANTISENSE OLIGONUCLEOTIDES

Another interesting lead is provided by the construction of antisense oligonucleotides. These molecules are designed to inhibit selectively the translation of mRNA from aberrant genes in cancer cells (e.g. the mutant Ha-*ras* oncogene). Oligonucleotides can also be targeted to the gene itself rather than to its mRNA, thereby blocking transcription. Current efforts are focused on antisense RNA, ribozymes, oligonucleotides linked to intercalating, nucleic acid-cleaving or crosslinking agents and strategies to improve the cellular uptake and transport of these agents.

SOMATIC CELL GENE THERAPY

An area of high potential, not restricted to the cancer field, is somatic cell gene therapy. Cells defective in the production of a specific enzyme or other protein may be compensated for, or replaced by cells into which the respective functional gene has been introduced. Although at present limited mainly to genes in cells of the haematopoietic system, this kind of "replacement therapy" may soon become feasible in other types of cells (e.g. fibroblasts) which could be re-implanted after gene transfer *in vitro*. The delivery of proteins of therapeutic value (e.g. interleukin-2 or tumour necrosis factor) via gene transfer might be more efficient than their systemic administration. Efforts in this area should be directed towards the construction of recombination-deficient retroviral vectors, together with the use of suitable promotors, in order to achieve cell type-specific expression and targeting of genes to tumours.

ANTICANCER DRUG DESIGN AND DEVELOPMENT

The field of anticancer drug design and development, although traditionally well established in several European countries, now needs to incorporate new approahes and methodologies. On the basis of studies of biomolecular conformation and dynamics, these include, for example, the modelling and molecular engineering of proteins with predetermined conformation and function and the use of antibody-directed drug design. New molecular targets for anticancer agents should be sought and exploited; these include critical genes and their products, cell surface constituents and molecules associated with signal transduction. Inhibitors of protein kinases may be of particular interest for interference with the latter pathways. In conjunction with anticancer drug design, investigation of drug resistance must be intensified, in close association with basic research on the molecular mechanisms underlying the capacity of mammalian cells for adaptive genotypic and phenotypic variation, including the control of genes that encode repair enzymes for different kinds of drug-mediated DNA damage. A mechanistic basis might thereby be provided for the circumvention or active modulation of drug resistance.

The procedures currently in use for preclinical evaluation of drugs should be extended to include new assay systems for evaluating the upcoming generation of anticancer agents developed on the basis of recent advances in molecular genetics and cell biology. More intensive collaboration between groups involved in "traditional" drug design and molecular cell biology appears necessary in this area. Likewise, a closer collaboration should be facilitated between chemists involved in drug synthesis on the one hand and pharmacologists and cell biologists working on new drug targets on the other.

NEW DRUG DEVELOPMENT PROGRAMME OF THE EORTC

Central to anticancer drug development and evaluation within the EORTC are the EORTC New Drug Development and Coordinating Committee (NDDCC) and its executive office, the EORTC New Drug Development Office (NDDO) in Amsterdam. Since its establishment in 1981, this programme has covered drug acquisition, *in vitro* and *in vivo* screening, drug formulation, animal toxicology, pharmacology and phase I and early phase II clinical trials. Efforts to intensify cooperation in preclinical research are exemplified by increasing collaboration in drug screening and by the establishment of an EORTC Joint Formulation Working Party. Preclinical research projects coordinated through the NDDO are channelled via the EORTC Research Groups, and initial clinical trials with new anticancer agents are performed within the framework of the EORTC Early Clinical Trials Cooperative Group (ECTG), with investigators in 15 European countries. Current attempts by NDDO to unify clinical monitoring procedures are encouraging, as is the close cooperation between NDDO and the Cancer Research Campaign in the UK and the US National Cancer Institute. Data produced in Europe can thus be easily transferred to the USA and *vice versa*.

The NDDCC has played a major role in establishing guidelines for the proper execution of the various steps in anticancer

drug development, including phase I and II clinical trials of investigational anticancer agents. On this basis, common guidelines should now be approved by health authorities throughout Europe (including European guidelines for somatic cell gene therapy). As a longer term strategy, both NDDCC and NDDO should further strengthen their interactions with other groups, in order to exploit more efficiently the expertise available in research institutions inside and outside Europe. The lack of a European equivalent of the National Cancer Institute (and the Food and Drug Administration) in the USA remains a matter of concern. Reorganisation and substantial upgrading (both scientifically and financially) of the EORTC New Drug Development Programme into the form of a European Coordinating Centre for the preclinical and clinical evaluation of anticancer agents will be important goals to achieve in the future.

EORTC RESEARCH GROUPS: INITIATING ELEMENTS FOR JOINT EUROPEAN ANTICANCER DRUG DEVELOPMENT AND EVALUATION

The work of several research groups within the EORTC Research Branch concentrates on the development and evaluation of potential anticancer agents in terms of molecular mechanisms, pharmacology and screening. These groups, although presently severely underfunded, represent a critical mass from which new directions for joint research in Europe can be initiated (e.g. European Task Forces focusing on particularly important new leads).

Thus, the Screening and Pharmacology Cooperative Group (SPG) identifies and evaluates potential anti cancer agents, often by a multicentre approach encompassing a range of *in vitro* and *in vivo* model systems. Structure–activity studies and the development of analogues are carried out in close collaboration between chemists and biologists, in order to ensure that only agents with real potential are carried on to clinical trials. In the Pharmacology and Molecular Mechanisms Project Group (PAMM), pharmacological principles are applied to the design and development of novel anticancer drugs. Pharmacokinetically guided dose escalation is used for reaching the maximum tolerated dose rapidly, but safely, in clinical phase I trials. Bioreductive agents are a particular interest. Cell membrane molecules and signal transduction pathways are being considered as drug targets. The Receptor Study Group (RSG) is concerned with the development and validation of methods that will permit reproducible determination of cellular receptors and the corresponding ligands (e.g. steroid hormone receptors, epidermal growth factor receptors and products of cellular proto-oncogenes). Moreover, the RSG determines the clinical value of new tumour markers and the interlaboratory reproducibility of their quantification. The Cell Culture and Characterization Group (CCCG) evaluates the cytotoxic activity of new anticancer agents on human cancer cells *in vitro* and the effects of cytokines (including haematopoietic growth factors) on the cells of solid tumours.

EUROPEAN BIOMEDICAL DATA BANKS AND CLINICAL TRIALS REGISTRIES

The establishment of a European computerised registry for recording ongoing clinical trials (phase II and phase III trials in particular) is urgently needed. At present, clinical oncologists in Europe usually have to consult the data bank of the US National Cancer Institute where many European trials are not registered (particularly those performed outside the EORTC). Of similar urgency is the establishment of a European data bank for normal and malignant human and animal tissues and cells (including hybridomas) as well as for nucleic acid probes stored by different institutes and clinics in Europe. An encouraging initiative in this direction has recently been taken in the form of the Data Bank for Biomedical Research (Interlab Project) financed by the Italian Ministry for University and Scientific and Technological Research.

PERSPECTIVES

Basic research in Europe has traditionally been conducted separately from clinical medicine. Joint projects to tackle scientific problems and efforts to transduce new results and leads into the clinic across the borders of individual European countries have unfortunately been exceptions rather than the rule. The EORTC Research Branch intends to strengthen European interactions in the cancer field by establishing means and structures for more effective collaborative research on topical problems in the basic sciences with potential applications in clinical oncology. There is no doubt that combined European efforts, with common strategies based on the efficient exchange and exploitation of information and expertise, avoiding unnecessary competition and duplication of work, will ensure more rapid advances and put European cancer research into an improved position *vis à vis* the USA and Japan. Appropriate funding of inter-European joint research programmes will be imperative in the future.

These objectives will require both that individual researchers be open-minded about international cooperation and that there be intra-European transparency with respect to national research programmes, e.g. by recruiting European groups of reviewers for local research projects and institutions. A further vital driving force will be provided by an intensification of the exchange of scientific personnel within Europe, particularly of young reseachers and clinicians. This can be achieved by upscaling European fellowship programmes, and by adjusting teaching and training programmes to common high quality standards that are recognised mutually on a European scale.