Pergamon

European Journal of Cancer Vol. 32A, No. 8, pp. 1300–1302, 1996

Printed in Great Britain
0959-8049/96 $15.00+0.00

PII: S0959-8049(96)00109-8

Meeting Highlight

Cancer Research in the European Community—Report from a Meeting in Stockholm in 1995

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A JOINT meeting between the European Commission and the Karolinska Institute was held in Stockholm in the Autumn of 1995. The aim of the meeting was to highlight cancer research projects supported by the European Commission. Basic science and clinical studies were presented, and the idea was to delineate important areas for support by the European Commission in the future. Here we have tried to highlight some of the topics, well aware of the fact that many that are not mentioned may be as important as those mentioned in this overview.

In terms of basic science, the role of viruses and genes were discussed. A presentation by Dumont (Brussels) focused on the control of proliferation and differentiation of normal and malignant thyroid cells in the search for new protooncogenes. Thyroglobulin, thyroperoxidase and TSH receptors as differentiation markers were shown to be decreased in various human adenocarcinomas and almost absent in anaplastic carcinomas. These markers were used for diagnosis and differential screening of cDNA libraries from thyroids. This allowed the identification of several unknown clones involved in cell regulation. Systematic screening of hereditary medullar thyroid carcinoma was performed in eight families with multiple endocrine neoplasia. Ret protooncogene point mutations were found in all affected members of each family.

Plachov's (Münster) presentation focused on the role of the human PAX2 and PAX8 transcription factors. These genes are expressed in the embryonic kidney, the developing CNS and the adult thyroid. Furthermore, overexpression of both PAX2 and PAX8 was demonstrated in Wilms' tumours that originate from human embryonic kidney. The function of the wild type and mutated versions of these genes was further analysed in transfection experiments. PAX8 appears to be part of the cAMP signalling pathway and mediates TSH dependent gene activation of thyroid cells.

Poupon (Paris) analysed Homeobox-containing genes and showed that malignant transformation is associated with altered *HOX* gene expression in kidney and colon cancer cells. *HOX* gene expression was further analysed in primary and metastatic human small cell cancers (SCLS) by Northern blotting. The combined results were in favour of the involvement of *HOX* genes in tumour progression.

Lower animal species can be useful genetic models for cancer research and tumour suppression (Mechler, Heidelberg). Genetic analysis of *Drosophila* has revealed that gene inactivation can lead to tumorigenesis in specific tissues during the fruit fly's development. Five tumour suppressor genes, controlling growth of haematopoietic organs, were cloned and analysed. Some of these genes display homologies with nuclear carrier proteins found in *Xenopus* (importin 60), yeast (SRP1), or humans (RCHI). Analysis of the *oho 23* gene showed that this gene encodes the S21 ribosomal protein, whose absence may cause tissue specific neoplasia, a hitherto unknown mechanism of tumorigenesis.

Invading foreign DNA or RNA molecules are also involved in tumour formation (e.g. HBV, HCV, HPV, EBV, HIV). Epidemiological studies by Brechot (Paris) revealed a very close relation betwen Hepatitis B virus (HBV) and Hepatitis C (HCV) infection and primary liver cancer. This was based on serum and liver samples obtained from patients with primary liver cancer in different European countries.

Graessmann/Levrero (Berlin/Rome) analysed in more detail the function of the HBV-X protein *in vitro* (cell culture) and *in vivo* (transgenic animals). Microinjection and transfection experiments confirmed that the X-protein is mainly found in the cytoplasm of the recipient cells, and intranuclear localisation of the X-protein was not demonstrable. Furthermore, tumour revertant rat cells (REV), which still synthesise the wt SV-T antigen, but not the grant-parental cells (REF52), became fully transformed again by the HBV-X protein. This indicates that expression of the X-protein *per se* is not sufficient for maximal cell transformation, but that the X-protein may have a tumour-promoter function. Transgenic animals that express the X-protein in mammary gland cells during late pregnancy and lactation exhibited an altered gland morphology.

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The human T-cell leukaemia virus type I (HTLV-I) is causally associated with adult T-cell leukaemia/lymphoma (ATLL). From HTLV-I infected patients, only 5% develop ATLL. It is unknown whether HTLV-II, which is commonly found in association with HIV-I in Europe, contributes to the morbidity and mortality of ATLL patients. A network was established (Weber, London) for the diagnosis of HTLV-I/II infection and more than 4 million blood samples were collected in different European countries.

That viruses are not only associated with tumour formation but may also cause oncosuppression was further discussed by Rommelaere (Heidelberg). It was demonstrated that the preferential killing of tumour cells by the parvoviruses is in part due to the greater extent of virus replication in transformed cells, compared to normal cells. The P4 promoter directed NS/Rep proteins are not only essential for virus replication, but these proteins are also cytotoxic.

Progress reports were presented concerning 13 Concerted Actions on childhood cancers or haematological disorders.

The search for the molecular defect in *Fanconi anemia* (FA) by E. Gluckman and associates has revealed that at least five complementation groups exist (based on fusion of EBV transformed cell lines). Complementation group A (*FAA*) is the largest group. Genetic linkage studies have demonstrated that the affected gene in FAA is located on 16q24.1-24.3. The affected gene in complementation group C (*FAC*) has been cloned and so far eight different mutations have been identified. Geographic clustering of FA subtypes has been found in Europe, e.g. the *FAA* gene is most frequently involved in Germany, whereas in The Netherlands, *FAC* gene aberrations are more frequently found. Interestingly, all the studied FAC patients in The Netherlands had the same mutations. The first prenatal diagnosis of a (healthy) dizygotic twin-pregnancy, being a carrier of one mutated FAC allele, was reported.

H. Jürgens and associates utilised a PCR-based staging in Ewing's sarcoma. The characteristic molecular aberrations in Ewing's sarcoma involve the *EWS* gene on chromosome 22 and concern t(11;22) in about 90% of cases and t(21;22) in about 5% of cases. Both aberrations are detectable by PCR analysis and were used for precise staging, thereby allowing better discrimination between localised tumour and metastatic disease in bone marrow and/or blood. The PCR studies were also used for detection of residual disease during or after treatment in patients with disseminated Ewing's sarcoma.

G. Gahrton and colleagues acted in several directions to optimise bone marrow transplantation with unrelated donors. Bone marrow transplantation (BMT) has proven to be an efficient treatment in many haematological malignancies and is also becoming important in the treatment of solid tumours. In 1994, approximately 9000 marrow transplantations have been performed in Europe, mainly for leukaemia, malignant lymphoma and solid tumours. In two-thirds of cases, it concerned autologous BMT and in one-third allogeneic BMT. Most donors (about 2700) were HLA-matched siblings, but matched volunteers were used in increasing numbers—now 600. A European database of HLA-typed volunteer donors is being built, and a registry of "Bone Marrow Donors Worldwide" now contains 2.7 million typed volunteers. Comparison of unrelated donor (UD)-BMT with identical sibling (ID)-BMT revealed that HLA-matching is extremely important for results of UD-BMT to approach those of ID-BMT. UD-BMT studies in chronic myeloid leukaemia revealed that conditioning, graft-versus-host disease prophylaxis (without T-cell depletion), short interval between diagnosis and BMT, and young age are important factors for outcome.

Studies by P.G. Pelicci and associates on acute promyelocytic leukaemia revealed that transfection-induced expression of the APL-specific PML-RARA fusion protein blocks differentiation and inhibits programmed cell death, but makes the cells sensitive to retinoic acid (RA). The PML-RARA fusion protein appears to contain most functional characteristics of the normal PML and RARA proteins. Apparently, the combined functional characteristics are responsible for the differentiation block and oncogenic potential on the one hand and for the sensitivity to RA-induced differentiation on the other hand.

The study by B. Van Camp and colleagues investigated the immunopathogenesis of multiple myeloma. Molecular studies of immunoglobulin (Ig) gene rearrangements have demonstrated that multiple myeloma patients have peripheral blood monoclonal B cells with identical hypermutated V-D-J gene rearrangements, but without Ig class switch. Based on these findings, it is assumed that the multiple myeloma cells originate from germinal centre B-cells, which migrate from the lymph node to the bone marrow, where they differentiate into mature tumour cells, due to interaction with marrow stroma.

L.M. Fischer and associates tried to unravel the molecular basis of anticancer drug action on topoisomerase II (topo II), especially the role of the ternary DNA complex in drug sensitivity and resistance. Information from the project might lead to design of selective more potent drugs and the development of strategies to circumvent cellular resistance. Topo II is an important target for anticancer agents, such as anthracyclines. Topo II catalysed chromosome segregation by double strand break mechanisms. Two types of topo II exist in man: topo IIα (gene on chromosome 17q21-11) is expressed during G2 and M phases of the cell cycle, while topo IIβ (gene on chromosomes 3p24) exhibits continuous expression.

A. Hagemeijer and colleagues tried to bridge the gap between research units and diagnostic laboratories working on molecular cytogenetics in haematological malignancies. Special attention is paid to multi-target translocations such as 11q23 aberrations (*MLL* gene) and 12p13 (*TEL* gene). Important aims are the design of powerful probes for fluorescence *in situ* hybridisation (FISH), such as for *BCR, ABL, MLL, ETO*, etc., and the development of multiplex PCR for rapid detection of chromosome aberrations.

Detection of minimal residual disease (MRD) in acute leukaemia patients is essential to obtain insight in the effectiveness of cytostatic treatment. Such information can be used to determine whether MRD detection can be applied for stratification/adaptation of treatment protocols. J.F. San Miguel and colleagues tried to develop and standardise methods for MRD detection. Two main types of techniques were used: (1) immunological marker analysis using multiparameter flow cytometry with triple antibody combinations; and (2) PCR analysis of leukaemia-specific sequences. The participating centres for flow cytometric MRD detection now use the same antibody panels and have agreed on the methodology for sample preparation techniques, data acquisition and data analysis. The first results of the centres are highly comparable. Progress in the molecular MRD studies include the central production of DNA probes and PCR primers and their evaluation for detection of junctional regions of rearranged Ig and TcR genes as PCR targets. The develop-

ment of standardised primer sets for detection of chromosome aberrations is under investigation.

One aim with the Stockholm meeting was to bridge from basic to clinical research. However, a number of obstacles were encountered in trying to obtain this goal. Two examples are provided to clarify the point.

The first is based on the report by P. Herdewijn (Leuven, Belgium) concerning the anticancer activity of antisense oligonucleotides targeted against mutated *RAS*. The limiting factors in the activity of such compounds are their instability and their poor cellular uptake. The authors therefore modified the structure of the oligonucleotides by making partially complementary duplexes to increase stability and by coupling the duplexes to a propanediol moiety to increase cellular uptake. This modified oligonucleotide was shown to be 400 times more effective in inhibiting cancer cell growth *in vitro* than the unmodified one. It was also found to be effective *in vivo* in animal models after direct injection in the tumour mass. This compound could now be tested in human malignancies carrying appropriate mutations of *RAS*.

However, in the academic field, the cost of running such a trial might be prohibitive. *RAS* mutations, as well as the antisense technology, are not patentable because they are in the public domain, and therefore no private company is willing to invest in clinical trials. This raised the more general issue of the funding of clinical trials with compounds that are promising for the treatment of cancer but which, for economic reasons, are of no interest to private companies. These are cases in which public funding would be the most appropriate, and there the support of the European Community could be very helpful.

The other example is related to the need for a co-ordinating structure at the European level for new types of clinical trials. Recent developments in tumour immunology allowed the identification, mainly by the group of T. Boon in Brussels, of the molecular nature of a number of human tumour antigens that are the targets of a cytolytic T cell response of the host against its own tumour cells. These antigens are presented to cytolytic T lymphocytes by HLA class I molecules, and consist of small peptides derived from proteins that are exclusively expressed in the tumour cells. Because of their tumour-specificity, these antigens could constitute the basis of future cancer vaccines. Small pilot studies with such vaccines are under way in melanoma patients, and preliminary results seems very promising. If this is confirmed, the next step will be the running of large-scale multicentre trials.

The organisation of large-scale multicentre trials is not easy because the recruitment of patients is based on very specific selection criteria: eligible patients have to express a given HLA molecule and their tumour must express the relevant antigen. Therefore, the patients have to be HLA-typed, and their tumours tested for the expression of the gene encoding the antigen by PCR on RNA extracted from the tumour. To organise such complex studies, there is a need for co-ordination at the international level, which raised the more general issue of the need for a supranational structure that should be responsible for such co-ordination. In Europe, a structure like the EORTC would be well suited to this. In this vein, H. Zwierzina (Innsbruck) reported the recent creation within the EORTC of a new working group called "Biological Therapeutics of Development Study Group", which is devoted to the study of new treatments based on biological response modifiers in cancer.

A related issue was raised by D. Gabel (Bremen, Germany) when he asked: "How to conduct clinical trials on European schemes rather than on National schemes". This question referred to the huge differences in the national policies of the different European countries on clinical research and more particularly on the review process and insurance coverage. These differences definitely impede fruitful international collaboration between European clinical researchers. To harmonise the different national policies, a solution would be to support a European co-ordinating organisation that would have the means to propose common guidelines on this matter.

European countries differ not only in their policies on clinical research, but also in their efficiency in applying well-known cancer treatments, and this was clearly reflected in the results of a large epidemiological study called "Eurocare" reported by F. Berrino (Milan, Italy). This study, which was based on the cancer registries of 12 European countries, unmasked striking differences in the survival rates of cancer patients depending on the country in which they were treated. The origins of these differences are not yet clear and are presently being studied. Once again this highlights the need for translational research including education of physicians and optimal procedures to disseminate the results of high quality clinical trials.

Also, there is currently an increasing number of clinicians involved in clinical research who face numerous obstacles such as cost of clinical research, workload, legal and administrative matters, lack of support from hospital directors and difficulties in obtaining adequate local data management. Less than 10% of medical doctors have ever participated in clinical trials and less than 5% of cancer patients are currently included in clinical trials.

Therefore, support from the European Commission is important. As shown by the results of the projects, highlighted in this review, with moderate funding European cancer research can flourish.