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Meeting Report

17th meeting of the European Association for Cancer Research in Granada, Spain, 8–11 June 2002

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The 17th Meeting of the European Association for Cancer Research (EACR), held in the spectacular Palacio de Exposiciones y Congresos in association with ASEICA, the Spanish Association for Cancer Research, attracted almost 700 delegates. This excellent modern facility was a perfect counterpoint to the beautiful old Alhambra, Granada's most famous landmark. The Conference Chairman, J.C. Lacal and Scientific Co-Chairs E. Olah and R. Rosell, together with the Scientific Organising Committee and the able FECS Secretariat, produced a packed and varied programme. Plenary Sessions were combined with parallel Basic Science and Translational Symposia, world-renowned Keynote speakers, plus 60 oral proffered papers and 315 posters. The venue being Spain, it goes without saying that the hospitality was also exceptional, with many social events continuing long after midnight. Indeed, the only complaint heard was: "we are on Spanish time at night, and British time in the mornings!" We hope that the following report gives a flavour of the event, and encourages you to attend the 18th Meeting of the EACR, 3–6 July 2004, Innsbruck, Austria.

1. Plenary symposium: cancer epidemiology

The first session of the meeting set the scene with a lecture by **Paul Kleihues** on cancer control in Europe. There was good news and bad news. On the positive side, cancer-related mortality is declining in the Nordic countries; France, Switzerland and the UK and much of this can be ascribed to a reduction in tobacco smoking, particularly amongst men. Survival rates have increased significantly for most cancer types. On the negative side, many Central and Eastern European countries are still seeing sharp increases in cancer mortality. Cancers associated with affluence, such as those of breast, prostate, colon and endometrium are on the increase in all European countries, presenting a challenge for public health measures.

F. Kadlubar gave a comprehensive overview of molecular epidemiology, listing the familiar biomarkers of genetically increased cancer susceptibility, polymorphisms in carcinogen metabolism, in DNA repair and genes controlling cell growth and survival. Current studies are aimed at elucidating multi-gene–environmental interactions in breast and prostate cancer, and as an example a study of DNA adducts in mammary epithelium in relation to the metabolic genotypes *NAT1* or *NAT2* and the exposure to hair colouring products was described. The final talk on infection and cancer was presented by **N. Munoz**. It is estimated that infections account for 25% of cancers in developing countries compared with 9% that are related to tobacco. These figures are exactly reversed in developed countries. It is now widely accepted that certain types of human papilloma virus (HPV) are not only the main cause of cervical cancer, but that infection with the virus is necessary for its development and this fact is now being used in vaccine prevention trials.

2. Basic science symposium: signalling: from the cell surface to the nucleus

In this symposium, **J.L. Bos** (Utrecht, NL) described the Ras-signalling pathway and the connection between Ras and its close relative Rap1. One of the prevailing models of Rap1 function is the regulation of the ERK pathway by cyclic adenosine monophosphate (cAMP). However, using a novel cAMP analogue that specifically

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activates Epac, a cAMP-responsive guanidine nucleotide exchange factor for Rap1, it was found that activation of Rap1 does not modulate ERK activity. The most prominent effect of Rap1 activation on cells is increased cell adhesion mediated by integrins, but how Rap1 activates integrins is still unclear. The epidermal growth factor (EGF) receptor family was discussed by **W. Gullick**, who described nuclear localisation of one of the ligands for this receptor family, NRG1. The function of this localisation is still unclear. In addition, a very nice simulation of receptor clustering was presented which can be viewed at http://www.cs.ukc.ac.uk/people/staff/cgj/researchreceptors.html.

M. Pierotti discussed the Ret receptor signalling pathway. This receptor is mutated in Hirschsprung's disease (loss-of-function) and in multiple endocrine neoplasia (MEN2A and 2B). One mutation (C620R) is particularly interesting since the result is both MEN2A and Hirschsprung's disease. This mutant is largely a gain-of-function mutant like the other MEN2A mutations, but it fails to induce cell migration, most probably through its inability to activate Src. **H. Grunicke** described the mechanism by which the novel protein kinase C zeta regulates the ERK pathway via interaction with Ksr. In addition, atypical protein kinase Cs (PKCs) may be involved in cell survival. For instance, an interfering PKC iota mutant strongly induces apoptosis, particularly in synergy with cisplatin (*cis*-Pt). In addition, PKC zeta induces cell survival by phosphorylation of the pro-apoptotic Bad protein.

3. Translational research symposium: genetic susceptibility to cancer: recognition and application to prevention

Dr Peter Devilee (Netherlands) presented evidence for the existence of modifiers of the phenotype—in particular, age at onset—in carriers of mutations in the genes *BRCA1* and *BRCA2*. He then summarised current data in the search for these modifying genes. His conclusion was that there is preliminary evidence for a number of candidates, but none of this evidence is yet conclusive. **Dr Mary-Claire King** (Seattle) summarised the results of her population-based studies in Long Island, relating to the penetrance of *BRCA1* and *BRCA2* mutations, and the impact of non-genetic factors. The most striking finding was that, in contrast to the results from other groups, she found that the age-related risk of *BRCA1* and *BRCA2* mutations in individuals with no (or a modest) family history of cancer was very similar to the risk in members of extended families. The reasons for this divergence from the results of other groups were not clear.

Dr Bruce Ponder (Cambridge UK) addressed the question of the kinds of genes that might account for the approximately 80% of familial clustering of breast cancer that is not explained by mutation of *BRCA1* and *BRCA2*. He presented the results of epidemiological modelling which suggested that the effects of common genetic variance might account for a substantial proportion of this risk. In this model, there may be as much as a 40-fold difference in risk between women at the high end of the risk spectrum and at the low end. This has potentially important implications for the planning of interventions. The search for the common variants underlying the risk distribution is at a very early stage and is proving difficult.

4. Keynote lecture: the priorities of translational cancer research in the 21st century

Professor Paul Workman presented his view on the future of cancer research and how scientists can benefit from the latest technological developments. In the next 5–10 years, all cancer genes will be defined, the impact in the provision of novel potential therapeutic targets will be great, as will improvements in the use of biomarkers for detection, diagnosis, prognosis and monitoring disease. The use of tools such as microarrays, proteomics and molecular imaging will lead to individualised therapies. Thus, the future will involve personalised, genomically driven, long-term management of cancer as a chronic disease. Professor Workman also reported on new inhibition strategies already in clinical use (e.g. Trastuzumab (Herceptin) and STI-571 (Gleevec)) or under development (Raf kinase inhibitors and the Hsp90 inhibitor 17AAG). Hsp90 is a molecular chaperone that is often overexpressed in human tumours. It is essential for the stability and function of several oncogenic proteins (e.g. c-*erb*B2, Raf, CDK4, mutant p53, hTERT) and thus has the potential to be a one-step combinatorial therapeutic target in many cancers.

5. Basic science symposium: programmed cell death: the mechanisms, pathways, role in cancerogenesis and drug resistance

P. Krammer discussed the signals emanating from stimulated death receptors, particularly CD95 (APO-1/Fas). Upon receptor stimulation, a death-inducing signalling complex (DISC) is formed which leads to the initiation of a caspase cascade. Drug treatment of liver carcinoma cells causes CD95 (p53-dependent) and CD95L (ligand; AP-1-dependent) upregulation and apoptosis. CD95L-positive tumours might be able to counter-attack and kill attacker lymphocytes. **G. Kroemer** discussed the second important pathway of apoptosis signalling involving the mitochondria, the release of pro-apoptotic molecules from them (e.g. cytochrome

C) and the formation of the apoptosome and consecutive caspase activation. The major emphasis of his presentation was on the life and death regulation at the mitochondrial membrane.

C. Dive described the regulation of Bcl-2 family members in drug sensitivity and resistance in detail and discussed data with drug-sensitive and -resistant cell-lines.

B. Burgering reported the role of protein kinase B (PKB)/c-Akt in protecting from apoptosis, its role in reactive oxygen species (ROS)-induced cell death and how forkhead transcription factors are involved in life–death switching. Overall, the session focused on molecular events in the regulation of drug sensitivity and resistance and on molecules that determine survival.

6. Translational research symposium: angiogenesis: from basic to therapeutic applications

R. Giavazzi (Milan) described preclinical studies evaluating inhibitors of neoangiogenesis which may act at different stages of angiogenesis and on different cellular compartments. Combining antiangiogenic inhibitors such as SU6668 or matrix metalloproteinase (MMP) inhibitors with conventional therapy (e.g. taxanes) gave more effective control than either agent alone. Using drug-resistant cell lines, it was also clear that even 'cytotoxic' drugs could have indirect anti-angiogenic effects. **D. Marmé** (Freiburg) focused on the TIE-2 receptor and its ligands, angiopoietin 1 and 2, which act antagonistically to regulate blood vessel stability. He presented evidence that inhibition of this system could be as potent as inhibition of the vascular endothelial growth factor (VEGF)-FLK-1/FLT-1 pathways, and again the possibility exists to target both for a maximum effect. Finally, Dr Marmé reported on early clinical trials with a VEGF-receptor tyrosine kinase inhibitor (PTK 787/ZK222684) which showed evidence of biological activity and some stabilisation of disease.

P. Vermeulen (Antwerp) gave a pathologist's viewpoint and stressed the importance of objective, measurable criteria of response. Recognising the diversity of targets, he stressed the need for a multiplicity of predictive markers, and noted that 'vascularity' is not a measure of 'angiogenesis'. International consensus on angiogenesis quantitation is shortly to be published in the *EJC* [1] , and will emphasise the fact that integrated information can be obtained from simple pathological observations. It is also increasingly recognised that some tumours can develop angiogenesis-independent patterns of growth. **G. Gasparini** (Rome) concluded the session with an overview of tumour angiogenesis and therapeutic opportunities. He described the science of 'angiomics'—attempts to define the molecular phenotype of proliferating endothelia—and the search for new targets downstream of activated VEGF receptors. Again, the theme of targeting multiple angiogenic pathways emerged. He nevertheless reminded us that optimism must be tempered with caution, since some anti-angiogenic therapies had resulted in serious adverse events.

7. Plenary symposium: new approaches and targets for cancer intervention

All contributions in this session focused on means to interfere with disease-promoting signalling mechanisms. **Axel Ullrich** (Martinsried, Germany) stressed the role of the EGF receptor as a master switch of growth-promotion in tumours. EGFR can be trans-activated by numerous other receptors including serpentine receptors for thrombin and bombesin. In part, this is mediated by the activation of a membrane metalloproteinase which releases membrane-bound EGF. This appears an attractive therapeutic target, and a metalloprotease inhibitor (BB94, batimastat) revealed promising results. He has also identified a germ-line mutation in the *FGFR-4* gene. Carriers of this mutation (G388R) bear a significantly higher risk of developing a more aggressive type of breast cancer with early nodal metastases. Thus, this mutated fibroblast growth factor (FGF) receptor represents another target for the selective blockade of a cancer-promoting signalling mechanism.

L. Meijer (Roscoff, France) reported on new highly selective inhibitors of cyclin-dependent kinases which interfere with the adenosine triphosphate (ATP)-binding site of the enzymes. Despite high *in vitro* selectivity, some of these compounds exhibit a surprising cross-reactivity against glycogen synthase kinase 3 (GSK-3ß). In addition to their cytostatic effect, these agents trigger apoptosis in proliferating cells, whereas differentiated cells such as thymocytes or neuronal cells were protected from apoptosis by a still unknown mechanism. **Thomas Blundell** (Cambridge, UK) emphasised the relevance of multiprotein complexes in signalling cascades for maintaining sufficiently high signal: noise ratios. By employing X-ray analysis of 3-D structures of multiprotein complexes, he characterised the interacting surfaces in signalling complexes such as the FGF1: FGF2: heparin complex, cdk6: p19 INK4d and an Xrcc4 dimer with DNA ligase IV. Dr Blundell suggested consideration of these highly specific, well-defined protein surfaces as novel targets in rational drug development.

8. Basic science symposium: molecular mechanisms of invasion and metastasis

C. Lopez-Otin (Oviedo, Spain) gave an excellent overview of the importance of matrix metalloproteinases in cancer, and introduced the new ADAM-TS family. Tumour and host cells produce multiple

enzymes (and inhibitors), and it is their net effect which influences invasion and angiogenesis. He stressed the role of host-derived stromelysins, which are especially prominent at invasive edges, and are induced by a 'reciprocal dialogue' between tumour and host. Indeed, Dr Lopez-Otin left us with the exciting concept of aiming to define the 'degradome'—i.e. the proteolytic profile of individual tumours—which may enable better predictions of their metastatic potential and responsiveness to selective MMP inhibitors. **J.P. Thiery** (Paris, France) gave a very lively talk on the mechanisms by which growth factor signalling pathways, in concert with cell adhesion systems, influence epithelial–mesenchymal transitions (EMT) and cell motility. This requires Ras and Raf activity and involves transcription factors such as twist, slug and snail. He showed how autocrine signalling through FGFR and other tyrosine kinase receptors can mediate EMT. Interestingly, in human bladder cancers, loss of FGFR2b is associated with a poor prognosis, whereas FGFR3 is often mutated. He concluded with a model of two distinct routes of bladder cancer progression in which different molecular phenotypes predominate (see *Nat Rev Cancer* 2002, **2**, 442).

D. Welch (Pennsylvania, USA) gave a fascinating insight into the trials and tribulations of hunting metastasis suppressors, using the gene on chromosome 6q22 in melanoma as an example. Initially, *KiSS1* was identified as the likely candidate, but was found to be located on chromosome 1; so is KiSS1 regulated by the chromosome 6 gene(s)? By comparing metastatic and non-metastatic clones using microarrays, another gene (*V-Dup-1*) was found, but this also mapped to 1q. The story so far is that a gene called *DRIP130* has now been identified in the 6q22 region which may be able to regulate KiSS1 and V-Dup1 and which, when transfected, suppresses melanoma metastasis. This intriguing and complex series of experiments teaches us that we may need to consider suppressor *pathways* rather than single genes. **S. Eccles** (London UK) concluded with an overview of some molecular mechanisms of metastasis using the c-erbB family of oncogenes as an example. She listed six 'hallmarks of metastasis' and showed these could all be induced by erbB receptor activation, e.g. by altering cell–cell and cell–matrix adhesion, selectively upregulating MMPs and also increasing production of VEGF-A and VEGF-C. Although the receptors are good targets for therapy, shared downstream signalling pathways may yield pivotal points for intervention and provide a two-pronged attack on both tumour and host cell functions which contribute to invasion and angiogenesis.

9. Translational research symposium: new therapies

J.P. Armand described a journey of 6 years—from deriving active compounds from a variety of marine organisms to phase I and phase II trials. Ecteinascidin-743, an alkaloid isolated from the tunicate *E. turbinata*, interacts with DNA and nuclear proteins. It has shown 50% response rates, for example, against advanced drug-resistant sarcomas, although liver toxicity has been observed. There is evidence for synergism with other drugs such as platinum and taxanes. Aplidin, a depsipeptide isolated from another tunicate, *A. albicans*, has anti-angiogenic properties and is in phase I trials against colorectal and renal cancer, but has shown muscular toxicity. Kahalalide F from the mollusc *E. rubefescens* appears to act against lysosomes and a phase I trial in patients with androgen-independent prostate cancer is ongoing.

J.C. Lacal and co-workers have been investigating the Kennedy pathway which involves the generation of phosphorylcholine (PCho) by the activity of the PCho-specific phospholipase D followed by phosphorylation by choline kinase. This pathway is activated in Ras-transformed cells and 20% of tumour samples overexpress choline kinase. Compounds with inhibitory activity against choline kinase were synthesised, yielding the lead compound hemicholinium 3. Although this proved highly toxic, other compounds showed a selective and reversible inhibition of proliferation in oncogene-transformed cells and inhibitory effects against human tumour xenografts.

I. Judson addressed the major challenge of the switch from unselected cytotoxic therapy towards molecularly targeted and individualised therapy and identifying whether the proposed mechanism is occurring in a patient's tumour. He described how serial tumour biopsies have been used to verify the ability of the Hsp90 inhibitor 17-AAG to cause depletion of Raf-1 in tumour tissue. This is, however, not a very practical approach. He went on to demonstrate that new non-invasive imaging techniques such as magnetic resonance spectroscopy (MRS) can be used to detect changes in energy balance and membrane composition inside tumours in response to therapy.

J. Soria focused on carcinogenesis and premalignant changes used to monitor chemo-preventive treatments. Telomerase activation was frequently observed in multiple bronchial biopsies from smokers, regardless of the histopathological appearance. Results from a placebo-controlled retinoid chemoprevention trial suggest that telomerase reverse transcriptase expression may be a useful surrogate biomarker of efficacy, but others, such as p53, Fhit, EGFR and RAR-beta also need to be considered.

10. Keynote lecture: key signalling pathways in colon cancer

Hans Clevers showed how mutations in components of the Wnt signalling pathway contribute to oncogenic

transformation in colorectal cancer (CRC). Beta-catenin forms a complex with the transcription factor T cell factor (TCF), but the genetic programme induced has not been fully characterised. In elegant experimental studies, Clevers showed that β-catenin and TCF maintain the undifferentiated status of crypt progenitor cells, and this programme is aberrantly reactivated in CRC cells. Significantly, inhibition of the β-catenin–TCF activity restored the differentiation programme, in spite of the presence of other genetic defects, identifying this pathway as a promising target for therapy.

11. Basic science symposium: DNA repair: cancer associated deficiencies

Dr J. Hoeijmakers gave an overview on DNA damage repair mechanisms not only identified in experimental systems, but also those found to be of clinical significance. This insight into the underlying molecular mechanisms should provide opportunities for new therapeutic strategies to both pre-empt and overcome resistance to DNA damaging agents including radiotherapy and cytotoxic drugs. **P. Cejka** reported on the seven mismatch repair genes so far identified in man and attempted to elucidate their function in hereditary non-polyposis colon cancer. To this end, the peptides were expressed and used to complement extracts from different mismatch-repair-deficient cell lines. The ultimate goal of this group is to reconstitute the mismatch repair (MMR) process from its individual recombinant components, and to identify missing components using proteomic analyses.

Y. Shiloh reviewed the role of the ataxia telangiectasia (ATM) protein kinase which phosphorylates multiple substrates in response to DNA double-strand breaks. This gene is mutated in ataxia telangiectasia and linked to a predisposition to cancer. Although its role in DNA damage detection is well known, evidence is emerging that it may also be involved in other aspects of cellular homeostasis such as responses to growth factors and hormones, and maintenance of the cytoskeleton. It is therefore now seen as a key front-line element in the cell's defence against environmental stresses that may be linked to cancer risk. **A. Venkitaraman** concluded this session by describing the functions of the *BRCA2* tumour suppressor gene. He produced evidence that this gene is essential to maintain chromosomal integrity. This is mediated via regulation of RAD51 during homologous recombination. However, loss of *BRCA2* function leads to cell death, and secondary mutations in genes such as those involved in the spindle assembly checkpoint are required for malignant transformation.

12. Translational research symposium: immunotherapy and monoclonal antibodies

C. Huber began by describing how allogeneic stem cell transplantation for the treatment of leukaemia is limited by Graft versus Host Disease (GvHD) or alternatively by relapse or opportunistic infections due to immunodeficiency. His goal is to change the repertoire of transplanted T cells in an effort to avoid both these problems. The strategy employed is first to selectively eliminate alloreactive T cells by means of activation-induced cell death and secondly to break immunodeficiency to pathogens of leukaemic cells by T receptor transfers. These pioneering studies hold great promise for future therapeutic success. **G. Parmiani** described the activity of a vaccine containing autologous tumour-derived heat shock protein (HSP) peptide complex-96 (Oncophage) in metastatic melanoma patients. HSPs chaperone a repertoire of melanoma-derived peptides and hence can induce activated T cells to recognise melanoma antigens. Some encouraging clinical responses were observed, with evidence of tumour-specific T cell responses post vaccination (see *Annu Rev Immunol* 2002, **20**, 395).

B. Groner reviewed the use of bifunctional recombinant proteins in cancer therapy. He first described monoclonal antibody (scfv) fragments linked to an effector function (e.g. *Pseudomonas* exotoxin) for targeting c-erbB-2-over-expressing tumours in preclinical and clinical studies. A more novel approach is the use of peptide aptamers which are designed to interfere with protein–protein or protein–DNA interactions; targets notoriously difficult to inhibit with small molecules. Described as the biologist's "combichem", peptides selected for desired binding specificities and linked to a protein transduction domain can be delivered with high efficiency into cells. They then can inhibit key signalling pathways such as those of EGFR and Stat3, resulting in cell growth arrest. **J. Baselga** gave an overview of monoclonal antibody based therapies, focusing on the EGFR/c-erbB receptor family as targets. In particular, he described the combinatorial effects of trastuzumab (Herceptin) (which can downregulate c-erbB2 expression and cleavage, partially inhibit dimer formation, inhibit angiogenesis and potentiate the effects of chemotherapy) and determinants of responsiveness. Recent clinical results were discussed, and the need for better regimens and earlier intervention. He then concluded with the challenges that remain; *viz* determination of optimal doses, how to select patients, meaningful study endpoints and appropriate trial design.

13. Plenary symposium: genomics, proteomics and beyond

There were three excellent presentations in this session. The first two partially overlapped in that both **Dr**

C. Cordon-Cardo (New York) and **Dr J. Celis** (Copenhagen) discussed the use of proteomic approaches and gene expression profiling to determine deregulation of protein and mRNA expression on the transition of normal tissues and cells to cancer. Proteins which were identified were studied in more detail using immunohistochemical staining of tumour tissues.

Professor John Lindon (London) described a new method of analysis of body fluids (or biopsies) using nuclear magnetic resonance spectroscopy, called 'metabonomics'. This can be focused on particular molecular components, but has been more commonly applied to groups of small molecules such as amino acids, sugars and metabolites. Changes in the concentrations of sets of these substances were observed during disease processes and treatment; in the latter case, changes were predictive of response. This technology, although complex and expensive, is rapidly developing and will complement other better known strategies such as proteomics and gene expression profiling.

14. Basic science symposium: genomics/bioinformatics

Michael Stratton (Cambridge UK) opened the symposium and outlined the strategy that the Cancer Genome Project at the Sanger Centre has taken to develop and apply technologies for high throughput detection of mutations in cancer. The methods used are a polymerase chain reaction (PCR)-sequence tagged site (STS) Taqman assay for detection of large homozygous deletions and a capillary-based heteroduplex analysis to detect small mutations. He estimated that within 4 years approximately 32000 genes will have been analysed. The group initially focused on genes in the ERK-MAP-kinase pathway, and demonstrated somatic mis-sense mutations in the *BRAF* gene in more than 66% of malignant melanomas and at a lower frequency in other cancers. All mutations were within the kinase domain. A single mutation, accounting for 80% of those detected, had elevated kinase activity and transformed NHI3T3 cells. Professor Stratton clearly demonstrated that the strategy has already proven fruitful and holds promise for the future identification of novel genes that might provide new therapeutic targets for cancer therapy (see *Nature* 2002, **417**, 949).

Olli Kallioniemi (NCI, Bethesda and Turku, Finland) described the different technologies used to detect genome wide gene copy number alterations and expression profiles of tumours. His group has also pioneered the use of tissue microarrays. He elegantly showed the path from detection of an amplicon in 17q23 in breast carcinomas to identification of the putative target gene (S6K) and immunohistochemical screening of tissue arrays to evaluate its clinical implications. He discussed the challenges cancer researchers now face and the need for large-scale analyses at all levels. This will be enabled by human genome sequence information, high-throughput DNA sequencing and transcriptional profiling, large-scale capabilities in proteomics and advances in bioinformatics. Access to large samples sets and associated clinical data will be critical in order to translate genomic information to the benefit of human health. He also discussed new research strategies for analysing the phenotypic consequences of identified target genes using gene transfection and RNA interference in cultured cells in an array format. This type of approach for functional studies using 'transfection' arrays was recently described by Sabatini and colleagues (*Nature* 2001, **411**, 107) and Kallioniemi and his group clearly demonstrated the potential of this technique in studying the effect of overexpressing or inactivating specific genes (see *Nature Med* 1998, **4**, 844; *PNAS* 2001, **98**, 5711).

Anne-Lise Børresen-Dale (Norwegian Radium Hospital) described pioneering expression studies of breast carcinomas using high-density cDNA microarrays. The expression patterns provided a remarkably distinctive molecular portrait of each tumour, and the gene expression patterns in tumour samples from the same individual, before and after treatment, were generally more similar to each other than other samples. Five subtypes of tumours (two luminal epithelial derived oestrogen receptor-positive tumour subtypes, a basal epithelial-like, an ERBB2+ group, and a normal breast-like group) were identified. Survival analyses showed significantly different outcomes including poor prognosis for the basal-like cancers and differences between the two luminal/ER+ subtypes. Differences in *TP53* mutation frequency between the subtypes indicated an important role in determining the gene expression patterns in the various tumours. Analyses using the same cDNA arrays showed that at least 12% of all the variation in gene expression is directly attributable to underlying variation in gene copy number, and that 62% of the genes with high level amplification ($\geqslant 4$ copies) showed mRNA levels $\geqslant 2$-fold enhanced. These findings set the stage for future studies aimed at identifying specific patterns of gene activation that may predict important clinical features, such as sensitivity to specific therapies and metastatic potential (see *Nature* 2000, **406**, 747; *PNAS* 2001, **98**, 10869).

15. Translational research symposium: mechanisms of drug resistance: new developments

This translational research symposium gave an overview of drug resistance mechanisms identified in the laboratory and their possible involvement in the lack of response or relapse in the clinic. In an overview of 'Old and new drug resistance mechanisms', **H.J. Broxterman**

(Amsterdam) argued that the distinction between old and new is arbitrary, and that many old resistance mechanisms, such as low oral bioavailability, low tissue penetration (e.g. by high interstitial pressure in tumour) or efflux by drug pumps may in fact contribute to clinical resistance. In addition, 'new', molecularly targeted drugs such as STI-571 (Gleevec) or 2D 1839 (Iressa) can be subject to 'old' drug resistance mechanisms. It may be that many drug resistance mechanisms, such as P-glycoprotein or mutations in signal transduction pathways or in apoptotic execution pathways all contribute to overall drug resistance. However, Broxterman emphasised that, with the exception of one clinical study with a PgP reverter (by A. List *et al.*) the clinical relevance of this type of agent is still unclear.

Dr R. Perona Abellón (Madrid) highlighted possible roles of the Jun kinase (JNK) pathway and the NFκB transcription factor in chemotherapy-induced apoptosis. She concluded that in human tumours there is often an increase in the MKP-1/CL100 phosphatase, which negatively regulates apoptosis induction by a net decrease of JNK activity. However, mutation of Ras or overexpression of EGFR or Her2/neu induces increased NFκB transcriptional activation of target genes such as X-linked inhibitor of apoptosis (XIAP) leading to a higher apoptotic threshold.

Dr C. Gambacorti-Passerini (Milan) gave an overview of the remarkable results with the Bcr/Abl fusion protein-targeted drug imatinib (STI-571) in chronic phase chronic myeloid leukaemia (CML) patients. Over 97% patients who reached a complete cytogenetic response maintain this for at least 2 years. There is concern about the development of resistance which includes a pharmacodynamic component: the Bcr/Abl tyrosine kinase should be blocked long enough to elicit cell kill. Secondly, plasma concentrations do not always reflect the tissue (and intracellular) concentrations of drug. Other causes of resistance that have been identified include amplification of the *Bcr/Abl* fusion gene (in this case a dose increase makes sense) and at least 10 different mutations which affect STI-571 binding. Despite these problems, STI-571 treatment of CML signifies a breakthrough in clinical oncology.

Dr B.K. Tsang (Ontario) discussed his work on the role of XIAP in the resistance to cisplatin in ovarian cancer. XIAP is highly expressed in many ovarian cancers, and is related to high proliferating cell nuclear antigen (PCNA) and low Tdt-mediated dUTP-biotin nick-end labelling (TUNEL) staining. Its importance was clearly shown by antisense XIAP, which induced cisplatin sensitivity in resistant cells. The anti-apoptotic effect of XIAP is related to pro-caspase-3 cleavage, MDM2 stabilisation and associated p53 breakdown. Cisplatin cytotoxicity is caused by XIAP down-regulation, which involves the phosphatidyl inositol 3 (PI3) kinase pathway since the PI3 kinase inhibitor, LY294002 + cisplatin combination reverted XIAP-induced chemoresistance.

16. Keynote lecture: pharmacogenomics in the clinic

R. Rossell's lecture on 'Pharmacogenomics as a way to improve cytotoxic chemotherapy' illustrated how cancer chemotherapy will be moving from the crude 'dark box' approach to carefully selected therapy based on the genetic characteristic of each cancer as well as patients' polymorphisms in metabolic genes such as glutathione S-transferase. The new approach will rely on the development of PCR-based methods of analysis of tumour-derived mRNA and DNA in serum or plasma. Several trials are underway examining for, for example, the influence of the level of expression of the DNA repair gene *ERCC1* on the response to cisplatin and the effects of mutations and expression of different isotypes of beta-tubulin on microtubule stability and resistance to paclitaxel.

17. Basic science symposium: cell cycle

The speakers in this session on the basic science of the cell cycle had a number of provocative and surprising results, some of which challenged our views of cell cycle regulation. **Dr M. Serrano** (Madrid) presented his results on a novel protein, Ris1 that he identified as important in the cellular response to oncogenic stress. He also showed that increasing the number of copies of the p53 tumour suppressor gene in a mouse from the normal two to three copies conferred enhanced resistance to oncogenic stimuli. **Dr Kristian Helin** (Milan) has been studying the influence of the E2F1 transcription factor upon the initiation of DNA replication (S phase). He showed that E2F1 alone is not sufficient to induce S phase in diploid mouse and human fibroblasts, it requires the suppression of the p53-mediated G1 checkpoint, or in primary mouse fibroblasts loss of pRB. Thus, in addition to acting as an E2F-dependent transcriptional repressor, pRB is also required for the cells to retain the G1 checkpoint in response to un-programmed proliferative signals.

Dr M. Malumbres (Madrid) presented the entirely unexpected phenotype of a mouse lacking the cyclin-dependent kinase 2 gene. Despite being implicated (in partnership with cyclin E) as the major kinase required for initiating DNA replication, mice lacking CDK2 are viable (albeit sterile). At present, it is unclear whether, as seems likely, another kinase, such as CDK1, compensates for CDK2, or, more heretically, whether CDK activity is not required for DNA replication. Dr Malumbres also showed that mice lacking the kinase part-

ners of the D-type cyclins, CDK4 and CDK6, have tissue-specific defects. Mice lacking CDK4 are small, mostly infertile and diabetic, whereas mice lacking CDK6 are also small and have haematopoeitic defects. In these mice the females, but not the males, are infertile. Eliminating both CDK4 and CDK6 is lethal, as is eliminating the major mitotic kinase, CDK1. Lastly, **Dr J. Pines** (Cambridge, UK) showed how live cell imaging using green fluorescent protein (GFP)-fusion proteins could be used to understand how mitosis is coordinated by proteolysis. By analysing the changes in fluorescence of these proteins, the exact time at which they began to be degraded could be assayed. This analysis showed that the timing of the destruction of the mitotic regulators cyclin A, cyclin B1 and securin was set by the spindle assembly checkpoint.

18. Translational research symposium: predictive/prognostic markers

S. Menard described a detailed analysis of the role of HER2/c-erbB-2 in breast cancer. She noted that HER2 overexpression was not predictive in node-negative patients, and that in the node-positive patients, the largest survival differences between HER2+ and HER2- subsets were in the early years. Patient response to cyclophosphamide, methotrexate, 5-fluorouracil (CMF) was unaffected by HER-2 status, but it seemed that HER2+ tumours were more sensitive to doxorubicin. Experimental studies showed that growth factors released during surgery may selectively potentiate the growth of HER2+ tumours, but that this effect could be reversed by Trastuzumab (Herceptin). **M. Schwab** reported on the role of *MYCN* mutations in neuroblastoma. Although often a rapidly progressing tumour, spontaneous regressions can occur, and it is important to discover the mechanisms underlying this phenomenon. However, *MYCN* amplifications signal particularly poor prognosis and identify the need for more aggressive therapeutic intervention (see *Cancer Lett* 2001, **167**, 115).

H. Cortes-Funes discussed the use of the shed extracellular domain (ECD) of HER2 as a predictive factor in breast cancer. Serum levels of HER2 ECD correlated with tumour levels, the extent of metastatic disease and poor response to chemotherapy. HER2 ECD+/ER+ positive patients were also more refractory to treatment with the aromatase inhibitor letrozole; trials are underway to determine if the addition of trastuzumab (Herceptin) to HER2 ECD positive patients will improve response rates. B. Chabner (replacing **E. Rowinsky**) gave a historical overview of the changes in our understanding of cancer and hence our approaches to therapy. In the 1950s, cancer was seen as a disease of excessive proliferation and drugs primarily targeted DNA synthesis and integrity. In the 1990s, the emphasis has shifted to a realisation that cancer is linked to multiple genetic defects in cell cycle regulation, apoptosis and DNA repair. Primary mutations do affect cell proliferation and survival, but also genetic stability and loss of regulation, leading to secondary mutations which influence invasion, angiogenesis and metastasis. Dr Chabner concluded with examples of new targets that are showing promise in non-Hodgkin's lymphoma and other cancers.

19. The Mike Price lecture: targeting drugs against cancer: the EGF receptor

John Mendelsohn noted that at the beginning of the 20th century the major causes of death in the USA were infectious diseases whereas a century later these have been replaced by cardiovascular diseases and cancer. Once the causes of infectious diseases were discovered, they were overcome by antibiotics. The causes of cardiovascular diseases and cancer have been identified to the point where specific targeted therapies and preventive measures are being developed. Four premises define cancer: it is caused by (1) malfunctions of key genes controlling cell proliferation or DNA integrity; (2) failures of pathways that regulate cell proliferation and function; (3) cancer is influenced by the biological and molecular environment in the patient; and (4) cancer treatment aims to induce cytotoxic and pro-apoptotic pathways. Strategies are being developed to circumvent each one of these premises. One example is the use of monoclonal antibodies which bind to EGF receptors, block ligand binding, inhibit signal transduction and hence prevent cell proliferation. Prof. Mendelsohn concluded that future targeted cancer treatments will be more effective and less toxic than at present. Collaboration and open exchange of information are essential. Scientists and clinicians seek to speed up and improve the process of drug development by changes in policy related to basic and preclinical research priorities, intellectual property, clinical trial design and implementation, and regulatory changes.

Other Award Lectures included the Mühlbock Memorial Lecture given by **Sir Walter Bodmer** on 'The somatic evolution of colorectal cancer', the Anthony Dipple Carcinogenesis Award lecture given by **Web Cavanee** on 'Genetics and new approaches to cancer therapy' and the Carcinogenesis Young Investigator Award given by **Steve Jackson** on 'Sensing and repairing DNA double-strand breaks'. All gave superb and beautifully presented talks on their chosen topics, readily intelligible to an audience with very different backgrounds. The latter two papers are published in the May 2002 issue of *Carcinogenesis*.

Look out for the report of the EACR Young Cancer Researcher Award winner, Dr Maria Blasco, to be published in volume 38, issue 17 of the *EJC*. Her talk was entitled 'Telomeres and telomerase in cancer, ageing and DNA repair'. Congratulations also to the EACR-MSD Meeting Award winner, S. Aznar Benitah, with a presentation on 'Regulation of Stat3 and Stat5a transcription factors by Rho GTPases: implication in Rho-mediated neoplastic transformation'.

Thanks to those who kindly contributed summaries for this report.

References

1. Vermeulen PB, Gasparini G, Fox SB, *et al*. Second international consensus on the methodology and criteria of evaluation of angiogenesis quantification in solid human tumours. *Eur J Cancer* 2002, **38**, 1564–1579.