were differentially expressed, Fibronectin, basic breast conserved gene (BBC 1) and ubiquitin enzyme variant -1 (UEV-1/CROC1) which all Inocalise to regions of chromosomal aberration in prostate cancer (2q 3.4, 16q 24.3 and 20q 13.2 respectively). Further data on the expression of these genes in prostate cancer will be present.

We have also examined the relationship between androgen-regulated gene expression and all cycle regulation and apoptosis using an AR-cell line, doubly transfected with the AR gene and bcl-2 gene, (the latter being closely associated with the AI state). Bcl-2 overexpression confers and antiapoptotic effect against both androgen deprivation and cytotoxic chemotherapy.

An understanding of the mechanisms of AI prostate cancer will provide new therapeutic options to treat this presently incurable disease.

1566

Meta analysis of the randomised trials in prostate cancer

Otilia Dalesio. *On behalf of the Prostate Cancer Trialists' Collaborative Group, Biometrics Department, Netherlands Cancer Institute, Plesmanlaan 121-1066 CX Amsterdam, Netherlands*

The Prostate Cancer Trialists Collaborative Group (PCTCG) is performing an overview of randomised trials of the treatment of prostate cancer. The aim of the cycle 1997–2000, is to bring together mortality results from randomised trials that began before 1991. Two hundred randomised studies were identified that included 40,000 patients. Data from each individual patient in each study was requested. Three main questions were addressed by many of these studies and for these an overview was considered relevant:

MAB vs castration alone;

Immediate vs deferred hormone treatment;

Chemotherapy vs no (or delayed) chemotherapy;

In 1995, a first cycle an overview by the of the results of these trials organised by the PCTCG found no significant overall survival advantage by MAB as compared to androgen suppression alone. However, the confidence limits were rather large and, therefore, the possibility of a small improvement in survival was not excluded. This second cycle of the overview includes in excess of 40% more information (8,000 patients with almost 6,000 deaths).

For the question of immediate vs deferred hormonal treatment 15 trials were identified including more than 7,000 patients. Analyses of mortality and cause specific mortality were performed.

Concerning the question of (first line) immediate chemotherapy vs no (or delayed) chemotherapy 9 studies were identified including circa 2000 patients. Results of this cycle will be presented.

1567

Biology of lung tumours: Targeting bcl-xL and EGP-2

R.A. Stahel[1], U. Zangemeister-Wittke[2]. [1]*Universitatsspital Zurich, Abt. med. Onkologie, Zurich, Switzerland*

We have previously demonstrated the anti-apoptotic protein Bcl-2 to be a promising target for antisense-based therapy of small cell lung cancer. In contrast to small cell lung cancer, in non-small cell lung cancer the Bcl-2 homologue and related cell death antagonist Bcl-xL is more commonly overexpressed. Using the secondary structure of the bcl-xL mRNA to predict the most promising target sites for antisense binding a series of dodecamer phosphorothioate antisense oligonucleotides were designed. Oligonucleotide 3011 was found to most effectively downregulate Bcl-xL protein levels and to induce apoptosis in non-small lung cancer cell lines. However, sequence control oligonucleotides also revealed a certain degree of unspecific cytotoxicity, making it difficult to discern the true antisense effect. To overcome this limitation, novel 2'-O-methoxyethly modified gapmer oligonucleotides with improved binding affinity and stability were designed. Three compounds were tested, including oligonucleotide 4259 (identical sequence as 3011), 4625 (with specificity for bcl-2 and 3 mismatches to bcl-xL) and 4627 (with one mismatch to bcl-2 and 2 mismatches to bcl-xL). Although oligonucleotide 4259 reduced bcl-xL message and protein levels in a dose dependent manner in non-small cell as well as small cell lung cancer cell lines, its effect on cell viability was more pronounced in non-small cell compared to small cell lung cancer cell lines. In contrast to the deoxy compounds the 2'-MOE modified sequence control oligonucleotides did not significantly reduce cell viability. Induction of apoptosis in non-small cell lung cancer cell lines was demonstrated by induction of caspase-3-like activity. The potentially bispecific antisense oligonucleotides 4625 and 4627 inhibited both bcl-2 and bcl-xL expression and reduced cell viability of non-small cell and small cell lung cancer cell lines. Our results imply the importance of the Bcl-xL protein in the tumorigenesis of non-small cell lung cancer and suggest the use of gene therapy approaches to counteract the expression of this cell death antagonist. Moreover, we show for the first time that it is possible to target the expression of Bcl-2 and Bcl-xL with a single antisense compound.

The epithelial glycoprotein-2 (EGP-2) is a surface antigen highly expressed in small cell and lung adenocarcinoma. Based on the monoclonal antibody MOC-31, we developed a single-chain antibody fragment (scFv) against EGP-2. To stabilize the unstable scFc we chose rational loop grafting of the binding residues on a stable antibody framework with favorable biophysical properties. The resulting molecule 4D5MOCB retained the high binding affinity of 10-9 M, while exhibiting a better expression behavior and serum stability, and demonstrated good tumor to blood ratios in a xenograft model. 4D5MOCB new serves as a basis to develop better targeting molecules directed against small cell and non-small cell lung cancer, as well as other solid tumors.

1568

Early non small cell lung cancer: The need for combined treatments

G. Giaccone[1]. [1]*Vrije Universiteit, Department Medical Oncology – 10 Oost, Amsterdam, Netherlands*

As result of several randomized studies and the large meta-analysis published in 1995 on the BJC, locally advanced non-small cell lung cancer (NSCLC) is nowadays mainly treated by combined modality treatments. Chemotherapy including a platinum compound is generally part of the combined modality treatment. The local treatment varies depending on the staing of the disease, but also by country. In stage III technically resectable, the addition of neoadjuvant chemotherapy has been showed to improve survival in 3 small randomized trials. However, whether radical radiotherapy can achieve results similar to surgery is still a matter of debate. A large EORTC randomized trial is addressing this question in patients with histologically verified stage IIIaN2 disease who respond to platinum-based chemotherapy. The study has now accrued over 400 patients and randomized over 200 of them; the response rate to the newest combination chemotherapy (e.g. cisplatin-gemcitabine or carboplatin-paclitaxel) is well over 50% in this patient population. Other similar studies are running the the US. However, in the US chemotherapy is usually given concomitant to radiation. This leads to an improved response rate, with over 20% pathological complete response rates (it is less than 10% in chemotherapy only treatments), but also increased toxicity and morbidity of operation. For inoperable stage III (usually stage IIIb) radiation therapy has been standard treatment in several institutions. The combination of chemotherapy and radiation has now become standard in many centers. However, the optimal way of combining these modalities still needs further investigation. In particular the timing of the treatments, the radiation dose and volume, the type of chemotherapy are still under evaluation.

Recently combined modality treatment has been evaluated also in early stage (stage I, II, IIIa) resectable NSCLC, and promising results have been recently reported. Neoadjuvant chemotherapy seems feasible in this patient category with very few progressions before surgery. Response rates are very high and the only randomized study so far presented appears to be positive for some subgroups of patients. Tolerability of the chemotherapy, together with its effectiveness are major issues in this type of studies. It is however clear that the compliance to neoadjuvant chemotherapy is higher than that to post-operative chemotherapy.

1569

Post-operative treatments in resected NSCLC

M. Tonato[1]. [1]*Policlinico Monteluce, Divisione di Oncologia Medica, Perugia, Italy*

Five-year survival rate of patients (pts) with non-small cell lung cancer (NSCLC) who undergo complete surgical resection is only 40–69%, depending on the stage. It is well known that distant metastatic disease is the dominant site of recurrence in such patients and this observation served as the basis for trials of postoperative systemic therapy. The earliest trials of adjuvant chemotherapy, which consisted of single alkylating agents, could not achieve this goal or, even worse, showed a detrimental effect of chemotherapy on survival. The introduction of more active drags, such as cisplatin and vinca alkaloids, made it possible to obtain more promising results in terms of delayed recurrence of the disease. A recent meta-analysis including all randomized trials with accrual from January 1965 to December 1991 showed that the absolute risk of death was reduced by 3% at two years and by 5% at 5 years for pts who were treated with postoperative cisplatin-con-

taining regimens compared with pts who were treated with surgery alone (P = 0.8). Although the results of this meta-analysis suggest that postoperative cisplatin CT regimens may result in a slight survival improvement, adjuvant CT in NSCLC cannot be considered a standard therapy and it is important that large, carefully conducted randomized trials be performed in this group of pts. Four such randomized trials are being conducted in Europe. One of them, the ALPI trial recently completed its accrual with more than 1,200 pts. The IALT, ANITA and MRC trials are till ongoing. The results of such trials are eagerly awaited and it is hoped that, once the value of postoperative CT is well ascertained, future developments can improve further the results of combined treatment. In such direction the recently reported results of PORT meta-analysis evaluating the role of RT are of great contribution in selecting the proper population for future studies. In fact, only patients with pN2 disease seem to have a beneficial effect in terms of survival, especially if they have a good PS, while RT is not justified in NoN1 patients. The optimal integration of CT and RT when both therapies are indicated represent another goal for future research.

1570

Induction treatments in marginally operable non-small cell lung cancer

Thierry Le Chevalier, Federico Cappuzzo. *Institut Gustave Roussy, Villejuif, France*

Patients (pts) with marginally operable non-small cell lung cancer (NSCLC) have a poor 5-year survival when treated with surgery alone. To improve the outcome of these pts, several authors have evaluated the feasibility and the effectiveness of induction chemotherapy (CT), and numerous phase II studies have reported promising results in terms of response and resectability. Three randomized trials evaluated the role of primary CT in operable NSCLC. The first, (R. Rosell et al), used three courses of CDDP, IFO, MMC (MIP regimen) and was stopped early after inclusion of 60 pts due to a significant improvement of survival in the neoadjuvant CT arm compared to the control arm (median survival of 26 months versus 8 months, $p < 0.001$). Both arms received post operative radiation therapy (RT). In the MD Anderson Study (J. Roth et al), three cycles of neoadjuvant CTX, VP 16, and CDDP followed by surgery were compared with surgery alone. RT was not a part of either treatment regimen. Sixty patients were randomized and median survival was 64 months in the neoadjuvant arm compared to 11 months in the control arm ($p < 0.008$). A recent follow-up confirmed these results. These two trials have shown encouraging results but the groups were small and populations heterogeneous. More recently, Depierre completed a phase III randomised trial comparing induction CT (MIP) followed by surgery to surgery alone in 375 patients with operable NSCLC. No significant benefit was observed in this trial with induction CT. The addition of preoperative concomitant RT to CT has also been evaluated in some phase II trials with encouraging results, and several studies have been recently initiated.

In conclusion, it is still unclear whether preoperative CT ± RT provide a significant and substantial survival advantage in marginally operable NSCLC. Further studies are needed to clarify the exact role of this therapeutic strategy.

1571

Radio-chemotherapy for stage III disease: From theory to practice

P. Van Houtte. *Institut J. Bordet, Brussels, Belgium*

Locally advanced non-small cell lung cancer (NSCL) still represents a challenge. Indeed, a combined approach including chemotherapy (CT) and radiotherapy (RT) has let to some slight improvements for some subsets of patients (i.e. good performance status, no weight loss...) but this was mainly achieved by a reduction of distant metastasis when a sequential approach was used; local control after RT remains dismal: less than 20% even after 65 Gy. Several drugs show great radiosensitizing properties both in vitro and in vivo and some results in phase II trials are quite promizing suggesting that a concurrent approach may be more effecient but with an increase in acute toxicity including acute esophagitis. During the last years, some large phase III trials have been launched to explore this approach. How to integrate those drugs with the new developments in the field of RT (conformal RT, modifications of the fractionation) is a major area for an intensive research but survival must remain the main objective with acute and late toxicities and pattern of failure as secondary endpoints. The latter may be only helpful to define new strategies including the place of surgery, a more useful staging system, the patient selection criteria for an aggressive approach or only to consider palliation and quality of life..

1572

Oncogenes and the cell cycle: Targets for modifying radiosensitivity

W.G. McKenna[1], R.J. Muschel[2]. [1]*University of Pennsylvania, Department of Radiation Oncology, Philadelphia;* [2]*University of Pennsylvania, Department of Pathology and Laboratory Medicine, Philadelphia, United States*

Our laboratories have shown that the ras oncogene expression causes tumors to become increasingly resistant to radiation. The expression of the ras oncogene in tumor cells both renders them resistant to apoptosis and allows a prolongation of the G2 delay. There are three major goals relating to this work currently being pursued and which will be discussed, 1) to develop methods of radiosensitizing tumors that have ras mutations, 2) to determine which of the ras signaling pathways leads to alterations in radiosensitivity and 3) to understand the mechanisms underlying the G2 delay induced by radiation.

Toward the first goal we have shown that the use of farnesyl-transferase inhibitors, pharmacological agents that block activity of ras, allows tumors to be radiosensitized. These experiments have shown that treatment of tumors with ras mutations, but not those with wild type ras leads to increased radiosensitivity and increased susceptibility to apoptosis. This work has now led to a phase 1 clinical trial using these agents in patients with pancreatic cancer, lung cancer and cancer of the head and neck. The outline of this trial will be discussed.

Ras is known to signal through a variety of pathways including raf, PI3 kinase, rac and rho. This is a major focus of the lab because identification of the pathways that can lead to radioresistance could potentially allow the identification of targets that could be used for radiosensitization clinically. The current view of the invovlement of these downstream pathways will be discussed.

Lastly, the laboratory is investigating the effect of the G2 delay on radiosensitivity. Radiation causes cells to delay in G2 and there is suggestive evidence that this delay is important for radiation survival. We are attempting to characterize the G2 delay in order determine whether it is directly involved in radiation survival or control of apoptosis. We will discuss the role of cyclin expression, cdk activation and nuclear localization of the phenomenon of radiation-induced G2 delay.

1573

DNA damage-dependent checkpoints in yeasts and human cells

Noel F. Lowndes. *ICRF, United Kingdom*

The DNA damage-dependent checkpoint pathway is believed to be of critical importance to cancer as it is involved in the maintenance of genetic stability and, in human cells, mutation of components of this pathway result in cancer predisposition. In addition to the use of yeast model systems to study this pathway we have identified and are studying human homologues of yeast genes with roles in this pathway.

In yeast the DNA damage-dependent checkpoint pathway is composed of two upstream branches both of which either sense DNA damage or interact with specific DNA damage sensors. This information is transduced to the downstream biological consequences of checkpoint pathway activation: cell cycle arrest and transcriptional induction of a regulon of genes with roles in DNA repair. We have biochemically analysed two of the components of the yeast pathway. Rad9 is phosphorylated during cell cycle progression and hyperphosphorylated after DNA damage. Rad24 has been purified to homogeneity and interacting proteins identified by mass spectroscopy.

Expression of the human homologue *(HRAD1)* of the fission yeast checkpoint gene *rad1*$^+$ in *rad1* mutant fission yeast cells partially rescues the G2/M checkpoint defect of these cells. The hRad1 protein is overproduced in testis and some human cancer cell lines and interacts with meiotic prophase chromosomes at both synapsed and ansynapsed regions. Thus hRad1 interacts with chromosomes at sites where double strand breaks are present or being processed.