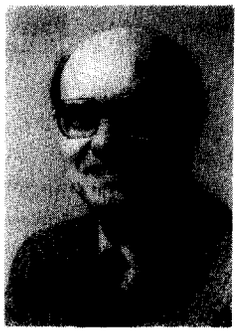
Eur J Cancer Clin Oncol, Vol 24, No. 6, pp. 945–949, 1988.
Printed in Great Britain

0277-5379/88$3.00 + 0.00
Pergamon Press plc

Symposium in Honour of Prof. L.M. van Putten on 'Preclinical Evaluation of Anti-cancer Drugs' and Workshop on 'Experimental Models for Non-small Cell Bronchial Cancers' on the Occasion of His Retirement from the Radiobiological Institute TNO at Rijswijk, The Netherlands

October 29 and 30 1987



AFTER 31 years of research at the Radiobiological Institute, Luke van Putten has retired. Following the completion of his medical studies at the State University of Leyden, he trained as an internist under Prof. Andries Querido at the University Hospital Leyden. During his training, Luke became interested in research and in 1952 he passed his Ph.D. thesis on the subject of 'Experimental hypothalamic obesity'. In 1956 he joined the small group of scientists who were assembled to form the nucleus of the Radiobiological Institute. In preparation for his new task he worked on a WHO Fellowship for six months with Dr. J.F. Loutit in the Radiobiological Research Unit of the Medical Research Council at Harwell and under Prof. S. Mitchell in the Department of Radiotherapy at the University of Cambridge. When the Radiobiological Institute became operational in 1960, Luke van Putten was appointed as assistant director. For a period of 25 years he helped to organize and run the Institute until illness made him resign from these demanding duties in 1985. As an Eleanor Roosevelt Fellow of the U.I.C.C. he studied in 1964–1965 with the late Henry Kaplan and with Bob Kallman in the department of Radiology at Stanford Medical Center, Palo Alto. His research centred on the influence of oxygenation on the response of tumours to radiotherapy. Among his many talents were an extraordinary memory for relevant data and the ability to transfer and explain these in discussions, which made him a gifted teacher. He carried a substantial share of the teaching programme of the staff of the Institute which is partly performed under the auspices of the J.A. Cohen Institute for Radiopathology and Radiation Protection at Leyden University. In 1970 he was appointed to the chair of Applied Radiation Biology at the State University Leyden and became director of the Educational Programme of the J.A. Cohen Institute, while continuing his work at the Radiobiological Institute.

Luke van Putten's broad interests are reflected by the diversity of his many contributions to the scientific literature. During the early years of his association with the Radiobiological Institute he worked on the late effects of strontium-90 and other bone seeking isotopes and on methods to counteract their toxicity. He also soon joined the experimental bone marrow transplantation programme by assisting in setting up the preclinical studies in rhesus monkeys, and contributing to the development of cryopreservation techniques and the unraveling of Graft-versus-Host Disease. After his return from Stanford he began to study cell kinetics in irradiated tumours and in particular the role of the hypoxic fraction in certain tumours continued to fascinate him.

Towards the end of the 60s it became clear that chemotherapy would become an important modality in the treatment of cancer and that combination therapy with radiation had to be investigated. At that time chemotherapy was in its infancy in The Netherlands. It was decided that

the Institute would include experimental chemotherapy in its programme, and that Luke was to organize this. While setting up tumour systems and *in vitro* cultures for testing the effects of chemotherapeutic agents he joined the Groupe Européen de Chimiothérapie Anticancéreuse (which in 1975 was renamed European Organization for Research on Treatment of Cancer, E.O.R.T.C.) which he served with never ending enthusiasm as Councillor and later as Treasurer and Member of the Board, as well as in numerous subcommittees, most notably the Screening and Pharmacology Group. To promote the dissemination of scientific information on the new drugs and their proper introduction into clinical oncology he founded the Chemotherapy Club in The Netherlands which became the informal meeting centre for all the young pioneers entering this new field. Soon he developed an exceedingly varied research programme which comprised both the testing of agents and the study of their mechanism of action on primary tumours and metastases. Over the years this programme has attracted many fellows both from abroad and from our own country. More than 80 papers on chemotherapy have appeared from his hand and his lectures in particular those on the theoretical aspects of chemotherapy were in high demand. His experience and judgement were equally valued as member of the editorial board of several oncological journals, among which was the *European Journal of Cancer and Clinical Oncology*.

While exploring the possibilities and limitations of chemotherapy and laying the foundations for many projects in the Radiobiological Institute on the combination effects of radiation and chemotherapy, Luke managed to maintain his expertise in radiation biology and radiation protection at a high level. In this capacity he served on the National Committee that organized and analysed the Public Dispute on Nuclear Energy in The Netherlands during 1981 and 1982. For his outstanding services to the State and society he received the distinction of Officer in the Order of Orange Nassau.

His colleagues from abroad and from the Institute expressed their appreciation for Luke van Putten by organizing a Symposium on 'Preclinical Evaluation of Anti-cancer Drugs' on the occasion of his retirement. During the first part of the meeting, a number of distinguished friends reviewed progress in areas to which Luke has contributed. Dr. Bob Kallman (Stanford University, U.S.A.) discussed the selection of animal tumour models. Dr. Gordon Steel (Sutton, U.K.) dealt with the use of human tumour xenografts. Dr. Peter Twentyman (Cambridge, U.K.) lectured on *in vitro* clonogenic assays and Dr. Henry Tagnon (Brussels, Belgium) presented an overview of the achievements of the EORTC and their significance for oncology in Europe. On the next day experimental models for non-small cell bronchial cancers were discussed in a workshop attended by 30 radiobiologists and clinicians.

THE CLINICAL PROBLEM

The clinical problem of bronchial cancer was discussed by Noordijk (Leiden, The Netherlands). Among all cancers, bronchial cancer is:

1. the most common: in men it is the main cause of death due to cancer, in women it is the second after breast cancer and in some states of the U.S.A. it is already the first cause of cancer death.
2. still the least treatable, due to local failures and distant metastases.
3. most easily preventable, about 80–90% of lung cancer can be prevented by not smoking.

The overall cure rate of bronchial cancer is about 10%, regardless whether patients received surgery, radiotherapy or chemotherapy [1]. Combined modality treatments have not been able to overcome the two major causes of failure, local progression and distant metastases. Although complete remissions and response rates up to 50% can be reached in metastases of non-small cell lung cancer with combination chemotherapy (e.g. vindesine/*cis*-platinum), the duration of remissions is short and the toxic effects of the therapy are considerable. Loco-regional failure is mainly a problem of insufficient radiotherapeutic possibilities. Although occasionally tumours smaller than 4 cm are cured by radiotherapy, most tumours are larger or radioresistant.

From a radiobiological point of view the total dose should be as high as possible, using a short overall time to prevent repopulation and using small fractions to prevent late damage on normal tissues, such as lung, heart and oesophagus. The combination of hyperfractionation and accelerated fractionation, i.e. multiple daily fractionation, seems attractive.

IN VIVO MODELS FOR BRONCHIAL CANCERS

Kal (Rijswijk, the Netherlands) reported that in the Radiobiological Institute TNO attempts were made to develop realistic animal models for human bronchial cancers. Using intrapulmonary implantation of I-125 seeds or Ir-192 wires, several transplantable squamous cell carcinomas in the WAG/Rij rat were obtained. From these tumours the growth characteristics and responses to treatment were studied with implants growing subcutaneously in the flank of syngeneic rats [2]. The responses to a variety of chemotherapeutic drugs were different for each tumour line and a similar tumour

specific pattern of response was observed when these tumours were grown as xenotransplants in nude mice [3]. These results indicate that the responsiveness to drugs is determined by the intrinsic properties of the tumour cells and not by host influences. In addition, results were discussed of experiments with squamous cell carcinomas growing intrapulmonarily. Results indicate that the intrapulmonary tumours are less sensitive to drug treatment than subcutaneously growing implants. This observation contrasts with those of others [4] who treated lung tumours at smaller tumour volumes. With the models described, realistic testing of regimens involving radiation doses and cytostatics can be performed. A comparison of the results obtained with intrapulmonary implants with those of subcutaneous implants will be of interest to determine the role of the tumour bed in response to treatment.

Fergusson (Edinburgh, U.K.) discussed results of interferon–drug combinations tested in a series of human bronchial carcinomas in CBA mice rendered immunodeficient by neonatal thymectomy and total body irradiation [5]. Interferon has not been shown to have activity in patients with lung cancer. The combination of interferon and cytostatic drugs was tested in two non-small cell tumours. Interferon alone had no effect. The activity of both *cis*-platinum and ifosfamide was enhanced by the addition of interferon. The effectiveness of other drugs (adriamycin, TCNU, etoposide, platinum analogues, vindesine) in combination with interferon in these tumours was studied. Although single agent drug activity was low, most combinations proved more active than a single agent. No such effect was seen in two small cell xenografts. A pilot clinical study assessing the efficacy and toxicity of combination therapy with *cis*-platinum and interferon in patients with non-small cell lung cancer is ongoing. Laboratory studies attempting to elucidate the mechanism of interaction of interferon and drugs using xenografts and cell lines derived from these tumours are under way.

HUMAN LUNG CANCER LINES *IN VITRO*

Duchesne (Sutton, U.K.) discussed results on radiation sensitivity assessments of human lung cancer cell lines. A panel of human lung carcinoma lines representing the four main histological types was established [6] to determine their radiosensitivities and relate these to biological properties. A determination of *in vitro* single-cell responses in a soft agar clonogenic assay showed that the tumour types fell into two main classes, small-cell lines and some adenocarcinomas being relatively radiosensitive, and the other cell types radioresistant. The examination of the survival curve parameters suggested that hyperfractionation might usefully be employed in the clinical management of the sensitive tumours to increase the maximum tolerated tumour dose.

The *in vitro* response was modified by hypoxia in xenograft and spheroid models, increasing the cell survival relative to oxygenated cells, but in the spheroid model a progressive hypoxia-mediated cell loss was also observed over 10 days following irradiation, with amplified radiation-induced cell death.

It was observed that the degree of radiosensitivity correlated well with the extent of expression of the neuroendocrine phenotype, and suggested that identification of neuroendocrine markers (such as intermediate filaments) in biopsies from patients might allow a selection of those patients most likely to respond to irradiation.

Responses of human lung cancer cell lines to drugs and radiation were discussed by Carmichael (Newcastle, U.K.). Thirty human lung cancer cell lines were tested for chemosensitivity using the MTT colorimetric assay [7]. These cell lines came from all the major histological sub-types, including 15 small cell lung cancer cell lines both from previously untreated and previously treated patients; 15 non-small cell lines, including adenocarcinoma, large cell carcinoma, adenosquamous carcinoma and squamous carcinoma lines. The lines were tested with seven drugs, adriamycin, BCNU, cisplatin, melphalan, vinblastine, vincristine and VP-16. Small-cell lines from previously untreated patients were the most sensitive to the majority of drugs tested. Minimal differences were found between treated small-cell and non-small cell lines. The MTT assay represents an excellent screening method for chemosensitivity testing of human lung cancer cell lines.

In addition, 14 non-small cell lines were tested for radiosensitivity using a clonogenic assay. All major histological sub-types were tested. Variability in radiation response was observed in adenocarcinoma lines, which could be of clinical importance. The remaining cell lines were generally resistant to radiation, with the surviving fraction following a 2 Gy dose considered the best indicator of clinical radio-responsiveness.

MULTI-DRUG RESISTANCE

The problem of multidrug resistance in lung cancer was discussed by Twentyman (Cambridge, U.K.). Drug resistant variants of three human lung cancer cell lines were obtained by growth *in vitro* in increasing concentrations of adriamycin [8]. The lines are NCI-H69 (small cell), MOR (adenocarcinoma) and COR-L23 (large cell carcinoma). The lines each show a cross-resistance pattern which is

characteristic of the multi-drug resistance phenotype and each resistant line shows a reduced ability to accumulate adriamycin compared with its parent line. The molecular changes in the resistant lines have been shown to be multi-factorial. Several anthracyclines were identified (e.g. aclacinomycin and Ro 31-1215) which maintain good activity in adriamycin-resistant cells. The calcium transport blocker, verapamil, and the immunosuppressive drug, cyclosporin A, are effective modifiers of adriamycin and vincristine resistance. In NCI-H69, the resistant variant (LX4) may be sensitized in the absence of sensitization of the parent lines. In MOR and COR-L23, however, both parent and resistant lines show sensitization to adriamycin by these agents. More potent, and non-immunosuppressive analogues of cyclosporin A have now been identified as resistance modifiers.

DISCRIMINATING DIAGNOSTIC PROCEDURES

The expression of the fur gene as a discriminating marker for small cell and non-small cell lung carcinomas (SCLC and NSCLC) was discussed by Schalken (Nijmegen, The Netherlands). The recently discovered fur gene encodes a membrane associated protein with a recognition function [9]. The fur gene appeared to be differentially expressed, relatively high levels of fur mRNA being present in specimens of liver and kidney, low levels in brain, spleen and thymus, and very low levels in heart muscle, lung and testis. mRNA levels in specimens of human lung tissue without neoplastic lesions were also very low. A similar analysis of primary human lung carcinomas of different histopathological types revealed a highly selective and strong elevation of fur expression in non-small cell lung carcinomas, but not in small cell lung carcinomas.

The differential expression pattern of fur in SCLC and NSCLC also implied that fur gene expression could be used as a discriminating marker in studies on human lung cancer. At present, SCLC cells can be identified using a number of biomarkers. For example, gastrin-releasing peptide is shown to be a suitable marker for SCLC cells. A similarly useful marker for NSCLCs, which account for 75–80% of all cases of primary lung cancer, is not available at present. The identification of fur as a potential marker for NSCLCs could, therefore, be of importance for lung cancer diagnosis. The analysis of the fur gene has so far revealed promising characteristics of the gene and its product furin. First of all, evidence that furin represents a cell surface receptor is in favour of relatively easy accessibility of the protein. Moreover, the fact that in NSCLCs the levels of fur transcripts are selectively and strongly elevated may further facilitate furin detection. Both these characteristics could make the fur gene a valuable object for the development of reagents to detect NSCLC at an early stage.

The use of monoclonal antibodies to cytokeratins [10] and neuroendocrine markers to differentiate between lung cancer subtypes was discussed by Broers (Nijmegen, The Netherlands). More than 300 cases of the major lung cancer types, i.e. squamous cell carcinoma (SQC), adenocarcinoma, small cell lung cancer and lung carcinoids, were immunohistochemically investigated for their expression of cytokeratins and neurofilaments. Cytokeratins were present in all lung cancer cases examined, while in some lung carcinoids, some SCLC and some poorly differentiated SQC, neurofilaments were detected. The presence of neurofilaments seems to be related to the occurrence of the variant-type of SCLC as detected previously for variant SCLC cell lines. The neuroendocrine cell-surface antigen MOC-1 was present in all lung carcinoids, all SCLC and some poorly differentiated SQC, suggesting that this antigen is characteristic for neuroendocrine tumours, but may also be used to detect neuroendocrine differentiation in non-neuroendocrine carcinomas.

A panel of monoclonal antibodies, each specific for a different cytokeratin polypeptide was used on each 10 cases of histologically well-differentiated SQC, moderately differentiated SQC, poorly differentiated SQC, adenocarcinoma, SCLC and lung carcinoids. Most lung cancer subtypes appeared to contain certain cytokeratins characteristic for their type of differentiation. Cytokeratin 10 was specific for well-differentiated SQC, while cytokeratin 13 also occurred in moderate to well-differentiated SQC and not in poorly differentiated SQC. Cytokeratin 7 was present in all adenocarcinomas, while cytokeratin 18 was present in adenocarcinomas, in SCLC and in lung carcinoids. Apart from these general cytokeratin expression patterns, however, in many histologically homogeneous lung tumours heterogeneity was detected immunohistochemically, as observed by the occurrence of cytokeratin 7 in parts of SQC, SCLC and lung carcinoids, and the detection of cytokeratin 18 in most SQC in a variable number of tumour cells.

The use of a panel of cytokeratin, neurofilament and neuroendocrine antibodies therefore allows the detection of the main subtypes of lung cancer, but also to determine the degree and type of heterogeneity within each type of carcinoma. Therefore, the use of this panel of antibodies in the diagnosis of lung cancer may become of clinical significance.

D.W. VAN BEKKUM
H.B. KAL
Radiological Institute TNO
Rijswijk, The Netherlands

REFERENCES

1. Noordijk EM. Classificatie, stadiumindeling en radiotherapie van het bronchuscarcinoom. Ph.D. Thesis, University of Leiden, Leiden, 1983.
2. Kal HB, Berkel AH van, Zurcher C, Smink T, Bekkum DW van. A rat lung cancer model based on intrapulmonary implantation of tumour material. *Radiother Oncol* 1986, **6**, 231–238.
3. Kal HB, Bekkum DW van. Comparison of chemosensitivity of transplantable non-small cell bronchial tumours of rats in syngeneic hosts and in nude rodents. *Eur J Cancer Clin Oncol* 1988, **24**, 999–1003.
4. Smith KA, Begg AC, Denekamp J. Differences in chemosensitivity between subcutaneous and pulmonary tumours. *Eur J Cancer Clin Oncol* 1985, **21**, 249–256.
5. Fergusson RJ, Carmichael J, Smyth JF. Human tumour xenografts growing in immunodeficient mice: a useful model for assessing chemotherapeutic agents in bronchial carcinoma. *Thorax* 1986, **41**, 376–380.
6. Duchesne G. The biology of lung cancer: experimental observations and a review of current concepts. *Radiother Oncol* 1988 (in press).
7. Carmichael J, DeGraff WG, Gazdar AF, Minna JD, Mitchell JB. Evaluation of a tetrazolium-based semiautomated colorimetric assay: assessment of chemosensitivity testing. *Cancer Res* 1987, **47**, 936–942.
8. Twentyman PR, Fox NE, White DJG. Cyclosporin A and its analogues as modifiers of adriamycin and vincristin resistance in a multi-drug resistant human lung cancer cell line. *Br J Cancer* 1987, **56**, 55–57.
9. Schalken JA, Roebroek AJM, Oomen PPCA *et al.* Fur gene expression as a discriminating marker for small cell and non-small cell lung carcinomas. *J Clin Invest* 1987, **80**, 1545–1549.
10. Broers JLV, Ramaekers FCS, Klein Rot M *et al.* Cytokeratins in different types of human lung cancer as monitored by chain-specific monoclonal antibodies. 1988 (submitted).