Eur J Cancer, Vol. 26, No. 7, pp. 777–779, 1990.
Printed in Great Britain

0277-5379/90$3.00 + 0.00

Fax Communications

Absence of Abnormalities of the c-*erbB*-1 and c-*erbB*-2 Proto-Oncogenes in Human Thyroid Neoplasia

Nick R. Lemoine, Fiona S. Wyllie, Johan R. Lillehaug, Susan L. Staddon, Christine M. Hughes, Rein Aasland, Jonathan Shaw, Jan E. Varhaug, Christopher L. Brown, William J. Gullick and David Wynford-Thomas

The c-*erbB-1* and c-*erbB*-2 proto-oncogenes are frequently activated by gene amplification and overexpression in a variety of human cancers. In an analysis of a large series of benign and malignant thyroid tumours, no abnormalities of structure or expression of either of c-*erbB*-1 or c-*erbB*-2 were found. Activation of these oncogenes is not a necessary event in neoplasia of this epithelial system.
Eur J Cancer, Vol. 26, No. 7, pp. 777—779, 1990.

INTRODUCTION

THE c-*erbB*-1 oncogene encodes the receptor for epidermal growth factor [1] and transforming growth factor (TGF) alpha [2] and is overexpressed in several types of carcinoma, particularly squamous carcinoma [3, 4] and brain tumours [5]. The c-*erbB*-2 proto-oncogene (also called HER-2 or NEU) encodes a putative growth factor receptor for which a ligand has not yet been identified. Overexpression of the protein product, usually associated with gene amplification, has been demonstrated in a wide range of human adenocarcinomas (breast [6–8], ovary [6], stomach [9, 10], colon [11], lung [12] and pancreas [13]).

In a small series of thyroid tumours, expression of c-*erbB*-1 and c-*erbB*-2 was reported to be elevated in some follicular and papillary tumours [14]. For breast and ovarian cancer, amplification and overexpression of these genes can give prognostic information [6] and there are therapeutic possibilities of using specific inhibitors in cases with overexpression of growth factor receptor. We have therefore analysed the true frequency of abnormalities in the c-*erbB*-1 and c-*erbB*-2 proto-oncogenes in thyroid tumours with Southern blot hybridisation to detect gene amplification or rearrangement and immunocytochemistry to detect overexpression of c-*erbB*-2 oncoprotein. Similar to rat *NEU*, the human c-*erbB*-2 protein can be activated by aminoacid substitutions in the transmembrane region to produce oncoproteins with transforming potential [15]. It is obviously of great interest to determine whether such mutations occur in human tumours *in vivo*, and so we used polymerase chain reaction (PCR) amplification and sequence-specific oligonucleotide hybridisation to screen for possible activating point mutations of the transmembrane-encoding region of this gene.

MATERIALS AND METHODS

Southern blot analysis

Fresh tissue samples (Table 1) from thyroid glands or metastatic deposits were snap-frozen in liquid nitrogen immediately after surgical removal and stored at −80°C. High molecular weight DNA was extracted and *Eco*RI digestion was followed

Correspondence to N.R. Lemoine.
N.R. Lemoine, S.L. Staddon, C.M. Hughes and W.J. Gullick are at the ICRF Molecular Oncology Group, MRC Cyclotron Building, Hammersmith Hospital, London W12, U.K.; F.S. Wyllie, J. Shaw and D. Wynford-Thomas are at the CRC Thyroid Tumour Biology Research Group, Department of Pathology, University of Wales College of Medicine, Cardiff, U.K.; J.R. Lillehaug, R. Aasland and J.E. Varhaug are at the Laboratory of Biotechnology, Departments of Biochemistry and Surgery, University of Bergen, Norway; and C.L. Brown is at the Department of Morbid Anatomy, Institute of Pathology, London Hospital, London E1, U.K.

Table 1. *Thyroid tumour DNA samples analysed by Southern blot of c-erbB-1 and c-erbB-2 proto-oncogenes*

Follicular adenoma	9
Follicular carcinoma	7
Papillary carcinoma	17
Undifferentiated carcinoma	5

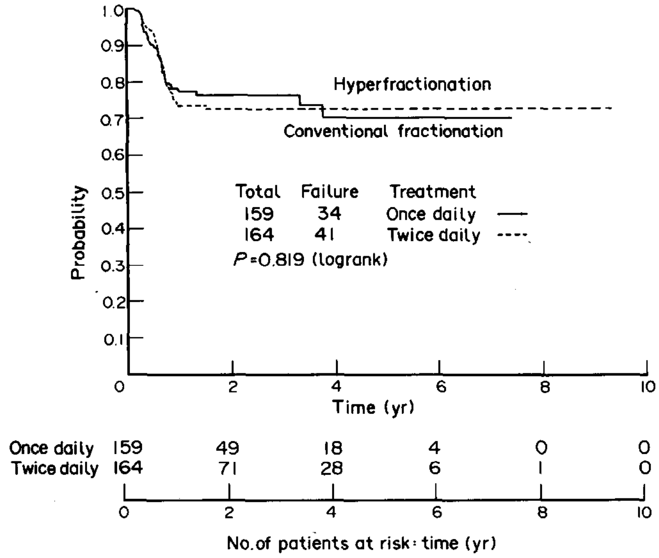

Fig.1. Probability of avoiding late side-effects of grade 2 or 3.

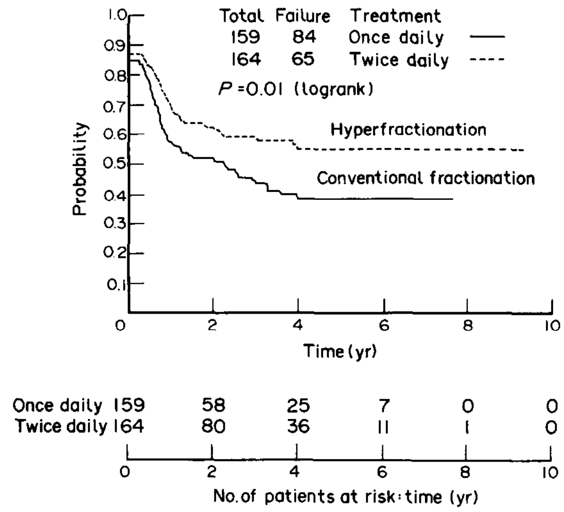

Fig.2. Probability of remaining free of locoregional disease.

PATIENTS AND METHODS

Patients had T2 T3 N0 or N1 oropharyngeal squamous cell carcinoma under 3 cm in size (except for primaries arising from the base of the tongue). Patients were randomly allocated to conventional frationation (70 Gy in 35 fractions over 7 weeks) or hyperfractionation (80.5 Gy in 70 fractions over 7 weeks). From 1980 to 1987, 356 patients were entered by 28 institutions from seven European countries. 90% of the patients were evaluable for the final analysis.

Acute and late tolerance

Objective acute tolerance based upon the scoring of acute mucosal reactions (according to the EORTC scoring scales for acute and late radiation damage) was significantly decreased in the hyperfractionation arm ($P = 0.007$ logrank, chi square test for trend), resulting in a prolongation of the overall treatment time in 13% of the patients. Only 6% of cases did not reach the prescribed dose.

Late damage to normal tissues was evaluated with an actuarial estimate of the freedom from grades 2 and 3 late tissue damage (Fig. 1). No difference was observed between the two treatment arms, which confirms the accuracy of the radiobiology prediction for normal tissue tolerance in the head and neck area. These results are of particular interest for the slower proliferating normal tissues such as bone and connective tissues.

Locoregional control

The locoregional control was significantly higher ($P = 0.01$ logrank) after hyperfractionation compared with conventional fractionation (Fig. 2). At 5 years, 56% of patients are locoregional disease-free in the hyperfractionated arm, as compared to 38% in the conventional fractionation arm. This advantage was observed only in the 217 patients with a good initial performance status (Karnofsky index 90–100%). The superiority of hyperfractionation was also demonstrated in patients staged T3 N0 T3 N1 but not in T2. The Cox model confirmed that the treatment regimen was an independent significant prognostic factor for locoregional control ($P = 0.007$, logrank).

Survival

Survival was not an end-point in our study. However, the improvement of locoregional control was responsible for a trend to an improved survival ($P = 0.07$, logrank).

CONCLUSIONS

This is the first controlled trial of the benefit of delivering 2 fractions per day instead of 1. We stress the importance of selecting patients in good general condition and with moderately advanced tumours to assess improvements of radiotherapy regimens in head and neck carcinoma. These positive results strongly support clinical research of new schemes of radiotherapy designed after cooperation between radiobiologists and radiotherapists. The present trials (ref. 2 and EORTC protocol 22851) include comparisons of conventional fractionation and accelerated fractionation (same total dose within a shorter overall treatment time) with individual measurements of tumour kinetics. Future trials should try to demonstrate the respective indications for conventional fractionation, hyperfractionation and accelerated fractionation, based upon clinical presentation and cell kinetics.

Correspondence to J.C. Horiot.

J.C. Horiot is at the Centre G.F. Leclerc, 21034 Dijon; R. Le Fur is at the Centre H. Becquerel, Rouen; T. N'Guyen is at the Institut J. Godinot, Reims; C. Chenal is at the Centre Eugène Marquis, Rennes; S. Schraub is at the CHU Jean Minjoz, Besancon; S. Alfonsi is at the CHU La Timone, Marseille, France; G. Gardani is at the National Tumor Institute, Milano, Italy; W. Van den Bogaert is at the University Hospital Saint Rafael, Leuven, Belgium; S. Danczak is at the Tumor Institute Mari Curie, Warszawa, Poland; M. Bolla is at the CHU La Tronche, Grenoble, France; and M. Van Glabbeke and M. De. Pauw are at the EORTC Data Center, Brussels, Belgium.

1. Withers R, Horiot JC. Hyperfractionation. In: Withers R, Peters LJ, eds. *Innovations in Radiation Oncology*. Berlin, Springer, 1987, 223–230.
2. Begg AC, Hofland I, Moonen L, *et al.* The predictive value of cell kinetics measurements in a European trial of accelerated fractionation in advanced head and neck tumors: an interim report. Communication at ASTRO meeting, Miami Beach, 15–20 October, 1989. *Int J Radiat Oncol Biol Phys* (in press).

of human breast tumours with amplified *neu* DNA. *Oncogene* 1988, **2**, 175–178.
9. Yokota J, Yamamoto T, Miyajima N, *et al.* Genetic alterations of the c-*erbB*-2 oncogene occur frequently in tubular adenocarcinoma of the stomach and are often accompanied by amplification of the v-*erbA* homologue. *Oncogene* 1988, **2**, 283–287.
10. Falck VG, Gullick WJ. c-*erbB*-2 oncogene product staining in gastric adenocarcinoma. An immunohistochemical study. *J Pathol* 1989, **159**, 107–111.
11. D'Emilla J, Bulovas K, D'Ercole K, Wolf B, Steele G, Summerhayes IC. Expression of the c-*erbB*-2 gono product (p185) at different stages of neoplastic progression in the colon. *Oncogene* 1989, **4**, 1233–1239.
12. Schneider PM, Hung MC, Chiocca SM, *et al.* Differential expression of the c-*erbB*-2 gene in human small cell and non-small cell lung cancer. *Cancer Res* 1989, **49**, 4968–4971.
13. Hall PA, Hughes CM, Staddon SL, Richman PI, Gullick WJ, Lemoine NR. The c-*erbB*-2 oncogene product in human pancreatic cancer. *J Pathol* 1990, **161**, 195–200.
14. Aasland R, Lillehaug JR, Male R, Josendal O, Varhaug JE, Kleppe K. Expression of oncogenes in thyroid tumours: coexpression of c-*erbB*-2/neu and c-*erbB*. *Br J Cancer* 1988, **57**, 358–363.
15. Segatto O, King CR, Pierce JH, Di Flore PP, Aaronson SA. Different structural alterations upregulate in vitro tyrosine kinase activity and transforming potency of the *erbB*-2 gene. *Mol Cell Biol* 1988, **8**, 5570–5574.
16. Wyllie FS, Lemoine NR, Williams ED, Wynford-Thomas D. Structure and expression of nuclear oncogenes in multistage thyroid tumorigenesis. *Br J Cancer* 1989, **60**, 561–565.
17. Xu YH, Ishii S, Clark AJL, *et al.* Human epidermal growth factor receptor cDNA is homologous to a variety of RNAs overproduced in A431 carcinoma cells. *Nature* 1984, **309**, 806–810.
18. Ullrich A, Coussens L, Hayflick JS, *et al.* Human epidermal growth factor receptor cDNA sequence and aberrant expression of the amplified gene in A431 epidermoid carcinoma cells. *Nature* 1984, **309**, 418–425.
19. Hung MC, Thompson KL, Chiu IM, Rosner MR. Characterization of rodent epidermal growth factor receptor transcripts using a mouse genomic probe. *Biochem Biophys Res Commun* **141**, 1109–1115.
20. Semba K, Kamata K, Toyoshima K, Yamamoto T. A v-*erbB* related proto-oncogene, c-*erbB*-2, is distinct form the c-*erbB*-1/EGF receptor gene and is amplified in a human salivary gland adenocarcinoma. *Proc Natl Acad Sci USA* 1985, **82**, 6497–6501.
21. Lemoine NR, Mayall ES, Wyllie FS, *et al.* High frequency of *ras* oncogene activation at all stages of human thyroid tumorigenesis. *Oncogene* **4**, 159–164.
22. Higuchi R. Simple and rapid preparation of samples for PCR. In: Erlich HA, ed. *PCR Technology: Principles and Applications for DNA Amplification.* New York, Stockton Press, 1989, 31–38.
23. Lemoine NR, Staddon SL, Dickson C, Barnes DM, Gullick WJ. Absence of activating transmembrane mutations in the c-*erbB*-2 protooncogene in human breast cancer. *Oncogene* 1990, **5**, 237–242.
24. Fusco A, Grieco M, Santoro M, *et al.* A new oncogene in human thyroid papillary carcinomas and their lymph-nodal metastases. *Nature* 1987, **328**, 170–172.
25. Bongarzone I, Pierotti MA, Monzini N, *et al.* High frequency of activation of tyrosine kinase oncogenes in human papillary thyroid carcinoma. *Oncogene* 1989, **4**, 1457–1462.
26. Suarez HG, Du Villard JA, Severino M, *et al.* Presence of mutations in all three *ras* genes in human thyroid tumors. *Oncogene* 1990, **5**, 565–570.
27. Williams DW, Williams ED, Wynford-Thomas D. Loss of dependence of IGF-1 for proliferation of human thyroid adenoma cells. *Br J Cancer* **57**, 535–539.
28. Jasani B, Wyllie FS, Wright PA, Lemoine NR, Williams ED, Wynford-Thomas. Immunodetectable TGF-beta associated with malignancy in a human epithelial neoplasm. *Growth Factors* 1990, **2**, 149–155.

Acknowledgements—This study was part of the concerted action programme "Molecular Genetics of Human Thyroid Cancer" sponsored by the Commission of the European Communities. N.R.L., S.L.S., C.M.H., and W.J.C. thank the Imperial Cancer Research Fund; F.S.W., J.J.S. and D.W.T. thank the Cancer Research Fund of Great Britain; and J.R.L., R.A., and J.E.V. thank the Norwegian Cancer Society and the Faculty of Medicine, University of Bergen, for support, and Dr Lars A. Akslen for tumour classification.

Eur J Cancer, Vol. 26, No. 7, pp. 779–780, 1990.
Printed in Great Britain

0277-5379/90$3.00 + 0.00
Pergamon Press plc

Hyperfractionated Compared with Conventional Radiotherapy in Oropharyngeal Carcinoma: an EORTC Randomized Trial

J.C. Horiot, R. Le Fur, T. N'Guyen, C. Chenal, S. Schraub, S. Alfonsi, G. Gardani, W. Van den Bogaert, S. Danczak, M. Bolla, M. van Glabbeke and M. de Pauw

INTRODUCTION

IN PURE hyperfractionation, radiotherapy is given in a higher number of fractions with a smaller dose per fraction, within the same overall treatment time as in conventional fractionation regimens (1). Division of the daily dose into two fractions (with an 8 h interval between them) allows for better recovery of normal tissues, which determines the late radiation tolerance. This gain in tolerance can be exploited by giving a 15% higher total dose in the same overall time. Whether such an increase in dose improves locoregional control without increasing the complication rate was the question addressed by trial 22791 of the EORTC Cooperative Group of Radiotherapy.