Molecular biology and respiratory disease · 4 *Series editor: R A Stockley*

Cancer genes

Karol Sikora, Gloria Ong

Bronchial carcinoma is one of the most common malignant tumours and its incidence continues to rise. Although smoking and other environmental factors remain risk factors, little is known about its genesis. Treatment, other than surgery or radiotherapy for local disease, is rarely curative and remains at best palliative.

With the incidence of lung cancer continuing to rise, ways of predicting susceptibility and new therapeutic approaches are urgently required. The techniques of molecular biology have started to clarify some of the mechanisms of tumorigenesis and tumour growth. The application of these techniques to lung cancer has enormous potential and should make a major contribution to the management of this disease.

In this article we review some of the recent advances in our understanding of the mechanisms of tumour development and growth. The problems of disordered growth are not limited to one tumour type, and our information now comes from many studies on a wide range of tumour types.

Cancer as a disorder of genes

Changes within the structure and function of the DNA of certain cells allow them to escape normal growth control processes. There are three types of gene that may be concerned in the generation of malignancy.

The first—the dominantly active *oncogene*—was discovered through the RNA tumour viruses. These genes encode proteins vital for signalling transduction from cell surface to nucleus and when they are expressed aberrantly, either quantitatively or qualitatively, cancer may emerge.

The second group consists of the *genes concerned in the production of proteins essential to maintaining the integrity of the genome* through its faithful reproduction. Defects cause syndromes associated with an unusually high cancer incidence; examples include ataxia-telangiectasia and Bloom's syndrome.

In the third and most recently understood group are genes whose encoded proteins are responsible for slowing down growth. These are the tumour suppressor genes or *anti-oncogenes*. When defects arise at a gene or protein level abnormal growth patterns may result.

Growth control genes

The original evidence that specific genes have a role in carcinogenesis came from the studies of RNA tumour viruses. RNA tumour viruses in general possess only three genes: two encode structural proteins and a third encodes reverse transcriptase—the enzyme that produces a DNA copy of the viral RNA, allowing incorporation of the viral genome into the host DNA. The addition of a fourth gene confers on RNA tumour viruses the ability to induce tumours rapidly in vivo and to convey malignant properties to cells in vitro. Transformed cells lose contact inhibition and they grow in an unregulated fashion, piling up on the culture plates instead of forming the usual single layer. These transforming genes are called viral oncogenes (*v-oncs*). Viral oncogenes share common sequences with cellular genes, which were thus termed cellular oncogenes (*c-oncs*).[1]

The extensive part played by cellular oncogenes in normal cellular growth and differentiation indicates the origin of oncogenes as normal growth control genes that may be hijacked by certain viruses and carried in an activated state. Cellular oncogenes do not usually possess transforming potential in their native state and so are often termed proto-oncogenes.[2] The confusing nomenclature has arisen because the viral genes were named after the tumours in which they were first described. For example, *v-src*, one of the first oncogenes studied, causes sarcoma in chickens, and *v-sis* causes a simian sarcoma.[3] Figure 1 illustrates genetics of the Rous sarcoma virus with its typical viral oncogene (*v src*) that can be incorporated into cellular DNA. The gene labelled *pol* codes for reverse transcriptase to facilitate this process. The viral structural proteins are then produced by the *gag* and *env* genes whereas *src* codes for a protein that activates protein kinase and hence increases cell replication.

Oncogene function

The control of cell growth and development is a complex process. Oncogene products play a part at each level of this process. Alterations to this finely balanced system may result in unregulated cell growth. The factors that influence cell phenotype are indicated in figure 2, with examples of the oncogenes that code for them.

Growth factors

Growth factors are extracellular proteins that modulate growth (for example, c-sis, fig 2).

Imperial Cancer Research Fund Oncology Group, Department of Clinical Oncology, Royal Postgraduate Medical School, Hammersmith Hospital, London
K Sikora
G Ong

Address for reprint requests: Professor K Sikora, Department of Clinical Oncology, Royal Postgraduate Medical School, Hammersmith Hospital, London W12 0HS.

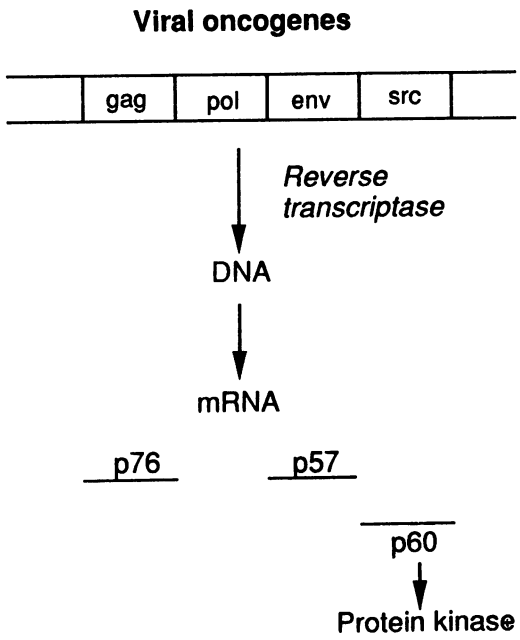

Figure 1 Genetics of the Rous sarcoma virus. The oncogene v-srx encodes the 60 kilodalton protein p60 src, which is responsible for transduction. mRNA—messenger RNA; p—protein.

They have some properties that indicate a role in carcinogenesis. Some growth factors will transform normal cells in vitro. Conversely, the induction of cell transformation may result in an increased production of growth factor. The observation that growth factors may both initiate and be a product of transformation suggests the possibility of an autocrine loop with unregulated cell division as the consequence. This phenomenon has been implicated in various conditions where there is rapid growth, such as wound repair and embryogenesis as well as malignant transformation. Certain small cell lung cancer lines are known to produce the growth stimulating peptide bombesin, which self stimulates. An autocrine loop is shown diagrammatically in figure 3.

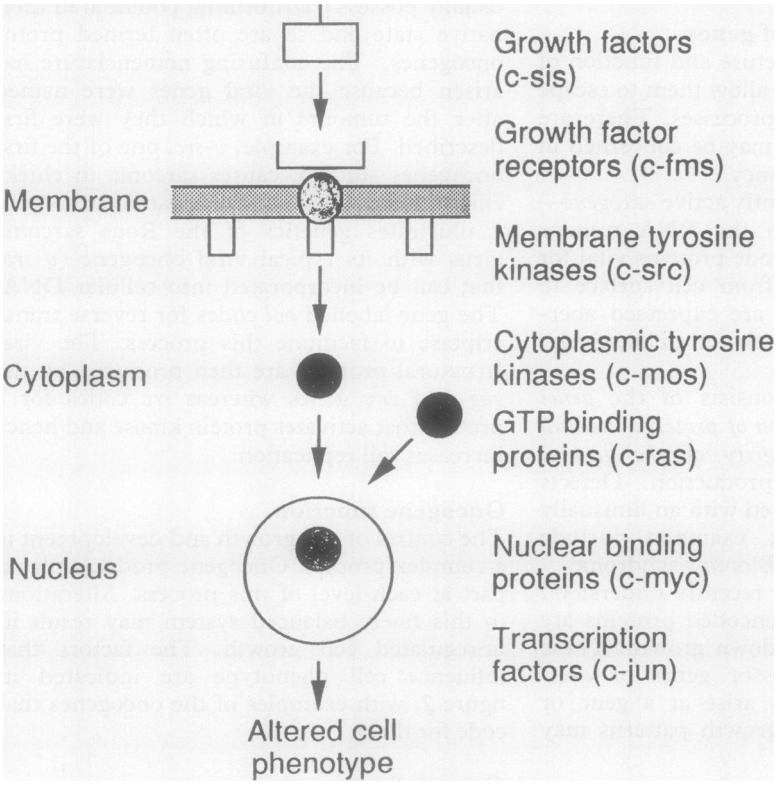

Figure 2 The cascade of growth control molecules encoded by various cellular oncogenes (c-sis etc). GTP—guanosine triphosphate.

Growth factor receptors

Several proteins encoded by oncogenes form part of a cell surface receptor (*c-fms*, fig 2). The presence of the appropriate growth factor causes structural changes that switch the receptor "on," with a resultant increase in tyrosine kinase activity within the cell. The kinase can also be regulated via an internal regulatory region in its molecular structure, allowing responses to intracellular events (*c-src* and *c-mos*, fig 2). The molecular consequences of increased kinase activity are many and such activity is a hallmark of transformed cells.

Small alterations in the cell surface receptor may cause defects in the regulation of tyrosine kinase activity. *c-erb 1*, for example, codes for the cellular receptor for epidermal growth factor (EGF). The equivalent viral gene, *v-erbB*, which has transforming activity, codes for a truncated external receptor that has an alteration in the internal regulatory region of the tyrosine kinase component. Transformation occurs if the viral gene product assumes a "locked on" configuration, tricking the cell into rapid growth.[4] A second mechanism is a direct alteration in tyrosine activity. A gene that acts in this way is the *c-fms* oncogene, which encodes the receptor for the colony stimulating factor CSF-1 in differentiating macrophages. The product of the transforming viral gene *v-fms* possesses greater kinase activity than its cellular counterpart, *c-fms*.[5]

Intracellular messengers

The best candidates for oncogenes acting at the level of intracellular messengers are the *ras* family of genes. The gene products are similar in structure to proteins termed G and N proteins that control adenylate cyclase activity. The products encoded by *ras* also have guanosine triphosphatase activity.[6] These proteins are all thought to be important in the "second messenger" system, thus providing a link between events at the cell membrane and the nucleus. C-ras mutations expressed at amino acids 12, 13, and 61 have been associated with various tumour types.[7] In about half of lung adenocarcinomas activated point mutations were found in codon 12 of Ki-ras.[8] No such mutations were found in squamous cell tumours. Why this particular mutation should be so specifically associated with this single histological type is not clear.

Oncogenes acting on the nucleus

Several oncogenes, such as *myc*, *myb*, and *fos*, code for proteins associated with the nucleus. Their precise localisation and function are unknown, but these oncogenes are concerned in the control of gene expression and may act to initiate the production of growth factors. Transcription factors, which control the binding of RNA polymerase to DNA and hence

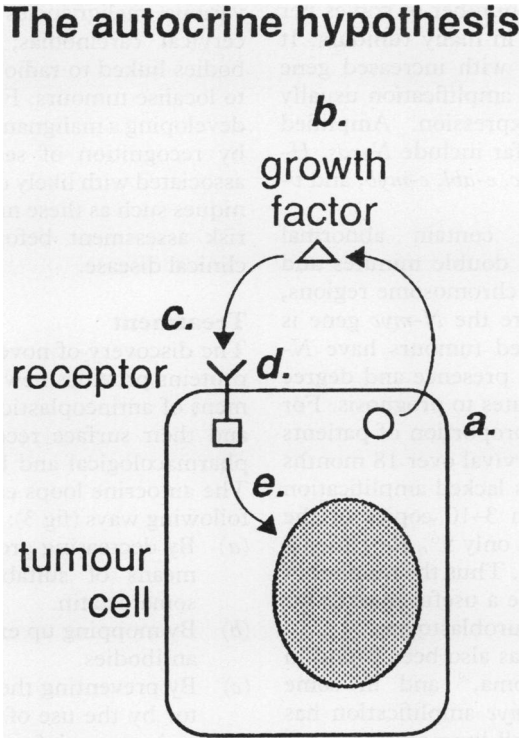

Figure 3 The hypothesis of an autocrine loop. Potential therapeutic strategies include: (a) decreasing growth factor secretion; (b) mopping up excess secretion of growth factor; (c) displacing growth factor from its receptor; (d) blocking signal transduction by receptors; (e) interfering with the surface to nuclear transduction pathway.

regulate gene expression, have recently been found to be oncogenes.

Tumour suppressor genes (anti-oncogenes)

The oncogenes mentioned above clearly possess the potential to alter regulation of cell growth, leading to tumour production. Increased expression of these genes could therefore be the key to the development of cancers. Increased expression may be a direct result of their activation within the genome, or their insertion into another active gene. More recently a third possibility for their increased expression has arisen with the discovery of anti-oncogenes or tumour suppressor genes. It is now clear that the defective expression of certain genes may result in tumour development. There are several potential mechanisms, including the activation of an oncogene or its product. Under some conditions the fusion of benign and malignant cells will result in the loss of the features of neoplasia. The discovery of inherited and acquired defects at a certain position on the long arm of chromosome 13 led to the mapping and eventual cloning of the retinoblastoma gene (*Rb*).[9] The defective expression of this gene at a critical stage of retinal development in childhood leads to a high risk of this exceedingly rare retinal tumour. The defective function of the *Rb* gene has now been implicated in breast cancer and a range of other common human tumours.

The gene for familial polyposis coli fits a similar model. This gene was mapped on to the short arm of chromosome 5 by genetic "fingerprinting" of patients and relatives. This technique can identify precisely the area or the genome that relates to a particular inherited characteristic. Minisatellite DNA probing showed that a second mutation at this site, making the individual homozygous for the defective gene, results in progress to adenocarcinoma. Mutations are present at this locus in 30% of sporadic colorectal cancers, suggesting that defective gene function at this site is important in many tumours besides those arising in patients with a background of polyposis.[10]

In lung cancer, especially the small cell type, effects have been noted consistently on chromosome 3. So far no single gene has been identified but loss of alleles at this point suggests the possibility of a tumour suppressor gene located here whose defective product may result in aberrant growth control and therefore tumour development. Deletion of a segment, p14–p23, on chromosome 3 results in loss of expression of at least one of the genes known to reside in this area.[11] Furthermore, the deletion is confined to the tumour cells and not the normal tissues from the same patient.[12] The implications of these observations are uncertain, but some DNA segments are susceptible to damage, particularly in smokers who develop lung cancer. The loss of function of this missing segment of DNA may result in loss of a tumour suppressor gene, leading to activation of a remaining oncogene. Indeed, the *raf* oncogene has been identified in a nearby region of the remaining DNA.[13] An alternative explanation could be that the deleted segment is inserted into another part of the genome, resulting in activation of its own oncogenes. Clearly, further studies are required to elucidate the mechanisms.

Clinical relevance

Oncogene changes occur in almost all the human malignancies analysed so far and may well have diagnostic and therapeutic potential. Malignancies such as Burkitt's lymphoma and chronic myeloid leukaemia are associated with specific chromosome aberrations. Translocation of the *c-myc* oncogene into the IgG locus[14] promotes tumorigenesis in Burkitt's lymphoma, and in chronic myeloid leukaemia the Philadelphia chromosome translocation from chromosome 9 to chromosome 22 alters the structure of both the *c-abl* gene transcript and its protein.[15]

Another feature commonly observed in tumorigenesis is increased gene expression. In a study of 54 different tumours Slamon *et al*[16] found no expression of erbA, erbB, mos, rel, sis, or yes in any of their tumour types but increased expression of fos and myc in renal cell carcinoma, ovarian adenocarcinoma, and colonic carcinoma. Breast carcinoma showed expression of fes, fos, and myb and high levels of fms, myc, H-ras, and Ki-ras. Increased expression of myc, H-ras, and Ki-ras was also detected in lung carcinomas. Spandidos and Agnantis[17] found a 4–15 fold increase in cellular H-ras expression in all 12 breast tumours that they examined. High levels of p21 ras expression have also been found in various tumours, including ovarian, lung, gastrointestinal, kidney, and lymphatic tumours. On the basis of many such studies it is now thought that the abnormal expression of more than one cellular oncogene is required for transformation. Gene

amplification (increased number of copies per cell) has been observed in many tumours. It should not be confused with increased gene expression, though gene amplification usually results in increased expression. Amplified oncogenes identified so far include *N-ras*, *H-ras*, *Ki-ras*, *c-erbB*, *c-myc*, *c-abl*, *c-myb*, and *v-myc*.

Neuroblastoma cells contain abnormal chromosomes known as double minutes and homogeneously staining chromosome regions, which are the sites where the *N-myc* gene is amplified. Most advanced tumours have *N-myc* amplification.[18] The presence and degree of gene amplification relates to prognosis. For instance, the estimated proportion of patients with progression free survival over 18 months was 70% where tumours lacked amplification and 30% for those with 3–10 copies of the oncogene, whereas it was only 5% for patients with more than 10 copies. Thus the number of copies of *N-myc* could be a useful marker for assessing prognosis in neuroblastoma.

N-myc amplification has also been found in small cell lung carcinoma,[19] and in some retinoblastomas, and *c-myc* amplification has been detected in small cell lung carcinomas,[20] breast carcinoma, and colonic carcinoma and carcinoma of the uterine cervix. We have also performed immunohistological staining, cloning a monoclonal antibody to *c-myc* protein in 31 patients with cervical carcinoma.[21] The indications are that *c-myc* positive patients had a poorer prognosis and a shorter relapse free survival. Amplification of the *c-erbB-2* gene has been found in many malignancies, including breast carcinoma and adenocarcinoma. An extensive study by Slamon *et al*[22] showed 28% amplification in 53 out of 189 tumours. *c-erbB-2* gene amplification has also been shown in stomach, kidney, and salivary gland carcinomas but not squamous cell tumours. Of 96 colonic adenocarcinomas, only one tumour showed *c-erbB-2* gene amplification. Thus *c-erbB-2* amplification appears to be confined to certain tumours. From immunohistological staining of breast tumours with specific antibodies, Venter[23] found that amplification of the *c-erbB-2* gene also results in overexpression of the protein. Overexpression of this protein, however, may also occur in tumours without gene amplification. Evidence for this came from studies of eight breast cancer cell lines, which showed that a 4–8 fold overexpression of mRNA was present in four that lacked gene amplification; but a 64–128 fold level of overexpression was present in the four cell lines that had gene amplification. Thus overexpression of *c-erbB-2* alone without gene amplification does occur, but at a lower level than when gene amplification is present.

Diagnosis and prognosis

Monoclonal antibodies directed against oncogene products may prove valuable as a diagnostic tool. Released oncogene products could be useful tumour markers for screening, diagnosis, or follow up. Immunocytochemical techniques have already shown potential in various malignancies, including breast and cervical carcinomas, and monoclonal antibodies linked to radionuclides have been used to localise tumours. Finally, patients at risk of developing a malignancy may well be identified by recognition of sequences in their DNA associated with likely oncogenic change. Techniques such as these might provide an accurate risk assessment before the development of clinical disease.

Treatment

The discovery of novel sets of growth control proteins provides new targets for the development of antineoplastic agents. Growth factors and their surface receptors are accessible to pharmacological and biological manipulation. The autocrine loops can be interrupted in the following ways (fig 3):

(*a*) By decreasing growth factor secretion by means of suitable inhibitors, such as somatostatin.

(*b*) By mopping up excess growth factor with antibodies.

(*c*) By preventing the binding of growth factor by the use of analogues that displace active growth factor from its receptor or of antireceptor antibodies to prevent access.

(*d*) At the receptor level, by blocking function by the use of suitable ligand analogues that interfere with signal transduction. The experience with luteinising hormone releasing hormone analogues in the management of prostatic cancer suggests that down regulation of surface receptors is possible even clinically. The mechanism for this well documented effect is not clear. The activation of a receptor results from the binding of ligand to the external domain and then the aggregation within the cell membrane of groups of receptors. This aggregation could potentially be blocked by small peptides that mimic the intramembranous portion of the receptor and sterically interfere with aggregation.

(*e*) Inside the cell, by interfering with the signal to grow in several ways: (i) tyrosine kinase inhibitors, such as suicide peptides (which bind and are phosphorylated by this enzyme, but which become covalently linked to it, irreversibly destroying enzyme activity) have been developed; (ii) nucleoside analogues, which bind to the *ras* gene product but prevent further activation, are also being investigated; (iii) the nuclear oncoproteins also provide fertile ground for the search for inhibitors with therapeutic potential; the mechanism by which c-fos, c-myc, and c-jun bind to DNA or other nuclear structures is currently being elucidated, and once these mechanisms are understood low molecular weight compounds might be developed to interfere with this binding and shift the partition between nucleus and cytoplasm, so altering growth control.

A major problem in the clinical use of these types of growth control agents will be to determine the best way to destroy a tumour selectively. Growth regulators have a physio-

logical role in the maintenance of normal cellular activity, so interference is likely to have profound effects on normal structure and function. The only way to determine how best to harness such compounds may be by painstaking clinical trials—in other words, by the empirical method by which we have discovered the combinations of cytotoxic drugs that are used successfully for the treatment of certain tumours.

During the 1990s clinicians are likely to be able to manipulate and control, in a sophisticated manner, abnormal growth in a range of cellular systems. This would appear to be the most promising avenue for future therapeutic discoveries, which should be in use in the early part of the next century.

1 Bishop JM, Varmus HE. Functions and origins of retroviral transforming genes. In: Weiss R, Teich N, Varmus H, Coffin J, eds. *Molecular biology of tumour viruses*. Part III: RNA tumour viruses. New York: Cold Spring Harbour Press, 1982:999–1108.
2 Bishop JM. Cellular oncogenes and retroviruses. *Ann Rev Biochem* 1983;**52**:301–37.
3 Bishop JM. Viral oncogenes. *Cell* 1985;**42**:23–38.
4 Hunter T, Cooper JA. Protein-tyrosine kinases. *Ann Rev Biochem* 1985;**54**:897–930.
5 Woolford J, Rothwall V, Rohrschweider L. Characterisation of the human c-fms gene product and its expression in cells of the monocyte-monophage linage. *Mol Cell Biol* 1985;**5**: 3458–65.
6 Tanaka T, Slamon DJ, Battifora H, Cline MJ. Expression of p21 ras oncoprotein in human cancers. *Cancer Res* 1988; **46**:1465–70.
7 Lemoine N. Ras oncogenes in human tumours. In: Sluyer M, ed. *Molecular biology of cancer genes*. Chichester: Harwood, 1989:82–118.
8 Rodenhuis S, Van De Wetering ML, Mool W,. Bos JL. Mutational activation of the K-ras oncogene. *N Engl J Med* 1987;**317**:929–35.
9 Benedict WF, Murphree AL, Banerjee A. Patient with 13 chromosome deletion evidence that the retinoblastoma gene is a recessive cancer gene. *Science* 1983;**219**:973–5.
10 Solomon E, Voss R, Hall V, *et al*. Chromosome 5 allele loss in human colorectal carcinomas. *Nature* 1987;**328**:616–9.
11 Miller YE, Minna JS, Gazdar AF. Lack of expression of aminoacylase 1 in small cell lung cancer. *J Clin Invest* 1989;**83**:2120–4.
12 Johnson BE, Sakaguchi A, Gazdar AF, Minna J, Marshall A, Naylor SL. Restriction fragment length polymorphism slides show consistent loss of chromosome 3 alleles in small cell lung cancer patient's tumours. *J Clin Invest* 1988;**82**:502–7.
13 Bonner T, O'Brien SJ, Nash WG, Rapp UR, Leder P. The human homologues of the raf/mil oncogene are located on chromosome 3 and 4. *Science* 1984;**223**:71–4.
14 Taub R, Kirsh I, Morton C, *et al*. Translocations of the c-myc gene into IgG heavy chain locus in Burkitt's lymphoma and in murine plasmacytoma cells. *Proc Natl Acad Sci USA* 1982;**79**:7837–41.
15 Shtivelman E, Lifshitz B, Gale RP, Canaani E. Fused transcript of abl and bcr genes in chronic myelogenous leukaemia. *Nature* 1985;**315**:550–4.
16 Slamon DJ, de Kernion JB, Verma IM, Cline MJ. Expression of cellular oncogenes in human malignancies. *Science* 1984;**724**:256–62.
17 Spandidos DA, Agnantis NJ. Human malignant tumours of the breast, as composed to their respective normal tissue, have elevated expression of the Harvey ras oncogene. *Anti Cancer Res* 1984;**4**:269–72.
18 Schwab M, Varmus HE, Bishop JM, *et al*. Chromosome localization in normal human cells and neuroblastomas of a gene related to c-myc. *Nature* 1984;**308**:288–91.
19 Nan MM, Brooks B, Corney D, *et al*. Human small cell lung cancers show amplification and expression of the N-myc gene. *Proc Natl Acad Sci USA* 1986;**83**:1092–6.
20 Little CD, Nan MM, Carney DN, Gazdon AF, Minna JD. Amplification and expression of the c-myc oncogene in human lung cancer cell lines. *Nature* 1983;**308**:194–6.
21 Sowani A, Ong G, Dische S, *et al*. C-myc oncogene expression and clinical outcome in carcinoma of the cervix. *Molecular and Cellular Probes* 1989;**3**:117–23.
22 Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER-2 neu oncogene. *Science* 1987;**235**:177–82.
23 Venter DJ, Tuzi NL, Kumar S, Gullick WJ. Overexpression of the c-erB-3 oncoprotein in human breast carcinomas: Immunohistological assessment correlates with gene amplification. *Lancet* 1987;ii:69–72.