The present example of preliminary closure of a trial on ethical grounds is not unique. However, the publication of mature data of this kind is important, particularly since it is more difficult to publish negative results after initial announcements of very promising results. Indeed, final reports are often lacking in such situations. A recent example that has attracted considerable attention [2] is the closure of the US AIDS Clinical Trials Group study in which zidovudine (AZT) has shown a beneficial effect. A critical look at the results of this study reveals that, despite significant differences between the treatment arms, the proportion of patients who have developed AIDS so far was relatively small (only 7% of the total patient population). Theoretically it would not be a surprise if, with longer follow-up, the statistical significance of the difference is lost.

Preliminary publications on the presumed positive effects of adjuvant therapy of common tumours like breast or colon cancer may cause harm in the long term. Assuming that 10% of randomized studies include a therapy that represents benefit and adopting a 5% false-positive rate and 20% false-negative rate, 36% of the reported positive outcomes from clinical trials will be false-positive [3]. When the false-positive rate is raised to 20% because of the frequent and often premature analysis of the data, the proportion of false-positive outcomes rises to 65%.

Even adoption of a 1% false-positive rate in a clinical setting will result in a 14% false-positive rate among all reported positive trials. These far-reaching consequences of early termination of a trial or announcement of preliminary results may outweigh the presumed ethical basis that led to study interruption.

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1. Tait DM, Thornton-Jones H, Bloom HJG *et al.* Adjuvant chemotherapy for medulloblastoma, the first multi-centre control trial of the International Society of Paediatric Oncology (SIOP I). *Eur J Cancer* 1990, **26**, 464–469.
2. Editorial: Clinical trials of zidovudine in HIV infection. *Lancet* 1989, **i**, 415–416.
3. Editorial: Early stopping, interim analysis, and monitoring committees: what are the tradeoffs? *J Clin Oncol* 1987, **5**, 1314–1315.

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The Clinical Relevance of Tumour Hypoxia

IT IS generally accepted that oxygen deficiency is a common occurrence in many human tumours. However, to what extent tumour hypoxia influences treatment response is still unclear. A detailed review of the state of knowledge on tumour hypoxia was the subject of a meeting convened by the Radiotherapy Group of the EORTC in Leuven in December 1989. The meeting addressed basic laboratory studies in molecular, cellular and tissue radiobiology, the measurement of tissue hypoxia, and new developments in the field of radiation sensitizers and bioreductive drugs. Clinical studies of methods for eradicating tumour hypoxia and exploiting the presence of hypoxia for new approaches to diagnosis and therapy were also reviewed.

Clinical evidence from some of the early hyperbaric oxygen trials indicates that tumour hypoxia may adversely influence treatment outcome, particularly in cancer of the cervix and head and neck. Recent results suggest that variation in oxygen levels in individual head and neck tumours directly influences treatment outcome. However, tumour heterogeneity is clearly a major confounding factor in the interpretation of clinical trials. In addition to tumour size, efficiency of re-oxygenation and haemoglobin status are probably important determinants of treatment outcome in some situations. Anaemia is clearly an important factor and there is increasing evidence that haemoglobin levels within the normal range can influence prognosis.

The clinical measurement of tumour hypoxia includes the use of *in situ* ultra-fine oxygen electrodes. The observations indicate considerable heterogeneity of oxygenation within individual tumours and even within normal tissues. This type of approach has promise for the identification and possibly classification of tumours where hypoxia has an adverse effect on treatment outcome. However, less invasive techniques for measuring individual variations in the hypoxic status of tumours would have obvious advantages.

In this context, the use of isotopically labelled bioreductive agents for detecting tumour hypoxia shows promise and the phenomenon of bioreductively activated binding of nitroimidazole drugs in hypoxic tissue is under active investigation as a means of localizing iodine-131 for scintigraphic identification of tumour hypoxia *in situ*.

Manoeuvres designed to improve tumour oxygenation include the use of blood flow modifiers for increasing oxygen levels in tumours. The vasoactive drugs flunarizine and nicotinamide significantly reduce the levels of hypoxia in some experimental murine tumours. An alternative approach is to manipulate the binding affinity of oxygen and haemoglobin. Clofibrate, for example, substantially right-shifts the oxy-haemoglobin association curve and increases the radiation sensitivity of some murine tumours.

The oxygen carrying properties of blood can be significantly increased by perfusion with some fluorocarbon emulsions. These agents can dissolve large quantities of free oxygen and consequently improve tissue oxygenation. Human tumour xenografts treated with fluorocarbon emulsions before irradiation show increased sensitivity. The effect, although significant, was not large, although somewhat greater when fluorocarbon was combined with carbogen.

The clinical hyperbaric oxygen trials carried out during the last 2–3 decades were reviewed. The large multi-centre MRC trial in carcinoma of the cervix showed a clear benefit for patients receiving oxygen; retrospective analysis indicated that a small subgroup of anaemic patients transfused before treatment with hyperbaric oxygen showed a high local control rate. Evidence of the significance of hypoxia from some hyperbaric oxygen trials has led to renewed interest in radiotherapy under conditions of normobaric oxygen (1 atmosphere of pure oxygen). In an experimental murine tumour (Ca NT) treated with fractionated X-rays in normobaric oxygen, enhancement ratios were 1.45 for 30 fractions given over 42 days and 1.30 for 36 fractions in 12 days. Preliminary results showed that nicotinamide combined with normobaric oxygen in a ten-fraction schedule increased the enhancement ratio to 1.8.

The current status of bioreductive drugs used as hypoxic cytotoxins or as hypoxic cell radiation sensitizers was reviewed. Bioreductive drugs are converted intracellularly by anaerobic biochemical reduction to form potent cytotoxins. Since bioactivation is favoured by a hypoxic environment, there is a sound basis for enhanced activity in tumours relative to normal tissue. Many of these agents can also function as hypoxic cell radiation sensitizers although there are clear differences in the mechanisms involved. Two types of bioreductive agent, the benztriazene di-N-oxides, e.g. SR 4233, and derivatives of the dual function alkylating nitroimidazoles, e.g. RSU 1069, were discussed. New data were presented on the radiosensitizing activity of the compound RB 6145, a bromoethylamino-substituted 2-nitroimidazole. This compound is a pro-drug for RSU 1069 and a potent radiosensitizer of murine tumours treated *in vivo*. It can be given orally, has good bioavailability and appears to be substantially less toxic than RSU 1069. Data on related analogues were reviewed.

The hypoxic cytotoxic property exhibited by bioreductive drugs can often be observed more easily *in vivo* when tumour hypoxia is enhanced. It is well known for example, that treatment with some vaso-active drugs, such as hydralazine, can induce severe reduction in tumour blood flow which in turn, increases oxygen deficiency in some murine tumours. The anti-tumour activities of various bioreductive agents, used either in tumours where the blood supply is occluded by clamping, or in tumours in mice treated with blood flow modifiers, show considerable variation. Of the two agents that induce the greatest differential toxicity *in vivo*, RSU 1069 demonstrated the greater anti-tumour effect in KHT tumours either clamped or in mice treated with hydralazine. Conversely SR 4233 showed the greater response in tumours in mice treated with flavone acetic acid (FAA).

Therapeutic strategies mediated by damage to the tumour vasculature were reviewed with particular attention to the effects induced by FAA. The pronounced anti-tumour activity observed in some murine tumours is believed to be due to the onset of coagulation cascades leading to induction of haemorrhagic necrosis. Clinical trials of treatment with FAA have so far proved disappointing.

In a dynamic study of tumour vasculature, tumour-bearing mice were infused at different times with two short-lived stains, fluorescein di-acetate and Hoechst 33342, substances that fluoresce at different wavelengths. Histological examination of tumour sections taken at various times after treatment showed patterns of stain uptake identifying those blood vessels open at the time of treatment. In principle, this method should permit detailed examination of the relative importance of acute and chronic hypoxia in tumours.

Investigations of the combined effects of hyperthermia, hydralazine and RSU 1069 in experimental murine tumours are based on the increased sensitivity of hypoxic cells to the hypoxic cytotoxic action of hyperthermia, and the anticipated sensitizing effect of hydralazine which substantially increases tumour hypoxia. Such an effect was observed in implanted mouse mammary tumours: incorporation of RSU 1069 into the treatment caused an even greater enhancement of tumour response.

The meeting concluded with discussion of current prospective randomized trials and pilot studies of hypoxic cell sensitizers including multi-centre trials of etanidazole (SR 2508) in the radiotherapy of head and neck cancer. Discussion was confined to toxicological considerations since information on treatment outcome is not yet available. To date, neurological complications are fairly mild as predicted by earlier phase I and II studies. Trials of other hypoxic cell sensitizers are in progress including the Danish trial of nimorazole, a 5-nitroimidazole, in the radiotherapy of advanced supra-glottic and pharyngeal tumours. The drug is well tolerated; interim results suggest a superior response rate in the sensitizer arm. Further, as was found in the previous DAHANCA trial of misonidazole, there appears to be a significant influence on response rate of haemoglobin status within the normal range.

Pimonidazole (Ro 03–8799) a 2-nitroimidazole, is under clinical trial as a radiosensitizer. Preliminary toxicity data on the combined use of etanidazole and pimonidazole was discussed and a progress report on the Medical Research Council trial of radiotherapy and pimonidazole for advanced cervic cancer was presented.

Bioreductive drugs which may enhance the effect of single doses of radiation may be ineffective when they are combined with fractionated radiation. In the SCC7 murine tumour, the sensitization by etanidazole is almost completely lost when the drug is given with each fraction of a ten-fraction radiation treatment. A possible explanation is re-oxygenation of hypoxic cells during treatment. Conversely, SR4233 gave substantial sensitization with fractionated irradiation.

Tumour hypoxia remains an important research topic in clinical radiotherapy and applied radiobiology. Hypoxic cell resistance is almost certainly a major factor influencing the clinical response of some tumours although tumour heterogeneity complicates the interpretation of data. Further development and clinical application of methods for identifying appropriate subgroups will remain one of the top priorities in the field.

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