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Editorial

Bioreductive Agents, Hypoxic Cells and Therapy

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EXPERIMENTAL CHEMOTHERAPISTS are aware of a rule in drug development which states that the chance of an anticancer agent being successful clinically, is not directly proportional to the elegance of the working hypothesis and the amount of time and effort put into its development. In fact, some cynics hold that the relationship is an inverse one. This rule reflects the major problem of experimental cancer chemotherapy, namely that the laboratory models used to investigate new chemicals do not accurately predict the behaviour of any type of human cancer. Thus, agents with excellent activity against transplanted rodent tumours, such as the nitrosoureas, have been quite disappointing in the clinic. It is no coincidence that some of the important advances in cancer chemotherapy, such as the discovery of the co-ordination complexes of platinum, have come not from planned drug development programmes based on screening or rational design, but initially from a chance observation made in the course of experiments unrelated to cancer chemotherapy.

Nevertheless, at first sight, it is disappointing that Dirix and associates [1] report in this issue on the failure of EO9, a bioreductive agent, to have activity in four independent phase II clinical trials [1] (pages 2019–2022). The aim of bioreductive agents is to act as prodrugs and by selective activation in hypoxic cells, remove a source of chemotherapy resistant cells that could otherwise regrow after treatment and lead to failure of therapy. The very nature of cancer means that most solid tumours, even when small, will be expected to contain necrotic zones on the borders of which will be chemotherapy resistant hypoxic cells. Thus, solid rodent tumours should be reasonably good models for the study of bioreductive agents. The existence of hypoxic cells in tumours and cell spheroids, and their resistance to conventional therapy has been amply proven, particularly by the efforts of the radiobiologists over the past 30 years, not only by physical and biochemical measurements but also by direct visualisation. Chapman and colleagues injected tumour bearing animals with a labelled nitroimidazole and showed covalent binding in autoradiographic sections exactly where they had been predicted, namely in cells close to central necrotic zones [2]. More recently, perfluorocarbons have been developed which contain up to twenty equivalent fluorine atoms and are highly sensitive reagents for NMR. Experiments on tumour bearing animals have shown that, under ideal conditions, hypoxic zones can be clearly visualised, and that these diminish in animals breathing carbogen [3]. There is a limited amount of clinical evidence that abolition of the hypoxic fraction of tumours can improve responses to therapy. In some trials, a beneficial effect of hyperbaric oxygen and electron affinic radiation sensitisers on, for example, radiotherapy of head and neck cancers has been reported. An anecdotal report also claimed that tumours infected with anaerobic bacteria were particularly sensitive to nitroimidazole radiation sensitisers, suggesting that, besides sensitising as electron affinic agents, they were also acting as prodrugs, being converted, to covalent binding electrophilic reactants by the bacterial nitroreductases (as noted in the aforementioned Chapman studies). The concept of bioreductive prodrugs has been well validated and many different classes have been investigated, including a range of nitro compounds, N-oxides and chemicals acting by EO9 type mechanisms [4].

In the paper by Dirix and associates, EO9 given as a 5 minute i.v. infusion at a weekly dose of 12 mg/m^2 to 22 patients with breast cancer, 26 with colon cancer, 24 with pancreatic cancer and 20 with gastric cancer gave no evidence of antitumour activity. In retrospect, it is surprising that so many phase II trials were carried on EO9 as a single agent when theoretically one might not expect activity. It is possible, of course, that the inactivity was due to inappropriate pharmacokinetics, failure to reach hypoxic zones or even because the drug was not behaving as a prodrug as predicted. However, it is also a possibility that the drug is acting as a perfect bioreductive prodrug, in which case measurement of antitumour effect using conventional techniques might well give negative results.

Many years ago, experiments were carried out on subcutaneously implanted tumours in rodents which could be accurately measured by calipers. A dose of cyclophosphamide which caused a small reduction in the volume of the tumour, nevertheless caused 99% tumour cell kill, followed by rapid regrowth, when measured by a clonogenic assay [5]. Since the hypoxic fraction of tumours, is unlikely to approach 90%, conventional measurements would be unable to detect any significant response, even if the hypoxic fraction was being totally removed by the action of EO9. This is another example where a working hypothesis followed by research uncovers a new class of potentially useful agents which enter clinical trials that are not designed to validate the mechanism of action. Although no significant

responses occurred, had the hypoxic fraction been found to be eliminated, then there would have been a stimulus to carry out trials in combination with radiotherapy or chemotherapy with a selectivity for oxygenated cells.

Much work has gone into the development of hypoxia activated prodrugs and it is essential that, before they are forgotten, more appropriate clinical trials are carried out to see if this potentially valuable class of agents has an important role in both radio- and chemotherapy. Certainly, the recent report of synergism between the bioreductive tirapazamine and cisplatin in clinical trials suggests that they may well have a role in the combination therapy of solid tumours [6].

A definitive clinical trials to offer proof of the bioreductive concept should contain the following features.

1. A tumour which shows some response to radio- or chemotherapy and which is known to have a significant hypoxic fraction at the time of treatment.
2. A tumour which is relatively accessible to needle biopsy.
3. Measurement of tumour hypoxic fraction prior to therapy, and, if possible, during therapy. Non-invasive techniques which use NMR and perfluorocarbons, described above, and related techniques, which use labelled imidazoles, should soon be available for clinical measurement [7]. A technique which is already available uses an antibody to theophylline. Patients are injected with theophylline chemically linked to a nitroimidazole, and the presence of the bound theophylline is visualised in biopsy specimens [8].
4. Measurements of the enzymes involved in the activation of bioreductive agents such as cytochrome P450 reductase or DT diaphorase. These can be determined in needle biopsy samples [9].
5. Highly sensitive methods to measure tumour response, particularly PET scanning using fluorodeoxyglucose methionine or thymidine [10].
6. Trials in which bioreductive is used at the appropriate time in combination with conventional therapy, for example, radiotherapy in head and neck cancer.

So many new classes of agent are at the stage of early clinical trial that is important that some form of sensitive marker of response is available other than standard measurements. The new agents entering the clinic are not broadly toxic, like the alkylating agents, but are designed to be very much more selective, for example, inhibitors of ras or specific tyrosine kinase receptors. At the time of treatment, most solid tumours will be heterogeneous for these pathways, and these agents may not kill sufficient cells to produce a large tumour response. However, proof of the hypothesised action would allow further laboratory research to optimise structures and to design rational combinations for further clinical trials.

1. Dirix LY, Tonnesen F, Cassidy J, *et al.* EO9 phase II study in advanced breast, gastric, pancreatic and colorectal carcinoma by the EORTC Early Clinical Studies Group. *Eur. J Cancer* 1996, **32A**, 2019–2022.
2. Chapman JD, Franco AJ, Sharplin J. A marker in hypoxic cells for tumours with potential clinical applicability. *Br J Cancer* 1981, **45**, 546–550.
3. Sotak CH, Hees PS, Huang M-H, *et al.* A new perfluorocarbon for use in fluorine 19 magnetic resonance imaging and spectroscopy. *Magn Reson Med* 1993, **29**, 188–195.
4. Workman P. Enzyme directed bioreductive development revisited. *Oncol Res* 1994, **6**, 461–475.
5. Wilcox WS. Concepts concerning the relationship of population kinetics to tumour growth. In Hall TC, Karrer K, eds. *Pharmacological Principles in Antitumour Chemotherapy.* Verlag der Wiener Medizinische Akademie, Vienna, 1968, 25–38.
6. Kilpatrick D, Johnson CA, Langer C, *et al.* Phase I multiple dose trial of tirapazamine (WIN 59075, SR4233) in combination with cisplatin. *Proc Am Assoc Cancer Res* 1996, **37**, 604.
7. Cherif A, Wallace S, Yang DJ, *et al.* Development of new markers for hypoxic cells ^{131}I iodomisonidazole and ^{131}I iodoerythronitroimidazole. *J Drug Targeting* 1996, **4**, 31.
8. Hodgkiss RJ, Stratford MRL, Dennis MF, Hill SA. Pharmacokinetics and binding of the bioreductive probe for hypoxia NITP; effect of route of administration. *Br J Cancer* 1995, **72**, 1462–1468.
9. Ross D, Beall HD, Siegel D, *et al.* Enzymology of bioreductive drug activation. *Br J Cancer* 1996, 74 (Suppl XXVII), S1–S8.
10. Jansson T, Westlin JE, Ahlstrom H, *et al.* Positron emission tomography studies in patients with locally advanced and/or metastatic breast cancer; a method for early therapy evaluation? *J Clin Oncol* 1995, **13**, 1470–1477.