

conceptually tumours shrink due to the metabolic consequences. This is categorised as vascular targeting; the effect of isolated limb perfusion might be due to selective tumour vessel toxicity. An agent such as CM101 binds to tumour endothelium, activates complement and causes selective endothelial damage. Targeting tissue factor to tumour endothelium results in selective and rapid necrosis. The second approach interferes with the processes ECs undergo during neovascularization: basal membrane degradation and matrix invasion, migration, proliferation and tube formation. Different agents are given anti-angiogenic properties because they inhibit EC proliferation in vitro. This might result in tumour growth inhibition. This class harbours compounds such as TNP-470, PF-4, metalloproteinase inhibitors, and endostatin. Our current cytotoxic drugs have also anti-angiogenic activity in vitro. This should lead to the distinction between EC-selective and non-selective compounds. Different agents as diverse as growth factor antagonists, antintegrins and endostatin, however do result in tumour regression. This suggests an ongoing remodelling of tumour vasculature with induction of drug induced EC apoptosis and/or a critical role of activated EC for tumour growth. The difficulties in the clinical development of these compounds will be discussed.

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### Antisense oligonucleotides in cancerology

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The concept of antisense oligonucleotides in cancerology is a direct consequence of the results of molecular biology. These small synthetic DNAs (often with chemical modifications) are used in tumor cells to target genes which are active to support the cell proliferation. These genes can be proto-oncogenes, growth factors, transcription factors, factors involved in the signal transmission from the cell membrane to the nucleus... The aim is to prevent specifically the protein synthesis of one gene through a Watson Crick interaction between the oligonucleotide and the RNA transcript. Therefore with molecular weights comparable to the ones of some classical anticancer agents, oligonucleotides are expected to be much more specific and less toxic. Many oligonucleotides have been described in the last 10 years as efficient against transformed cells in culture. In the last 4 years they have also been shown to inhibit, with efficiency, the growth of human tumors grafted to mice after local or systemic administration. These results already demonstrate that in various instances it is possible to greatly reduce the tumor growth by targeting one single genetic event. However we have still to learn a lot at the basic level about how oligonucleotides work and how to improve their efficiency. Among other questions a major one is how are they delivered to their site of action in the cell? Vectorization and/or serum deprivation are required in cell culture to obtain gene inhibition. However oligonucleotides already display activity in animal as free injected molecules. Actually 4 clinical trials are taking place in phase 1 with oligonucleotides targeting specific genes involved in cancers (PKC, p53, c-myc, c-rat).

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### Identifying tumour hypoxia

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There is experimental and clinical evidence that hypoxic tumour cells create resistance to several cancer therapies. The definition of tumour "hypoxia" is dependent on the assay used.

Recently, direct identification of hypoxia in human tumours has become feasible using either polarographic oxygen sensitive electrodes or by the use of hypoxia marker assays, such as detection of nitroimidazole labelling by the use of antibody techniques, <sup>18</sup>F PET, or <sup>123</sup>I SPECT. Also indirect estimates of tumour hypoxia, such as tumour blood perfusion by laser Doppler, vascular staining techniques and functional MRI or <sup>31</sup>P MRS energy measurements, have been reported.

It is now becoming clear, from a large number of clinical studies using different assays, that hypoxia exist in most tumours but not in all. The level of hypoxia is heterogeneous both within and between tumours, and data obtained with oxygen electrodes indicate that the variability between tumours is larger than the variability within a tumour. Moreover, the oxygenation status is independent of histopathological tumour type and tumour size.

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### Tumour hypoxia and treatment outcome

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Tumour oxygenation is a factor influencing the response of human solid tumours to radiotherapy or to certain cytotoxic drugs. Despite technical limitations, needle pO<sub>2</sub> probes were first used to assess the oxygenation status of human tumours in the 1950's.

This invasive technique was improved in the late 1980's with the appearance of new polarographic equipment's, using fast responding electrodes movements programmed to minimise the effects of tissue compression. The pO<sub>2</sub> values recorded in normal tissues were in general lower than in tumours. Most of tumours had low pO<sub>2</sub> values (defined as values below 1.33 kPa, 10 mmHg): these values were found in 83% (29/35) of the patients with an ENT tumour and 46% (6/13) of the patients with melanoma.

The differences observed between tumours have long suggested that pO<sub>2</sub> measured by polarography could be a discriminant factor for treatment response. From the end of the 60's, data have been published showing that pre-radiotherapy measured pO<sub>2</sub> was of predictive value for treatment outcome. However, different parameters have been used to define tumour hypoxia (median, hypoxic fraction, % <2.5 mmHg), making comparisons difficult. The results of the more recent studies will be presented together with proposals on oxygen manipulation to sensitize solid human tumours to treatment.

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### The influence of the tumor microenvironment on malignant progression

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Angiogenesis, the development of new blood vessels, is a highly regulated process that is genetically controlled by alterations in tumor suppressor gene function and physiologically controlled by oxygen tension (hypoxia). In this study, we investigated how low oxygen conditions influenced the expression of the anti-angiogenic gene, thrombospondin 1 (TSP-1) and the pro-angiogenic gene, vascular endothelial growth factor (VEGF) in cells that differ in their expression of the p53 tumor suppressor gene or the bcl-2 anti-apoptotic gene. We found that hypoxia increased the transient induction of TSP-1 in cells containing a wild-type p53 genotype and that the basal and inducible expression of TSP-1 was undetectable in cells lacking p53. In contrast, VEGF was induced under hypoxic conditions, regardless of the cellular p53 genotype. In cells expressing a conditionally inducible myc proto-oncogene, hypoxia also transiently induced the expression of the TSP-1 which was undetectable by 12 h post-treated. Although hypoxia also increased the expression of VEGF, it only remained elevated in cells containing bcl-2, suggesting that decreasing the apoptotic responsiveness of cells to hypoxia permitted sustained expression of VEGF. Sections from tumors derived from these same cells indicated that VEGF and hypoxic regions co-localized, but that TSP-1 levels were low and did not co-localize with hypoxic regions. These studies suggest that fluctuating oxygen tensions play an important role in driving tumor progression both by influencing cell death and stimulating angiogenesis. Supported by NCI grant P01CA67166

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### Hypoxic modification in radiotherapy

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It is well established that solid tumors may contain oxygen deficient hypoxic areas and that cells in such areas will cause tumors to be resistant to ionizing radiation. Experimental clinical studies during the last 30 years have shown that this source of radiation resistance can be eliminated or modified by a variety of procedures that include high oxygen content gas breathing and use of nitroaromatic radiation sensitizers. By 1997 over 10,000 patients in 82 randomized trials had undergone treatment designed to modify tumor hypoxia prior to radiation therapy. Although a number of these trials showed no benefit, an overview analysis showed that modification of tumor hypoxia significantly improved the loco-regional tumor control after radiotherapy. The treatment benefit could mostly be related to an improved response in head and neck. Similarly to the local control benefit, the overall survival rate