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 neoangiogenesis: 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, antiintegrins 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-myb, c-raf).

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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, ^{18}F PET, or ^{123}I SPECT. Also indirect estimates of tumour hypoxia, such as tumour blood perfusion by laser Doppler, vascular staining techniques and functional MRI or ^{31}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_2 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_2 values recorded in normal tissues were in general lower than in tumours. Most of tumours had low pO_2 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_2 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_2 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 sensitise 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 I (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 PO1CA67166

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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

was improved. The overall results thus demonstrated that the biological issue related to hypoxia appears to be a sound rationale, which may impact the outcome of radiotherapy, especially in head and neck carcinoma. Yet, despite this wealth of positive data, "hypoxic modification" still has no impact on general clinical practice.

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Exploiting hypoxia: Bioreductive drugs and gene therapy approaches

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Background: Hypoxia is known to play a major role in determining resistance to conventional therapy of solid tumours. However, hypoxia mediated changes in gene expression can also influence treatment outcome.

Purpose: To develop two complimentary strategies *a)* selectively kill and/or inhibit the function of hypoxic cells in tumours by the use of bioreductive drugs *b)* exploit the presence of hypoxia in tumours to deliver highly selective therapy.

Examples: Tirapazamine kills hypoxic cells via a reductase mediated mechanism. In breast carcinoma cells, cytotoxicity is exquisitely dependent on P450 reductase. Further in human breast tumour biopsies, P450 reductase levels are sufficiently high that tirapazamine should cause substantial toxicity. We have identified a novel hypoxia mediated drug delivery system based on the indoloquinone nucleus. This, under hypoxic conditions will selectively release diffusable cytotoxic species, enzyme inhibitors etc. Exploiting hypoxia is further demonstrated by taking advantage if genetic sequences (HREs) that allow increased gene expression under hypoxic conditions. Therapeutic genes that are controlled by HREs will only be expressed under hypoxic conditions, thus providing a novel method for delivering selective gene therapy of tumours. The transcription factor which binds to HREs to promote gene transcription is HIF-1. Data will be presented to show that inhibition of HIF-1 inhibits tumour growth, thus identifying HIF-1 as a novel therapeutic target.

Clearly hypoxia is still a problem to be overcome but it is also a physiological abnormality of tumours that can be exploited.

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Anaerobic bacteria as a potential tumour gene transfer system

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To have a highly specific vector system for gene therapy in cancer, we propose the use of apathogenic clostridia. This use of strictly anaerobic bacteria as vector for specific tumour targeting is based on several observations. (1) Hypoxic-necrotic regions are unique to solid tumours. (2) Spontaneous and deliberate infiltration of anaerobic bacteria has been shown both in animal and in human tumours. To test the feasibility of using *Clostridium* as a tumour specific transfer system, we have used WAG/rij rats with rhabdomyosarcomas as a model. Our data showed that after intravenous administration of at least 10^8 spores, *Clostridium* could colonise the tumour model; the most efficient species being *Clostridium acetobutylicum* and *C. oncolyticum*. Spores could survive a few weeks in normal tissues, they did not germinate in these tissues. In tumours *Clostridium* spores started germination already after 2 days. We found that *C. acetobutylicum* and *C. oncolyticum* are not sensitive to therapeutic doses of the prodrug 5-fluorocytosine or the drug 5-fluorouracil to be obtained under the influence of the suicide gene cytosine deaminase that we plan to express in *Clostridium*. To this order, we are able to transform *C. acetobutylicum* by electroporation. Furthermore, repeated injections of *Clostridium* do not provoke any change in body temperature. In conclusion, it seems likely to use these bacteria as a selective transfer system. This strategy would be quite tumour specific.

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The biology of Hodgkin's disease

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Due to the scarcity of Hodgkin-Reed Sternberg (H-RS) cells their genetic analysis is difficult to perform. The recent establishment of micromanipulation of single H-RS cells from lymph node biopsies with subsequent gene amplification by polymerase chain reaction (single cell PCR) allows the molecular characterisation of these cells for the first time. Using this new approach analysis of immunoglobulin (Ig) gene rearrangements revealed evidence that Hodgkin's disease (HD) represents a B-cell disorder in the majority of cases. The sequences of rearranged Ig genes contain multiple somatic hypermutations indicating that the H-RS cells are derived from the germinal centre of lymph follicles. Moreover, mutations appear which prevent the expression of the antibody. Physiologically these cells would undergo apoptosis. A possible mechanism to keep the H-RS cells from apoptosis could be the activation of the oncogene bcl-2 which might be induced by EBV infection. The transformation process thus might take place in EBV-infected B-lymphocytes. Loss of EBV after initiation of the malignant transformation could explain the occurrence of EBV negative Hodgkin's lymphomas. Karyotype analysis of H-RS cells revealed a heterogeneous pattern including a broad spectrum of numerical and structural abnormalities. No specific chromosomal marker could be found. Nevertheless the analysis of HD by fluorescence in situ hybridisation (FISH) demonstrated clonal numerical aberrations in 100% of immunophenotyped H-RS cells. In addition the analysis of a recently established H-RS cell line L1236 revealed loss of heterozygosity (LOH) at several chromosomal loci. Newly established methods such as LOH, FISH and single cell PCR will reveal new insights into the molecular structure of H-RS cells. This might help to identify the remaining 20% of patients with a poor prognosis for early aggressive treatment.

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Management of early stage Hodgkin's disease (HD) in 1997

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In Europe, exploratory laparotomy and splenectomy is no more considered as a routine staging procedure for early stage HD, so that we will concentrate on the management of clinically-staged (CS) stages I–II HD.

Most groups involved in HD therapy agree that (at least) two subgroups should be defined among those patients; "favourable" (or good prognosis) and "unfavourable" (or good prognosis).

For the favourable group, a competition still exists between subtotal nodal irradiation (STNI) alone and combined modality (chemo-radiotherapy) treatment (CMT), both modalities having been shown to achieve similar long-term survival in several large-scale trials. However, the usually lower event-free survival (EFS) and the late complication risk linked to STNI presently make more and more attractive specific CMT schemes which are both reducing the number of chemotherapy courses (down to 3–4) and the extent of irradiation.

For the unfavourable group of CS I–II HD patients, most data in the literature suggest that the optimal treatment is a combination of chemotherapy and radiotherapy. Nevertheless, the optimal delivery of both chemotherapy (schedule, number of courses, timing) and irradiation (volumes, dose) is still debated.

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The Classification of the non-Hodgkin's lymphomas. Results from the International Lymphoma Classification Project

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The REAL classification (Harris 1994) provides a listing of the clinico-pathologic, lymphoma entities which pathologists can recognise. It represents an attempt to develop a common language between North American and European pathologists which would have obvious advantages in clinical trials of lymphoma therapy.

The ability of pathologists to apply the REAL classification had not been tested prior to publication nor was there any information about the clinical value of this proposal. In order to address these questions the International Lymphoma Classification Project was carried out under the chairmanship of Prof. J.O Armitage (University of Nebraska).

A cohort of 1403 consecutively accrued cases of lymphoma, from nine different study centres around the world was assembled. Five expert haematopathologists visited each site and classified cases using the REAL and Kiel classifications and the Working Formulation. Each expert re-reviewed a random selection of 20% of the cases from each site.

The inter-observer reproducibility was over 85% for most of the major lymphoma subtypes. The intra-observer reproducibility, when clinically insignificant divergences were discounted, was 94%.

The REAL classification could be readily applied and identified clinically