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

Ian J. Stratford. *School of Pharmacy and Pharmaceutical Sciences, University of Manchester, Oxford Road, Manchester M13 9PL, UK*

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

P. Lambin[1], J. Theys[1,2], S. Nuyts[1], A. Van der Kogel[3], W. Landuyt[1], J. Anné[2]. [1]*Lab. of Radiation Oncology, U.Z. Gasthuisberg;* [2]*Lab. of Bacteriology, Rega Institute, K.U. Leuven, 3000 Leuven, Belgium;* [3]*Institute of Radiotherapy, Univ. Hosp. St. Radboud, Nijmegen, The Netherlands*

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

A. Jox, M. Kornacker, J. Wolf, V. Diehl. *Department of Internal Medicine I, University of Cologne, Germany*

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

J.M. Cosset. *Institut Curie, Paris, France*

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

K.A. MacLennan. *I.C.R.F. Cancer Medicine Research Unit, St. James's University Hospital, Leeds, UK*

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