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Methylation inactivates critical pathways in tumourgenesis

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Genetic alterations are a hallmark of human cancer. In addition to these genetic alterations, changes in DNA methylation, an epigenetic modification present in mammalian cells, are frequent in human cancer. The promoter regions of many genes contain CpG islands which, with the exception of genes on the inactive X chromosome and imprinted genes, are protected from methylation in normal cells. Work in the past several years has demonstrated that the silencing of tumor suppressor genes associated with promoter hypermethylation is a common feature in human cancer, and serves as an alternative mechanism for loss of tumor suppressor gene function, since promoter region hypermethylation leads to transcriptional repression. The genes targeted for this tumor specific change include genes important for tumor development and progression. For example, promoter region methylation inactivates genes regulating cell cycle control (Rb, p16, p15, p73) as well as those inhibiting invasion (E-cadherin) and apoptosis (DAP kinase), and genes involved in DNA repair (hMLH1, MGMT, GST-p). Patterns of inactivation for some of these genes have striking specificity, implicating particular importance to inactivation of these pathways in some tumors. This specificity is most striking for the inactivation of genes associated with inherited predisposition to cancer. Hypermethylation of these loci occurs in sporadic tumors of the same types as those which develop in the familial syndromes. The functional consequences of loss of function of these genes will be explored. In addition, promoter region hypermethylation is a promising new biomarker for early detection and disease monitoring in human cancer.

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RAS-oncogene interacting agents

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The *RAS* supergene family is comprised of 21–29 Kd GTP-binding proteins, that play a central role in the integration of the regulatory signals that govern cell cycle and proliferation. In addition, they are involved in cellular differentiation, apoptosis, cytoskeletal re-arrangement and nuclear import of proteins. To function as such, RAS must localise to the plasma membrane and be activated. Mutations in the RAS gene leading to an almost continous activation of RAS have been extensively reported in a wide variety of tumor types. Thus, interefering with RAS activation in theory would lead to inhibition of tumor cell proliferation. Recently, inhibitors of farnesylprotein transferase, the rate-limiting enyzme in the activation of RAS have entered clinical development. Although they have been developed as selective inhibitors, to a varying degree the FTase inhibitors suppress farnesylation of a number of proteins other than RAS. For an optimal efficacy it is likely that these agents will have to be administered in a continuos schedule. To date clinical experience has been reported for 3 FTIs, the peptidomimatic L778, 123 the tricyclic inhibitor SCH 66336, and finally R115777. H-RAS can also be targetted by antisense agents such as ISIS 2503. For all drugs it seems that at the recommended doses their toxicities are relatively minimal. The most recent findings will be discussed as well as issues on clinical trial design.

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Anti-angiogenesis drugs

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Growth and survival of cancer cells beyond initial stages is critically dependent upon oxygenation and nutritional supply. Tumor-directed neoangiogenesis thus is an important step for tumor progression, invasion, and metastasis and involves the multifactorial development of an angiogenic phenotype characterized by overexpression of both stimulatory and inhibitory growth factors. Antitumor strategies targeting tumor-neoangiogenesis are worldwide actively pursued. Targets include angiogenic growth factors and their receptors [eg vascular endothelial growth factor ($\alpha_v\beta_5$ receptor), basic fibroblast growth factor ($\alpha_v\beta_3$ vitronectin receptor: EMD 121974, S 836))] as well as other components involved in angiogenesis. Strategies currently investigated comprise: 1. monoclonal antibodies (eg. against VEGF, VEGF receptors, endoglobin), 2. ribozymes (eg. against VEGF receptors), 3. Antisense (eg. against mRNA of VEGF, VEGF receptors, angiogenin), 4. tyrosine kinase inhibition (eg. SU 5416, PD 166 285, PD 173074, CGP 79787), 5. antiangiogenic proteins (eg. angiostatin, endostatin), and 6. others (combretastatin-4, irsoglandine, fumagillin analogues). Since these compounds are not prima designed to directly cause tumor regression traditional endpoints of clinical shay design will have to be revisited. Supported by a grant from IND-Synergen Inc. and Verein z. Förderg. d. Krebsforschung e.V.

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Polymers and anti-cancer agents

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Our inability to selectively target an anti-cancer drug to the tumour and away from the organs of toxicity has two major consequences. Firstly, non-selective cell killing severely limits the amount of drug we can give in total by the toxic effects on normal tissues, in other words, cytotoxics have a narrow therapeutic index. Secondly, in some cases, insufficant drug will reach the site of the tumour to allow for therapeutic effect. This can be presnt de novo or can be aquired and is termed "pharmacological resistance". As with other forms of resistance it is difficult to assess just how important this might be in each individual patient.

The general structure of polymerics allows for maximum chance to engineer in characteristics that should be useful in targeting. Thus it is possible to manipulate molecular weight (and thus renal handling), charge, shape, lipophilicity, poly dispersion and other physico-chemical characteristics. Monomers may be introduced which have direct chemical bonding to cytotoxic moieties. More commonly "linker" substitutions can be introduced which not only allow for attachment of the cytotoxic component, but may in themselves be dependent on cleavage within specific cellular micro-environments. Thus leading to a further level of targeting.

The clinical application of polymer technology is limited at present. Gliadel wafers and slow release preparations of gonadotrophins are perhaps the most widely used at present. PK1 was jointly developed between the UK Cancer Research Campaign and Pharmacia Upjohn. Full results of phase 2 evaluations in colorectal, non-small cell lung and breast cancer are awaited. There are also a variety of agents which are in or about to enter phase 1 which show encoraging pharmacokinetic advantages in preclinical testing. The next generation will be polymers that can be tailor made with an intrinsic degradation and drug release profile, are non-immunogenic and completely biodegradable. This can be catalysed by the incorporation into the polymer of a fixed percentage of its own (non-mammalian) cleavage enzyme. These features are now achievable in the laboratory and urgently require translation into clinical practice.

If these systems eventually fulfill their promise it is concievable that all cytotoxics would be administered as part of a selective delivery mechanism in future.

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Hemopoietic stem cell transplantation (HSCT) in Europe

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The European Group for Blood and Marrow Transplantation (EBMT) was founded in 1975 to bring together scientists involved in hemopoietic stem cell transplants (HSCT): originally there were less than 10 Centers performing 16 transplants/year, now we have 361 Centers from 31 Countries, who performed over 14.000 transplants in 1996. Of these 4369 (30%) were allogeneic and 9988 autologous (70%). Allogeneic stem cells can be obtained from different sources: in 1996 these were bone marrow in 74% and peripheral blood in 26%. A small number of cord blood transplants are also being performed at present. The donor was a family member in 3422 and an unrelated individual in 947.

Major indications were leukemias (34%), lymphomas (38%) and solid tumors (24%). Emerging indications now exist, like breast cancer.

The procedure has become rather sophisticated and its complexity is likely to increase in the future: we are slowly moving from unmanipulated

bone marrow transplants, to minimally processed cell gafts, to manipulated cell transplants.

The EBMT has elaborated Standards for specialist units undertaking blood and marrow stem cell transplants (Bone Marrow Transplantation 16: 733–736, 1995), Standards for Blood and Marrow Progenitor Cell Processing, Collection and Transplantation (EBMT Operational Manual 1998), in collaboration with ISHAGE and Standard Indications in 1998 (Allogeneic and autologous transplantation for haematological disease, solid tumours and immune disorders: current practice in Europe 1998 (Bone Marrow Transplantation, 21: 1–7, 1998).

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Hematopoietic stem cell transplants (HSCT) in leukemia in Europe

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Hemopoietic stem cell transplantation is increasingly utilized for the treatment of leukemia. In the EBMT file there are registered 22,000 transplants for acute leukemias (AL), 9500 for chronic myeloid leukemia (CML), 2600 for myelodisplasia (MDS) and 450 for chronic lymphocytic leukemia (CLL). The results have improved considerably over time. Allogeneic (A-HSCT) between HLA compatible sibling for AL has reduced the transplant related mortality (TRM) from 40% to 20% if performed in 1CR; the leukemia free survival (LFS) after 1990 for acute myeloid leukemia (AML) exceeds 60% at 5 years. In most studies A-HSCT has shown to be never inferior to other therapeutic options. Current results of Autologous (AU-HSCT) for AML indicate approximately 45–50% LFS. AU-HSCT has been demonstrated superior to chemotherapy in the majority of randomized studies conducted on intent-to-treat basis. For acute lymphoblastic leukemia (ALL) the LFS after A-HSCT is 55% at 5 years. The role of autograft in ALL is unclear. Matched Unrelated donor (MUD) transplant results are improving; for AL patients beyond second remission, A-HSCT or MUD transplant represents, in adults, the only chance of cure. For CML patients, A-HSCT remains the only proven curative approach. A-HSCT from an HLA compatible sibling is able to produce 50–65% LFS. The trend in CML is to reduce the toxicity of the conditioning regimen in view of the efficacy of donor lymphocyte infusion (DLI), capable of rescuing 70% of relapsed patients. Matched unrelated donor (MUD) transplant are expanding and the results are rapidly improving; however, in view of the results with IFN treatment, the therapeutic choice for low Sokal risk patients remains difficult. For MDS HSCT can provide a possibility of cure although these patients are very fragile and adjustments in the conditioning regimen are needed. In the next years we shall know the role of HSCT in CLL.

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Bone marrow transplantation for multiple myeloma

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The place of stem cell transplantation in the management of myeloma remains controversial. The largest body of data relating to allogeneic transplantation has been collected by the EBMT registry and totals over 400 patients transplanted at various stages of the disease. Overall there is a high transplant related mortality of 30–40% and relapse incidencer of over 50%. However the survival curve beyond 5 years approaches a plateau, with a projected long-term survival for all patients of around 35%. In a multivariate analysis of pre-transplant features, two were identified as found to be poor prognostic factors i.e. transplant after more than one line of therapy and male gender. More recent results from the registry show that overall survival has improved over the last 5 years. Autologous transplant is now an extremely safe procedure for patients under the age of 65 years. However autografting is not curative with a median event free survival of about 2 years. A number of randomised studies have been designed to address the potential benefit of autografting over conventional therapy. The results of one such study, by the French IFM group have shown a survival benefit with the 5 year overall survival and EFS of autograft recipients being 52% and 28% compared to 12% and 10% in patients treated conventionally. A subsequent study from this group compared a single transplant with a double procedure. At this time there is trend in favour of two procedures for patients with normal β_2M levels at diagnosis. Patients with high β_2M concentrations tend to do poorly with all current forms of therapy and more innovative approaches are required for this group of patients.

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High-dose chemotherapy in lymphoma

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Lymphoma is the most frequent disease of patients undergoing high-dose therapy (HDT) followed by transplantation of hemopoietic stem cells (HSC) in Europe. The Working Party (WP) Lymphoma of the EBMT currently contains information on 20455 patients (pts) transplanted between 1978 and 1999. The diagnoses of these pts were: high-grade NHL (n = 3890, 21.3%), intermediate grade NL (n = 3184, 17.4%), low grade NHL (n = 2404, 13.2%), lymphoblastic lymphoma (n = 1761, 9.7%), Burkitt lymphoma (n = 524, 2.9%), unclassified NHL (n = 1171, 6.4%), or Hodgkin's disease (n = 5314, 29.1%).

The status of the disease at the time of transplantation was: CR1 (n = 3976, 21.3%), CR2 (n = 3235, 17.3%), CR $\geq$ 3 (n = 735, 3.9%), PR (n = 3605, 19.3%), VGPR (n = 1350, 7.2%), untested relapse (n = 748, 4%), sensitive relapse (n = 2595, 13.9%), resistant relapse (n = 1147, 6.1%), primary refractory disease (n = 1248, 6.7%), at diagnosis (n = 19, 1%). 11611 (62.4%) pts received autologous HSC and 328 (20.1%) pts received allogeneic HSCs; a major switch from bone marrow to peripheral blood stem cells as the source of hemopoietic stem cells occurred during the last decade. 1405 (18%) of all autologous grafts were purged by various methods. Overall survival and progression-free survival of pts vary widely with the type of lymphoma and the status of disease at the time of transplantation. Within the major disease categories status of disease was the overriding prognostic factor determining the success of the transplant. As with other diseases there are few prospective randomised trials directly comparing the results of HDT/transplantation with conventional therapy. EBMT together with the German Hodgkin's Lymphoma Study Group has recently analysed such a trial for pts with relapsed Hodgkin's disease; other trials are currently accruing pts.

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Lights and shadows in high-dose chemotherapy for solid tumours

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HDC is being offered to an increasing number of patients with solid tumors as in Europe as in North America. In 1997 nearly 2,600 patients with breast cancer received this treatment modality in Europe (60% with high-risk primary disease). In the last decade autologous marrow has nearly universally been replaced by PBPCT (peripheral blood progenitor cells transplantation) and toxic death rate has decreased from 15–18% in the mid and late eighties to the present 1–2%. The reasons might lie in the use of PBPC, hematopoietic growth factors, better knowledge of the procedure, but certainly also to a better patients' selection. In the treatment of high risk breast cancer (>10 positive nodes) several phase II studies have produced 3–5 year disease free-survival in the range of 50–70% which seems to compare favorably with the results achievable with standard anthracycline containing regimen even if conflicting results have been presented at the 1999 ASCO meeting so we still have to wait for more mature follow-up. For metastatic disease, patients intensified in CR1 show 30% DFS at 3 years from registry data. More unclear and non-homogenous results have been produced in ovarian cancer and SCLC, while for germ cell tumors data from the EBMT show a 50% DFS for patients autografted in sensitive relapse. Is more better? The answer is coming out from randomized phase III studies, but the definitive one has not yet be given. Other open questions are: Which drugs? Which regimens? Which strategies?

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Bone marrow transplantation in children

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Allogeneic transplantation (BMT) or better hematopoietic stem cell transplantation has been used successfully for the first time in 1968 in children with severe combined immunodeficiency. Since that time, inborn errors of the function of the immune system can be corrected by this procedure. In the meantime it is used also for the correction of inborn defects of the myelopoiesis, erythropoiesis, osteoclasts, osteoblasts, and monocytes, as well as for aplastic anemia. In pediatric ontology the indications for allogeneic BMT are very much dependent on the success rate of conventional