therapy alone by introducing additional less toxic chemotherapy up front.

Combined modality therapy is the standard therapy for patients in localised stages with one or more of the aforementioned adverse factors. However, the optimal duration of the chemotherapy regimen as well as the right dose and extent of radiotherapy of such combined modality programmes are ill defined. A special risk for patients treated with combined modality are second neoplasias [8]. Current strategies aim at reducing treatment-related toxicity without compromising efficacy. The HD1 trial of the German Hodgkin's Lymphoma Study Group (GHSG) compared 40 Gy extended field versus 20 Gy extended field plus 20 Gy bulk irradiation after 2 cycles COPP–ABVD. Survival and time to treatment failure were identical after a median follow-up time of 48 months. These data suggest that 20 Gy irradiation may be sufficient in the extended field after chemotherapy to control subclinical disease. The ongoing HD8 trial is now comparing extended versus involved field radiation after 2 double-cycles COPP–ABVD.

The treatment of choice for patients in advanced stages IIIB and IV is combination chemotherapy. Several chemotherapy regimens are capable of inducing complete remissions in over 80% of patients. However, as patients are followed, up to 30% relapse. Thus, the primary treatment regimen should only result in the cure of approximately 50% of advanced stage patients [9]. One approach to improve treatment results is intensification of chemotherapy by support of haemopoietic growth factors.

The optimal salvage therapy for patients relapsing after polychemotherapy depends on the primary chemotherapy regimen used and the duration of the response. Accumulating data suggest that high-dose chemotherapy (HDC) followed by stem cell transplantation can improve the treatment results for patients with relapsed HD. In order to better define the role of HDC in patients with relapsed HD, the ongoing HDR-1 trial of the GHSG compares HDC plus stem cell support with repeated courses of a dose escalated salvage chemotherapy (DexaBEAM) Plus G-CSF support.

HD may be an ideal target for immunotherapy. Current experimental strategies involve bispecific monoclonal antibodies, anti-idiotypic antibodies and immunotoxins. Immunotoxins are currently being evaluated in clinical phase-I trials in patients with refractory Hodgkin's Disease [10].

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Breast Cancer Angiogenesis: Therapy Target and Prognostic Factor

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BREAST CANCER is the commonest cancer in women in Europe. Improvement in current treatments and the development of new approaches to breast cancer therapy are most likely to come from advances in the basic understanding of breast biology. Angiogenesis is essential for tumour growth and metastasis [1]. It is a complex multistep process involving extracellular matrix remodelling, endothelial cell migration and proliferation, capillary differentiation and anastamosis which is regulated by angiogenic peptides [2]. However, the relative importance of each of these key events or their regulating factors in tumour angiogenesis is unknown. Similarly, although there is increasing evidence that endothelial cell adhesion molecules play a significant role in angiogenesis [3] and tumour-endothelial cell adhesion [2, 4], their role in breast cancer is unknown.

PROGNOSTIC ROLE OF ANGIOGENESIS

Weidner *et al.* [5] first demonstrated the association of angiogenesis, assessed by factor VIII associated antigen, with metastasis

in breast cancer. We optimised vessel detection and showed CD31 (platelet endothelial cell adhesion molecule, PECAM) was a more sensitive endothelial marker, that showed smaller vessels [6, 7]. Our group and Weidner's then both showed the prognostic importance of high vessel counts in breast cancer and the independent association with poor prognosis [6, 8]. In node negative patients, it was more important in predicting poor prognosis than *TP53* or labelling index [9]. Several studies have since confirmed angiogenesis to be a powerful prognostic tool in several tumour types [6, 8, 10, 11]. However, current methods of vascular quantification are time consuming and laborious, making it unsuitable for diagnostic practice. For routine diagnosis, a rapid method of measuring angiogenesis is required. Therefore, we examined a variety of alternative techniques including computerised image analysis and the Chalkley eyepiece microscope grid [12]. Direct comparison of these methods with microvessel density showed a highly significant relationship with both computerised image analysis and Chalkley count, but the latter was more rapid.

MECHANISMS OF ANGIOGENESIS

We showed that there was 10–50-fold increase in mitotic rate in tumour endothelium compared to normal breast endothelium, and this was not proportional to the number of blood vessels detected or the labelling index of the tumour [13]. The conclusion was that different growth factors were regulating endothelium and epithelium, and that processes in addition to cell division were important for angiogenesis.

In view of the role of adhesion molecules in both capillary morphogenesis [3] and metastasis [4] we studied the expression of the selectin and immunoglobulin adhesion molecules in breast tumour endothelium. E selectin and P selectin were selectively upregulated at the tumour periphery. ICAM 3 was less frequently upregulated, but was not observed in normal endothelium. Unexpectedly, adhesion molecules were also upregulated in some tumour epithelium.

We analysed expression of seven major vascular growth factors (VEGF, PDECGF, PLGF, TGFb1, pleiotrophin, bFGF, aFGF) in 64 primary breast tumours. All factors were expressed but VEGF and PDECGF were most abundant. Immunolocalisation with PDECGF antibodies that we have generated and VEGF antibodies is being performed. Initial studies indicate that PDECGF is in macrophages and tumour cells and is often upregulated in the stroma, whilst VEGF is mainly detected on endothelium and in clusters of tumour cells.

ANTIANGIOGENIC THERAPY

We have carried out studies involving different aspects of antiangiogenesis therapy, involving antiangiogenic drugs, left shifters, hypoxic active agents and combinations with chemotherapy.

BB94

This is a low molecular weight collagenase inhibitor produced by British Biotech. Dr F. Balkwill (ICRF Cytokine Group) showed intraperitoneal administration had major therapeutic effects in xenografts. We are carrying out the first Phase I trial using intrapleural therapy.

Left-shifting of the oxygen dissociation curve

BW12C is an anti-sickling agent that shifts the oxygen dissociation curve to the left. This could produce selective tumour hypoxia and activation of hypoxically activated drugs, and may synergise with anti-vascular approaches.

Gene therapy with hypoxia promoters and prodrug activation genes

The recently described hypoxia-regulated elements in the erythropoietin gene, VEGF and phosphoglycerate-kinase genes share consensus sequences that are regulated by a hypoxia activated transcription factor. Hypoxia, being more profound and extensive in tumours than normal tissues, provides a mechanism for tumour selective gene expression.

Inhibitors of angiogenic growth factors

We showed that there were suramin analogues with selective toxicity against endothelium which were antiangiogenic *in vivo* and inhibited tumour growth with less toxicity than suramin. Two of these compounds, 12 and 14, have been selected for further development.

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