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Monoclonal antibodies in the detection and treatment of micrometastatic or minimal residual disease; an overview

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Submitted
Tumor immunotherapy is referred to as the fourth modality for the treatment of human neoplasms alongside surgery, chemotherapy, and radiotherapy [1]. Technologies to produce large quantities of recombinant immunobiological agents have now been available for about 20 years. During this period, a plethora of laboratory and clinical investigations have been performed to better define the role of these molecules in both diagnosis and treatment of human malignancies. This report reviews the use of monoclonal antibodies in both the detection and treatment of micrometastatic or minimal residual disease.
Introduction

Tumor immunotherapy is defined as treatment that mediates tumor lysis through the action of the host's immune system [2]. Its concept is based on the assumption that tumor cells express unique proteins, known as tumor antigens, which can be recognized as foreign or 'non-self' by the host, resulting in tumor rejection. In essence, two more or less complementary immunotherapeutical approaches can be distinguished. The first approach is to cause the tumor to appear more 'foreign' compared to surrounding normal tissues and thus create a suitable target for natural host defense mechanisms [3]. The second approach involves the amplification of relatively weak immune responses to immunogenic tumors, such as renal cell carcinoma and malignant melanoma [4]. Ever since Paul Ehrlich's envision of a 'magic bullet', selectively combating malignant cells while leaving healthy cells untouched, a myriad of methods have been used in an attempt to optimize both pillars of tumor immunotherapy [5]. At present, the identification and modification of tumor antigens parallels the exploitation of cellular and humoral components of the host’s immune response, such as leukocytes [6], cytokines [7], and monoclonal antibodies (MoAbs) [8, 9]. In this report, brief attention will be given to the general role of tumor antigens and MoAbs in human cancer, after which the use of MoAbs in both the clinical detection and treatment of micrometastatic or minimal residual disease will be reviewed.

Tumor antigens

Tumor cells have endured a series of genetic changes and therefore are likely to express novel or altered proteins, i.e., tumor antigens [10]. The first evidence for the presence of such antigens came from animal models consisting of inbred strains of rodents and the use of syngeneic transplantable tumors. In 1943, Gross was the first to recognize that inbred mice could be immunized against a tumor that was developed in a mouse of the same inbred strain [11]. Utilizing a spectrum of agents to induce tumors in animals, this observation was extended by others [12, 13]. Much research has since focused on the identification and characterization of tumor antigens in human neoplasms. In the last decade, human tumor cells have actually been demonstrated to harbor a wide range of antigens, encoded by oncogenes and suppressor genes that have undergone some structural alterations resulting from point mutations, chromosomal translocations, inversions, deletions and (or) viral insertional mutagenesis [14]. Although it remains questionable whether these tumor antigens are sufficiently recognized as foreign to evoke a strong anti-tumor response, several lines of evidence suggest that they really are immunogenic in some cases. In these cases, the anti-tumor response can be taken as a guide to find the matching antigens [15]. Until now, it has been difficult to detect human tumor antigens that have as high a degree of tumor specificity as shown for most tumor antigens in
animals. The majority of antigens identified in human cancers can be classified as tumor-associated rather than tumor-specific, indicating that they are preferentially though not exclusively expressed by the tumor [16, 17]. Known tumor-associated antigens (TAA) involve normal cellular gene products such as carcinoembryonic antigen (CEA), α-fetoprotein (AFP) and CA-125; differentiation antigens such as gangliosides; and overexpressed normal cellular determinants such as the folate receptor and the proto-oncogene product HER-2/neu [15]. Clearly, TAA and tumor-specific antigens (TSA) allow the discrimination of normal and neoplastic cells, and should provide a suitable target for MoAb-based immunotherapy [18]. More often than not, however, human tumor antigens will also exhibit a certain degree of heterogeneity, as demonstrated in both experimental models and in biopsies from human malignancies [19-21]. Since biological response modifiers such as human recombinant interferons have been shown to induce and amplify the expression of tumor antigens on the surface of a variety of human tumor cell lines [22], the clinical usefulness of MoAb-therapy in concert with interferon and other biological response modifiers is object of study [3].

Monoclonal antibodies

Antibodies have the ability to recognize and remove abnormal or 'non-self' structures, while leaving normal or 'self' components unscathed. For this reason, it has long been hoped that they could be utilized to eradicate tumor cells more selectively than do conventional treatment modalities such as chemotheraphy. The literature is replete with animal and human studies utilizing impure and poorly characterized polyclonal antibody preparations raised against the host's tumor. Overall, a limited number of successes in treating patients with hematopoietic malignancies have been described [5, 23]. Reports on the treatment of solid malignancies involved small numbers of patients, providing only partial and nondurable remissions [24, 25]. Its rather disappointing therapeutic efficacy together with its potential danger in use eventually resulted in a waning of interest in polyclonal antibody-based immunotherapy. In the mid-1970s, Köhler and Milstein published their discovery of a method of producing antibodies by in vitro hybridization of a malignant myeloma cell line in continuous culture with mouse splenic B cells [26]. This hybridoma technique was a breakthrough in antibody research since it allowed for large-scale isolation of an infinite range of (murine) MoAbs against predefined target epitopes, and resulted in a resurgence of interest in tumor immunotherapy. At present, several approaches have been reported utilizing MoAbs alone (i.e., unconjugated or 'naked' MoAbs) [27], or as vectors for a diversity of molecules, such as radionuclides [28], enzymes [29, 30], toxins [31, 32], superantigens [33-36], and immune effector cells [37-39].
Clinical use of MoAbs

In the first 20 years of clinical trials in patients with advanced malignancies, MoAbs did not live up to their initial promise: overall efficacy remained disappointing. Only recently, the advent of Panorex™ (anti-17-1A), Herceptin™ (anti-HER2) and Rituxan™ (anti-CD20; rituximab; IDEC-C2B8) has brought a glimmer of hope in treating respectively colorectal carcinoma [40], breast carcinoma [41-43] and non-Hodgkin's lymphomas [44-49]. Administered either alone or in combination with chemotherapeutical agents, these MoAbs produce response rates that compare favorably to those reported for conventional treatment modalities. Phase III studies evaluating their clinical value are currently underway.

Major obstacles

Major obstacles for the clinical application of MoAbs involved host-related factors such as rapid plasma clearance [50] and the development of human anti-murine antibody (HAMA) responses [51, 52], as well as factors associated with the tumor itself. Rapidly growing, solid neoplasms are generally not amenable to immunotherapeutical regimens, which is not surprising since the majority of MoAbs do not distribute uniformly in large tumor masses after systemic administration. In part, this observation reflects antigenic heterogeneity and modulation of the tumor population [19-21]; in part, it represents poor penetration of tumor tissue. The latter may be attributed to a variety of physiological factors, such as the nature of tumor vasculature [53], relatively high interstitial pressure in tumor centers [54], and the supposed ‘binding site barrier’ phenomenon, i.e., the binding of high affinity MoAbs to target epitopes on the outer rim of the tumor, whereas less high affinity MoAbs diffuse into the tumor nodule straight-away [55-61]. In fact, these factors have been demonstrated to dramatically reduce the amount of MoAbs that penetrate tumor tissue and saturate epitopes to approximately 0.01% of the injected dose per gram of tumor [62]. More encouraging results of MoAb detection and treatment have been reported in the case of hematopoietic malignancies, although even there, with better accessibility of the tumor cells, successes may be substantially limited by high tumor load [63, 64].

Overcoming obstacles

Until now, a host of strategies to overcome the major obstacles encountered with MoAb-guided detection and therapy of human malignancies have been tested both in vitro and (or) in vivo. HAMA responses can be considerably reduced or even avoided by the genetic engineering of humanized [65], or ‘resurfaced’ [66, 67], murine MoAbs or human MoAbs [68, 69]. The problem of tumor heterogeneity and modulation can be circumvented by the induction and augmentation of TAA expression with recombinant human interferons [70], or by the use of effector arms of MoAbs that exert a bystander effect [71, 72]. The physiological barriers can be conquered by
increasing the concentration of MoAbs at the tumor site or by reducing bulky tumor masses before the application of MoAbs. Conceivable ways to enhance MoAb uptake by impenetrable tumors involve the intra- or peritumoral administration [73], as well as the implementation of F(ab')2 and Fab' fragments [74, 75], or even smaller molecules that readily enter tumor tissues, including single-chain Fv (scFv) fragments as such [76], or in the format of diabodies [77-81] and chelating recombinant antibodies (CRAbs) [82, 83]. Finally, to meet the condition of reduced tumor mass, MoAbs can be given in an adjuvant setting, i.e., after achieving clinical remission (CR) with conventional modalities. As early as 1978, Ghose et al. hypothesized that lack of efficacy of MoAbs in human cancers is due to increased tumor load [84]. One of the keys to success with MoAbs lies therefore in careful selection and treatment of cancer patients with micrometastatic or minimal residual disease (MRD), the stage when tumor cells are few and dispersed, since individual cells are likely to be more promising targets in terms of optimal tumor to MoAb ratio and accessibility.

**Minimal residual disease**

MRD is defined as the tumor burden that is present after a course of treatment that has resulted in CR [85]. Techniques used for the detection of MRD include sensitive immunological assays with MoAbs that are capable of identifying extremely low numbers of neoplastic cells in otherwise normal bone marrow, peripheral blood or lymph nodes [86], as well as molecular methods based on the extensive amplification of a specific (c)DNA sequence by the polymerase-chain reaction (PCR) [87]. Moreover, to directly locate tumor sites in the body, MoAbs can be given following radiolabeling (radioimmunoscentigraphy, RIS) [9, 88]. This process involves the systemic application of a MoAbs that have been bridged to an γ-emitting isotope, after which external imaging with a γ-camera will be performed [89]. Whatever technique is chosen, the detection of MRD is only worthwhile as long as its presence is strongly associated with the occurrence of clinical relapse and as long as treatment options are available. In that case, the observation of single neoplastic cells will allow identification of patients at high risk of clinical relapse, as well as earlier detection and subsequent treatment of relapse [90]. For most human malignancies, however, the prognostic significance of the detection of MRD is currently not known. First, it remains questionable whether the MoAbs used for the immunological detection of MRD are tumor-specific or not, since some degree of false-positivity will clearly limit their prognostic value. Second, the presence of MRD can be regarded as a single step in the cascade of metastasis and does not necessarily indicate the formation of clinically relevant metastatic deposits [91, 92].
MoAb-guided detection of MRD

The expression of tumor antigens offers a means to identify small numbers of neoplastic cells against a normal background. In the case of hematopoietic malignancies, however, markers are neither specific for the malignant state per se, nor for a given lineage of differentiation. Therefore, the exact characterization of leukemic populations must rely on a combination of atypical or aberrant antigens [93]. Moreover, since abnormal antigen density might also represent a characteristic feature of malignant cell populations, quantitative marker evaluation seems to be another important mean for the detection of MRD in leukemias and lymphomas [94]. In the case of solid malignancies, the antigenic repertoire leaves much to be desired as well: except for a limited number of virus-encoded and hybrid proteins [15], useful TSA are currently lacking. To date, the set of frequently targeted TAA consists of cytokeratin (CK), epithelial glycoproteins (EGP), epithelial membrane antigen (EMA), the first-generation ovarian cancer marker OC-125, and the gastrointestinal cancer markers CEA and 17-1A. In addition, the oncogene product HER-2/neu (p185 or c-erb-B2), being overexpressed in a diversity of human neoplasms [95-103], receives much attention. Preclinical data indicate that this oncoprotein may be valuable for receptor-targeted therapeutics [104-110]; its potential use for MoAb-guided clinical detection and treatment of cancer is currently under intense investigation and trials are now underway to develop optimum treatment strategies. This is true also for newly discovered molecules responsible for extracellular matrix degradation, tumor cell migration as well as angiogenesis, like numerous growth factors (GF), e.g., vascular endothelial GF [111-114], epidermal GF [115], platelet-derived GF, transforming GF-α [116, 117] and angiogenin [118]; various tyrosine kinases [119] and membrane-associated (metallo)proteases [120-122]; urokinase-type plasminogen activator [123, 124]; and cyclo-oxygenase [125].

Hematopoietic malignancies

At present, aberrant patterns of antigen expression allow the immunological identification of MRD in a significant portion of patients with acute leukemia [126], hairy cell leukemia (HCL) [127-129] and T-NK cell lymphoma [130]. Nevertheless, the clinical relevance of MRD in leukemias and lymphomas remains difficult. In general, hematological patients with demonstrable MRD tend to do worse than patients without detectable tumor cells, but results depend on the timing of the assay and whether the detectable cells appear to be increasing in number with subsequent assays [85]. Larger, prospective studies are therefore needed to determine if MRD will be predictive of clinical relapse.
Acute myeloid leukemia
For acute myeloid leukemia (AML), it was observed that 'late' myeloid differentiation markers (CD15) and 'early' markers (CD10, CD20, CD34) were co-expressed on the same cells only in bone marrow from AML patients and not in normal bone marrow. Marker combinations were present in 17 of 19 cases examined during CR, indicating the presence of MRD [131]. Although strongly associated with AML, however, the CD34+CD15+ phenotype has also been described as a rare phenotype in normal bone marrow. To investigate whether this finding interferes with the detection of MRD in AML, Venditti et al. analyzed both normal and leukemic bone marrow utilizing a triple immunofluorescence assay. They confirmed the presence of CD34+CD15+ cells in normal bone marrow, albeit at statistically lower frequency than in leukemic bone marrow, and monitored two patients in CR, of whom one relapsed after showing a progressive increase in leukemic bone marrow cells. Thus, the unusual combination of CD34 and CD15 may distinguish a leukemic population and allow monitoring of 'early' AML relapse [132]. In childhood AML, CD34+CD56+ cells representing the t(8;21)(q22;q22) karyotype, are present in approximately 20% of cases. Coustan-Smith et al. detected double positive cells in bone marrow from two of three CD34+CD56+ AML children in CR, whereas no positive cells could be found in normal bone marrow. In one of them, MRD detection anticipated overt clinical relapse [133]. In another study on AML, a clonal population of CD117+/CD15+ cells was identified in bone marrow from 34 of 71 patients in CR, but not or at extremely low frequencies in normal bone marrow [134]. Recently, San Miguel et al. investigated in sequential studies the impact of the detection of MRD on the outcome of 53 AML patients that had achieved CR. Patients were monitored for the presence of aberrant or uncommon phenotypic features in bone marrow by flow cytometry with a panel of 35 MoAbs. The level of MRD at the end of induction therapy, and particularly after intensification therapy, was found to be significantly related to the number of relapses, relapse-free survival and overall survival [135].

Acute lymphatic leukemia
Using flow cytometry, peripheral blood cells from 50 patients with acute leukemia were studied for leukemia-associated antigen combinations that are absent or extremely rare among normal hematopoietic cells. In 44 of 50 cases, aberrant co-expression allowed the detection of MRD. Specifically, mCD7+cCD3+ and mCD19+CD22+ cells were shown to be suitable for tracing scarce leukemic cells in T-ALL and B-ALL, respectively. Marker combinations of mCD33/cCD13 and mCD13 or mCD33/cCD11, respectively, were demonstrated to characterize single AML cells. At relapse, relevant antigen combinations were found to be preserved [136]. In ALL, MoAb KOR-SA3544, recognizing leukemic cells positive for Philadelphia-chromosome-1, was demonstrated to detect neoplastic cells in bone marrow samples from patients, but not in normal bone marrow. No data on the clinical impact of MRD in ALL were included [137].
Hairy cell leukemia
Six to 64 weeks after the start of treatment with interferon-α2 or 2'deoxycoformycin, bone marrow leukemic cells from 26 HCL patients were found to display strong surface membrane staining with the MoAb B-ly7, while leukemic cells from only one of 63 patients with other proliferative disorders were positive. This fact, as well as the finding of very low numbers of B-ly7 positive cells in normal bone marrow, provided the basis for the detection of even single hairy cells [138]. In another study on HCL, five of 24 patients in CR after treatment with 2-cholorodeoxyadenosine (2-CdA) were demonstrated to have MRD, as detected by MoAb L26. Follow-up at one year showed that one patient relapsed, two remained positive and one became negative by immunostaining alone; one patient was not re-evaluated. These data suggest that some HCL patients in CR after 2-CdA therapy may have MRD [139], a finding that has been confirmed by others [127]. Recently, Wheaton et al. investigated to what extent the presence of MRD predicted clinical relapse in HCL patients. They performed serial bone marrow analysis after 2-CdA treatment and found three relapses in six (50%) patients with MRD detected at any time after therapy, and only one relapse in 25 (40%) patients without MRD. It was concluded that the presence of MRD after 2-CdA therapy might predict tumor relapse [128]. However, others found no correlation between the presence of MRD and clinical outcome in HCL [140].

T-NK cell lymphoma
Recently, Tsang et al. evaluated the sensitivity and specificity of the MoAb 123C3, recognizing CD56, using 32 CD56+ T-NK cell lymphomas, one CD56+ B lymphoblastic lymphoma, 24 CD56− T cell lymphomas and 50 CD56− B cell lymphomas. All CD56+ but none of the CD56− tumors were found to stain with 123C3. Moreover, in normal of reactive lymphoid tissues from a variety of sites only a few small cells were positive, indicating that 123C3 can be used not only to support the diagnosis, but also to identify MRD in patients with CD56+ T-NK cell lymphomas [130]. Data on the clinical impact of MRD in T-NK cell lymphoma are currently missing.

Solid malignancies
Since MRD is missed by conventional staging procedures like bone scanning and radiological skeletal survey, immunological methods using MoAbs have been successfully applied to detect individual tumor cells disseminated to bone marrow, lymph nodes and other tissues in case of solid malignancies [141]. With exceptions [142-145], detection of MRD in bone marrow and (or) lymph nodes has been found to yield prognostic information for recurrent disease in carcinomas from breast [146-148], colorectum [149-151], esophagus [152, 153], pancreas [154, 155], stomach [156], and lung [157-160].
Breast carcinoma

Most data on the clinical significance of MRD is derived from analysis of patients with primary breast carcinoma. In 95% of the patients, the extent of micrometastatic disease cannot be proven by conventional techniques, but relapse develops in 50% within five years. Initial studies using MoAbs recognizing CK and EMA revealed that some patients have tumor cells in bone marrow without overt metastases [161, 162], as well as that MRD+ patients appear to relapse sooner than those patients with normal bone marrow [161]. Shortly afterwards, using MoAbs against membrane and cytoskeletal antigens expressed by epithelial cells (C26, T16, AE-1), Cote et al. detected MRD in bone marrow samples from patients with operable breast carcinoma, while no MRD was found in control bone marrow. MRD was significantly associated with early recurrence, but studies involved only a few patients with short follow-up [146, 147].

Using a polyclonal EMA antibody, Mansi et al. investigated bone marrow samples from 307 patients in CR and detected MRD in 81 cases, with relapse-free interval and survival time being significantly shorter for MRD+ patients after a median follow-up of 28 months [148]. Diel et al. confirmed and extended these data by examining bone marrow aspirates from 128 breast cancer patients with MoAbs recognizing CK and the tumor-associated glycoprotein-12 (TAG12). MRD was observed in 41 (32%) patients. After a median follow-up of 39.5 months, 45% of MRD+ and 11% of MRD- patients relapsed, the relapse-free interval being significantly shorter for MRD+ patients. The presence of MRD was found to be a strong and independent indicator of clinical relapse, but data on overall survival were not given [163]. In contrast, others failed to find a significant correlation between the presence of MRD and relapse-free survival [142-145].

Recently, Brandt et al. managed to isolate and characterize hematogeneously spreading HER-2/neu+ epithelium-derived cells from the peripheral blood of breast carcinoma patients, using a combined buoyant density gradient and immunomagnetic separation with MoAbs to CK and HER-2/neu. Twenty-nine of the 49 patients tested had either CK+(24/29) or CK+HER-2/neu+ clustered cells in their peripheral blood. The frequency of CK+HER-2/neu+ clustered cells was on average 10 times higher than that of CK+HER-2/neu+ single cells. The numbers of clustered cells were positively correlated with tumor stage; data on the correlation with clinical relapse, however, were not included [164].

Colorectal carcinoma

Reports on the detection of MRD in colorectal carcinoma have been reviewed by Calaluce et al. [165]. Using MoAbs to CK and 17-1A, bone marrow analysis of 57 patients with colorectal carcinoma and 155 patients with breast carcinoma at the time of primary surgery revealed the presence of MRD in 9.5-20.5% of patients. Schlimok et al. found a significant correlation between the presence of MRD on the one hand, and risk factors such as distant metastases or lymph node involvement on the other [149]. In an ensuing study, bone marrow samples from 156 patients with colorectal carcinoma were examined at the time of operation of the primary
tumor utilizing CK MoAbs. In 42 cases (27%), MRD was detected. The incidence of positive findings varied considerably with the size and localization of the primary tumor, the involvement of regional lymph nodes, and the presence of clinically overt metastases. Clinical follow-up studies on 85 patients revealed a significantly higher relapse rate in MRD+ patients [166]. Lindemann et al. looked for MRD preoperatively in bone marrow from 88 patients with radically resected colorectal carcinoma and detected individual tumor cells in 28 (32%) of cases using CK MoAbs. The prognostic value was assessed in a follow-up study with a median follow-up of 35 months. MRD+ patients showed a significantly shorter relapse-free survival. Multivariate analysis demonstrated that the finding of MRD in bone marrow is an independent determinant of relapse [150]. Recently, Schott et al. examined the clinical outcome of MRD in bone marrow as well as in the peritoneal cavity of 109 colorectal and 84 gastric cancer patients using a panel of MoAbs. They concluded, in contrast to Lindemann et al., that positive results in bone marrow revealed only little prognostic significance. However, the finding of MRD in the peritoneal cavity was significantly associated with better survival rates [167].

In a retrospective trial, Greenson et al. examined 50 cases with 568 lymph nodes obtained from colorectal cancer patients at the time of primary surgery using MoAbs against CK and TAG2 (CC49). Six of the 14 patients with CK+ lymph nodes died within 66 months, while only one of the 36 patients with CK- lymph nodes died in the same period. However, no significant difference in survival between TAG2+ and TAG2- groups was observed [151]. Utilizing radioactive MoAb CC49, Cote et al. were able to detect MRD intraoperatively in lymph nodes from patients with primary or recurrent colorectal carcinoma, but they did not report on the clinical relevance of this finding [168].

Recently, Arnold et al. evaluated the prognostic value of radioimmunoguided surgery in 31 patients with primary colorectal cancer receiving 125I-labeled MoAb CC49. Using a γ-camera, patients were classified as to the presence or absence of MRD+ tissue. After a follow-up ranging from 30 to 54 months, survival of 11 stage I or II patients was significantly longer than in 20 stage III or IV patients. Moreover, all 14 patients cleared of MRD were alive at last follow-up, while 15 of MRD+ patients died. Results indicate that radioimmunoguided surgery provides the surgeon with immediate prognostic information and supplements traditional pathological staging [169].

**Esophageal carcinoma**

In a prospective case-control study, Thorban et al. were capable of identifying esophageal carcinoma cells disseminated to bone marrow, utilizing an immunocytochemical assay with MoAbs recognizing epithelial CKs (CK2, KL1 and A45-B/B3). CK+ cells were detected in 36.8% of patients in CR and in 48.5% of patients with extended disease. After a median follow-up of 9.5 months, 64% of the patients in CR but with MDR in bone marrow presented with tumor relapse compared to 10% of corresponding MRD+ patients. Thus, the finding of MDR in bone
marrow is not uncommon in esophageal cancer and indicates that hematogenous dissemination of viable tumor cells occurs early in tumor progression [152].

Izbicki et al. examined 1308 lymph nodes from 68 pancreatic cancer patients without overt metastases who had undergone radical en bloc esophagectomy. A total of 399 lymph nodes were found to be free of tumor by routine histopathological analysis and were studied further for isolated tumor cells using the anti-epithelial cell antibody Ber-EP4. Of these ‘tumor-free’ lymph nodes, 67 (17%) contained Ber-EP4+ tumor cells. The presence of MRD was independently predictive of significantly reduced relapse-free and overall survival. On the contrary, the finding of MRD in bone marrow had no additional prognostic value [153].

Pancreatic carcinoma
Thorban et al. investigated the presence of MRD in bone marrow samples from 42 patients with operable pancreatic carcinoma using CK MoAbs (CK2, KL1, A45-B/B3). CK+ cells were identified in 14 (58%) of 24 patients in CR and in 10 (56%) of 18 patients with extended disease. MRD+ patients in CR had relapsed more frequently at follow-up than corresponding MRD- patients. In addition, MDR+ patients showed a significantly shorter overall survival [154].

Horsch et al. investigated lymph nodes from patients with pancreatic carcinoma following radical tumor resection. Lymph nodes judged as ‘tumor-free’ by routine histopathology were further studied for MRD utilizing the anti-epithelial MoAb Ber-EP4. The presence of Ber-EP4+ cells in ‘tumor free’ lymph nodes were found to be significantly associated with reduced relapse-free and overall survival. All patients who were re-staged as lymph node-negative by histopathology as well as immunohistochemistry survived follow-up without recurrence [155].

Gastric carcinoma
With regard to gastric carcinoma, immunocytochemical analysis utilizing CK MoAbs revealed the presence of MRD in 34 of 97 patients examined at the time of primary surgery. The finding of MRD was correlated to established risk factors like histological classification and regional lymph node involvement. Clinical follow-up studies on 38 patients demonstrated a significantly shorter relapse-free survival time in MRD+ patients, but numbers were too small to perform multivariate analysis in order to define independent prognostic factors [170]. A second study revealed the presence of MRD in bone marrow from 55 (50%) of 109 patients who underwent curative tumor resection. After a median follow-up of 30.6 ± 5.2 months, the extend of MRD was found to be significantly correlated with relapse-free and overall survival. It was therefore concluded that the presence of MRD in bone marrow might be a selection criterion for adjuvant treatment [171].

Bone marrow data were extended with data on the clinical relevance of MRD in perigastric lymph nodes from patients with lymph node-negative early gastric carcinoma who died of tumor relapse. Using the MoAb CAM 5.2. to CK-polypeptides, CK+ cells were detected in 15 (3.6%) of
420 dissected lymph nodes and in 8 (23.5%) of 34 patients at the time of primary operation. In retrospect, patients with MRD were found to have poorer prognosis than patients without MRD [156].

**Lung carcinoma**

Accurate assessment of the presence and absence of tumor in the regional lymph nodes is critical for the prognosis of lung cancer. To date, the clinical relevance of MRD in the case of non-small cell lung cancer (NSCLC) has been proven in both retro- and prospective clinical trials; data on its role in small cell lung cancer are currently missing. In a retrospective study, 588 lymph nodes from 60 patients with NSCLC confined to the lung and 72 lymph nodes from five patients with metastatic disease were examined using a polyclonal anti-keratin antibody. Single tumor cells and occult micrometastases not visible on routine evaluation were readily detected in 38 (63%) of 60 patients with clinically localized NSCLC. The median survival of MRD+ patients was shorter than that of MRD- patients, but longer than that of patients whose lymph nodes contained metastases detectable on routine histological examination [157]. In a prospective study, the prognostic relevance of lymph node MRD in NSCLC was evaluated. A total of 391 regional lymph nodes from 72 patients, in CR after radical tumor resection, were examined using MoAb Ber-Ep4. Isolated tumor cells were detected in 15.2% of patients. After a median follow-up of 26.0 months, lymph node-positive patients showed a significantly shorter relapse-free survival time than lymph node-negative patients. The presence MRD in lymph nodes turned out to be an independent indicator of the disseminatory capacity of an individual primary tumor [158]. In a second prospective study, lymph nodes from 93 patients with completely resected NSCLC were screened for the presence of MRD using MoAb Ber-Ep4. Twenty of 73 patients (27.4%) with disease staged as pN0 and nine of 20 patients (45.0%) with disease staged as pN1 had MRD. Mean relapse-free survival was significantly decreased in stage pN0 and pN1 patients with MRD compared to corresponding patients without MRD; the prognosis of pN0 and pN1 patients with MRD strongly correlated with the prognoses of a control population of NSCLC patients with disease staged as pN2 [160]. In a retrospective study, all lymphoid tissues from five lymph node stations from 49 patients with T1-2N0 NSCLC were immunohistochemically examined for MRD using MoAb MNF116, a broad-spectrum anti-keratin MoAb. In five cases, lymph nodes contained positively staining cells; two cases proved to be false positive, i.e., benign mesothelial inclusions. Follow-up on 46 of 49 patients revealed recurrence in 27% and overall survival of 68%. However, all three cases with MRD were free from relapses, suggesting that the use of immunohistochemistry adds little valuable information above that of routine histopathological examination of lymph nodes [172].

Using MoAb CK2, Pantel et al. detected MRD in bone marrow aspirates from 83 (59.7%) of 139 NSCLC patients without evidence of distant metastases, while 1 positive cell was found in each of 6 out of 215 controls. Patients were followed up for a median of 39 months after
surgery. The presence of MRD was found to be an independent predictor of clinical relapse [159].

**Prostatic carcinoma**
To test the hypothesis that MRD does occur in lymph nodes of patients with prostatic carcinoma, Moul et al. studied archival pelvic lymph node specimens from radical prostatectomy utilizing the pan-CK MoAb SB-3, as well as MoAbs against prostate-specific antigen (PSA). They found a 3 percent incidence of unsuspected pelvic lymph node MRD, not related to serum PSA. It was concluded that the finding of MRD in lymph nodes not appreciated by standard methods appears to be uncommon in clinically localized prostatic carcinoma [173].

**Squamous cell carcinoma of head and neck**
To investigate the incidence of MRD in squamous cell carcinoma of the head and neck (HNSCC), Ambrosch et al. evaluated 1020 lymph nodes from 67 neck dissection specimens of 60 patients utilizing a MoAb to pan-CK. In approximately 6% of cases, MRD was detected, resulting in upstaging. The prognostic significance of MRD was not investigated [174]. For HNSCC, preliminary data indicate that the radiolabeled MoAbs U36 and E48 are capable of selectively targeting neoplastic cells [175].

**Neuroblastoma**
Based on bone marrow studies in 37 patients with stage IV neuroblastoma, Frappaz et al. concluded that two MoAbs, claimed to be specific for the identification of MRD in bone marrow, could detect MRD in some cases, but were unreliable in the remaining cases [176]. Gussetis et al., using 11 MoAbs, were able to detect MRD in bone marrow from 11 of 16 patients with neuroblastoma. The phenotype of the neuroblastoma cells at the time of diagnosis and during MRD revealed a unique pattern in all patients but one. This led to the conclusion that MoAbs provide a valuable tool to detect MRD in bone marrow samples [177]. Data, however, on the prognostic significance of MRD in the case of neuroblastoma were not involved.

**MoAb-guided eradication of MRD**
Since MRD is regarded as the major cause of clinical relapses in a variety of hematopoietic and solid malignancies, immunotherapeutical strategies utilizing MoAbs or their conjugates to target ‘dormant’ neoplastic cells have increasingly attracted scientific interest. Several approaches to prolong remission and prevent tumor relapses have been evaluated in a vast array of preclinical models on MRD, mimicking hematopoietic malignancies such as acute leukemia [178, 179], multiple myeloma [180, 181] and thymic lymphoma [182], as well as solid malignancies such as
neuroblastoma [183-185], malignant melanoma [124, 183, 186, 187] and carcinomas from breast [188], colorectum [124, 189-194], ovary [195], lung [196, 197], and head and neck [198]. Collectively, preclinical data show that MoAbs, whether or not conjugated to other molecules, are capable of eradicating MRD and thus may be relevant for the adjuvant treatment of cancer patients.

**Clinical trials - hematopoietic malignancies**

As predicted by preclinical models, MRD is an attractive target for immunotherapeutical trials. The majority of clinical studies on the use of MoAbs in eliminating MRD have focused on colorectal and ovarian cancers but, because of the introduction of new-generation MoAbs with greater specificity and the ongoing identification of novel tumor antigens, the indications for adjuvant MoAb treatment are expanding.

**Acute leukemia**

Juric et al. found that the MoAb $^{131}$I-M195, reactive with CD33, could safely reduce MRD and prolong remission and survival durations in seven patients with relapsed acute promyelocytic leukemia (APL) after they had attained CR with all-trans retinoid acid. MRD was identified by reverse transcription PCR. No immediate toxicity was observed and late toxicity was limited to myelosuppression. Median relapse-free and overall survivals were found to be 8 and 28 months, respectively. This regimen compared favorably to chemotherapy-based treatment regimens for relapsed APL [199] and data support further study of MoAb-based treatment for MRD in acute leukemia [200].

**Chronic leukemia**

Dyer et al. assessed the role of Campath-1H, a MoAb that reacts with cells presenting CD52, in six patients with chronic lymphocytic leukemia (CLL) treated to maximal response with purine analogues, in whom persistent leukemic infiltration of blood and bone marrow had precluded autologous stem cell transplantation. Five patients achieved both hematological and histological complete remission following and one had minimal focal residual CLL in bone marrow. Treatment with Campath-1H may be of value in eradicating MRD in CLL [201].
Clinical trials - solid malignancies

Colorectal carcinoma
One of the first clinical indications that MRD might serve as a suitable target for adjuvant MoAb therapy was provided by Schlimok et al., who demonstrated that the infused MoAbs CK2 and 17-1A can label individual tumor cells in bone marrow in vivo [149, 202]. The most comprehensive proof of therapeutical efficacy in MRD has been published in the mid-1990s by Riethmüller et al. [203]. In a multicenter prospective trial, 189 patients with radically resected Dukes’ C colorectal carcinoma were randomly assigned to an observation regimen or to treatment with 500 mg MoAb 17-1A, followed by four 100 mg infusions each month. The application of MoAb 17-1A significantly reduced the overall mortality rate by 30% (95% CI, 1-53%) and decreased the recurrence rate by 27% (95% CI, 5-46%). These results compared well to the benefit obtained with the two most accepted treatment regimens including 5-fluorouracil (5-FU) in concert with levimasol [204] and 5-FU in combination with semustine and postoperative radiotherapy [205]. Adverse effects involved mild constitutional and gastrointestinal symptoms, requiring oral or intravenous intervention in only a minority of instances [203]. A recent report showed that the therapeutical effect obtained with MoAb 17-1A is maintained after 7 years of follow-up evaluation [206]. A phase III trial evaluating treatment of 2700 colorectal carcinoma patients with MoAb 17-1A alone, MoAb 17-1A in concert with chemotherapy, and chemotherapy alone is currently underway; up to now, approximately 2000 patients have been entered.

In a pilot, prospective randomized study, 40 patients with breast or colorectal carcinoma presenting with metastatic CK+ tumor cells in bone marrow were given either six doses of 100 mg of MoAb Lewis Y during two weeks or a placebo, i.e., six infusions of human serum albumin. CK+ cells in bone marrow were monitored prior to and after commencement of treatment. Reduction of tumor cells could not be conclusively determined in 30 colorectal carcinoma patients presenting with relatively low tumor cell counts, but was remarkable in ten breast carcinoma patients presenting with high tumor cell counts. This immunocytochemical assay therefore needs to be improved for patients with lower tumor cell counts before it can be applied as a surrogate test for adjuvant therapies [207].

Ovarian carcinoma
Since the early 1980s, the possibility of tumor targeting and treatment by intraperitoneally administered radiolabeled MoAbs in patients with ovarian carcinoma has been object of study [208]. Only in 1993 appeared the first comprehensive survival data of ovarian carcinoma patients treated with an intraperitoneal radiolabeled MoAb in an adjuvant setting. Hird et al. published an extended phase I-II trial on the single-dose administration of 25 mg 90Y-labeled MoAb HMFG1, recognizing polymorphic epithelial mucin, following conventional treatment.
Twenty-one of 52 patients showed no clinical evidence of residual disease and were regarded as receiving MoAb HMFG1 as adjuvant treatment. Two of these patients died after a median follow-up of 35 months, indicating that patients with advanced ovarian carcinoma who achieve CR with standard management may benefit from further treatment with intraperitoneal radiolabeled MoAbs. Although survival data showed a remarkable difference with data on 70 historical controls who presented with at least stage IIb disease and who had no evidence of disease at second-look laparoscopy, patient numbers are small and need to be substantiated by larger phase III trials. Treatment was generally well tolerated. Toxicity consisted of reversible myelosuppression at high doses and was reduced by the use of more stable chelating agents [209]. Survival data were confirmed by another trial, in which 25 patients with stage Ic-IV ovarian carcinoma were given a single intraperitoneal dose of radiolabeled MoAb HMFG1 after achieving CR on conventional chemotherapy. The observed survival rate at five years was 80%, compared to 55% for a close-matched historical control group [210]. Crippa et al. reported on 16 stage III (FIGO classification) ovarian carcinoma patients with MRD following surgery and first-line chemotherapy, as demonstrated by second-look evaluation. Thirty to 40 days after the second-look procedure, patients received a mean intraperitoneal dose of 14 mg (range, 8-21 mg) radiolabeled MoAb 131I MOv 18, recognizing a membrane folate-binding glycoprotein of 38 kDa present on 90% of ovarian carcinomas. Treatment gave complete responses in five (31%), stable disease in six (38%) and progressive disease in five (31%) patients. Follow-up showed a long-term maintained complete response (34 months) in one patient and relapses in the other four after a mean relapse-free period of 10.5 months, whereas five patients with stable disease progressed after a mean relapse-free period of 13 months. Clinical efficacy of this treatment regimen is, if not superior, at least comparable to that of adjuvant regimens incorporating second-line chemotherapy [211, 212]. Side effects involved mild bone marrow depression in one patient, with spontaneous recovery [213]. In a retrospective study, Osmers et al. investigated to what extent patients with treated ovarian carcinoma benefit from the administration of MoAb OC-125 in terms of survival. Collectively, data on 120 paired patients, of whom 60 underwent RIS for diagnostic purposes, showed that at favorable stages (II) the patients benefited more from MoAb OC-125 application than at prognostically unfavorable stages (IV). Even within stage III, authors have been able to demonstrate that patients without clinical signs of disease benefited significantly more from adjuvant MoAb treatment in terms of survival than those with residual disease [214].

**Pancreatic carcinoma**

The use of MoAbs in the treatment of advanced and micrometastatic pancreatic carcinoma has been reviewed by Friess et al. [215]. The first controlled clinical data on passive adjuvant immunotherapy of pancreatic carcinoma are based on a randomized trial, in which 61 patients received IV treatment with 370 mg MoAb BW 494, recognizing a carbohydrate epitope on
human pancreatic carcinoma cells, or no systemic treatment following Whipple resection. Median survival times in patients with (428 days, range 248 to 510 days) and without (386 days, range 296 to 509 days) MoAb BW 949 treatment were comparable, leading to the termination of further clinical studies with this MoAb. One of the reasons for the negative outcome of this trial might be the relatively low MoAb dose used, resulting in an insufficient saturation of target epitopes despite good vascularization. Thus, for therapeutical effectiveness, the dosage to be used in humans might have to be higher. Side-effects of MoAb treatment consisted of nausea, vomiting, fever, chills and abdominal pain, all responsive to drug administration and not necessitating interruption [216]. New clinical studies using humanized MoAbs in conjunction with immune response modifiers should be initiated to further evaluate immunotherapy as a treatment option in pancreatic cancer [215].

Other carcinomas
In a phase I study, the feasibility of treatment with radiolabeled MoAb NP-4, raised against CEA, was assessed in patients with MDR of colorectal cancer (8), lung cancer (3), pancreatic cancer (1), and medullary thyroid cancer (1). Disease stabilization ranging from 3.5 to 7 months was observed in 6 of the 13 patients who previously had clear evidence of progressive disease [217]. In a first clinical trial, eight patients with poorly differentiated gastric carcinoma received 20 or 30 mg of the apoptosis-inducing human MoAb SC1 intravenously, followed 24 or 48 hours later by gastrectomy and lymphadenectomy. In seven cases, a significant induction of apoptotic activity was measured in primary tumors as compared to earlier biopsies, with significant tumor regression in five. These data hint at a potential role of MoAb SC1 in the adjuvant treatment of gastric carcinoma [218, 219].

HNSCC
The improvement of detection and eradication of MRD, to reduce local and distant relapse after primary therapy, is one of the major challenges in the management of HNSCC. The application of the radiolabeled MoAbs E48 and U36, directed against HNSCC-associated antigens, has proven to be quite valuable [220].

High grade glioma
Fifty-nine patients with primary presentation of high-grade gliomas of the brain, 13 with astrocytomas with anaplastic foci and 46 with glioblastoma multiforme, were treated with surgical intervention and radiotherapy, followed by multiple intravenous administration of the $^{125}$I-labeled MoAb 425. At one year, 34 (58%) of the 59 patients in the trial were alive. The overall survival for both groups was 13.5 months [221].
Concluding remarks

In cancer patients, clinical relapses occur frequently despite the absence of residual tumor after conventional treatment. It therefore has been hypothesized that common staging procedures fail to detect the presence of MRD, which might be the seed for subsequent metastatic relapse. It is important to realize, though, that the mere presence of isolated tumor cells in otherwise normal bone marrow, peripheral blood or lymph node will not automatically lead to the development of clinically relevant metastases [91, 92]. The majority of tumor cells entering the blood stream are cleared and do not survive; animal data suggest that only 0.01% of circulating tumor cells will eventually produce metastatic deposits. Moreover, other factors are now being discovered as important in the formation of metastases, such as chromosomal defects and the expression of particular adhesion molecules and proliferation markers [86].

Detection of MRD

Using sensitive immunological methods, MRD can be detected in a vast array of hematopoietic and solid malignancies. Nevertheless, the prognostic value of MoAb-guided MRD detection is limited by lack of MoAb specificity. Recently, progresses in the detection of MRD have been brought by more sophisticated and sensitive techniques such as PCR and reverse-transcriptase PCR (RT-PCR). These techniques are currently on their way to eclipse immunological detection methods, at least in the case of solid malignancies, although false-positive interpretation of data can occur. In most published studies, however, the PCR assays have been highly specific and consistently negative in control populations [222]. Whatever approach is utilized, the clinical relevance of MRD remains contested and has to be validated as an independent predictor of clinical relapse by further studies. Up to now, clinical trials have lacked uniformity in their protocol, preventing adequate comparison of data [87, 223].

Eradication of MRD

Until now, significant improvements in relapse-free and overall survival have been achieved by treating MRD in selected tumors such as leukemia, colorectal carcinoma and ovarian carcinoma. However, the clinical value of MoAbs in eradicating MRD has to be determined in the setting of phase III studies before their general use as adjuvant agents can be recommended. The advent of potentially powerful MoAbs such as Panorex™, Herceptin™ and Rituxan™ might mark the beginning of a novel, exciting period in clinical antibody research. By now, Panorex™ has been approved for the adjuvant treatment of colorectal carcinoma. Clinical studies evaluating the role of Herceptin™ and Rituxan™ in advanced malignancies are currently underway; their value in the elimination of MRD remains to be judged.
References


