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# Prevention of Manifest Metastasis with Monoclonal Antibodies: A Novel Approach to Immunotherapy of Solid Tumours

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**Until now, surgery, chemotherapy and radiotherapy have remained the mainstay of current cancer therapy. The major limitation of chemo- and radiotherapy is their narrow therapeutic index between cancer and normal cells. In the search for less toxic and more specific therapies, various modalities of immunotherapy have been tried. It is now increasingly recognised that patients presenting with minimal cancer burden or micrometastatic disease will experience the greatest benefit from treatment with monoclonal antibodies (mAbs). The first proof of efficacy of a monoclonal antibody in minimal residual disease has recently been published, with mAb 17–1A in patients with colorectal cancer stage III after complete resection of the primary tumour. After a median follow-up of 5 years, antibody therapy reduced the overall death rate by 30% and decreased the recurrence rate by 27%. This result is similar to the benefit obtained in (radio)chemotherapy trials, however, with notably lesser toxicity. It is clear from past experience that all currently available treatment modalities for cancer are far from perfect. However, because the mechanism of action or target cells of different treatment modalities may be complementary in the control of tumour growth, the next logical step is to rationally design clinical trials that combine conventional chemo-, hormonal or radiation therapy with immuno- or biotherapy.**

**Key words: immunotherapy, monoclonal antibody, 17–1A, micrometastases, solid tumour**
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UNTIL NOW, surgery, chemotherapy and radiotherapy have remained the mainstay of current cancer therapy. The major limitation of chemo- and radiotherapy is their narrow therapeutic index between cancer and normal cells. In the search for less toxic and more specific therapies, various modalities of immunotherapy have been tried. Among those modalities, adoptive strategies based on monoclonal antibodies (MAb) have been applied to a broad spectrum of malignancies [1, 2]. Because MAbs can bind to antigens selectively expressed on the surface of malignant cells, optimistic projections proclaimed that these agents could be used to specifically target and destroy tumour cells. Furthermore, by exploiting cytotoxic mechanisms distinct from those of chemo- or radiotherapeutic agents, additional therapy with mAbs was supposed to improve overall efficacy, and to circumvent tumour cell resistance induced by radiochemotherapy.

Ideally, a mAb should specifically target the tumour cell and have minimal crossreactivity with normal tissues. If, however, the antibody crossreacts with normal tissues, it should not impair vital functions of the body. When the antibody reaches the tumour cell, its ability to exert a cytotoxic effect depends on the nature of the antigen as well as on the biological activities of the antibody itself, such as its ability to activate endogenous cytotoxic mechanisms, for example, antibody-dependent, complement-mediated cytotoxicity (ADCMC) or antibody-dependent cell-mediated cytotoxicity (ADCC). The latter depends on lymphocytes, macrophages and granulocytes recognising the $F_c$ region of cell-bound antibody which triggers killing of the target cell.

In the past 15 years, the clinical development of mAbs has essentially followed the same path as other small molecules intended for therapy of cancer, i.e. entry into Phase I trials with terminally ill patients who present with large tumour burden. Thus far, only a few anecdotal objective clinical responses have been reported in such patients treated with antibodies [1, 2]. This is not surprising since immunoglobulins with their molecular weight of 150 kDa can hardly penetrate the solid parenchyma of bulky tumour masses [3]. In fact, studies [4] have shown that less than 1% of an infused radiolabelled mAb reaches the tumour. This restricted accessibility of tumour cells seems to be one of the major reasons why antibodies have so blatantly failed in this patient group. Speculation that the inaccessibility of a large tumour mass is a function of antibody characteristics has led to controversy [5]. The binding site hypothesis suggested that high antibody affinity and increased target antigen density prevent mAbs from penetrating into tumour tissue. Evidence obtained *in vitro* on spheroids [6] and *in vivo* in nude mice [7] supports this hypothesis. Other experiments, however, employing radiolabelled mAbs against the same antigen demonstrated that penetration into tumour tissue was independent of affinity [8].

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More encouraging results were reported with particular mAbs rationally chosen for the treatment of leukaemias and lymphomas [9–11], although even here, with better accessibility of the target cells, the delicate balance of tumour mass to administered antibody needs to be considered.

It is now increasingly recognised that patients presenting with minimal cancer burden or micrometastatic disease will experience the greatest benefit from treatment with mAbs. One of the first indications that micrometastatic cells might be suitable targets for passive antibody therapy was provided by Schlimok and associates [12], who demonstrated that tumour cells in the bone marrow of patients with colorectal cancer can be labelled with mouse immunoglobulin *in vivo*. In a subsequent report [13], the therapeutic effects of antibody infusions over a period of several months up to 2 years were monitored by immunocytochemical analysis of bone marrow. In 12 of the 23 Dukes' C patients with clinically manifest metastases, micrometastatic cells were repeatedly identified and clinical relapse occurred in 9 of these patients.

Furthermore, in a pilot study, the intravenous application of mAb ABL 364 resulted in specific clearance of dispersed metastatic cells in the bone marrow [14]. ABL 364 is a murine monoclonal $IgG_3$ antibody directed against a Lewis Y blood group-related antigen on the cell membrane of carcinoma cells from gastrointestinal, breast and lung tumours [15]. The application of 6 doses of 50 mg mAb over 2 weeks (3 doses/week, total 300 mg) in patients with advanced breast cancer, induced significant eradication or reduction of dispersed CK18-positive tumour cells in bone marrow; at the same time solid metastases in any of the treated patients did not show objective shrinkage. A similar decrease of disseminated cells was not observed in the placebo-treated group or in patients who received mAb, but presented with cells lacking the Lewis Y-related antigen. The antibody exerted a marked cytotoxicity on tumour cell lines when tested *ex vivo* in serum taken from these patients after antibody infusion. As inactivation of the serum by heat treatment at 56°C for 30 min abolished the cytotoxicity completely, the observed reduction of individual tumour cells is most likely to be due to a complement-mediated cytotoxic effect of the infused antibody.

The most comprehensive test of efficacy of a mAb in minimal residual disease has recently been published, with mAb 17–1A in patients with colorectal cancer stage III (Dukes' C) after complete resection of the primary tumour [16]. In this adjuvant trial, patients received a total of 900 mg mAb 17–1A in 5 infusions over 20 weeks starting at 2–3 weeks after surgery. After a median follow-up of 5 years, antibody therapy reduced the overall death rate by 30% and decreased the recurrence rate by 27% (Figure 1a and b). This result is similar to the benefit obtained in (radio)chemotherapy trials [17–21]. Compared with those trials, it is noteworthy that the toxicity of 17–1A treatment was considerably lower [16].

The 17–1A antibody is one of the earliest and best published monoclonal antibodies [22]. It recognises a 40 kDa antigen expressed on the cell surface of a wide variety of tumours, derived from different simple epithelia, that is also present on epithelial cells of various normal tissues [23–27]. This was surprising at the time, since earlier *in vitro* studies on tumour cell lines had led to the belief that this mAb was highly specific [28]. Quantitative differences observed in antigenic expression between normal and malignant tissues were not evident in comparative immunohistochemical studies of colorectal carcinomas and normal mucosa obtained from the same patients. A possible structural heterogeneity of the 17–1A antigen when expressed on normal and malignant cells cannot as yet be ruled out from these studies.

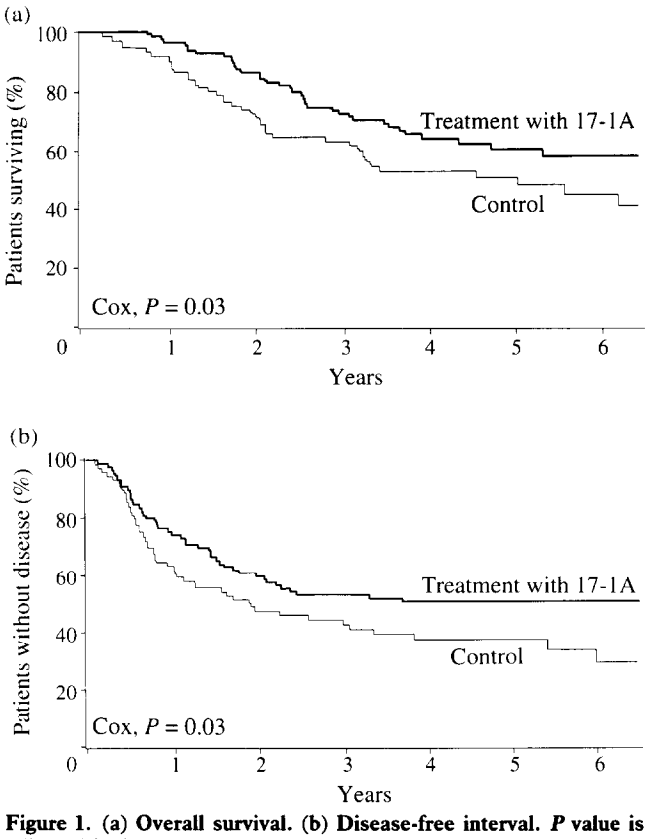


**Figure 1. (a) Overall survival. (b) Disease-free interval. *P* value is adjusted for imbalances in prognostic values with Cox's proportional hazard model.**

The function of the 17–1A antigen has been claimed to be related to cell–cell adhesion of epithelial cells [29]. It is highly conserved, thoughout evolution, supporting the importance of its function [30]. The broad reactivity of the 17–1A antibody with epithelial cells in *in vitro* studies must be contrasted with its apparent selectivity *in vivo*. Biodistribution studies with radioactively labelled 17–1A in humans have demonstrated radiolocalisation to tumour, with specificity indices ranging up to a factor of seven [31, 32].

Another clue to 17–1A's "operational" *in vivo* specificity can be derived from its rather benign profile of adverse events. The overall clinical safety profile and human anti-mouse antibody (HAMA)-response of patients have been studied in many trials with 17–1A administered in single doses of up to 1000 mg and cumulative doses of up to 12000 mg [33, 34]. Adverse experiences considered to be related to treatment with 17–1A are predominantly gastrointestinal or allergic in nature, and have been easily controlled or reversed without prolonged tissue damage. Generally, approximately half of all adverse experiences reported are gastrointestinal, with frequently experienced nausea with and without vomiting, diarrhoea, abdominal pain and cramping and less frequently noted occurrences of constipation, indigestion and bleeding. Less than 5% of adverse experiences were anaphylactic, and required cessation of treatment and medical intervention, but no hospitalisations. Otherwise, all adverse experiences were mild to moderate in nature, and resolved with routine outpatient measures.

When studying the pattern of adverse experiences after appli-

cation of a multidose regimen, it becomes apparent that most were observed following the first infusion (Figure 2) [16]. This may be due to the five times higher initial dose or higher infusion rate as well as to the relatively close proximity to surgery, but it also weighs against allergic drug reactions.

Interestingly, in other studies, a correlation has been identified between the severity of gastrointestinal adverse experiences and serum concentration of 17–1A. Therefore, these disturbances may be controlled by slowing the rate of antibody infusion. Thus, gastrointestinal symptoms depend on the duration of infusion and can be reduced by using a 2 h infusion [35].

A HAMA response has been observed in 80–100% of patients treated with 17–1A [16, 36]. It has been postulated that such a HAMA response favourably influences the prognosis of a patient, by inducing the idiotypic cascade leading to anti-antibody response ($ab_3$) of the patients that is directed against the tumour-associated antigen [36]. Others have argued that the development of HAMA may prejudice the efficacy of mAbs because of enhanced clearance of the antibody from the circulation, and/or neutralisation via formation of antigen–antibody complexes, which subsequently could lead to damage of organs such as kidney or lung. No side effects, however, have so far been seen in association with HAMA formation, perhaps because the absolute amount of HAMA formed is very low. This also explains why the putative presence of HAMA does not alter the pharmacokinetics of subsequent administrations of antibody when given at high doses, such as 100 mg or more [33].

It is clear from past experience that most of the currently available treatment modalities of cancer are far from being perfect. However, because many of them are somewhat effective in systemic and/or local control of tumour growth, the next logical step has, therefore, been to combine conventional chemo-, hormonal or radiation therapy with immuno- or biotherapy.

Rationales for combining treatment modalities are manifold, and a compilation of clinical trials employing such combination regimens in multiple permutations emphasises the necessity of designing future trials on a more rational basis [37]. The existing body of knowledge, together with experiences gained from preclinical models, as well as a better definition of surrogate endpoints in patients, should allow the design of regimens and schedules combining agents at optimal doses which would improve the overall results of cancer therapy.

For example, an argument for combining radiation with antibody therapy is that radiation is largely accepted as effective local tumour control, but as having no effect on overall survival when applied alone, while antibody therapy appears to be particularly effective against distant metastases. Thus, adjuvant systemic therapy, when combined with post-surgical immuno- or chemotherapy, may confer additional benefit by reducing the rate of distant metastases. In the trial cited above, the reduction of the proportion of distant metastases in patients with Dukes' C colorectal cancer has been particularly impressive where murine 17–1A was administered as an adjuvant to curative surgery [16]. Since murine 17–1A has been proven comparatively safe relative to systemic cancer chemotherapies, a carefully timed combination of these regimens may constitute a promising treatment alternative.

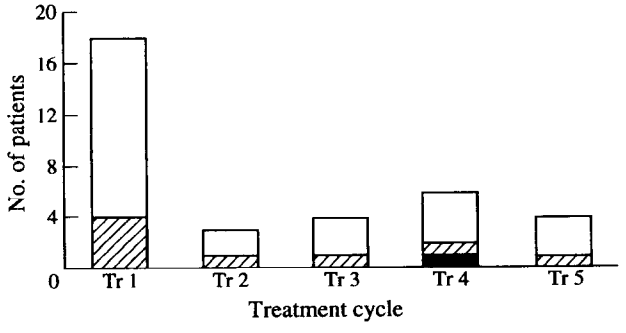


**Figure 2. Number of patients with adverse events per treatment cycle and number of adverse events: 1, □; 2, ▨; and 3, ■ occurring simultaneously in patients during and after consecutive treatments.**

A further argument for combining immunotherapy with chemotherapy can be made on the basis of information obtained from studying the biology of individual microdisseminated tumour cells via an immunocytochemical method [38, 39]. For example, isolated tumour cells in the bone marrow of patients with breast, non small cell lung (NSCL) or gastrointestinal cancer rarely express proliferation-associated markers, such as Ki 67 or p 120 [40, 41], and are in the $G_o$ phase of cell cycle. Although the authors were cautious with their conclusions, since the number of detectable carcinoma cells per patient was rather small, these observations may provide another argument for combining immunotherapy active on resting cells with chemotherapy, which is mainly directed against proliferating cells.

Finally, efforts to improve immunotherapy have led to the introduction of novel cytokines into preclinical and clinical studies of combinations of cytokines and monoclonal antibodies [42–44]. There is ample preclinical evidence that cytokines can enhance effector cell-mediated ADCC [45]. Also, the efficacy of mAbs can be augmented when the full cytotoxic capacity of effector cells is exploited by stimulation with cytokines [46–48]. For example, Ragnhammar and associates [46] activated effector cells (peripheral blood mononuclear cells (PBMC) or granulocytes) with granulocyte (macrophage) colony stimulating factor (G(M)-CSF) or MCSF and added these activated effector cells, together with mAbs, to various tumour target cells. MCSF and GM-CSF were the most effective in activating PBMC cytotoxicity against various human tumour cell lines. G-CSF and GM-CSF, but not M-CSF, occasionally increased the lytic capacity of granulocytes in ADCC. Chimeric mAbs with human $F_c$-portions seemed to be the most active when human effector cells were used.

Eisenthal and associates [42] studied the abilities of IL-1, IL-2, TNF, IFN-α and IFN-γ to induce ADCC *in vivo*. Using an immunotherapy model in mice, it could be shown that IFN-α significantly enhanced the antibody-mediated antitumour effect on established B16 melanoma liver micrometastases. Furthermore, when IL-2 and IFN-α were simultaneously administered in combination with mAbs, the number of established macrometastases in the liver was significantly reduced. This regimen led to prolonged survival of tumour-bearing mice. All these preclinical studies indicate that the administration of an appropriate cytokine combination may be a useful adjunct to mAb-based therapy of cancer patients.

In a Phase 1b clinical trial, using GM-CSF in combination with mAb R24, in patients with advanced metastatic melanoma [47], it was shown that GM-CSF can upregulate monocyte and granulocyte function as measured by direct cytotoxicity and ADCC against a melanoma cell line. Of 20 patients treated, two achieved partial responses, suggesting that this combination may have clinical activity. The authors concede, however, that it is unclear whether the addition of GM-CSF enhanced the response rate of R24, since this mAb had led to clinical responses as a

single agent [49]. The combination of GM-CSF plus R24 can produce a variety of toxicities, including diffuse urticaria, nausea and vomiting, fever, flu-like symptoms, hypertension and hypotension. In the study described [47], the dose-limiting toxicity was hypotension.

In another study [44], the combination of mAb 17–1A and GM-CSF was evaluated in 20 patients leading to 2 patients with a complete response and one patient with a partial response and an overall clinical response rate of 30%. These results were obtained with minor toxicity. Bajorin and associates [50] reported the use of low dose IL-2 together with R24, and observed one partial response in 20 treated patients with metastatic melanoma. When a higher dose IL-2 regimen was used together with R24 and monthly low dose cyclophosphamide in patients with melanoma [51], 10 of 23 evaluable patients had partial responses. However, toxicities related to IL-2 and reported in this trial were substantial and included hypotension, fever, renal and pulmonary toxicities.

Although this selection of clinical studies shows that there may be merit in combining mAbs and cytokines for the treatment of cancer patients, further studies are needed to define the optimal agents, their dose and schedule of administration. It will depend on a better understanding of the immunopharmacological actions of antibodies and cytokines, which will allow a more rational translation of preclinical results into clinical practice, which ultimately should lead to improved clinical responses with acceptable adverse side effects for the patients.


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