Monoclonal antibodies in cancer therapy

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A review of the clinical trials of antibody based cancer therapies reveals that this approach can, in rare cases, induce complete remissions in individual patients with cancer. Since these trials have usually involved patients with large tumor masses, tumor cell inaccessibility is probably a major reason for the prevailing failures. Minimal residual disease, the stage when tumor cells are few and dispersed, should therefore be a more promising target for therapeutic antibodies. This hypothesis is supported by a prospective randomized trial on patients with resected Dukes C colorectal carcinoma that resulted in increased survival and prolonged recurrence-free intervals. Thus, in addition to strategies designed to produce more effective, human-derived reagents, efforts need to be concentrated on directing passive antibody therapy towards the appropriate target.

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Introduction

Passive antibody therapy of cancer is one of the oldest and most prominent issues of tumor immunology. As early as 1895, a few years after von Behring's and Kitasato's discovery that antisera against diphtheria toxin could cure children with diphtheria, Hericourt and Richet reported on their attempts to treat cancer patients with antisera prepared in dogs and donkeys. Paul Ehrlich, with his antisera against plant toxins abrin and ricin, had shown the specificity of the newly induced serum substances and named them Antikörper (antibody). He became particularly intrigued by their potential use as specific weapons against cancer cells and coined for them the term Zauberkugel (magic bullets). Nevertheless, despite these early beginnings, antibody therapy of cancer has become a story of unending failures.

In 1975, a turning point seemed to have been reached with the invention of the hybridoma technique by Köhler and Milstein [1]. Monoclonal antibodies (mAbs) with their uniform and well-defined specificity and virtually inexhaustible supply, promised to bring a solution to the vexing problems of variable specificity and irreproducibility inherent in polyclonal antisera. Indeed, a spate of reports on mAbs with presumed tumor-restricted or demonstrated tumor-associated specificity appeared in subsequent years. While several of those mAbs were used in a clinical setting as valuable diagnostic tools, so far none of them has gained recognition as an established therapeutic against malignant disease. As reviewed by ourselves for solid tumors [2], numerous clinical trials have been performed with unmodified mAbs without any consistent pattern of response. An obvious conclusion to be drawn from these conspicuous failures was, that in spite of their exquisite specificity and their apparent ability to target tumor cells, antibodies alone were either not sufficiently cytotoxic or could not adequately harness the patients' own effector mechanisms. Consequently, a broad research effort was begun to improve the cytotoxicity of antibodies by conjugating them with radioactive isotopes, cytotoxic drugs or potent toxins. These efforts culminated in the development of single chain antibodies, consisting solely of covalently connected V_H and V_L peptides, to which toxin molecules had been fused by recombinant DNA techniques [3–5]. However, as accessibility of tumor cells in advanced stages of cancer to macromolecules may be strictly limited, this review focuses on the minimal residual disease stage as a much more promising target for antibody-based therapies.

A decade of clinical trials—some successes but more disappointments

Within the last year, several extensive reviews have appeared that describe results of phase I and phase II therapeutic trials using both unmodified mAbs as well as various antibody conjugates (Table 1) [2,6••,7•,8••,9].

A rough assessment of the reported successes and failures indicates that complete remissions have been most often observed in Non-Hodgkin lymphomas with radioimmunoconjugates, which appear to be superior to immunotoxins and unmodified antibodies. Moreover, in myeloid leukemia, the combination therapy with high-dose cytoxan and radiation therapy led to a sizeable rate of remissions. Antibody trials on solid tumors, how-

Abbreviations

CDR—complementarity determining region; **GM-CSF**—granulocyte-macrophage colony-stimulating factor; **mAb**—monoclonal antibody.

Table 1. Recent reviews on antibody-based tumor therapy.

Authors	Target	Antibody therapy	No. of trials*	No. of patients	Complete responses
Grossbard *et al.* [6••]	Leukemia and lymphoma	Unmodified antibodies Immunotoxin Radioimmunoconjugates with or without chemotherapy or radiotherapy	16 9 20	161 179 211	5 6 40
Riethmüller and Johnson [2]	Melanoma and carcinomas	Unmodified antibodies	12	196	2
Steffens *et al.* [7•]	Melanoma	Unmodified antibodies	8	74	3
Vitetta *et al.* [8••]	Melanoma and carcinomas	Various immunotoxins	16	375	9
LoBuglio and Saleh [9]	Lymphoma, melanoma, ovarian, breast and gastrointestinal cancer	Unmodified antibodies, radioimmunoconjugates, immunotoxins	16 9 10	not detailed	not given

*Some trials reviewed by two or more reviewers.

ever, have yielded complete remissions only in the rarest cases. This group of tumors comprises mainly cancers derived from simple epithelia which develop metastases that are composed of host-derived stromal tissue and of differentiated epithelial parenchyma growing within the envelope of a dense basement membrane. The epithelial tissue organization sets carcinomas quite apart not only from lymphoma and leukemia but also from melanoma, the metastases of which lack the typical coherent trabecular or adenomatous formations in which cells are connected by desmosomal intercellular junctions. Interestingly, melanoma appears to be one of the more susceptible neoplasms for antibody-based therapies.

The critical issue of tumor-cell accessibility

The rare complete tumor regressions observed with all of the various antibody-based modalities demonstrate that, in principle, unmodified antibodies as well as immunoconjugates can produce sufficient tumor-directed cytotoxicity. Why then do complete remissions only occur in rare individual patients? In the absence of any common immunogenic trait characteristic for the responder patients, the suspicion is warranted that peculiar conditions of the individual tumor are responsible for the antibody-induced regression. Among these, an abnormal vascularization highly permeable to intravenously injected antibodies, a homogeneous expression of the target antigen on the relevant clonogenic tumor cells, and accessibility as well as vulnerability to direct or indirect cytotoxic effects of antibodies, rank very high. Although the sporadic nature of antibody-induced regressions may be reduced to the rare coincidence of several of these factors, several lines of evidence point to the inaccessibility of cancer cells growing in solid tumor parenchyma as a leading cause of the observed therapy failures. The results of the trials themselves (Table 1), showing that responses are far more common in hematopoietic malignancies than in solid tumors, underscore this reasoning. Furthermore, an impressive amount of experimental data, much of which has been compiled in recent years by Jain and his colleagues [10,11], demonstrate that macromolecules, including mAbs, have difficulty reaching epithelial tumor cells. Elevated interstitial fluid pressure in solid tumor nodules is one of the major obstacles in the long list of vascular and interstitial barriers impeding the delivery of antibodies to cancer cells (Table 2). A recent report demonstrates that interstitial pressures as high as 33 mmHg can be directly measured in individual head and neck tumors *in situ* [12] and similar values have been determined for subcutaneously growing metastases of melanoma and primary cervical cancers [10,13]. Additional barriers for the free diffusion and convection of antibodies include the basement membrane enveloping the epithelial tumor trabeculae, the intercellular tight junctions and the long distances extravasated antibodies must travel through the dense intrastitial mesh of proteoglycans in order to reach their cellular targets.

The positive *in vivo* labelling data obtained by numerous immunoscintigraphic studies and the *ex vivo* autoradiographic analysis of labelled tumor biopsies do not refute this view of the ability of antibodies to penetrate into tumor tissue, as the majority of these studies attest to a heterogeneous uptake of the antibody by the tumor tissue [14].

Tumor cell accessibility is a parameter that cannot easily be assessed in model systems. The many reports of complete cures obtained after antibody treatment of nude mice transplanted with human tumors may convince the experimental novice, but not the skeptical clinician, as they frequently fail to work in patients. Marked differences exist between the vasculature of spontaneous

Table 2. Barriers and factors impeding free access of monoclonal antibodies to cancer cells growing in solid tumors.

Heterogeneous or poor vascularization of tumors, reduction of total vascular surface area compared with normal tissue
Elevated interstitial fluid pressure in tumor nodules
Shallow or reversed transvascular pressure gradient leading to decreased transvascular convection and diffusion
Long transport distances for extravasated macromolecules in interstitium of tumors
Radially outward directed interstitial fluid convection
Basement membranes surrounding epithelial tumor tissue
Shed or released tumor antigen present in peritumorous extracellular matrix
Intercellular tight junctions in tumorous epithelia.
Adapted from [11].

autochthonous tumors and transplants of these tumors [10,11], indicating that xenotransplantation models primarily measure antibody effector function.

If accessibility of tumor cells is indeed a major reason for the overall disappointing results of the clinical trials and if accessibility is negatively correlated with the volume of the tumor mass, the question must be asked whether therapy trials on patients with advanced malignant disease, i.e. with bulky epithelial tumor masses or with leukemic or lymphoma cells in excess of 10^{12} cells, will ever show a therapeutic efficiency of antibodies.

A much more appropriate target for assessing the efficacy of antibody therapy may well be minimal residual disease, a stage in which, after resection of all macroscopic tumor the remaining cancer cells are very few and dispersed as individual cells or small clusters in the interstitium of various distant organs.

Minimal residual disease — a target within reach

Minimal residual disease is present in roughly half of the patients with curatively resected solid tumors. Previously, the presence of hidden metastasis in these patients could be inferred only retrospectively from overt relapses occurring several years after curative surgery. In the last few years, however, novel immunocytochemical methods that allow the detection of small numbers of carcinoma cells in bone marrow have become available [15,16•,17]. As these cells do not express proliferation associated antigens they appear to be in a state of dormancy [18,19•]. Several studies have now shown that the presence of these micrometastatic cells during early stages of tumor dissemination can serve as a strong predictor of a later clinical relapse [20••,21,22].

Because of their low number, their presence in mesenchymal interstitium and lack of epithelial structures, these visible micrometastatic cells can be considered as ideal targets for therapeutic antibodies. Indeed, a previous study demonstrated that intravenously injected mAbs directed against a membrane-associated glycoprotein could be targeted to individual tumor cells in bone marrow [23].

Therefore, with these deliberations in mind, a multicenter randomized clinical trial involving 189 patients with resected colorectal carcinoma was initiated in 1985 and was completed in December of 1992 (G Riethmüller, E Schneider-Gädicke, G Schlimok *et al.*, unpublished data). Following surgery, the patients, all of whom had Dukes C stage carcinoma, were randomized to a control arm, i.e. observation only, and to a treatment group. The treatment group received 500 mg of mAb 17-1A within two weeks of surgery followed by four subsequent monthly infusions of 100 mg of antibody. After a median follow-up of 5 years, therapy with antibody was found to have decreased the overall death rate by 30% and reduced the recurrence rate by 27%. These data contrast with the results of numerous, non-randomized trials with 17-1A antibody in advanced tumors where anecdotal remissions were observed only in a few patients and no benefit for survival could be secured. Interestingly, in this adjuvant study, the reduction in recurrence rate was found to be restricted to the development of distant metastases, while local relapses were not reduced by the treatment. This altered pattern of recurrences can be interpreted such that local satellite tumors were already too big and/or inaccessible to the antibody, in contrast with the distant micrometastases which were destroyed by it. This trial shows that by carefully selecting the stage of tumor growth at which therapy is initiated, antibody therapy of colorectal carcinoma is comparable with other adjuvant therapies (Table 3) [24,25]. However, because of the remarkably low toxicity of unmodified antibody, this therapy can be administered to patients following curative surgery without exposing them to the current hazards of adjuvant chemotherapies.

As to the contentious issue of target antigens most suited for antibody therapy, it is notable that the antigen recognized by 17-1A mAb is by no means a tumor-specific antigen as it is widely expressed on various normal simple epithelia including, not only small and large intestine, but also bile ducts, kidney tubules and epithelial cells of thyroid and prostate [26]. The antibody, a murine IgG_{2a}, has been administered to more than 300 patients with advanced disease [27]. Both the the lack of toxicity and efficacy (some minor transient gastrointestinal effects excepted) of doses of antibody up to 12 g may be due to the poor delivery of the antibody to cells shielded by a dense basement membrane and other vascular and interstitial barriers [28]. Thus, one may arrive at the conclusion that absolute tumor specificity of an antibody is less important than homogeneous expression of the relevant antigen on as many tumor cells as possible, as long as their normal counterparts and the stem cells from which they are derived are either less accessible or do not express the antigen. It appears from the reviews in Table 1 that numerous therapy trials have been performed with antibodies, immunotoxins and radioimmunoconjugates recognizing absolutely normal differentiation antigens, e.g. in B-cell lymphoma, without intolerable toxicity for the recipient. The 17-1A antibody has a remarkably low affinity and induces only intermediate antibody-depen-

Table 3. Comparison of adjuvant therapies in colorectal carcinoma.

Therapy	% Reduction in mortality rate (with 95% confidence interval)	% Reduction in recurrence rate (with 95% confidence interval)
mAb 17-1A versus control Colorectal cancer, stage III Riethmüller *et al.*, unpublished data	31 (1–54)	25 (1–45)
Levamisole + fluoroacil versus control Colon cancer, stage III Moertel *et al.* [24]	33 (10–50)	41 (23–54)
Radiation + fluoroacil + methyl-CCNU versus radiation alone Rectum cancer, stage II or III Krook *et al.* [25]	29 (7–45)	34 (12–50)

dent cell-mediated cytotoxicity [29,30]. Whether these peculiar characteristics of the antibody are essential for its clinical efficacy is unknown so far. However, an argument can be made that low affinity antibodies penetrate the solid tumor more deeply [30,31]. This argument is contended by Schlom *et al.* [32] who in contrast favor high affinity antibodies as more efficient therapeutics.

New perspectives for antibody therapy

If minimal residual disease, a stage so frequent in patients with the most common solid tumors, is such a promising target for antibody-based strategies, then a further refinement of antibodies indeed makes sense.

As the target patient population is quite healthy and as at least half of them are already cured by surgery and/or local radiation therapy alone, the risk/benefit assessment of experimental therapies becomes critical. Thus, for the development of adjuvant therapies, unmodified antibodies with their low toxicity profile have clear advantages over immunotoxins or radioconjugates. In order to obtain steep transvascular concentration gradients towards mesenchymal tissue compartments, higher doses of antibodies may be required which, in turn, will favor the induction of counterproductive immune responses in patients. Indeed, in virtually all the trials cited in Table 1, human antibodies to the murine Ig reagents were produced. Although the number of reported anaphylactic reactions has been low, it is clear that for prolonged therapy regimens, the immunogenicity of antibodies should be as low as possible.

A number of clinical trials have clearly shown that replacement of the Fc region with human sequences can substantially reduce the immunogenicity of murine antibodies [33–36]. The least immunogenicity is expected to be obtained when only the complementarity determining regions (CDRs) of the murine antibody remain, and recent studies suggest that it may be possible to significantly simplify the production of these reagents [36,37•].

The use of antibodies derived entirely from humans and isolated from combinatorial libraries in bacteriophage [38,39] will most likely soon replace such engineered murine antibodies. This technique allows the isolation of high affinity, antigen-specific Fabs or Fvs, even from naive human B cells [40]. Furthermore, such antibodies may be generated from 'semi-synthetic libraries', which are produced by replacing the CDR3 region of a single human Ig with random oligonucleotides [41•,42•].

Table 4. Cell-directed effects of unmodified antibodies.

Activation or stimulation of cells
e.g. Signalling via receptor aggregation, mimicry of agonists such as cytokines, hormones, adhesion ligands
Inactivation of cells
Negative signalling
Blockade of functions of receptors or ion channels
Modulation of receptors/adhesion molecules
Induction of differentiation
Elimination of cells by
Complement mediated cytolysis
Opsonisation, sequestration and phagocytosis
Induction of apoptosis, directly via anti-Fas (Apo-I) antibodies or indirectly via antibody-dependent cellular cytotoxicity
Induction of cytotoxic T-cells against murine Ig, processed and presented by antibody labelled target cells
Induction of anti-idiotypic antibodies (ab_3) with anti-cellular activity

The therapeutic efficacy of mAbs may be further increased by a miniaturization of the antibody molecule. By linking the V_H and V_L sequences of such an antibody together on a single transcript, single chain antibodies (sFvs) can be produced. Because of their small size single-chain antibodies are deemed to penetrate more rapidly into tissues and interstitial spaces [43]. Single-chain antibodies can be easily engineered and produced in bacteria. A variety of effector moieties such as toxins, cytotoxic drugs, growth factors, functional receptor domains, and cytokines as well as Ig Fc regions can be fused to these mini-antibodies.

The type of linker used to couple the antigen-binding domains to effector domains is also of critical importance.

By coupling an anti-tumor antibody to doxirubicin using a linker that is stable in plasma but acid labile and, therefore, set free after internalization in lysosomes, Trail *et al.* [44•] were able to dramatically increase the effectiveness of the immunoconjugate.

Unmodified antibodies, which may be much more reasonable agents for treatment of minimal residual disease, rely on the various natural effector mechanisms of the host and the manifold ways they may interfere with cell function (Table 4). These functional characteristics of antibodies are generally determined by their Fc receptors, which can also now be exchanged at will. Several of the antibodies used in clinical trials appear to work by activating human complement. Activation of complement *in vivo* has been observed to occur in patients treated with a IgG_{2a} antibody directed against the ganglioside GD2 [45•]; this was shown by a decrease in C4, C3c and C3a during treatment. In addition, an IgG_3 antibody directed against the Lewis Y carbohydrate epitope, which has been shown to be very effective in the activation of human complement *in vitro* [46], has recently been shown to result in a reduction or eradication of antigen positive tumor cells in the bone marrow of five out of seven patients treated for two weeks with 6 × 100 mg antibody (G Schlimok, H Loibner, I Fackler-Schwalbe, K Pantel, G Riethmüller, unpublished data). The efficiency of the complement cascade may now be further increased by blocking the membrane proteins CD59, C8bp and decay accelerating factor that control the activity of homologous complement components [47].

Another important anti-tumor effect of unmodified antibodies is antibody dependent cellular cytotoxicity, which is mediated by various effector cells including neutrophils [48]. A recent study of 17-1A mAb in patients with advanced colorectal carcinoma suggests that cellular effector functions can be enhanced by the additional administration of cytokines such as granulocyte-macrophage colony-stimulating factor (GM-CSF) [49•]. Cellular effector functions may also be directed to the target by antibody-coupled cytokines [50] or by bi-specific antibodies designed to activate and orient cytotoxic cells to the tumor [51,52•].

Antibodies recognizing particular epitopes on functionally important cell surface molecules may also be effective in tumor therapy, even without the engagement of conventional host effector mechanisms. For example, antibodies directed to the Fas antigen have been shown to induce apoptotic cell death [53,54], and certain antibodies directed against the Her-2/neu cell surface receptor can induce differentiation of the tumor cells, which results in decreased growth rate both *in vitro* and *in vivo* [55•]. Antibodies against the Ig idiotype of B-cell lymphomas have been used in some of the most successful clinical trials [6••,8••]. Such antibodies can also induce regulatory changes in experimental B-cell lymphoma such that aggressive growth is abrogated and the tumor cells revert to a non-cycling dormant state [56•].

Passive antibody therapy versus active immunization strategies

A notable consequence of the general disappointment with antibody-based strategies is the recent surge of interest in active immunotherapy of cancer [57–60]. The identification and cloning of tumor associated cell surface antigens as well as of peptides recognized by MHC restricted T lymphocytes open up the possibility of specifically immunizing patients against defined antigens. In addition, vaccination with genetically engineered tumor cells that are transduced with lymphokine genes has yielded impressive results in transplanted tumor models [60], and recently a protective vaccination against B-cell lymphomas was shown to be improved when the lymphoma-specific Ig was fused with GM-GSF [61].

Active immunization, however, relies on an intact immune system and this is often compromised in advanced stages of cancer. Even more importantly, it requires that the target cell maintain MHC expression, proper antigen processing capability and the expression of any of a variety of additional accessory molecules. However, loss or downregulation of such molecules is a common trait of human tumors. Most spontaneous human tumors and the micrometastatic cells found in minimal residual disease have lost expression of one or more MHC class I products [62,63]. Interestingly, this may even be an early event in some tumors as it is observed in about half of benign colorectal adenomas [64••]. Human tumor cells defective in peptide processing and transport have also been identified [65•] and several studies now suggest that the expression of co-stimulatory molecules, such as B7, by the tumor cells may also be necessary for induction of immunity [66]. Clearly then, for active immunization to be effective, not only must the patient's immune system be more or less intact, but the tumor cells themselves need to express an entire array of gene products. These manifold and complex requirements for successful vaccination stand in stark contrast with passive antibody therapy, the only demand of which is that the tumor cells continue to express the target antigen.

Outlook or "Jester do oft prove prophets" (King Lear)

As long as the focus of current research is centered on the design of ever-new antibody constructs, employing the whole armamentarium of synthetic biology, and not centered on the judicious selection of more appropriate clinical targets and carefully designed therapeutic trials, one can foresee that another decade will be spent on 'misguided missiles' [8••] directed towards unassailable targets. For the adjuvant therapy situation, i.e. for the treatment of hidden metastatic cells, unmodified antibodies or antibody derivates that rely on natural effector mechanisms offer clear advantages over immunotoxins, because the intended cytotoxic reaction will be restricted to the target site where the antibody has bound. Moreover, cellular and humoral components of effector sys-

tems may be decreased or even absent in normal tissues such as simple epithelia shielded by a dense basement membrane. Thus, despite extensive crossreactivity between benign and malignant tissue, an operational specificity may be achieved *in vivo* even with a broadly cross-reacting anti-epithelial antibody. In addition, the use of such differentiation antigens with their more homogenous expression on cancer cells may circumvent the formidable problem of antigenic heterogeneity so often encountered with more restricted or tumor-specific antigens.

Humanization of rodent antibodies as well as generation of human mAbs from recombinant libraries will without doubt allow the best adaptation of therapeutic antibodies to the natural effector mechanisms. A major drawback of the naked antibody scenario is that it looks too simple and, therefore, runs against the current fashion for sophisticated immunoconjugates. Irrespective of the type of applied immunotherapeutics, the emphasis towards minimal residual cancer will require that the diagnosis of micrometastatic cells is refined. In the arduous area of adjuvant therapies, the pace of progress in immunological as well as chemical cancer treatment will critically depend on the availability of surrogate markers that allow a quick and reliable assessment of the particular therapeutic manoeuvre. The immunocytochemical diagnosis of micrometastatic epithelial cells in bone marrow of patients with various cancers is slowly gaining ground in the clinic [17]. Although the demonstration of their prognostic significance does not prove that they are the actual progenitors of later arising metastases, they clearly provide evidence for the disseminative capability of an individual tumor. Furthermore, it has been suggested by Schlimok *et al.* [17] that the elimination of such cells might give valuable information on the cytoreductive efficacy of a particular antibody. The further establishment of micrometastatic cells in bone marrow as surrogate targets can be envisaged as a crucial step towards a more rational design of immunotherapies of minimal residual disease. As long as primary prevention of cancer will remain an utopic goal the secondary prevention of metastatic disease by immunological means is a worthwhile and realistic option.

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