Monoclonal Antibody Therapy for Resected Dukes' C Colorectal Cancer: Seven-Year Outcome of a Multicenter Randomized Trial

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Purpose: **As previously shown, antibody treatment increased survival of patients with resected colorectal cancer of stage Dukes' C. Since the 5-year analysis was criticized because of the wide range (2.7 to 7.5 years) of follow-up time, we performed a 7-year analysis with only four of 189 patients monitored for less than 5 years.**

Patients and Methods: **A total of 189 patients with resected Dukes' C colorectal cancer were randomly allocated to infusions of a total of 900 mg 17-1A antibody, 500 mg postoperatively followed by 4 monthly doses of 100 mg (n = 99), or to observation only (n = 90). Primary end points were overall survival and disease-free interval. Patients were stratified by a dynamic randomization according to center, sex, location of tumor, number of affected lymph nodes, and preoperative carcinoembryonic antigen concentration.**

Results: **Randomization produced balanced distribution of risk factors. After 7 years of follow-up evaluation, treatment had reduced overall mortality by 32% (Cox's proportional hazard, $P < .01$; log-rank, $P = .01$) and decreased the recurrence rate by 23% (Cox's proportional hazard, $P < .04$; log-rank, $P = .07$). The intention-to-treat analysis gave a significant effect for overall survival (Cox's proportional hazard, $P < .01$; log-rank, $P = .02$) and disease-free survival (Cox's proportional hazard, $P = .02$; log-rank, $P = .11$). While distant metastases were significantly reduced (Cox's proportional hazard, $P = .004$; log-rank, $P = .004$), local relapses were not (Cox's proportional hazard, $P = .65$; log-rank, $P = .52$). This differential effect of 17-1A antibody on disseminated isolated tumor cells versus occult local satellites may explain the increased significance seen in the overall survival.**

Conclusion: **The now-matured study shows that 17-1A antibody administered after surgery prevents the development of distant metastasis in approximately one third of patients. The therapeutic effect is maintained after 7 years of follow-up evaluation.**

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THE CURRENT PESSIMISTIC VIEW of unconjugated monoclonal antibodies as cancer therapeutics has been largely influenced by the negative outcome of multiple clinical trials initiated shortly after introduction of the hybridoma technology. Thus, it comes as no surprise that recent reports on some successes obtained with antibody therapy in lymphoma, breast, and colorectal carcinoma were met with great reserve. Wondering what the characteristic features of these trials were, one notes that two of these trials, one in lymphoma[1] and one in breast carcinoma,[2] had been performed with engineered antibodies, ie, either human/murine chimeric or human complementarily defining regious (CDR)-grafted immunoglobulins, respectively, while the third stands out because of the target and end point used. In contrast to other trials aimed at patients with larger metastatic tumor masses and using regression or shrinkage as the end point, this one on colorectal cancer of stage Dukes' C required microscopically complete resection of the primary tumor for patients to be admitted.[3] As the primary end point, overall-survival was determined at 5 years.

Here, we report on the 7-year follow-up study of this prospective randomized two-arm trial in minimal residual disease that used the murine 17-1A antibody,[4] which recognizes a 34-kd glycoprotein of the cell membrane of epithelial cells.[5] The 5-year analysis had shown that a short, albeit intensive, postoperative course of antibody treatment (900 mg total dose) significantly reduced the appearance of distant metastases, but did not affect the rate of local relapses. However, local metastases may not be as innocuous as generally thought. As recently shown for breast carcinoma, without regional radiation therapy, occult local metastases can give rise to distant metastases after many

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years and thus affect long-term survival.[6,7] Therefore, with no effect of antibody therapy on the local relapse at 5 years, it was of interest to know how the overall survival evolved at the 7-year follow-up evaluation. This analysis also invalidated objections concerned about the maturity of the reported 5-year follow-up study. Now, after a median observation time of 7 years, with only four of 185 patients monitored for less than 5 years, the study has matured and continues to demonstrate a significant benefit with regard to survival.

PATIENTS AND METHODS

Patient Selection and Surgery

The multicenter study consisted of six academic centers in former West Germany A total of 189 patients were randomized, with the first patient entered in May 1985 and the last in April 1990 The study was closed in December 1992, and we present here the final status with median follow-up data of 7 years Eligible patients were required to have histologically confirmed adenocarcinoma of colon or the rectum with spread to regional lymph nodes (Dukes' C or International Union Against Cancer [UICC] stage III) In addition, histopathologic confirmation of curative resection (R0) was secured Radical tumor resection was performed by a standardized technique agreed upon by all participating centers When located within 15 cm of the anal verge, a tumor was classified as rectal carcinoma Patients were less than 70 years old and had a Karnofsky index greater than 50% Eleven of 99 patients randomized to treatment refused their treatment assignment, but were otherwise considered eligible for the study. According to Zelen,[8] these patients were included in the treatment group for final analysis, although they had not received antibodies

Stratification and Randomization

After confirmation of histology and R0 resection of lymph node metastasis, patients were randomized into two groups one received 17-1A treatment, whereas the other served as observation controls. All patients were stratified according to Zelen[8] by the following factors participating center, sex, location of tumor, stage (Dukes' C1 or C2 equivalent to pN1 or pN2-3), number of affected lymph nodes, and carcinoembryonic antigen (CEA) level before surgery (Table 1) In the treated arm, more patients had pT2 and pT3 tumors, and fewer had pT4 tumors However, treated patients appeared to have had higher numbers of involved lymph nodes (pN2) and tumors of less differentiated histology (grade 3). However, a Fishers' exact test, comparing the distribution of patients according to all prognostic variables, showed no significant differences Randomization before consent was performed and evaluated according to Zelen [9] On approval by legal experts in 1985, this procedure was accepted by the ethical review board Statistical analyses were performed according to SAS (Statistical Analysis System, Cary, NC)

To test for potential selection bias in the study, a retrospective analysis was performed at Medizinische Hochschule Hannover on 67 Dukes' C colorectal cancer patients selected from 107 patients seen between 1980 and 1985 They fulfilled the entry criteria for the 17-1A clinical trial and were monitored for at least 5 years each The survival analysis was performed by the Institut fur Medizinische Informatik und Biomathematik, Universitat Essen The overall survival according to Kaplan-Meier curve is nearly identical to the control group of the 17-1A trial (log-rank, $P = 97$), thus, this historical control does not show any selection bias

Table 1. Clinical and Pathologic Characteristics of Eligible Patients

Variable	17-1A		Observation		Total
	No	%	No	%	No
Age, years					
< 61	50	52	47	48	97
≥ 61	40	58	29	42	69
Sex					
Male	45	55	37	45	82
Female	45	54	39	46	84
Location of primary tumor					
Right colon	20	59	14	41	34
Left colon	33	53	29	47	62
Rectum	37	53	33	47	70
Depth of invasion					
T1-T2	16	64	9	36	25
T3-T4	74	52	67	48	141
Nodal involvement					
N1	61	54	52	46	113
N2-3	29	55	24	45	53
Histologic differentiation*					
Grade 1	6	60	4	40	10
Grade 2	55	50	55	50	110
Grade 3	21	64	12	36	33
Preoperative CEA (ng/mL)*					
≤ 5	54	53	48	47	102
> 5	27	53	24	47	51

*Data missing on 13 patients

The first 5-year analysis of the study performed by the Essen biometrical center was confirmed by two independent external audits, both of which relied on examination of original representative record samples

Protocol Management

Protocol management has been described in detail previously.[3] The study performance and protocol adherence was overseen by the trial's biometrical center located at the University of Essen. In brief, patients were staged after surgery and had a chest radiogram and abdominal ultrasound or computed tomography scan Patients in both groups were monitored in exactly the same way, with 14 prospectively defined follow-up visits, first on a quarterly and later on a half-year basis Follow-up evaluation continued beyond 5 years at yearly time points, but without formal protocol requirements.

For the end point analysis, standardized and objectively verifiable dates were used for all patients, ie, time point of surgery up to death of all cause or date of last contact alive for overall survival analysis In a separate analysis, cancer-related mortality was assessed, ie, death without recurrence was not counted as an event.

A documented histologic or radiologic diagnosis of disease was required to confirm local or distant recurrence, whereas abnormal CEA values were not used as evidence of relapse Recurrence was defined as time from date of surgery to date of first objective evidence of an abnormal diagnostic finding

Antibody and Antigen

The 17-1A antibody, a murine monoclonal immunoglobulin IgG2a antibody, originally described by Herlyn et al,[4,10] has a distinct antitumor effect on human xenografted tumors in a nude mouse model Clinical-grade antibody was purchased from the Wistar Institute

(Philadelphia, PA), with support by the German Cancer Aid (Bonn, Germany) During the last phase of the study, 17-1A antibody was provided by Centocor (Leiden, the Netherlands) Recent data on cell lines transfected with 17-1A cDNA suggest that the antigen is involved in cell-cell adhesion Therefore, it is now named epithelial cell adhesion molecule (EpCAM) [11]

RESULTS

Patients and Causes of Ineligibility

Patient characteristics and distribution of risk factors for both groups were well balanced (Table 1) [3] A total of 189 patients were randomized between May 1985 and April 1990. Four patients, two in each group (2.1%), were lost to follow-up evaluation from the start and have not been included in the trial. It could be verified that neither of the two patients randomized to treatment had actually received the antibody. Some patients have now been monitored for 10 years and only five patients were observed for a period of less than 5 years, with a range of 3.5 to 4.5 years. The presented 7-year follow-up data may therefore be considered mature and final. A final status of 185 patients could be obtained for this analysis. Nineteen patients (10.3%) were ineligible: seven in the treatment arm and 12 in the observation arm. The main cause of ineligibility (12 cases) was erroneous staging. Thus, eight patients had Dukes' B, four had Dukes' D, three were older than 70 years, one presented with polyposis coli, and one patient had residual tumor (R2) after surgery. One patient had a competing neoplasm and one patient received postoperative radiochemotherapy. Since ineligibility was not biased by treatment assignment, these patients were excluded from further analysis. However, eleven patients who refused their treatment assignment were considered eligible and were included in all statistical analyses as participants in the treatment group.

Survival

After 7 years, a total of 87 deaths were observed in 166 eligible patients. Since the 5-year follow-up evaluation, four additional deaths occurred in the treated group and nine additional deaths in the control group. The death rate of the observed group was 63% (48 of 76; 95% CI, range, 48 to 78) of patients and that of the treated group was 43% (39 of 90; 95% CI, range, 31 to 58). Thus, treatment with antibody led to a relative reduction of mortality by 32% (hazards ratio, 0.57; 95% confidence interval, 8% to 51%). The Kaplan-Meier survival curves (Fig 1A) showed a significant benefit for patients who received antibody over those who did not receive treatment ($P < .01$ with Cox's proportional hazard model corrected for influence of prognostic variables, and $P = .01$ with log-rank test for the univariate analysis). Also, the intention-to-treat analysis (Fig 1B) on all 185 patients showed a significant overall benefit for the treated group ($P < .01$ with Cox's multivariate analysis, and $P = .02$ with log-rank test for the univariate analysis).

Among all deaths recorded, six patients died without recurrence or secondary colorectal cancer and one observed patient of the control group died of secondary colon carcinoma, which was not counted as a recurrence. When cancer-related mortality was assessed, ie, death without recurrence was not counted as event, the benefit of treatment remained significant at $P < .01$ by Cox's multivariate analysis and $P = .01$ by log-rank test for the univariate analysis. The median survival time before death after recurrence was 1.4 years for patients randomized to treatment with 17-1A and 1.2 years for patients in the observed group.

Recurrence

According to the disease-free interval analysis, tumor recurred in 96 of 166 eligible patients after 7 years of

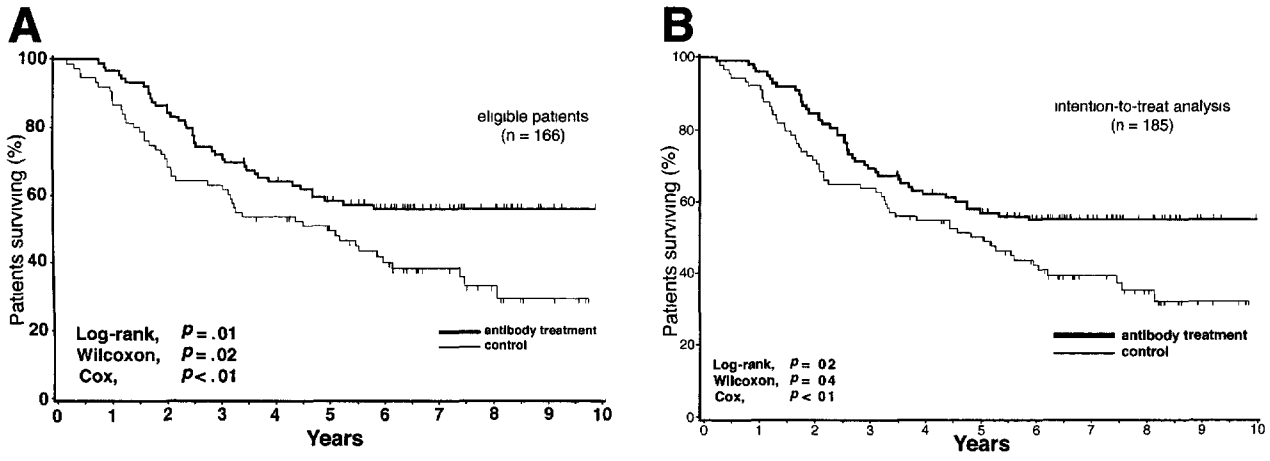

Fig 1. Overall survival of (A) eligible patients after 7 years of follow-up, and (B) according to intention-to-treat analysis. *P* is adjusted for imbalances in prognostic values with Cox's proportional hazards model; the other *P* values were calculated with the univariate log-rank test and Wilcoxon test.

follow-up evaluation; thus, three more recurrences were recorded in the treatment group and one more in the observation arm. The calculated recurrence rate was 68% (49 of 76; 95% CI, range, 53 to 82) in the control group and 52% (47 of 90; 95% CI, range, 39 to 67) in the 17-1A group. The reduction in recurrence was 23% (hazards ratio, 0.66; 95% confidence interval, 1% to 43%) Recurrence-free intervals were plotted according to Kaplan-Maier (Fig 2). Antibody treatment increased time to recurrence ($P = .04$ with Cox proportional hazards model corrected for influence of prognostic variables, and $P = .07$ with log-rank test for univariate analysis). In this analysis, data on patients who died without recurrence were censored. However, when death without recurrence was considered an event (recurrence-free survival), again a significant treatment advantage with 17-1A over control was obtained ($P = .03$ with Cox multivariate, and $P = .05$ with log-rank test for the univariate analysis). When the analyses of recurrence-free interval and recurrence-free survival were performed including all randomized patients, ie, according to intention-to-treat, 17-1A treatment again led to significant advantage over the untreated group (Cox multivariate, $P = .02$, log-rank, $P = .01$, respectively). In summary, 46% of patients (41 of 90) in the treatment group are at risk at 7 years and only 29% of patients (22 of 76) in the observed group are alive without recurrence.

Pattern of Recurrence

As most of the relapse events occur in the first 2 years after resection, it is not surprising that the pattern of relapses did not show gross changes during the sixth and seventh years. When the two groups were compared with regard to the site of first recurrence, the 7-year data similar to the 5-year analysis show that significantly fewer distant recurrences occur in patients treated with the antibody than in the control arm (Fig 3). Therefore, the proposition is holding up

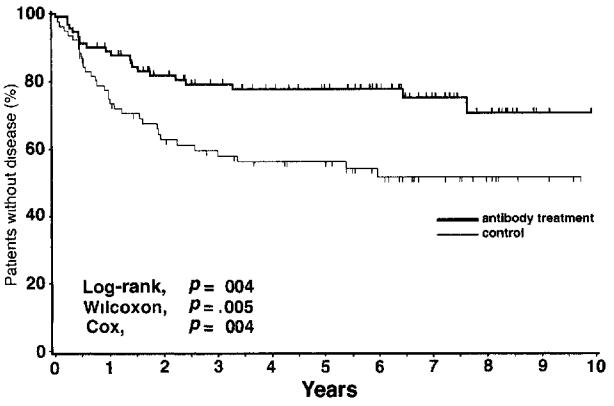

Fig 3. Kaplan-Meier analysis of distant metastatic recurrences as first event in eligible patients. Statistics were applied as in Fig 1.

that treatment with 17-1A may preferentially act on isolated tumor cells and thus prevent outgrowth of distant metastases However, local relapses that occurred as the first sign of progressive disease (Fig 4) were not reduced ($P = .86$ with Cox multivariate analysis). As shown in Table 2, there is an excess of local recurrences in the treatment group, which may partially be explained by the longer survival of treated patients. Another reason for this difference may be local radiation applied to rectum carcinoma patients in the control group, as detailed further later.

Toxicity

The rather mild toxicity of 17-1A antibody has been described in detail in the 5-year report. Since only acute and no chronic adverse effects were seen, the toxicity profile was not changed during the last observation period. There were no late treatment-related fatalities or life-threatening conditions, or any evidence for chronic drug-related side effects.

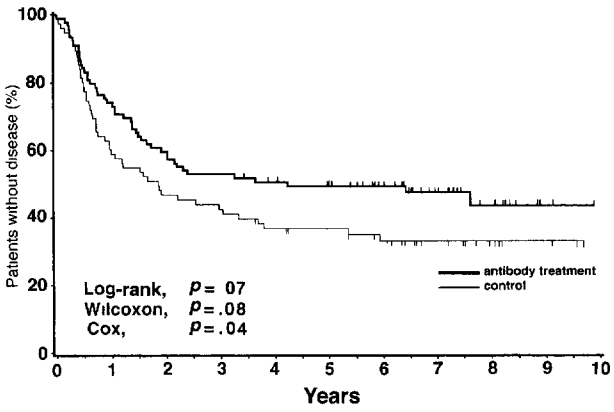

Fig 2. Recurrence-free survival after 7 years of follow-up (eligible patients). Statistics were applied as in Fig 1.

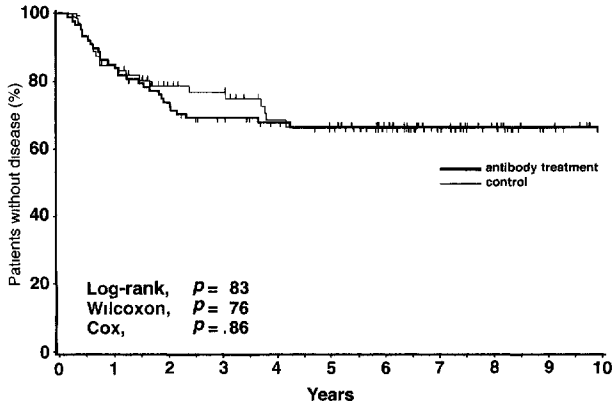

Fig 4. Kaplan-Meier analysis of local recurrences in eligible patients. Statistics were applied as in Fig 1.

Table 2. Pattern of Recurrences

Location of Recurrences (first events)	Treated	Observed
Liver	16	22
Lung	6	11
Abdominal*	6	7
Other distant sites†	1	3
Local recurrences	26	16

*Including lymph node and omentum
†Including brain and skeleton

Immune Response to 17-1A Antibody

When tested for antibody response against murine antibody (HAMA), 80% of treated patients developed a distinct HAMA response after the second or third infusion. Patients who developed recurrences and those who remained tumor-free did not show a difference in antibody titers. The kinetics of the HAMA response in the two subgroups have been described in the 5-year report. In brief, after the first two infusions, antibody titers were low and reached their maximum only after a fifth infusion at 18 to 20 weeks after surgery, but remained detectable for 2 more years. As will be reported elsewhere, the serum levels of antiidiotypic antibodies (Ab2) showed no significant differences between the analyzed patients who remained tumor free (n = 30) or experienced a relapse (n = 30).

DISCUSSION

As the 7-year median follow-up data confirm the previously reported 5-year findings, the conclusion seems warranted that antibody treatment improves the chances of curatively operated patients. In the univariate and multivariate analyses, the *P* values for overall survival are statistically significant for the eligible as well as intention-to-treat cohort. Also, the hazards ratios of the 7-year analysis support the 5-year data. Similarly, the rates of reduction in mortality and recurrence, 32% and 23%, respectively, are in alignment with the 5-year results. The conclusion seems warranted that some of the treated patients, who have not yet recurred, may indeed be cured. The conspicuous inefficacy of the antibody on local recurrences (Fig 4) may account for the smaller *P* values in the disease-free-interval analysis.

Local recurrences may not affect the overall survival curve to the same degree as distant metastases do (Fig 3); therefore, survival at 7 years appears to be superior to the disease-free interval. Also, the less significant univariate analyses may well be explained by the large treatment effect, which we expected at the onset of the trial in 1985, when we hoped for an absolute reduction of events from 50% to 30%, ie, a 40% relative reduction. The observed reduction of approximately 30% is clearly lower than expected, but still in the order of that obtained by Moertel et al[12] and Krook et al[13] after a similar follow-up period.

In the antibody trial, both colon and rectum cancer patients were enrolled onto the trial presented here. Radiation therapy now accepted as efficient to reduce local recurrences was not part of our clinical protocol, but was allowed for patients in the control group. Thus, irradiation was applied to 11 patients of this group (two received preoperative, four preoperative radiation and postoperative radiochemotherapy, and five postoperative radiation only). Since these forms of therapy were administered only to the control group, a bias may have been introduced that influenced the outcome against the antibody treatment, which explains the apparent difference seen in the local recurrence rate (Table 2).

It is noteworthy that in various reports, results recorded after a 5-year observation period do not necessarily hold up when patients are monitored for an additional time of 2 years. For example, the National Surgical Adjuvant Breast and Bowel Project (NSABP) conducted a study from 1977 to 1983 to compare a combination regimen that consisted of semustine, folic acid, and fluorouracil with bacillus Calmette-Guérin. While after 5 years of follow-up evaluation, a significant increase in disease-free and overall survival of patients was evident,[14] after 2 further years, the overall survival did not show significance. These findings were confirmed by another cooperative group.[15]

The pattern of first recurrences beyond 5 postoperative years shows that patients in each group still recur, with manifest distant metastasis as first sign of a relapse. This finding lends some support to the concept of long-lasting dormancy in early disseminated tumor cells. Indeed, nuclear proliferation markers such as Ki-67 or p120 nucleolar antigen were rarely detected on micrometastatic cells disseminated to bone marrow.[16]

Patients in the control group continued to die of progressive disease that probably had arisen from occult distant metastatic cells that were eliminated by the antibody in the treatment group. However, this argument rests on the assumption that the antibody exerts its antitumor effect by mobilization of direct effector mechanisms that are active only as long as the antibody is bound to the target.

Interestingly, in the most recent report (median follow-up time, 6.5 years), adjuvant therapy with fluorouracil plus levamisol administered to patients with Dukes' C colon cancer had only a minimal effect on local recurrences.[12] In that trial, only colon cancer patients were treated who were known to have fewer local relapses than rectum cancer patients and were included in our trial. Therefore, the less pronounced chemotherapeutic effect on local relapses may be due to the difference in patient populations.

The dose of antibody chosen deserves a special comment. In the planning stage of the trial, we were afraid of a strong anaphylactic response to repeated administration of murine immunoglobulin. We therefore resorted to a large, first intravenous dose that consisted of 500 mg highly soluble monomeric immunoglobulin G2a to induce tolerance to mouse immunoglobulin G. This strategy, originally proposed by Sears et al,[17] was only partially successful, as only about 20% of the recipients did not respond to mouse immunoglobulin, while the others exhibited a distinct, albeit slow rise of HAMA titers. Nevertheless, the high dose may have been critical to obtain a steep transvascular concentration gradient required for tissue penetration. Similar high doses were used in the lymphoma trial cited earlier.[1]

In view of the still viable hypothesis that antibody-induced tumor regression is brought about by antiidiotype-mediated immunization, we analyzed retrospectively HAMA positive sera. Antiidiotype antibodies were detected in most HAMA-positive samples. However, patients with and without manifest metastatic relapses did not differ in antiidiotype titers.[18] To detect ab3 antibodies, we applied a sensitive enzyme-linked immunoadsorbent assay (ELISA), which allowed us to determine human 17-1A autoantibody. Thus far, this assay has been negative. However, it may be insufficient to determine only a humoral antiidiotype response. Indeed, recent data presented by Fagerberg et al[19] indicate that also antiidiotype-specific T cells are induced by treatment with 17-1A antibody Thus far, in murine systems, breaking of tolerance against autoantigens via the antiidiotype route has not yet been shown in an unambiguous fashion

Why only one third of the patients at risk have responded to the antibody treatment may be explained by variations in accessibility, vulnerability, and/or antigen expression in individual disseminated tumor cells. Immunohistochemical staining of the primary tumor had shown that the antigen is expressed by most, but not all cells within an individual tumor.[5] By double staining of individual metastatic cells in bone marrow, it was shown that the 17-1A target is expressed by disseminated cells in only two thirds of the examined patients.[20] It is safe to assume that antigenic heterogeneity, a likely consequence of the marked genomic instability of human epithelial tumors, will be a major obstacle for single-antibody treatments, as well as for any other form of monotherapy.

Therefore, in anticipation of antigenic heterogeneity, a combination therapy that consists of antibodies of different specificities may forestall a selection of antigen-negative cells exerted inevitably by a one-antibody approach. Furthermore, humanized antibodies or entirely human antibodies generated from human immunoglobulin gene libraries that are less immunogenic than murine antibodies are now being tested. They do not only possess extended serum half-lives, but also harness human effector mechanisms much more efficiently than murine immunoglobulins can do. On the other hand, the peculiar immunogenicity of the murine antibody may be important for the recruitment of human T cells as mediators of an indirect antitumor effect. Direct tumoricidal effects are good candidates for the still controversial reaction mechanism of unconjugated antibodies. This view is supported by the immediate gastrointestinal toxicity, such as abdominal cramps, nausea, and transient diarrhea, observed in a substantial proportion of patients shortly after infusion of the antibody.[3] These reactions are best explained by cytotoxic effects of the antibody on normal epithelial cells that bear the 17-1A antigen to a similar degree as tumor cells.[5] Although we still think that normal epithelial cells of the intestinal mucosa are less accessible for immunoglobulin G and its effector mechanisms than for locally produced immunoglobulin A. The discovery of the adhesion function of the 17-1A molecule,[11] hence the new name EpCAM, raised the speculation that the antibody inhibited outgrowth of metastasis by interference with cell-cell adhesion. In other studies that also targeted a monoclonal antibody to an organ-specific, rather than a tumor-specific antigen, the tumor-specific biologic action induced by the applied antibody had been ascribed to the faster elimination of the bound antibody from normal than from malignant cells.[21]

For the immediate future, adding antibody to chemotherapy seems logical to attack both dormant, as well as proliferating, metastatic tumor cells. Two major trials using the 17-1A antibody with the same dose and similar regimen are currently under way to test this question in colon cancer patients. One of these trials, performed in Europe and Israel, includes a confirmatory arm with antibody as single treatment, with the other two arms applying chemotherapy or chemotherapy plus antibody, respectively. A pilot trial had shown that fluorouracil with folic acid overlapping with the three last infusions of the antibody regimen did increase overall toxicity.

In conclusion, the presented results still support the original hypothesis that minimal residual disease occurring so frequently in patients with solid tumors appears to be an indication for antibody therapy.

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APPENDIX

The following are contributors to the German Cancer Aid 17-1A Study Group Friedrich-W Eigler, Chirurgische Universitatsklinik Essen, Essen, Irene Fackler-Schwalbe, Klinikum Augsburg, Augsburg, Carl Gottfried Schmidt, Tumorklinik, Universitat Essen, Hans Schreiber, Chirurgische Universitatsklinik Eppendorf, Hamburg, and Leonhard Schweiberer and Bernolf Eibl-Eibesfeldt, Chirurgische Universitatsklinik Munchen, Germany

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