Newcastle Disease Virus-infected Intact Autologous Tumor Cell Vaccine for Adjuvant Active Specific Immunotherapy of Resected Colorectal Carcinoma

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ABSTRACT
An active specific immunization (ASI) procedure with two types of autologous tumor cell vaccines (ATVs) is tested for adjuvant immunotherapy of resected colorectal carcinoma to provide preliminary information on local immunological skin responses, side effects, and 2-year survival rates. For vaccine preparation, the tumor-derived freshly isolated and cryopreserved cells were thawed, purified by Percoll density centrifugation, and depleted of tumor-infiltrating lymphocytes by immunomagnetic beads. After inactivation by 200 Gy, the cells of this ATV were either infected by Newcastle disease virus (NDV) or they were admixed with *Bacillus Calmette Guérin* (BCG) organisms. Vaccination was performed in the arm beginning 6–8 weeks after operation, three times at 2-week intervals.

Of 57 patients that received ASI, 48 were treated by virus-infected ATV (ATV-NDV) and 9 were treated with the BCG-admixed vaccine (ATV/BCG). The mean value of delayed hypersensitivity skin reactions from ATV-NDV-treated patients was 18 mm for the first vaccination and 26 and 29 mm for the succeeding ones. Although the application of ATV-NDV was associated with only mild side effects, the ATV/BCG vaccine led to long-lasting ulcers and to more serious side effects. The 2-year survival rate obtained with ATV-NDV was 97.9%, whereas the survival rate with ATV/BCG was 66.7%. The mean survival of 661 patients from a historical control was 73.8%.

These data suggest that the type and quality of the tumor vaccine for ASI treatment is important. The findings with ATV-NDV necessitate corroboration in a prospective, randomized controlled study.

INTRODUCTION
The prognosis of colorectal carcinoma depends on its histological grade and on the stage of disease. The postoperative 5-year survival rates of UICC stage I (Dukes' A) is 90%, stage II (Dukes' B) is 60–80%, stage III (Dukes' C) is 30–60%, and stage IV (Dukes' D) is 5%. These values reflect the degree of systemic manifestation of the disease at the time of operation (1). Systemic treatment of colorectal carcinoma patients predominantly involves the use of drugs such as 5-FU<sup>4</sup> with or without its biochemical modulator leucovorin or combined with levamisol (2). Recently, combinations of 5-FU with cytokines as biological response modifiers such as IFN-α or interleukin 2 are being tested; however, in all of these studies toxicity is still a major problem.

Active immunotherapy aims at stimulating the patient's immune system to keep disseminating cancer cells and metastases under control as much as possible. One hundred years ago, Coley (3) reported on tumor regression following activation of the immune system by bacterial toxins, a finding which led to the discovery of tumor necrosis factor. The history of ASI, which involves the use of antigens from tumor cells, began in 1902 in Germany when von Leyden and Blumenthal (4) injected an oncolysate of autologous tumor cells into patients with incurable cancer. Since then many studies have shown an effect of ASI, but most of them were neither prospectively controlled nor randomized (reviewed in Ref. 5). In 1985, Hoover et al. (6) reported on early positive results of a prospectively randomized trial of adjuvant ASI for patients with colon cancer in Dukes' stage C. Positive clinical results were also described by us in 1982 from a Phase II trial of ASI with NDV-modified autologous tumor cells derived from resected liver metastasis in colorectal cancer (7). Forty percent of the ASI-treated colorectal cancer patients experienced an increased DTH reactivity against autologous tumor cells following vaccination, while only 17% or less showed an increased activity to Merieux test antigens, NDV, or autoantigens from normal liver cells. Recently (8), a 6.5-year median follow-up of a Phase III trial with 94 colorectal cancer patients treated postoperatively with an autologous tumor cell vaccine mixed with BCG revealed significant improvements in overall and disease-free survival for patients with colon carcinoma of Dukes' stages B2-C3 but not for treated rectal...
carcinoma patients. Also recently, a randomized trial of monoclonal antibody for adjuvant therapy of resected Dukes’ C colorectal carcinoma was reported to have resulted in prolongation of remission and life extension (9).

On this basis, a prospective clinical study with adjuvant ASI was started in January 1990 in the University Clinic of Mannheim in colorectal carcinoma patients with Dukes’ stages B2-C following R0 resection of the primary tumor. This study differs from the one of the University Clinic of Heidelberg (7) in which patients with established liver metastasis were treated with vaccines from the operated liver metastases. Although both studies involve the use of autologous NDV-infected tumor cells as vaccine, the study reported here uses vaccines from the primary tumor and an additional purification step to remove tumor-infiltrating lymphocytes from the tumor cell suspension by a magnetic bead procedure.

PATIENTS AND METHODS

Inclusion Criteria for Patients. Patients were included in this study if they had undergone a potentially curative R0 resection of the colon or rectum because of an adenocarcinoma histopathologically classified as T3 or T4 without positive lymph nodes or in case of positive lymph nodes irrespective of the tumor size and T staging. The patients were accrued for this study chronologically. The age limit was 75 years. All patients were operated on and treated in the Department of Surgery of the University Clinic of Mannheim. Patients with serious immune diseases and prior malignant diseases or treated by chemotherapy or radiation were excluded, and informed consent had to be given by the patients for inclusion.

Tumor Cell Dissociation. While a piece of the operated tumor was removed for pathological classification, the main tumor mass was placed in a tube with HBSS containing gentamicin and stored at 8°C. Within 8 h, the fresh tumor specimens were carried to the laboratory, where they were dissociated. For dissociation, the specimens were cut into small pieces (about 1 cm³) with a scalpel. Thereafter, they were incubated in an enzyme solution at 37°C. The combination of enzymes that was most effective in dissociating viable cells was a mixture of collagenase, DNase, and hyaluronidase as described (7). The resulting suspension was filtered through a nylon net with a pore size of 40 μm. The enzymatic digestion step was repeated with the rest of the tumor pieces until the main fraction of the specimen was dissolved. The resulting cell suspension was washed three times in HBSS.

Cryopreservation and Thawing. Tumors cells isolated in this manner were frozen in 10% human serum albumin and 10% DMSO and stored in aliquots of 10⁶ cells in liquid nitrogen. Cell freezing was performed in a freezing computer Kryo 10 Series II (Messer-Griesheim). On the day of the planned vaccination (6–8 weeks after operation), the cells were carefully thawed in warm medium with the addition of 10% human serum albumin and washed three times in this medium.

Purification Procedures. The thawed tumor cell suspensions normally consisted of varying numbers of tumor cells, leukocytes, erythrocytes, and cell debris. To separate viable tumor cells from cell debris, a Percoll density centrifugation step was included. Tumor infiltrating T and B lymphocytes were coated with anti-CD2 and anti-CD19 antilymphocyte monoclonal antibodies, respectively, and removed via magnetic beads (Dynabeads). In a preliminary study, the percentage of tumor-infiltrating lymphocytes could be reduced by Percoll density centrifugation alone by 76 ± 12%. After purification by magnetic beads, no lymphocytes could be detected. In between and after these purification steps, the cells were washed three times. Routinely, a sample of the vaccine was sent to the Institute of Microbiology, and drug resistance was determined when the cultures were positive.

Inactivation of the Tumor Cells, Infection by NDV, or Admixture of BCG. The tumor cells proliferative capacity was inactivated by irradiation with 200 Gy using a telecobalt source. Directly before vaccination, 10⁷ tumor cells were incubated with 250 hemagglutinating units of our standardized NDV nonvirulent, low pathogenic strain Ulster for 1 h at 37°C. Between January and June 1990, we also used 10⁷ live BCG units (BCG Trockenimpfstoff; Connaught, Fa. Medac) as adjuvant.

Cellular Yield and Quality of Vaccines. The mean tumor mass for vaccine preparation was 4.9 g. Of this mass, an average of 3.5 g could be dissociated. About 1.6 × 10⁷ viable tumor cells were obtained per g dissociated tumor mass. Only trypan blue unstained viable cells were counted. After controlled freezing, thawing, and purification, the vaccines contained an average of 2.7 × 10⁶ viable tumor cells per single vaccine.

Study Design, DTH Reactivity, and Follow-Up. All Dukes’ B and C colorectal carcinoma patients who fulfilled the inclusion criteria and whose tumor had enough cells for preparation of vaccine were included in the study. The tumor stage was classified according to the TNM system. If the tumor reached the serosa and or lymph nodes were positive, the necessity of an adjuvant therapy was discussed with each patient.

When informed consent to ASI was given, three vaccinations were given at 2-week intervals, beginning 6–8 weeks after the operation. The vaccines contained inactivated autologous tumor cells and NDV (n = 48) or BCG (n = 9). The vaccines never had both adjuvants together nor were cytokines added. The usual vaccination site was the skin of the forearm. Injections were done i.d. in a volume of 300 μl. DTH reactions consisted of an erythema with a palpable central induration. Two days after each vaccination, the DTH was measured by determining the maximum diameter of induration. The response was considered positive if a DTH >3 mm in diameter could be detected.

In the follow-up period, the patients underwent routine examinations every 3 months for the first year and in the next 4 years every 6 months. The routine checks consisted of a complete physical examination, blood tests including blood cell count, carcinoembryonic antigen, urea, creatinine, transaminases, and every 6 months chest X-ray, abdominal ultrasound, and colonoscopy or air-contrast barium enema. If metastases or relapses were diagnosed, further therapy was planned in an interdisciplinary discussion. The data of the first diagnosis of a relapse or of a metastasis were collected and statistically evaluated.
RESULTS

Characteristics of Patients Receiving ASI and of the Historical Control Group. Fifty-seven patients and tumors fulfilled all inclusion and quality criteria for ASI treatment, i.e., no macroscopic metastases, patients consent, good cellular yield, and good quality of the vaccine. Forty-eight patients were vaccinated with autologous tumor cells infected with NDV, and 9 patients had the vaccine admixed with BCG. No patient was lost during the follow-up. The key date for data evaluation was fulfilled all inclusion and quality criteria for ASI treatment, yield, and good quality of the vaccine. Forty-eight patients were enrolled in this Phase II ASI study are summarized in Table 1. The median follow-up period was 22 months, the minimum 6 months, and the maximum 43 months. All patients had received surgery between January 1, 1990 and February 28, 1993. In the ASI group, 39 tumors were located in the colon and 18 in the rectum. Thirty tumors were classified as Dukes’ B and 27 tumors as Dukes’ C. The tumors were graded as G1 (n = 2), G2 (n = 46), and G3 (n = 9).

A historical control group (Table 1) consisted of 661 patients who had surgery in the same clinic between January 1, 1985 and December 31, 1989 because of a cancer of the colorectum in Dukes’ stages B and C. The patients of the control group did not receive any adjuvant therapy. There was no significant difference between the ASI and the control group with regard to age or sex distribution, tumor grade, and stage. The 2-year survival rates did not change significantly during five successive periods of observation (1985-1989) in the historical control group (Table 2).

Side Effects of Vaccination. Erythema, swelling, and induration at the injection site (Fig. 1C) was seen in all patients except one. Eight patients responded with slightly increased temperatures, up to 38°C. One patient reported diarrhea 1 day after the vaccination. Five patients expressed tiredness on the evening of the vaccination day. In seven patients, we saw a generalized lymph node swelling 48 h after the first vaccination. Generally, the vaccination was well tolerated. In contrast to the mild side effects we saw with the NDV vaccine, the BCG vaccine led to more serious side effects. In all BCG-treated patients, long-lasting ulcers formed at the site of the injection (Fig. 1, A and B). In two patients, the abscesses had to be treated surgically. There were local lymph node swellings, and 6 of the 10 patients reported that they felt sick and fatigued.

DTH Skin Reactions to the Vaccine. In 31 patients, the skin reactions to the NDV vaccine were systematically recorded. Of these, 21 (68%) showed an increasing amount of DTH reactivity during the vaccination. In 8 (26%) patients, a skin reaction was seen, but no increase of the DTH could be observed during the three vaccinations. One patient showed no reaction at all, and one patient presented with a good DTH following the first vaccination, but showed a decreased DTH reactivity during the second and third vaccinations. The mean value of the recorded DTH reactions was 18 mm for the first vaccination and 26 and 29 mm for the succeeding ones. Fig. 1 shows typical skin reactions to the NDV vaccine and to the BCG vaccine, respectively.

Two-Year Survival Rates. The mean 2-year survival rates with SEs of colorectal carcinoma patients in the control group and in the ASI group are summarized in Table 2. In the control group, the 2-year survival rates at five different time intervals since 1985 were very similar and varied between 75 and 77% for patients with colon carcinoma and between 65 and 68% for patients with rectal carcinoma. No trend could be seen for any improvement within a time period of 5 years before the ASI therapy. For the ASI group, the data were evaluated separately for patients receiving the BCG vaccine (Figs. 2 and 3) and for those receiving NDV vaccine (Table 2; Figs. 2 and 3). For all ASI-treated patients, the overall survival rate was 93% and for the NDV vaccine group alone, 97.9%. The respective values for the patients with colon carcinoma and patients with rectal carcinoma (Table 2) treated with the NDV vaccine were 100% and 93%, respectively. All patients of the ASI group who died, died of recurrent disease. The site of recurrent disease of colon cancer was liver (one time), lung (one time), and lymph nodes and lung (one time). The site of recurrent disease of rectal cancer was liver (twice), liver and lung (one time), and pelvis (local recurrence) and liver (one time).

The Kaplan-Meier curves of overall survival rates for all patients are shown in Fig. 2. Without adjuvant treatment, there was a gradual decrease in survival with a nearly linear slope in the historical control group. The nine patients that were treated with the ATV/BCG vaccine did not seem to benefit from this treatment, and their 2-year survival was only 66.7%. In contrast, the survival of the 48 patients treated with the NDV vaccine was greater, 97%. The Kaplan-Meier estimates of the 2-year survival in the ATV-NDV group was 96.4 ± 3.5 for Dukes’ B and 95.0 ± 4.9 for Dukes’ C patients.

We also performed an analysis after stratification by stage. Fig. 3 shows the Kaplan-Meier 2-year survival curves of colorectal carcinoma patients with stage Dukes’ C only. Separate curves are shown for the historical control, BCG vaccine group, and NDV vaccine group. Although the NDV vaccine group showed 95% survival, the historical control had only 61.7% (33% less) and the BCG vaccine group only 57% (38% less) survival. The results obtained with the two vaccine types is likely to show an advantage for the patients who were vaccinated with ATV-NDV.

At the moment, no difference is seen between the prognosis of the NDV vaccine-treated patients with carcinoma of the
Table 2  Two-year survival rates at different intervals in the control group and ASI group

<table>
<thead>
<tr>
<th>Group</th>
<th>Year</th>
<th>n</th>
<th>Total&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Colon</th>
<th>Rectum</th>
</tr>
</thead>
<tbody>
<tr>
<td>I: Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>1985</td>
<td>141</td>
<td>71.8% ± 3.5</td>
<td>74.8% ± 3.2</td>
<td>66.1% ± 3.6</td>
</tr>
<tr>
<td>b</td>
<td>1986</td>
<td>135</td>
<td>69.9% ± 3.8</td>
<td>75.3% ± 3.1</td>
<td>65.2% ± 3.7</td>
</tr>
<tr>
<td>c</td>
<td>1987</td>
<td>122</td>
<td>74.1% ± 3.3</td>
<td>76.4% ± 3.0</td>
<td>67.4% ± 3.4</td>
</tr>
<tr>
<td>d</td>
<td>1988</td>
<td>138</td>
<td>74.4% ± 3.1</td>
<td>76.9% ± 3.1</td>
<td>64.5% ± 3.7</td>
</tr>
<tr>
<td>e</td>
<td>1989</td>
<td>125</td>
<td>72.9% ± 3.0</td>
<td>74.5% ± 3.0</td>
<td>68.3% ± 3.5</td>
</tr>
<tr>
<td>II: ASI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NDV vaccine</td>
<td>91-93</td>
<td>48</td>
<td>97.9% ± 2.8</td>
<td>100.0% ± 3.0</td>
<td>93.3% ± 6.4</td>
</tr>
</tbody>
</table>

<sup>a</sup> Percentage of colorectal carcinoma patients (Dukes’ B + C) surviving at 2 years after operation (mean ± SE) (I) or after operation and ASI treatment (II).

Fig. 1  Skin reactions of colorectal carcinoma patients to BCG-tumor cell vaccine (A and B) or NDV-tumor cell vaccine (C). A and B, patient B. B's reaction to first vaccination (A, March 27, 1990) and second vaccination (B, April 9, 1990). Photograph was taken on April 23, 1990; the ulcers can still be seen 4 weeks after first vaccination. C, patient E.L.'s reaction to second vaccination (March 4, 1991) 48 h later; diameter of induration, 19 mm; diameter of erythema, 36 mm.

rectum or colon. This contrasts with what was reported from the ATV/BCG vaccine study by Hoover et al. (6, 8).

The Kaplan-Meier estimates for 2-year relapse-free survival in the ATV-NDV group are summarized in Table 3. Because of the small number of patients and the relatively short period of follow-up, there is still a relatively high SEM.

DISCUSSION

Immunotherapy appears to be an old way of adjuvant cancer therapy. In spite of its early start in 1902 (4) and many reports of efficacy in individual patients, there has been no proof for its general effectivity in the form of large controlled studies. Meanwhile, the understanding of the cellular and molecular
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Fig. 2 Kaplan-Meier curves of the survival rates for all patients treated with ATV-NDV (n = 48) or ATV/BCG (n = 9) in comparison to the historical control group (n = 661). The patients of the control group did not receive any adjuvant therapy.

Table 3  Two-year relapse-free survival rates in the ATV-NDV-treated group

<table>
<thead>
<tr>
<th>Stage</th>
<th>Colon + rectum</th>
<th>Colon</th>
<th>Rectum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dukes' B + C</td>
<td>71.9 ± 9.5</td>
<td>79.9 ± 10.3</td>
<td>66.7 ± 12.2</td>
</tr>
<tr>
<td>Dukes' B</td>
<td>68.3 ± 12.8</td>
<td>78.8 ± 12.8</td>
<td>62.5 ± 17.1</td>
</tr>
<tr>
<td>Dukes' C</td>
<td>85.0 ± 8.0</td>
<td>92.3 ± 10.0</td>
<td>71.4 ± 38.2</td>
</tr>
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Fig. 3 Kaplan-Meier curves of the survival rates for patients with colorectal carcinoma stage Dukes' C only. Separate curves are shown for patients who were vaccinated with ATV-NDV (n = 20) or ATV/BCG (n = 7) and the historical control group (n = 315), whose patients did not receive any adjuvant therapy.

Theoretical basis of immunotherapy has increased considerably. The theoretical basis for active specific immunotherapy and some parameters of importance for effectivity have been worked out in two animal tumor models, the L10 hepatocarcinoma of the guinea pig (10) and the ESb lymphoma of the DBA/2 mouse (11-17). In the L10 hepatocarcinoma model, postoperative vaccination with syngeneic tumor cells mixed with BCG led to the elimination of lymph node metastases in about 45% of the animals. The effect of the therapy depended on the number of tumor cells used, the ratio of BCG organisms:tumor cells, and the viability of the tumor cells after controlled freezing, thawing, and inactivation by 100 Gy 7-irradiation. In the ESb mouse lymphoma model, postoperative vaccination with irradiated autologous tumor cells infected with NDV induced antitumor immunity and led to protection against micrometastases in approximately 50% of the treated animals (11, 12). The ASI-induced protective antitumor immunity was dependent on the activation of CTLs with specificity against a tumor-associated transplantation antigen (13). Tumor cell membrane integrity and viability were found to be important parameters, in particular for tumor-specific CTL activation by cancer vaccines (16).
cell lysates were able to elicit tumor-specific DTH reactivity but could not stimulate antitumor CTL reactivity (17). Infection of irradiated tumor stimulator cells by a low dose of an avirulent strain of NDV led to an augmented CTL response (18), which could be blocked by the addition of anti-IFN-α, β antibody (19). Furthermore, the NDV-derived HN molecule was shown to play an important role in this phenomenon. Using HN cDNA transfectants, it was demonstrated that the viral hemagglutinin augments peptide-specific CD8 T-cell responses in general (20). These findings suggested a role of HN in cell-cell interactions and in costimulation of specific T cells.

In animal tumor models and human tumors, tumor-reactive CTLs have been demonstrated to exist, and tumor-associated T-cell epitopes associated with distinct MHC class I molecules (e.g., HLA-A1, HLA-A2, and HLA-B5) have been molecularly cloned for human melanoma (21, 22). Peptide fractions have been isolated from human melanoma which can make suitable target cells sensitive to lysis by autologous tumor-specific T-cell lines (23). In the case of the tyrosinase gene product in melanoma cells, the recognition of their peptides as tumor antigens was restricted by HLA-A2 (24). Also, human CTL lines have been isolated with restricted specificity for squamous cell carcinoma of the head and neck (25). Tumor-infiltrating lymphocytes were reported to lyse human renal cell carcinoma in a HLA-A2-restricted mode (26). Adenocarcinomas of the pancreas, colon, breast, and ovary express epithelial cell mucin, encoded by the MUC-1 gene and mucin-specific T cells, have now been developed against mucin-transfected human fibroblast and B cells (27, 28). Also, HER2/neu-derived peptides were shown to be recognized by breast and ovarian cancer-specific CTLs (29). There are only few reports of T-cell recognition and immunotherapy of colorectal cancer, however (30). Hints that colorectal cancer could be immunogenic were found using leukocyte migration tests with peripheral blood leukocytes pulsed by extracts of different colorectal tumors (31). In 93% of these patients, the leukocytes displayed a positive migration reaction, irrespective of the tumor stage. In animal models, products of mutated oncogenes and tumor suppressor genes have been shown to be recognized by specific T cells. Mutated peptides derived from p21 ras were shown to be immunogenic (32, 33). Such mutations have been found in adenocarcinomas of the colon and rectum. The growth of a human colon cancer cell line expressing peptides derived from mutant p21 ras protein was inhibited by respective specific CD4+ T cells (33–35). This tumor inhibition was HLA class II restricted. Mutation of the p53 gene is the most commonly seen genetic change in human cancer (36). In an in vitro study, specific CTL responses were developed against a mutant p53 peptide presented by the HLA-A2.1 molecule (37). This could be a possible mechanism for many specific T-cell responses against human carcinomas.

In the last year, five antigens associated with human colon carcinoma cells were described that induce specific CD4+ T-cell responses following adjuvant vaccination with irradiated autologous tumor cells and BCG after resection of the primary tumor (38, 39). When we started with ASI we first used the protocol of Hoover et al. (6, 8) with the adjuvant BCG. However, the partly serious side effects led us to leave this concept. Use of the paramyxovirus NDV instead of BCG for vaccine modification appeared to have the following advantages (12):

1. The virus binds to the surface of the tumor cells via its HN glycoprotein which interacts with sialic acids on ubiquitous cell surface ganglioside receptors.
2. It replicates in infected tumor cells leading to an enhanced expression of viral antigens on tumor cell surfaces.
3. It induces the local production of the cytokines IFN-α, β as well as tumor necrosis factor α and stimulates the production of heat shock proteins, adrenocorticotrophic hormone, and tissue inhibitor of metalloproteases.
4. It has stimulating effects on T-helper cells, CTLs, natural killer cells, and macrophages.
5. It is well tolerated, and attenuated NDV strains used in humans showed antitumor activity (39–41) with hardly any pathology in treated patients.

Earlier studies with the avirulent NDV strain Ulster used here (42) showed a good compatibility of the vaccination for humans. No ulcers or abscesses were reported. The first clinical results were reported from patients following the resection of liver metastases (7). As was demonstrated in previous studies (42, 43) and also in this study, challenges with autologous tumor cells after vaccination with NDV-infected tumor cells showed an increase in the DTH response. This is interpreted as augmentation of systemic antitumor reactivity. The DTH response was not due to bacterial contamination nor to sensitivity to the virus.

This study for the first time applied a high-quality autologous live tumor cell vaccine. The quality was improved by Percoll density centrifugation to remove dead cells and cell debris and applying an immunomagnetic bead procedure to remove tumor-infiltrating lymphocytes. The purification was always associated with a loss of tumor cells and thus of total cell yield. At present, it cannot be decided whether the use of primary tumor cells offers an advantage over the use of metastatic tumor cells.

Shortly after starting this study, the results of adjuvant chemotherapy studies by Moertel et al. (2) and Krook et al. (44) were published. After preliminary analyses of our results, we decided to continue with the study. We informed the patients of the necessity and possibilities of adjuvant therapy and respected the patients' wishes. In comparison to the data of the study of Moertel et al. (2), our survival data show no disadvantage for the ASI group. The ASI treatment has clearly less side effects than the chemotherapy and also the combined radiochemotherapy.

During the accrual period of this study (January 1990 until February 1993), only 15 colon carcinoma patients decided for adjuvant chemotherapy. For these 15 patients (who met also the inclusion criteria of the ASI study and who received chemotherapy), the Kaplan-Meier estimates of 2-year-survival were 80.0 ± 14.6%. There was no statistically significant difference compared to the ASI group (Mantel-Haenzel test).

To understand the findings in this small number of adjuvant chemotherapy patients, one must know that the acceptance of chemotherapy during this period was not as high as in the United States. Until March 1994, only patients who were accrued for clinical studies received adjuvant chemotherapy or combined radiotherapy and chemotherapy. A consensus conference during the German Cancer Congress at Hamburg in March 1994 recommended adjuvant chemotherapy or combined radiotherapy and chemotherapy for patients with colon carcinoma.
stage Dukes' C or rectal carcinoma stage Dukes' B3 and C outside of controlled clinical studies.

In one way our results are in line with the work of Hoover et al. (6, 8), who suggested that ASI may have a beneficial effect as an adjuvant therapy in colon carcinoma. Because of better tolerability by the patients, we used NDV as adjuvant. On the basis of the small number presented here, it cannot be decided which form of ASI is more effective. With regard to rectal carcinoma, Hoover et al. (8) did not see a therapy effect with their protocol and assumed that rectal cancers may be intrinsically less immunogenic than colon carcinomas. However, their own results of the DTH reactivity did not support this hypothesis. Zöller et al. (31) could not demonstrate a difference between colon and rectal cancer extracts in their effects on leukocyte migration, suggesting that there is no principal difference in the immunogenicity of colon and rectal cancers. The concern of Hoover et al. (8) that the early onset of the radiation therapy could have interfered locoregionally with the effects of ASI seems to us more obvious.

We are now at a point where we don't have to rely on individual case reports any more and can investigate underlying mechanisms of anticancer immune responses and how these respond to immunotherapy. Also, at least in some instances, the interaction between tumor cells and autologous CTLs can now be traced to the peptide level. The data presented here from vaccination studies along with those from a monoclonal antibody trial (9) let us hope that immunotherapy may become established as a standard form of adjuvant treatment of minimal residual disease in human colorectal cancer.

REFERENCES

Newcastle disease virus-infected intact autologous tumor cell vaccine for adjuvant active specific immunotherapy of resected colorectal carcinoma.

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