Articles

Active specific immunotherapy for stage II and stage III human colon cancer: a randomised trial

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Summary

Background Colon cancer is curable by surgery, but cure rate depends on the extent of disease. We investigated whether adjuvant active specific immunotherapy (ASI) with an autologous tumour cell-BCG vaccine with surgical resection was more beneficial than resection alone in stage II and III colon cancer.

Methods In a prospective randomised trial, 254 patients with colon cancer were randomly assigned postoperative ASI or no adjuvant treatment. ASI was three weekly vaccinations starting 4 weeks after surgery, with a booster vaccination at 6 months with 10^7 irradiated autologous tumour cells. The first vaccinations contained 10^7 BCG organisms. We followed up patients for time to recurrence, and recurrence-free and overall survival. Analysis was by intention to treat.

Findings The 5·3 year median follow-up (range 8 months to 8 years 11 months) showed 44% (95% CI 7–66) risk reduction for recurrence in the recurrence-free period in all patients receiving ASI (p=0·023). Overall, there were 40 recurrences in the control group and 25 in the ASI group. Analysis by stage showed no significant benefit of ASI in stage III disease. The major impact of ASI was seen in patients with stage II disease, with a significantly longer recurrence-free period (p=0·011) and 61% (18–81) risk reduction for recurrences. Recurrence-free survival was significantly longer with ASI (42% risk reduction for recurrence or death [0–68], p=0·032) and there was a trend towards improved overall survival.

Interpretation ASI gave significant clinical benefit in surgically resected patients with stage II colon cancer. ASI has minimal adverse reactions and should be considered in the management of stage II colon cancer.

Lancet 1999; **353:** 345–50

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Introduction

Colon cancer is potentially curable by surgery; the cure rate is, however, moderate to poor dependent on the extent of disease.[1] In 1990, a National Cancer Institute consensus conference recommended fluorouracil plus levamisole given for 1 year for stage III colon cancer.[2,3] Intergroup studies in the USA and Europe have since led to an alternative recommendation of fluorouracil plus folinic acid.[4–6] Adjuvant chemotherapy for stage II disease has no benefit.[2–7] Our efforts have focused on active specific immunotherapy (ASI) that makes use of the patient's tumour to elicit a long-term cell-mediated immune response as adjuvant therapy. Clinical studies of ASI were designed from results obtained with a guinea-pig hepatocarcinoma line. In this model, BCG admixed with synergeneic tumour cells can induce systemic immunity that eradicates a limited disseminated tumour burden if the vaccine is carefully controlled for variables such as number of tumour cells, ratio of viable BCG organisms to tumour cells, viability of the tumour cells, and regimen.[8–11] These data led to initial clinical studies of ASI in stage II and III colon cancer.[12–14] ASI with autologous tumour-cell vaccines has been successfully applied to melanoma[15] and renal cell carcinomas.[16]

Three incremental clinical trials of ASI in colon cancer have been completed. The first two studies used a three-vaccination immune induction regimen after surgical resection of the primary tumour.[14–17] In the third trial, which we report here, we assessed a three-vaccination immune induction regimen with a booster immunisation at 6 months. The need for a booster was suggested by the results of the first two trials. The first study involved 98 colon and rectal cancer patients.[1,14] An intention-to-treat analysis showed no significant improvement in the rate of recurrence or survival, but a subgroup analysis of overall and disease-free survival in colon cancer patients showed a significant trend for ASI being superior to surgery alone. Equally important, these immunised patients showed delayed cutaneous hypersensitivity reactions to a skin test of autologous tumour cells that were stronger than background responses to autologous normal mucosal cells, which suggested tumour-associated immunity. The delayed cutaneous hypersensitivity responses waned over 6 months after vaccination.[12]

In the second study, by the Eastern Cooperative Oncology Group (ECOG),[17] 412 stage II and III patients were randomly assigned after surgery ASI with three vaccinations or no further treatment. An

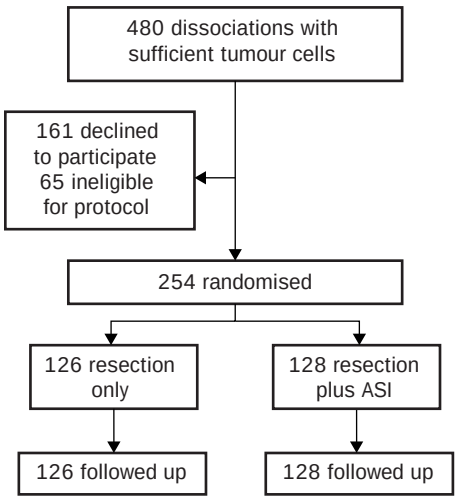

Figure 1: **Trial profile**

intention-to-treat analysis showed no significant differences in benefits between groups. In a subset of 307 patients who were immunised with vaccines that met specifications and had a substantial immune response (delayed cutaneous hypersensitivity to third vaccine >5 mm), ASI improved overall survival. Although the results were not significant, they suggested that an increased vaccination schedule over a longer period could achieve positive results.

We investigated in patients with stage II or III colon cancer whether ASI with four vaccinations (three induction immunisations plus boost) or no further treatment after surgery was beneficial.

Methods

Patients

254 patients were randomly assigned after surgery ASI or no further treatment (figure 1). Eligible patients had undergone curative resection for primary tumour classification or stage II or III adenocarcinoma of the colon. An adequate number of cells from the primary tumour and a performance status of 0 or 1 were also required. Patients with direct extension of tumour into the abdominal wall or an adjacent organ were eligible if en-bloc resection had achieved a tumour-free margin on microscopic examination. Patients who had intestinal obstruction that needed a colostomy before definitive surgery for the primary tumour were eligible if they were able to enter the study within 28–35 days of resection. We excluded patients whose carcinoembryonic antigen concentrations did not return to normal within 21 days of resection; who had perforation or evidence of peritoneal seeding; a previous malignant disorder other than carcinoma of the skin; rectal cancer, defined as tumour below the peritoneal reflection; ulcerative colitis, Crohn's disease, Gardner's syndrome, or Turcot's syndrome; or who were receiving steroids, cytotoxic, or immunosuppressive agents.

We stratified patients by the institution in which the surgery was done; location of the primary tumour—right or ascending colon, including the hepatic flexure, transverse colon, or left colon, including the splenic flexure, descending, and sigmoid colon; and tumour pathological stage.

Vaccination schedule

ASI involved administration of four autologous tumour-cell vaccinations, started 28–35 days after tumour resection to allow sufficient recovery from immunological suppression that may have been induced by anaesthesia and surgery. Treated patients received one intradermal vaccination per week for 2 weeks of about 10^7 viable, irradiated autologous tumour cells and 10^7 viable fresh-frozen BCG organisms (Organon Teknika, Durham, NC, USA). At 3 weeks and 6 months, patients received one vaccination of about 10^7 irradiated tumour cell alone. The first and second vaccines were injected intradermally, one on each anterior thigh about 100 m below the groin crease. The third and fourth vaccines were injected intradermally in the inner aspect of the upper arm.

Study design

The study was done at 12 hospitals in the Netherlands, with a single vaccine preparation site at the University Hospital, Vrije Universiteit, Amsterdam. 11 other hospitals within a 4 h radius of the University Hospital screened potential participants. Colon resections were done at one of the 12 sites, and the tumour sample was sent to the University Hospital's vaccine production laboratory for dissociation, cryopreservation, irradiation, and administration. Participating surgeons were experienced in colon resection. The protocol specified that the surgical procedure should involve wide removal of the involved bowel segment. Stratification of patients by institution kept the influence of surgical technique on outcome to a minimum. We obtained informed consent from all patients and ethical approval from all participating hospitals' medical boards.

The preparation of autologous tumour cell vaccine has been described previously.[9–11] The tumour samples were minced, dissociated with collagenase and DNase into a single-cell suspension, and frozen in a controlled-rate freezer. We tested quality of the bulk vaccine by cell number, viability, and sterility. About 60% of bulk vaccines contained micro-organisms typically associated with normal gastrointestinal flora. No vaccines were deemed unsafe for administration because of microbial contents. On the day of vaccination, the cells were thawed, irradiated with 200 Gy, and, for the first two injections, 10^7 viable BCG organisms added. We administered 0·2–0·4 mL vaccine.

Patients were observed after each vaccination for erythema and induration at the site of injection, fever, lymphadenopathy, or any adverse reactions. Immunised and non-treated patients were scheduled for monitoring every 3 months for years 1 and 2, every 6 months until year 5, and once yearly thereafter. We measured carcinoembryonic antigen concentrations at each follow-up. Chest radiography was done every 3 months for year 1, every 6 months for year 2, and once yearly thereafter. Computed tomography and colonoscopy were performed annually. Documented histological diagnosis by percutaneous, colonoscopic biopsy, or surgical exploration was required to confirm recurrence of tumour, except in cases of lung or liver metastases, which were confirmed by unequivocal radiography or scan. We listed the date of recurrence as the date of confirmation of disease recurrence.

Statistical analysis

Data management and statistical analyses were done by an independent monitoring agency (IKA, Comprehensive Cancer Center, Amsterdam) with SAS software (version 6.11). Recurrence-free interval (recurrences of any malignant disorder) and recurrence-free survival (free from any disease or cause of death) were the primary clinical endpoints. Disease-specific survival (deaths not related to disease were censored) and overall survival (death of any cause) were secondary endpoints. We did all main analyses by intention to treat. For recurrence-free interval and survival we generated Kaplan-Meier curves and used the log-rank statistic. We used Cox's proportional hazards model to calculate ratios of recurrence and survival and for all multivariate analyses. The protocol prespecified separate analyses by pathological stage (stage II and III). All statistical tests were two-sided.

The original protocol called for a sample size of 515 patients (377 stage II and 138 stage III) to be accrued over 5 years. Data on the degree of treatment effect was not available when the study started. During the course of the study, however, the final results of the phase II trial by Hoover and colleagues[14]

Characteristics	Control (n=126)	Vaccination (n=128)
Demographic		
Median (range) age (years)	65 (33–87)	66 (36–88)
Male/female	69 (54·8%)/ 57 (45·2%)	67 (52·3%)/ 61 (47·7%)
Location of primary tumour		
Right colon	48 (38·1%)	54 (42·2%)
Transverse colon	9 (7·1%)	6 (4·7%)
Left colon	69 (54·8%)	68 (53·1%)
Stage of differentiation		
Poor	24 (19·0%)	26 (20·3%)
Moderate	94 (74·6%)	92 (71·9%)
Good	8 (6·3%)	10 (7·8%)
Number of positive nodes in stage C		
≤4	101 (80·0%)	108 (84·0%)
>4	25 (20·0%)	20 (16·0%)
Pathological stages after final review		
TNM I, Dukes' B1	8 (6·3%)	4 (3·1%)
TNM II, Dukes' B2/B3	77 (61·1%)	81 (63·3%)
TNM III, Dukes' C	40 (31·7%)	43 (33·6%)
TNM IV, Dukes' D	1 (0·8%)	0

Percentages may not add up to 100% because of rounding.

Table 1: **Patients' characteristics**

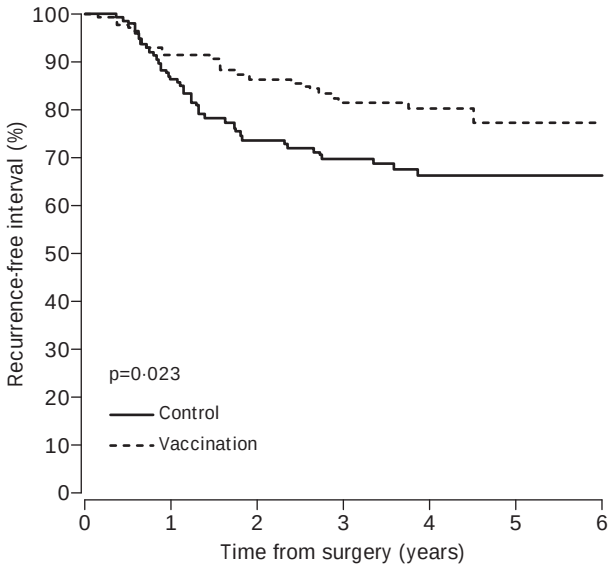

Figure 2: **Recurrence-free interval for all patients**

became available. This study reported hazard ratios of 4·0 for overall survival and 2·67 for disease-free survival. We therefore thought it appropriate to recalculate rates of 0·35 in stage II and 0·50 in stage III colon cancer. The weighting of these variables by 2:1, according to the observed accrual proportions and a hazard ratio of 2·67 by comparison of exponential parameters, implied a necessary number of at least 38 events to attain 80% power, type I error 0·05 for a two-sided test.

Results

126 patients were randomly assigned surgery alone and 128 surgery plus ASI between September, 1987, and February, 1996 (figure 1). At the time of statistical analysis in November, 1997, the median follow-up time was 5 years and 4 months (Range 8 months to 8 years 11 months). Characteristics of patients did not differ significantly between the two groups (table 1). 21 non-treated patients and 16 vaccinated patients were ineligible, mostly because of adjustments made in the final pathological review or secondary malignant disorders discovered later, but were followed up normally. Of the 128 vaccination patients, 101 had all four vaccinations.

The mean time from colon resection to the start of dissociation was 2·98 h (SD 0·28). The mean number of viable tumour cells was similar for tumours processed the same day as resection and those held overnight (n=13). The mean weight of tumour tissue was 8·0 g (4·2). The average yield of viable tumour cells per gram of tissue was $1{\cdot}89\times10^7$ ($0{\cdot}97\times10^7$). Sufficient cells to prepare an additional vaccine were obtained from more than half of the patients. There was no significant difference in yield between stage II and III tumours. The mean viability before freezing was 89·1% (3·9). The mean dose of viable tumour cells in all vaccinated patients was $1{\cdot}3\times10^7$ (range $0{\cdot}3\times10^7$–$1{\cdot}4\times10^7$) with a viability of 86% (6).

During the study, 965 contacts were made to obtain tumour samples. Of these, 130 (13%) had no tumour available after surgery for various reasons. 3 g tumour tissue is generally sufficient to produce enough cells for four vaccines. 730 samples had more than 3 g tissue and 99 had less than 3 g. Of those with less than 3 g, 25 yielded sufficient cells for four vaccines. Therefore, about 88% of patients could potentially have been vaccinated.

The delayed cutaneous hypersensitivity response was measured by testing for induration to the third and fourth vaccines, which consisted of irradiated autologous cells. 96% of patients had indurations of more than 5 mm 48 h after injection and 87% had indurations of more than 10 mm, with a mean induration of 16·9 mm (7·4), to the third vaccine. The percentages of patients with indurations of more than 10 mm to the fourth vaccine increased to 92% and the size of the indurations increased significantly (p=0·02) to 18·4 mm (7·8).

All patients developed some degree of induration and erythma at the site of the first two vaccinations containing BCG. Because of severe reactions at the first vaccination site, eight patients did not receive BCG in their second vaccination and two patients received only half of the dose of BCG at that time. More than 93% of patients developed some degree of ulceration at the first two vaccination sites, most of which healed within 3 months. Reactions to the third and fourth vaccines, without BCG, were of short duration, and ulceration occurred in only 7% and 4% of patients, respectively. Swelling of the lymph nodes closest to the vaccination site was more frequently seen with vaccines

Event	All patients		Stage II patients		Stage III patients	
	Non-treated (n=126)	Vaccinated (n=128)	Non-treated (n=85)	Vaccinated (n=85)	Non-treated (n=41)	Vaccinated (n=43)
Recurrences	40 (31·7%)	25 (19·5%)	23 (27·1%)	10 (11·8%)	17 (41·4%)	15 (34·9%)
Recurrences or deaths	48 (38·1%)	39 (30·5%)	31 (36·5%)	20 (23·5%)	17 (41·4%)	19 (44·2%)
Deaths	36 (28·6%)	32 (25·0%)	23 (27·1%)	17 (20·0%)	13 (31·7%)	15 (34·9%)
Disease-related deaths*	27 (21·4%)	18 (14·1%)	14 (16·5%)	7 (8·2%)	13 (31·7%)	11 (25·6%)

*Deaths of unknown causes were scored as disease related.

Table 2: **Summary of events by treatment and stage**

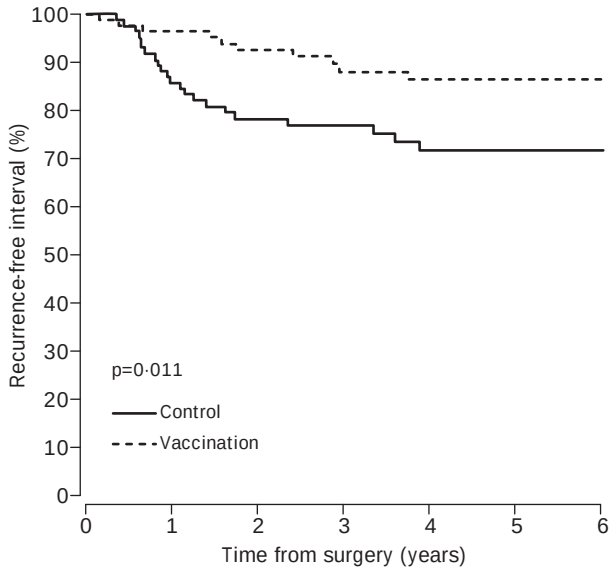

Figure 3: **Recurrence-free interval for stage II patients**

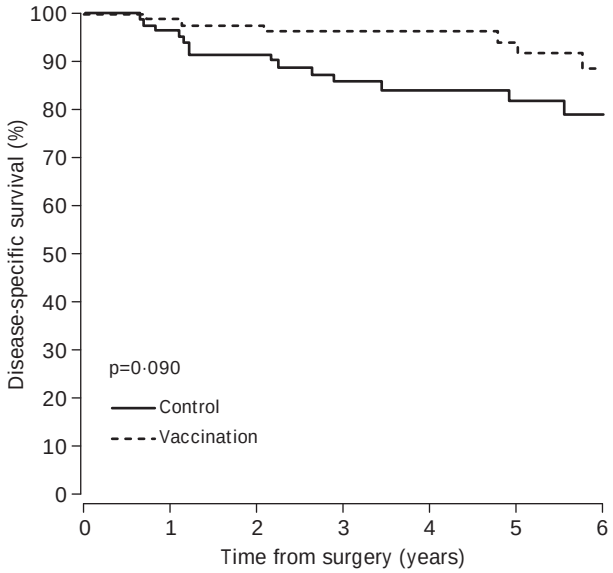

Figure 5: **Disease-specific survival for stage II patients**

containing BCG and occurred in 66% of patients. Systemic reactions, including fever, chills, or both in the first 24 h occurred more frequently with the BCG vaccines than with the vaccines without BCG (mild reactions 22·0 *vs* 7·0%; severe reactions 5·0 *vs* 0·5%). One patient developed a necrotic area at the site of an infection that required surgical excision. This patient received no further vaccination for medical reasons. One patient died 4 months after the first three vaccinations because of complications after reoperation to correct an anastomotic leak. No patient refused vaccination because of side-effects, and none needed to be admitted.

65 of the 254 randomised patients had recurrences—40 non-treated and 25 vaccinated patients (table 2). There was significantly fewer recurrences of any malignant disorder among vaccinated patients compared with non-treated patients (p=0·023, figure 2). The ASI vaccination decreased recurrence rate to 44% of that in non-treated patients (95% CI 7–66). Vaccinated patients had a non-significantly improved recurrence-free survival (p=0·110) and overall survival (p=0·330) compared with non-treated patients. Proportional decreases were, however, consistent with the lowered rate of recurrences. 34% of patients died from causes not associated with disease. The median ages of patients in the two groups who died from unrelated causes were 73 years and 74 years, respectively, and 68 years and 71 years, respectively, for those who died of related causes. The high proportion of deaths not related to the disease among these older patients may have contributed to the lack of significance for overall survival. The most common cause of death not related to cancer was cardiovascular disease.

The lower rate of recurrences of any malignant disorder among vaccinated patients was not seen for stage III patients alone (p=0·568). The proportion of recurrence-free patients at 4 years was, however, 59% for controls and 70% for vaccinated patients.

The significant rate of recurrences was, therefore, due to the effect of ASI in stage II disease. Ten vaccinated

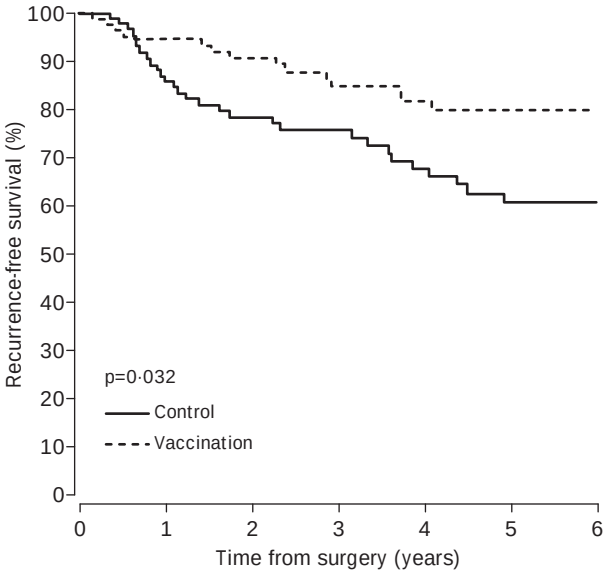

Figure 4: **Recurrence-free survival for stage II patients**

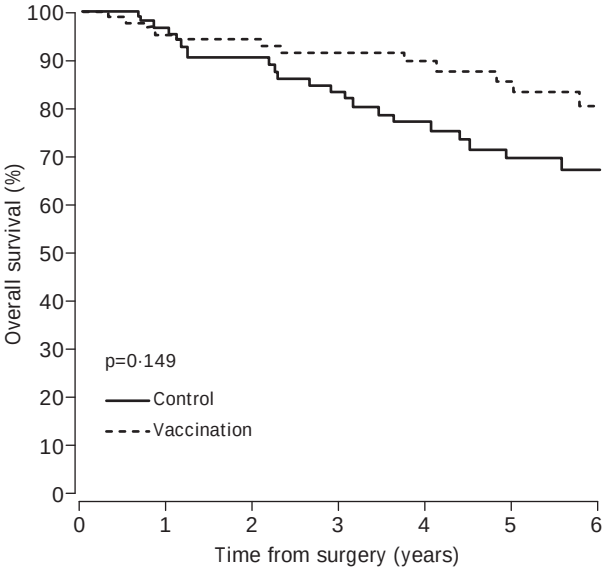

Figure 6: **Overall survival for stage II patients**

patients compared with 23 non-treated patients had tumour recurrences (table 2). The proportion of recurrence-free patients at 4 years was 74% for non-treated patients and 88% for vaccinated patients. Recurrence-free interval (p=0·011, figure 3) and recurrence-free survival (p=0·032, figure 4) were significantly improved by vaccination. The estimated overall decrease with ASI recurrence rate of any malignant disorder was 61% (95% CI 18–81). Disease-specific survival was non-significantly higher after vaccine than non-treatment (p=0·090, figure 5) but less so for overall survival (p=0·149, figure 6). There was a disproportionate number of non-disease-related deaths among non-treated patients (39%) compared with vaccinated patients (59%). Thus, the major risk factor for death other than colon cancer among vaccinated patients was age. When considering overall survival, age 70 years or older was associated with worse outcome, with cardiovascular disease being the prominent non-cancer related event. The number of disease-related deaths in the vaccinated group was 50% lower than that in the non-treated group (7 *vs* 14); with this small number of events, however, the study lacked the power to detect a significant difference.

Discussion

Adjuvant ASI with an autologous tumour cell and BCG vaccine significantly lowered the rate of tumour recurrences. The major impact was in stage II disease. Molecular characterisation of vaccines does not suggest any differences between the tumour cells derived from stages II or III tumours.[18] Furthermore, the proportion of patients who developed a positive delayed cutaneous hypersensitivity response to the third vaccine containing tumour cells alone was similar for stage II and III patients. Therefore, the difference in clinical outcome between patients with stage II and III disease is probably due to the extent of tumour burden, which is consistent with observations made in the guinea-pig model of ASI.[9,10] We undertook our study of ASI in colon cancer after two other clinical trials assessed vaccine specifications and treatment regimens.[14,17] We speculated that the significant outcome we found for ASI, compared with results from the ECOG study,[17] was probably attributable to two factors: the addition of the fourth booster vaccination at 6 months to enhance and maintain antitumour immunity, and the quality assurance of the vaccine preparations achieved by centralised vaccine production. The difference in clinical effects was not due to differences in the two study populations, since comparison of the survival curves for non-treated patients in the two studies (4·42 in EGOG *vs* 4·5 years of follow-up, our trial) showed no significant differences.

The three studies all showed that high-quality vaccines are needed and that treatment is most effective in patients with minimum residual disease (stage II).

Adjuvant chemotherapy of patients with stage III colon cancer with fluorouracil and low-dose folinic acid is standard treatment, based on the results of numerous cooperative and intergroup clinical studies.[4–7,19] None of these studies reported significant improvements in stage II colon cancer. With the use of ASI for stage II colon cancer, however, adjuvant therapy can provide benefit over surgery alone in more than 90% of colon cancer patients (64% stage II, 30% stage III).[20]

One of the most appealing aspects of ASI in our trial was the quality of life of the patients during treatment. No patient discontinued treatment early because of side-effects. Only one patient had treatment withdrawn by the treating physician after two vaccinations because of a severe local reaction. About 60% of vaccine preparations contained normal gastrointestinal flora, and between vaccines positive or negative for micro-organisms there were no systemic infections and no differences in the rate of local regional reactions. This finding is in contrast to that for fluorouracil and levamisole,[3] in which 30% of patients discontinued treatment early after a median of 5 months. About 20% of patients on fluorouracil and folinic acid did not receive the full course of treatment.[4] Common side-effects associated with chemotherapy include anaemia, neutropenia, diarrhoea, nausea, vomiting, and anorexia with grade 3 toxic effects reported in as many as 26% of patients in some investigations.[4] The improved overall survival benefit in stage III patients seems, however, to outweigh the side-effects.[2–7]

The logistics of providing tumour vaccine to colon cancer patients involves a network of hospital-based vaccine-manufacturing centres. The centres need to have good manufacturing practices and transport facilities to move tumour samples from hospital surgery units to the manufacturing facility. Two centres are presently established and operational. In the USA, the first centre exists at the Lehigh Valley Hospital in Allentown, Pennsylvania. In Europe, a centre is established at the University Hospital, Vrije Universiteit, Amsterdam, which we used in this study. Our future plans for ASI, in stage III patients, includes the assessment of ASI in combination with fluorouracil and folinic acid to test for synergistic improvement over chemotherapy alone.

Contributors

Jan B Vermorken was the study coordinator and coprincipal investigator. Herbert M Pinedo was the principle clinical investigator and was responsible for the the running of the study. Anke M E Claessen and Elizabeth Bloemena were the senior scientists who managed and coordinated the laboratory production of vaccines. Renate Ezinga and Harm van Tinteren were responsible for management and statistical analysis of clinical data. Sybren Meijer was the principle surgeon who operated on patients entering the study and reviewed and controlled quality of the surgery in the participating centres. Chris J L M Meijer was the head pathologist who provided overall pathology review of all patients and coordinated and standardised the pathology of the laboratory vaccine production. Rik J Scheper was an immunologist in the pathology department that supervised overall vaccine production. Janet H Ransom did quality assurance for the statistical analysis and clinical data coordination for the study sponsor and was involved in the writing of the paper. Michael G Hanna Jr was responsible for the preclinical concepts that led to this clinical trial, and developed the study design with Herbert Pinedo. Michael G Hanna also provided the technical resources for the study, including details of vaccine preparation and administration.

Acknowledgments

We thank Jane Brandhorst and Leona Peters for study coordination and quality assurance of vaccines, Herbert C Hoover for critical comments on the manuscript and study design. This study was a multicentre trial. Additional investigators include: R M J M Butzelaar, St Lucas Ziekenhuis, Amsterdam; H Steffelaar, Twenteborg Ziekenhuis, Almelo; J A C Brakenhof, Waterland Ziekenhuis, Pumerend; A van Bochove, Ziekenhuis De Heel, Zaandam; W W J Clarijs, Gemini Ziekenhuis, Den Helder; J G J Roussel, E J Hessenlink, Ziekenhuiscentrum Apeldoorn, Apeldoorn; E J Boerma, Kennemer Gasthuis DEO, Haarlem; A R Koomen, Kennemer Gasthuis, Elizabeth Gasthuis, Haarlem; C W Taat, Academisch Medisch Centrum, Amsterdam; B A van Driel, Streekziekenhuis Midden-Twente, Hengelo.

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