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The EORTC Early Clinical Trials Cooperative Group Experience with 5-Aza-2′-deoxycytidine (NSC 127716) in Patients with Colo-rectal, Head and Neck, Renal Carcinomas and Malignant Melanomas

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Abstract—*The Early Clinical Trials Cooperative Group of the EORTC conducted several phase II studies with a pyrimidine analogue of deoxycytidine, 5-aza-2′-deoxycytidine (DAC). The drug was given as three consecutive* 1 h *i.v. infusions of* 75 mg/m^2, *separated by intervals of* 7 h; *courses were repeated every* 5 *weeks. A total of* 101 *eligible patients were studied:* 42 *with colo-rectal adenocarcinoma,* 27 *with squamous cell carcinoma of the head and neck,* 18 *with malignant melanoma and* 14 *with renal cell carcinoma. Drug-induced toxicities consisted of moderate myelosuppression, and nausea and vomiting. One single partial remission was seen in a patient with malignant melanoma. DAC given in this dose and schedule is devoid of antitumour activity in adult patients with these refractory types of carcinomas.*

INTRODUCTION

5-Aza-2′-deoxycytidine (NSC 127716, DAC) is a pyrimidine analogue of deoxycytidine. It differs from deoxycytidine by the presence of a nitrogen in the 5 position of the heterocyclic ring (Fig. 1). DAC is converted into the nucleotide form by deoxycytidine kinase [1, 2]. Its exact mechanism of action is not certain, but the drug must be incorporated into DNA [3]. Further, inhibition of DNA methylase may lead to DNA hypomethylation. This observation has been associated with altered gene expression and induction of cell differentiation [4, 5]. On the other hand, direct cytotoxic effect and growth inhibition in leukaemic cells resistant to differentation have been recently demonstrated [6, 7].

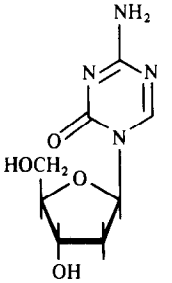

Fig. 1. Chemical structure of 5-aza-2′-deoxycytidine.

DAC showed antitumour activity *in vitro* and against murine leukaemias [1, 3, 8–10]. Animal toxicology was studied in mice, showing myelosuppression, intestinal mucosa damage and testicular atrophy [11, 12].

Phase I studies were performed initially in children with acute leukaemia, using prolonged i.v. infusions [13]. More recently, a phase I study was completed in 21 adults with solid tumours, using a schedule consisting of three consecutive 1 h

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infusions over 24 h. Dose-limiting toxicity was myelosuppression, with a delayed leukocyte nadir. Pharmacokinetic results showed very short half-lives, suggesting rapid elimination of DAC by metabolic processes [14].

Based on these results obtained by a member of the Group, the Early Clinical Trials Cooperative Group of the EORTC decided to further evaluate the activity of 5-aza-2′-deoxycytidine in simultaneously conducted phase II studies. This paper reports the results in colo-rectal adenocarcinoma, squamous cell carcinoma of the head and neck, malignant melanoma and renal cell carcinoma.

MATERIALS AND METHODS

Four separate studies were conducted by the investigators of the Early Clinical Trials Cooperative Group (ECTG) in patients with colo-rectal adenocarcinoma, squamous cell carcinoma of the head and neck, malignant melanoma and renal cell carcinoma.

Eligibility criteria were the same in the various studies: histologically proven and progressive malignant disease, not amenable to surgery and/or radiation therapy. Prior chemotherapy was not allowed, except for patients with head and neck and colo-rectal carcinoma. Serum creatinine and bilirubin levels were to be less than 125 and 25 μmol/l, respectively. Leukocyte and platelet counts were to be higher than 4000 and 100,000/ml. In all cases, measurable lesions in two diameters had to be present.

DAC was supplied in bulk by Pharmachemie, Haarlem, The Netherlands. Formulation was done by Y. Schoemaker (Slootervaart Ziekenhuis, Amsterdam, The Netherlands). Vials containing 50 mg DAC were reconstituted with 5 ml sterile water for injection. Reconstituted vials were further diluted in 250 ml 0.9% sodium chloride for i.v. injection. DAC was given at a dose of 75 mg/m^2 as a 1 h i.v. infusion, three times on day 1 with intervals of 7 h. Between administration of the three doses of DAC, the i.v. line was kept open. Treatment was repeated every 5 weeks. Dosage adjustments upwards or downwards were made according to the lowest haematologic values measured weekly during the previous course.

Criteria of response were those of WHO [15]. Patients had to receive at least two courses of DAC to be evaluable for response. Patients receiving only one course and being progressive were considered as 'early progressive'. Early death was defined as any death occurring within 28 days after DAC administration, without severe signs of toxicity.

RESULTS

Table 1 shows the characteristics of the eligible patients in the four studies. A total of 107 patients were entered: 43 with colo-rectal adenocarcinoma, 29 with squamous cell carcinoma of the head and neck, 20 with malignant melanoma and 15 with renal carcinoma. Six patients were considered as ineligible (4 with unallowed prior chemotherapy or radiation therapy, 1 with elevated serum creatinine and 1 without measurable lesions), leaving thus 101 eligible patients. Early deaths were seen in four patients: two because of malignant disease and two because of intercurrent causes.

The total number of DAC courses was 230. More than four courses of treatment were given to only five patients (colon 2, melanoma 2, renal 1) in case of tumour stabilization.

Response could be evaluated in 82 patients (81% of the eligible patients) by study chairpersons. Only one response was reported and reviewed by the Group investigators: a 57-year-old woman had a wide excision of a malignant melanoma (superficial spreading type, Clark level V) on the calf. Inguinal lymph node clearance was performed 20 months later because of metastases, followed by radiation therapy. DAC treatment was begun 3 months later because of the appearance of new skin lesions over the leg. Metastatic work-up in other organs was negative. She received three courses of DAC, with an almost complete regression of the skin lesions, lasting for 116 days. At that time, treatment was discontinued because of appearance of new skin metastases.

Tumour stabilization was reported in 7% of the patients with colo-rectal, in 15% of the patients with head and neck, in 22% of the patients with melanoma and in 14% of the patients with renal cell carcinomas. Early progressive disease and progression were seen in 74% of the patients with colon, 52% of the patients with head and neck, 67% of the patients with melanoma and 79% of the patients with renal carcinomas.

Toxicity

The haematologic toxicity is reported in Table 2. Considering all courses together, 82% of the patients had leukocyte values under 4000/ml, and 10% of the patients had thrombocyte values under 100,000/ml. Patients with head and neck primary tumours had lower nadir values, compared to other patients. In the former ones, WHO grade 3+4 toxicity for leukocytes was seen in 57% of the patients. The same figures for patients with other tumour types were 19% for colon, 25% for melanoma, 0% for kidney regarding leukocytes. As far as platelets are concerned, WHO grade 3+4 toxicity was seen in 7% of the patients with head and neck carcinomas, and 0% of the other patients.

Regardless of the type of the primary tumor, the day of lowest value was day 14 (range 6–38) for platelets and day 21 (range 7–31) for leukocytes

Table 1. *Characteristics of the eligible patients*

	Tumour types			
	Colon	Head and neck	Melanoma	Kidney
No. of patients	42	27	18	14
Median age (years)	61	56	53	54
range	29–76	39–75	21–75	28–73
Male : female	25:17	23:4	15:3	12:2
P.S. 0	12	6	12	2
1	21	12	5	10
2	9	8	1	2
3	0	1	0	0
Prior radiotherapy	3	24	2	4
Prior chemotherapy	19	22	0	0
Median no. of drugs	1.1	2.9		
range	1–3	1–9		

Table 2. *Haematologic toxicity*

	Tumour types				
	Colon	Head and neck	Melanoma	Kidney	All
Leukocytes					
median value*	3.35	1.75	2.45	3.30	2.92
range	0.5–9.1	0.6–4.0	1.1–5.6	2.6–5.8	0.5–9.1
Platelets					
median value*	250	148	190	244	201
range	74–467	50–243	66–322	126–339	50–467

* × 1000/ml.

Table 3. *Non-haematologic toxicities*

	Tumour types				
	Colon	Head and neck	Melanoma	Kidney	All
No. evaluable patients	33	16	17	12	78
Nausea + vomiting	56% (9%)*	31%	31%	58%	47% (4%)
Diarrhoea	6%	6%		8%	5%
Drug fever			6%	17%	4%
Local			6%	8%	3%
Alopecia			6%	8%	3%
Skin	3%				1%
Infection	3%				1%
Peripheral neuropathy				8%	1%

*WHO grade 3 + 4 toxicities.

through all courses. Time to recovery above 100,000 and 4000/ml was day 20 (17–22) and day 33 (range 13–50), respectively.

The most frequent non-haematologic toxicity was nausea and vomiting reported in 47% of the patients, usually of mild (WHO grade 1+2) intensity. Other toxicities were rarely encountered (Table 3).

CONCLUSIONS

Several phase II studies with DAC were performed simultaneously in various tumour types using a particular schedule of administration, which was a compromise between drug instability after dissolution and practicability.

The most frequent toxicity consisted of moderate

myelosuppression, with a late leukocyte nadir at day 21 (range 7–31). Drug-induced myelosuppression that was seen in patients with squamous cell carcinoma of the head and neck was more pronounced than in the other patients. Since patient characteristics were not different as far as age, sex and performance status were concerned, it is highly probable that this difference was related to other risk factors such as chronic alcoholism and dietary deficiency, commonly seen in these patients. It should also be noted that previous exposure to anticancer drugs was higher in head and neck patients than in patients with colo-rectal adenocarcinoma, and often included cisplatin.

The Early Clinical Trials Cooperative Group experience with DAC, given at a dose of 3×75 mg/m^2 every 5 weeks, is negative. We conclude that 5-aza-2′-deoxycytidine has less than a 5% chance of having $> 20\%$ activity in colo-rectal adenocarcinoma, squamous cell carcinoma of the head and neck, malignant melanoma and renal cell carcinoma.

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