Eur J Cancer Clin Oncol, Vol. 23, No. 12, pp. 1837–1843, 1987.
Printed in Great Britain

0277–5379/87$3.00+0.00
Pergamon Journals Ltd.

A Phase I Clinical Evaluation of 1-(2-Chloroethyl)-3-[2-(dimethylaminosulphonyl)ethyl]-1-nitrosourea (TCNU)

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Abstract—*TCNU (1-(2-chloroethyl)-3-[2-(dimethylaminosulphonyl)ethyl]-1-nitrosourea) is a newly developed water-soluble nitrosourea based on the endogenous aminoethanesulphonic acid taurine. TCNU was in an extended phase I trial given orally every 4–8 weeks using a stepwise dose escalation from 20 to 170 mg/m^2. One hundred and thirty-nine patients received a total of 323 courses. Minor haematologic toxicity was observed in 12 patients treated at dose levels < 70 mg/m^2. Thrombocytopenia WHO grades 1–4 occurred in 43% (55/127) and leucopenia WHO grades 1–3 in 45% (57/127) of the patients treated at dose levels ≥ 70 mg/m^2. Nausea and vomiting was recorded in about half the patients despite the use of metoclopramide. At the initial dose level 41 patients received ≥ 3 courses of TCNU. Cumulative leucopenia and thrombocytopenia occurred in 3/41 and in 12/41 patients, respectively, while reversible hepatotoxicity was observed in two patients. Antitumour activity was observed in patients with advanced squamous cell, adeno- and large cell carcinoma of the lung. The recommended starting doses for phase II trials with TCNU are as follows: heavily pretreated patients 90 mg/m^2, minimally/-moderately pretreated patients 110 mg/m^2 and previously untreated patients 130 mg/m^2 with TCNU given every 4–5 weeks, the repeated doses and intervals being adjusted to individual tolerance.*

INTRODUCTION

THE FIRST nitrosourea compounds were introduced clinically in the 1960s, and therapeutic activity was soon demonstrated both for BCNU [1,3-bis-(2-chloroethyl)-1-nitrosourea] and CCNU [1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea] in solid tumours and haematologic malignancies [1, 2]. Both compounds are highly lipophilic and the dose-limiting toxicity is haematologic of delayed type.

Since then a series of more hydrophilic nitrosourea derivatives have been synthesized, and among these ACNU [1-(4-amino-2-methyl-5-pyrimidinyl)--methyl-(2-chloroethyl)-3-nitrosourea] and HECNU [1-(2-hydroxyethyl)-3-(2-chloroethyl)-3-nitrosourea] have undergone clinical evaluation showing some activity in solid tumours [3].

In the search for other nitrosoureas with different toxicity and antineoplastic pattern TCNU (1-(2-chloroethyl)-3-[2-(dimethylaminosulphonyl)ethyl]-1-nitrosourea) was chosen for further clinical development (Fig. 1). The decision was based upon its unique biochemical structure founded on the endogenous aminoethanesulphonic acid taurine as carrier making the compound more hydrophilic. Pharmacokinetic studies of TCNU in dogs revealed a bioavailability after oral administration of 26% as a mean, peak concentration was reached after about 25 min and the plasma half-life was 15–20 min [4]. TCNU was furthermore selected because of its favourable antitumour activity in a number of *in vitro* and *in vivo* test systems [4, 5]. Antitumour activity has been demonstrated in cell cultures of human colon tumour cell lines, BE and

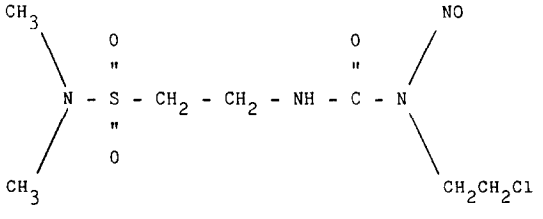

Fig. 1. Molecular structure of TCNU.

Accepted 13 May 1987.
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HT-29 and in five human small cell lung cancer cell lines [4, 5]. In five experimental murine tumours TCNU exhibited oral activity similar to or better than other nitrosoureas [4]. Against the Walker 256 carcinosarcoma the median effective dose (ED_{50}) of TCNU was only a fourth (0.25 mg/kg vs. 1.0 mg/kg) as compared to BCNU, CCNU and MeCCNU, whereas TCNU had comparable activity against L 1210 leukaemia, Harding–Passey melanoma, Lewis lung and Colon C 26 tumours.

In mice, rats and dogs the main dose-limiting adverse reactions recorded, after a single oral dose as well as repeated doses, have been bone marrow, gastrointestinal and hepatic toxicity. In comparison with CCNU TCNU induces in repeated toxicity studies a higher bone marrow toxicity but a lower hepatotoxicity. Thus the toxicity profile of TCNU has been shown to be similar to that of other nitrosourea compounds in clinical use [4]. Based on the preclinical data of TCNU it was decided to conduct a phase I study with the following aims: to establish the maximal tolerable dose (MTD) of TCNU as a single dose as well as after repeated doses, to characterize drug-induced toxic effects and to identify dose-limiting factors. In addition pharmacokinetic studies were performed. This report will describe the results of the clinical observations while the pharmacokinetic studies will be the subject of another publication [6].

METHODS AND MATERIALS

Patients aged 18–75 years with histologically or cytologically confirmed diagnosis of malignancy for which no effective antitumour therapy was available were eligible for the study, provided that the performance status was ≤ 3 (WHO scale) and life expectancy judged to be at least 8 weeks.

Previous antineoplastic therapy, including radiotherapy, should have been discontinued for at least 3 weeks or 6 weeks if other nitrosoureas or mitomycin C had been administered. Pretherapeutic values of platelets $\geq 100 \times 10^9$/l and white blood counts (WBC) $\geq 3.0 \times 10^9$/l were required just as normal liver (se-alkaline phosphatase, se-ASAT, se-LDH, se-bilirubin and prothrombin time) and renal (se-electrolytes and se-creatinine) function tests were demanded with the exception of abnormal values directly related to the underlying malignant disease. Informed consent from the patient, according to the Helsinki declaration, was obtained. Evaluable/measurable tumour lesions were *not* a prerequisite for inclusion into the trial, but when available, the WHO criteria were used in the evaluation of response [7]. The measurability of disease was based on chest X-ray, cutaneous metastases/lymph nodes with positive cytology at fine needle aspiration, CT-scan, ultrasound including positive cytology obtained by surecut biopsy or gynaecological examination including bioptially verified residual disease/relapse.

Complete blood counts (CBC: haemoglobin, WBC and platelets) were performed weekly. All patients were examined physically and laboratory evaluations consisting of a differential count, serum electrolytes, se-creatinine, se-urate, se-alkaline phosphatase, se-lactic dehydrogenase (LDH), se-ASAT, se-bilirubin, prothrombin time and blood sugar were performed before entry and repeated every 3 weeks for patients treated at intervals of 6 weeks and every 4 weeks for patients treated at intervals of 4 or 8 weeks. Before entry bone marrow aspiration from the posterior iliac crest, chest X-ray and ECG were also performed.

If patients died without toxicity during the first course of treatment they were categorized as 'early deaths' and excluded from the study.

TCNU was supplied, by the LEO Company, Helsingborg, Sweden, as tablets of 5, 10, 25 and 50 mg containing lactosum and amylum maydis as bulk. The tablets were protected from light and stored in a refrigerator. The dosage was calculated in mg/m^2 body surface area to the nearest 5 mg and administered on an empty stomach as a single oral dose (p.o.) every 4–8 weeks. Initially TCNU was given at intervals of 8 weeks but from dose levels of 40 mg/m^2 and above the stipulated interval was shortened to 6 weeks and later on to 4 weeks based on experience from the use of CCNU [8]. The starting dose was chosen to be slightly higher than 1/10 LD_{10} in mice (12.7 mg/m^2) or 1/3 MTD in dog (14.9 mg/m^2). The dosage was escalated from 20 to 170 mg/m^2 in 10 steps using a modified Fibronacchi search scheme (Table 1). The series was postponed with a repeat of the CBCs weekly until restitution if there was no full recovery of haematologic values (i.e. WBC $\geq 3.0 \times 10^9$/l and platelets $\geq 100 \times 10^9$/l) at the day of scheduled retreatment.

Three patients were entered per dose at the lowest four dose steps. Thereafter a relatively large number of patients were included as compared to usual phase I studies because during the trial it became evident that considerable variability in toxicity, depending on the degree of previous treatment, occurred at the same dose level. In addition surprising clinical activity was observed at 40 mg/m^2 thereby making it desirable even at a low non-toxic dose to achieve as much information as possible concerning the potential therapeutic activity of TCNU.

Each patient was scheduled to receive at least two courses of therapy. In the case of progressive disease (PD) the treatment was discontinued whereas no change (NC) or regression (PR + CR) after two courses resulted in continuation of therapy until PD was noticed. If the patients had shown a

Table 1. Number of evaluable patients per dose level

Dose	Previously untreated	Minimally/ moderately* pretreated	Heavily† pretreated	Total No. of patients
20	0	2	1	3
30	1	1	1	3
40	3	0	0	3
50	2	1	0	3
70	4	12	11	27
90	16	10	15	41
110	9	8	2	19
130	20	0	0	20
150	14	0	0	14
170	6	0	0	6
	75	34	30	139

*Patients previously having received ≤ 3 cytotoxic agents and/or minimal radiotherapy.
†Patients previously having received > 3 cytotoxic agents and/or extensive radiotherapy.

PR or CR at a non-toxic or slightly to moderately toxic dose level an increase in dose was permitted in order to give the patients with a TCNU-sensitive tumour the possibility of achieving the longest duration of response. The latter group of patients was only included into this analysis with respect to toxicity until the time of dose escalation.

At dose levels ≥ 40 mg/m^2 of TCNU patients were treated with metoclopramide 40–80 mg 30–40 min before TCNU and in case of nausea and vomiting the dose was repeated at intervals of 2–3 h.

Patient material

One hundred and sixty-nine patients were included in the study from December 1984 to November 1985. Twenty-seven were classified as early deaths, two were lost to follow up while one patient was included at an incorrect dose level leaving 139 patients evaluable for toxicity. The median age was 58 years (range 18–74), 102 patients (73%) had performance status (PS) 0 or 1 while the remaining had PS 2 (33 patients) or PS 3 (four patients). The male–female ratio was 72/67.

With respect to prior treatment the patients have been divided into three categories:

(a) previously untreated patients.
(b) minimally/moderately pretreated patients defined as patients having received ≤ 3 cytotoxic agents and/or *minimal radiotherapy*.
(c) heavily pretreated patients (patients having received > 3 cytotoxic agents and/or *extensive radiotherapy*).

RESULTS

One hundred and thirty-nine evaluable patients received a total of 323 courses with 98 patients (70%) having one or two courses of TCNU, while 41 patients were exposed to ≥ 3 courses of TCNU q 4–6 weeks at the primary dose level. Twenty-four patients received three courses, 10 patients four courses, five patients five courses and two patients six courses, respectively. Fifty-four per cent (75/139) of all patients were previously untreated. The details concerning the number of patients at each dose level with respect to prior treatment is given in Table 1.

Toxicity

Immediately observed adverse reactions secondary to TCNU consisted of nausea and vomiting, which occurred in 55% (77/139) of patients despite the fact that the majority had received metoclopramide before TCNU. The symptoms occurred within few hours after administration of TCNU lasting from a few hours until 24–48 h in rare cases. No allergic reactions were observed.

Haematologic toxicity

Acute haematologic toxicity was evaluated after an observation period of one and/or two courses. There were no drug-related deaths.

At dose levels < 70 mg/m^2 only minor haematologic toxicity was observed (Table 2). Anaemia (WHO grade 1) occurred in three out of 12 patients, whereas thrombocytopenia and leucopenia was not observed. In contrast and as illustrated in Table 2 and Figs 2 and 3 haematologic toxicity was found at all dose levels ≥ 70 mg/m^2. The nadir of the thrombocytopenia occurred 3–4 weeks after drug administration with recovery to pretreatment level after another 1–3 weeks. Thrombocytopenia WHO grades 1–4 was noted in 43% (55/127) of the patients treated at dosages ≥ 70 mg/m^2; of these eight patients showed grade 4 toxicity (thrombo-

Table 2. Toxicity of TCNU following one and/or two courses of TCNU

Dose (mg/m²)		< 70	70			90			110		130	150	170
Prior treatment			None	≤ 3 drugs	> 3	None	≤ 3 drugs	> 3	None	Plus	None	None	None
No. of patients		12	4	12	11	16	10	15	9	10	20	14	6
Thrombocytes													
WHO grade	0	12	4	8	6	12	8	9	7	5	7	4	2
	1	—	—	2	1	1	1	—	2	2	3	2	—
	2	—	—	1	1	2	—	—	—	2	3	5	1
	3	—	—	1	2	—	1	5	—	1	4	2	2
	4	—	—	—	1	1	—	1	—	—	3	1	1
Leucocytes													
WHO grade	0	12	4	7	7	12	6	8	4	5	6	8	3
	1	—	—	5	3	4	3	2	2	2	3	3	—
	2	—	—	—	1	—	1	3	2	3	6	2	2
	3	—	—	—	—	—	—	2	1	—	5	1	1
	4	—	—	—	—	—	—	—	—	—	—	—	—
Haemoglobin													
WHO grade	0	9	3	6	5	11	3	8	3	4	10	4	4
	1	3	1	5	4	4	5	4	4	5	7	5	2
	2	—	—	1	1	—	2	3	2	—	2	5	—
	3	—	—	—	1	1	—	—	—	1	1	—	—
	4	—	—	—	—	—	—	—	—	—	—	—	—
Nausea/vomiting													
WHO grade	0	1	3	7	3	8	6	6	4	4	12	6	2
	1	2	—	2	—	3	1	—	1	—	1	—	1
	2	5	—	3	5	2	1	4	2	2	4	3	1
	3	4	1	—	3	2	2	4	2	3	1	4	2
	4	—	—	—	—	1	—	1	—	1	2	1	—

cytes $< 25 \times 10^9$/l) necessitating thrombocyte-transfusions in six patients. The nadir of the leucopenia was usually recorded 5 or 6 weeks after treatment with recovery 1 week later. WHO grades 1–3 WBC toxicity occurred in 45% (57/127) of the patients, including 10 patients with grade 3 toxicity (WBC: $1.0–1.9 \times 10^9$/l). In Figs 2 and 3 a pronounced dose toxicity relation is observed with respect to both leucopenia and thrombocytopenia. WHO grades 1–3 anaemia occurred in 52% (66/127) of the patients, and 20 had anaemia WHO grades 2 and 3 (B-haemoglobin 4.0–5.5 mmol/l).

As can be seen from Table 2 the degree of haematologic toxicity was clearly related to the amount of prior treatment. This is also illustrated by Figs 4 and 5 showing the haematologic toxicity at a level of 90 mg/m² with the patients separated according to prior treatment.

Cumulative haematologic toxicity

Forty-one patients received ≥ 3 courses of TCNU at the initial instituted dose level. Cumulative thrombocytopenia (platelets $< 50 \times 10^9$ for ≥ 2 weeks after ≥ 3 courses) occurred in 12 of these (29%) whereas cumulative leucopenia (WBC $< 2.0 \times 10^9$/l for ≥ 2 weeks after ≥ 3 courses) occurred in three of 41 patients (7%). Tables 3 and 4 describe in detail the cumulative haematologic toxicity expressed by nadir values and the duration of cytopenia.

Other signs of toxicity

Reversible biochemical hepatic alterations occurred in two patients at a dose level of 130 mg/m² treated for 8 and 10 months, respectively. Observation of an otherwise unexplainable increase in se-ASAT, se-alkaline phosphatase and se-bilirubin resulted in an ultrasonic examination of the liver combined with a surecut biopsy. In both instances, the ultrasound evaluation was normal and the biopsy showed neither signs of malignancy nor any other pathologic changes. After termination of the treatment the biochemical values slowly returned to normal within a period of 6 and 8 weeks.

In addition to the above mentioned signs of toxicity attacks of sweating—especially at night—and tumour pain occurrred in about 20% of the patients. No cardiac, renal, pulmonary or CNS toxicity nor alopecia were observed.

Antitumour activity

The majority of the 139 evaluable patients had primary bronchogenic carcinoma (77 patients,

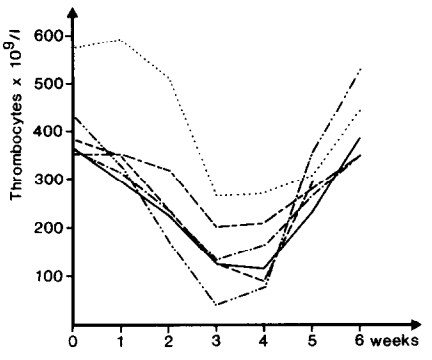

Fig. 2. Median values of thrombocytes following a single p.o. dose of TCNU at increasing dose levels.

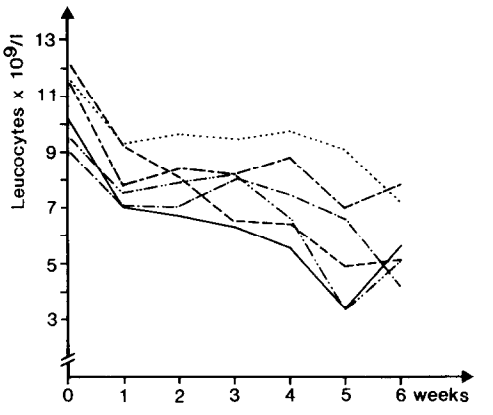

Fig. 3. Median values of leucocytes following a single p.o. dose of TCNU at increasing dose levels.

Haematologic toxicity — previously untreated patients.

····· 70 mg/m² (4 *patients*)
–·–·– 90 mg/m² (16 *patients*)
·—·—· 110 mg/m² (9 *patients*)
——— 130 mg/m² (20 *patients*)
– – – 150 mg/m² (14 *patients*)
–··–··– 170 mg/m² (6 *patients*)

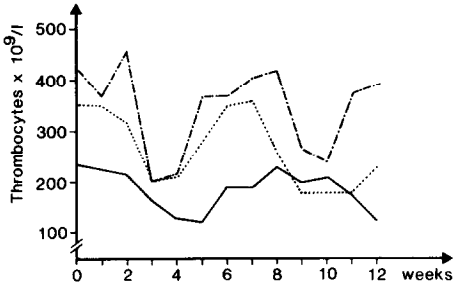

Fig. 4. Haematologic toxicity at a dose of 90 mg/m². Median values of thrombocytes following one and/or two courses at intervals of 6 weeks. ——— Heavily pretreated (15 patients); –·–·– minimally pretreated (10 patients); ····· previously untreated (16 patients).

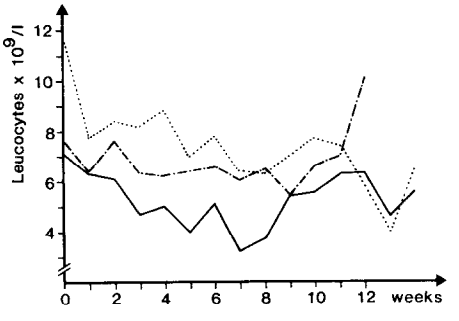

Fig. 5. Haematologic toxicity at a dose of 90 mg/m². Median values of leucocytes following one and/or two courses at intervals of 6 weeks. ——— Heavily pretreated (15 patients); –·–·– minimally pretreated (10 patients); ····· previously untreated (16 patients).

Table 5) while the remaining had breast cancer (five patients), gynaecological tumours (27 patients), urological tumours (nine patients), gastrointestinal tumours (eight patients) and miscellaneous tumours (14 patients), including one patient who had two malignant diseases. The histologic classification of responding lung tumours was performed by the Pathology Department of the referring hospital as well as by the specialized pulmonary pathologists at the Finsen Institute.

Overall 58 patients with non-small cell carcinoma of the lung (NSCCL) were evaluable for response and a total of one complete and 10 partial remissions were observed (Table 5). The first antitumour activity was recorded at a non-toxic level of 40 mg/m² (Table 5) in two patients with large cell carcinoma of the lung. In the responding patients, who were eventually exposed to dose escalation, the duration of response ranged from 4 to 72+ weeks with a median of 21 weeks.

DISCUSSION

The present study reveals that TCNU has a similar toxicity profile to other nitrosoureas hitherto evaluated clinically with delayed haematologic toxicity and especially thrombocytopenia being the dose-limiting factor. In addition cumulative haematologic toxicity and sporadic hepatotoxicity has been observed as described for other nitrosoureas. The data also indicate that the recommended doses for further clinical trials range from 90–130 mg/m²

Table 3. Cumulative thrombocytopenia for patients given ≥ 3 courses of TCNU

Dose (mg/m²)	No. of patients Total	+ Cumulative toxicity	Nadir of thrombocytes (× 10⁹/l) TCNU course No. 1	2	3	4	5	Duration* of cytopenia (weeks)
70	7	0						
90	12	4	29	24	57	97	18	10
			226	253	25			2
			42	80	55	67	23	13
			106	131	44			3
110	5	1	109	212	47			Died during 3rd course from PD
130	11	4	105	29	10			4
			87	201	32			3
			138	241	74	98	21	13
			72	286	11			6
150	5	3	56	110	187	36		4
			120	159	19			2
			55	169	8			6
170	1	0						

Cumulative thrombocytopenia: thrombocytes < 50 × 10⁹/l for ≥ 2 weeks after ≥ 3 courses.
*Stated for the last period of thrombocytopenia (platelets < 100 × 10⁹/l).

Table 4. Cumulative leucopenia for patients given ≥ 3 courses of TCNU

Dose (mg/m²)	No. of patients Total	+ Cumulative toxicity	Nadir of leucocytes (× 10⁹/l) TCNU course No. 1	2	3	4	5	Duration* of leucopenia (weeks)
70	7	0						
90	12	1	2.2	3.4	2.9	3.2	1.8	11
110	5	0						
130	11	2	4.9	3.1	1.9			2
			3.2	2.5	0.8			Unknown
150	5	0						
170	1	0						

Cumulative leucopenia: leucocytes <2.0 × 10⁹/l for ≥ 2 weeks after ≥ 3 courses.
*Stated for the last period of leucopenia (WBC < 3.0 × 10⁹/l).

Table 5. Responses in patients with bronchogenic carcinoma

Classification	No. of patients evaluable for Toxicity	Response	Dose (mg/m²) × courses of TCNU	*	Response	Duration of response (weeks)
WHO I	23	16	130 × 5	↓	PR	22
WHO II	3	3				
WHO III	37	30	70 × 2	↑	PR	20
			90 × 2	↑	PR	11
			90 × 5	↓	PR	20
			130 × 1	†	PR	4
			150 × 4	↓	PR	72+
WHO IV	14	12	40 × 2	↑	CR	34
			40 × 2	↑	PR	21
			90 × 3	↑	PR	34
			130 × 3	↑	PR	21
			130 × 1	†	PR	4

* ↑ Dose escalation; ↓ dose deescalation; † non-toxic death.

q 4–5 weeks with the starting dose depending on the degree of prior anti-neoplastic treatment. Dosages above 130 mg/m^2 produced regularly grade III + IV thrombocytopenia necessitating platelet transfusions and therefore cannot be recommended in general.

While TCNU thus does not differ in its toxicity profile from other nitrosoureas, the therapeutic results in this phase I trial suggest that TCNU might have a different antineoplastic profile, even though the mechanism of action appears similar to that of BCNU—at least based on flow cytometric analysis [5]. First of all therapeutic action was observed at non-toxic levels which is somewhat unusual for cytostatic agents. Secondly, overall high activity was seen in patients with non-small cell lung cancer, which otherwise is rather chemoresistant. It should be emphasized, however, that the majority of the latter patients included in the trial were untreated and in a good performance status, and thus preselected with relatively good prognostic characteristics. A direct comparison of the activity of TCNU with the established but low activity of other nitrosourea compounds in non-small cell carcinoma of the lung is thus not possible.

Only the results from ongoing phase II trials in solid tumours, especially in non-small cell lung cancer, will establish whether TCNU has clinically noteworthy activity. If that is the case, a reevaluation of the entire group of nitrosoureas is indicated, especially the influence of the changes in the chemical structure caused by the inclusion of taurine as carrier. It would also be interesting to investigate whether incorporation of taurine in other alkylating agents such as nitrogen mustard derivatives could induce not only a modulation of the toxicity but also an improvement in the antineoplastic pattern as suggested in pre-clinical systems by Pierson *et al.* [9].

Acknowledgements—The authors would like to express their appreciation to Merete Jacobsen and Merethe Larsen for typing the manuscipt.

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