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The Quantitation of Sister Chromatid Exchanges in Lymphocytes of Cancer Patients at Intervals after Cytotoxic Chemotherapy

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Abstract—*Venous blood was taken from patients with cancer, prior to and up to* 42 days *after the administration of cytotoxic chemotherapy. Lymphocytes were stimulated to divide* in vitro, *and examined for sister chromatid exchanges (SCEs). Cyclophosphamide rapidly increased the frequency of SCE, which returned to approximately double the control value* 24 hr *after administration. The remaining SCEs disappeared more slowly. There was a positive correlation between the dose of drug and the frequency of SCE measured immediately and* 4 hr, 20 hr *and* 21 days *after treatment. As patients received successive courses of treatment the number of SCEs generally increased from about* 0.14 *to* 0.25 *per chromosome. After this, further chemotherapy was often less effective in inducing them. The presence of SCEs in peripheral blood lymphocytes may be a useful indicator for the occurrence and persistence of alkylating metabolites, residual damage in the DNA and individual responses of patients to a standard regimen.*

INTRODUCTION

CYTOTOXIC chemotherapy is often used in the treatment of malignant diseases that surgery, radiotherapy or both have failed to cure [1–3]. Damage to the normal tissues is usually monitored by noting gross pathological changes (e.g. [4]) which may be difficult to quantify and irreversible. An early assessment of injury would therefore be a valuable aid to monitor chemotherapy.

Prolonged chemotherapy may result in changes in the effects of agents owing to acquired resistance [5] or increased sensitivity of cells [6]. Then the effect of a drug after many doses would be different from that at the start of treatment. If such changes could be measured, modification of treatment may be possible.

Sister chromatid exchanges (SCEs) in the lymphocytes of patients are a sensitive indicator of the effects of cytotoxic drugs such as cyclophosphamide [7–9]. Although the frequency of SCE in lymphocytes of treated patients decreases with time after chemotherapy [9, 10], they may persist for as long as chromatid and chromosome breaks and exchanges [11–13]. The aim of this study was to investigate whether a quantitative relationship exists between therapeutic dose and the frequency of SCE in patients.

Venous blood was collected from patients with cancer before and at various intervals during cycles of cytotoxic chemotherapy. Each cycle lasted about a month. The change in frequencies of SCE with time after a single dose or several doses of drugs given during successive cycles has been evaluated. The dose–effect relationship has also been determined at various times after treatment.

MATERIALS AND METHODS

The patients varied with respect to sex, age and disease (Table 1). Most had received previous radiotherapy; 8 had been treated with chemotherapy prior to this study.

The patients were given cyclophosphamide intravenously. The cannula was flushed with isotonic saline for the following 15 min. Women with breast cancer also received doxorubicin

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Table 1. Brief details of patients

Patient	Sex	Age (yr)	Carcinoma	Previous radiotherapy*	Previous cytotoxic chemotherapy†
7/78	F	43	breast	—	—
9/78	F	62	bronchus	chest, 30 Gy (6 weeks)	—
12/78	F	67	breast	—	—
16/78	M	64	bronchus	—	—
17/78	F	53	bronchus	chest and spine, 20 Gy (4 weeks)	—
19/78	F	56	breast	breast, 35 Gy (4 months)	—
23/78	M	39	seminoma	>1 yr	>3 yr
24/78	M	67	bronchus	chest, 24 Gy (2 months)	—
5/79	F	54	breast	lower spine, 30 Gy (11 months)	—
7/79	M	63	bronchus	chest, 30 Gy (8 weeks)	—
8/79	F	61	breast	—	—
11/79	M	60	bronchus	chest, 32 Gy (4 months)	CPM, 3 doses (2 days)
12/79	M	62	bronchus	chest, 24 Gy (3 months)	CPM, 2 doses (10 days)
18/79	F	49	breast	spine, 20 Gy (3 days)	—
27/79	M	56	bronchus	chest, 24 Gy (3 months)	—
29/79	F	44	bronchus	chest, 24 Gy (6 weeks)	—
41/79	F	51	breast	>1 yr	CPM, 3 doses (1 month)
42/79	M	70	bronchus	chest, 24 Gy (3 months)	CPM, 3 doses (21 days)
46/79	F	25	bronchus	upper chest, 40 Gy (3 months)	CPM, 1 dose (20 days)
50/79	F	57	bronchus	chest, 28 Gy (2 months)	CPM, 1 dose (21 days)
51/79	F	45	breast	>1 yr	CPM, 1 dose (7 days)
55/79	M	47	non-Hodgkin's lymphoma	axilla, 30 Gy (2 months)	CPM, 2 doses (21 days)
63/79	F	47	bronchus	chest, 30 Gy (6 weeks)	—
69/79	F	55	mesothelioma	—	—
2/80	M	41	bronchus	chest, 32 Gy (2 months)	—
3/80	F	37	breast	>1 yr	—
4/80	M	56	bronchus	lower chest, 8 Gy (same day)	—
8/80	F	55	bronchus	chest, 24 Gy (2 months)	—

*Total dose of low LET radiation and region irradiated described only if treated 6 months or less before giving blood. Time when fractionation regimen commenced relative to giving blood in parentheses.

†The only drug given before the study started which was likely to elevate the frequency of SCE was cyclophosphamide (CPM). Time of most recent treatment before first sampling blood given in brackets.

(adriamycin) and vincristine (oncovin). Patients with carcinoma of the bronchus were given methotrexate orally 9, 13, 16 and 20 days after receiving the cyclophosphamide. Both of these regimens were repeated every 3 weeks.

Samples of venous blood were collected from individuals via the cannula before each cycle of chemotherapy and at intervals starting immediately after the venous flushing with sodium chloride.

The procedures for obtaining samples of blood and the culture, harvesting and staining of lymphocytes and recording of SCEs were carried out as described previously [14].

Briefly, 0.5 ml of whole blood was added aseptically to 5.0 ml TC199 medium at pH 7.2, supplemented with ingredients for the culture of lymphocytes and including 10 μM 5-bromo2'deoxyuridine (BrdU, Sigma, London). Cultures were incubated in total darkness for 71 hr at 37 ± 0.5°C, when 0.1 μg/ml vincristine sulphate was added. After a further 3 hr of incubation the cells were harvested in a dark room illuminated through an Ilford S902 filter using standard procedures.

The cells were stained for 15 min with Hoechst 33258 at 0.5 μg/ml in distilled water. They were exposed to a 365 nm u.v. light source 13 cm away (APW Allen, London) and then stained for 6 min with Giemsa (BDH, 0.68% w/v solution in methanol glycerol) diluted to 2% with phosphate buffer (pH 6.8).

Cells at metaphase with clearly differentially stained chromosomes were chosen. For most samples more than 1000 chromosomes within 30 cells were examined. SCEs about the centromere were counted only when clearly distinguishable from chromosome twisting. The results are

presented as a frequency of SCE per chromosome for all the cells of that sample.

RESULTS

The frequency of SCE was monitored in four patients over a period of approximately seven months (Fig. 1). Donor 23/78 had an elevated level of SCE initially. Although the frequency of SCE increased as treatment progressed, particularly for donor 7/78, the degree of increase varied. Absence of chemotherapy resulted in a reduction of SCE. Twice, after transfusion with packed red cells, a relatively high level of SCE was noted afterwards.

Immediately after the injection of cyclophosphamide the frequency of SCE increased above pre-treatment levels (Fig. 2). Four hours later the increase continued. After 20 hr some values remained stationary but some continued to rise. However, the highest frequencies of SCE appeared to be between 4 and 20 hr. After 7 days the levels had fallen but were still above pre-treatment values. In the absence of further treatment with cyclophosphamide the decrease tended to continue during the next 6 weeks.

Two fractions of SCEs were distinguishable. One of these remained for about 24 hr, and a smaller proportion persisted for as long as 35 days after treatment (Fig. 2).

Dose-response relationships were derived for the induction of SCEs immediately and 4 hr, 20 hr

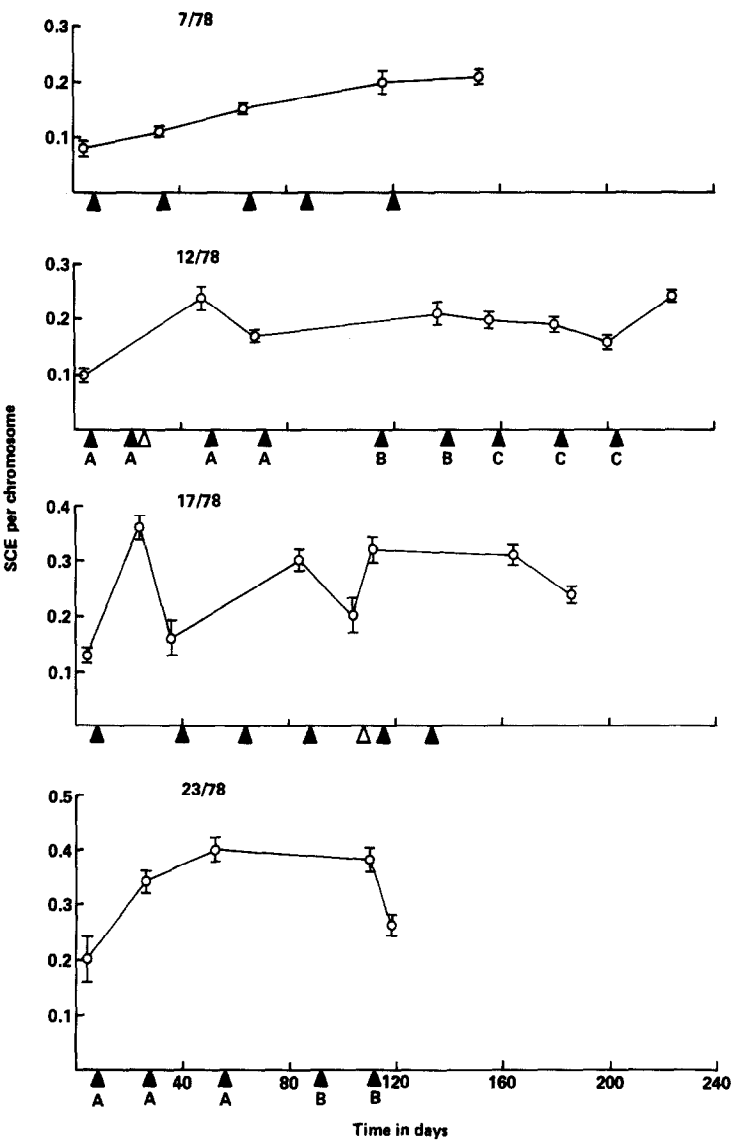

Fig. 1. Change with time of the frequency of SCE in lymphocytes of patients receiving cytotoxic chemotherapy. 7/78 carcinoma of breast ▲ 1.0 g *CPM* (+ 50 mg *doxorubicin + vincristine*). *12/78 carcinoma of breast* ▲A 0.5 g *CPM* (+ 25 mg *doxorubicin + vincristine*); ▲B 50 mg *CPM* (+ 25 mg *doxorubicin + vincristine*); ▲C *bleomycin + vincristine;* △ 4 *units packed cells. 17/78 carcinoma of bronchus* ▲ 1.4 g *CPM* (+ *sometimes methotrexate or prednisolone*); △ 2 *units packed cells. 23/78 seminoma of testis* ▲A 3.0 g *CPM;* ▲B 850 mg *CPM* (+ *actinomymycin D + vincristine*). *CPM = cyclophosphamide. Blood taken on same day before chemotherapy given. Results* ± 1 *S.E.*

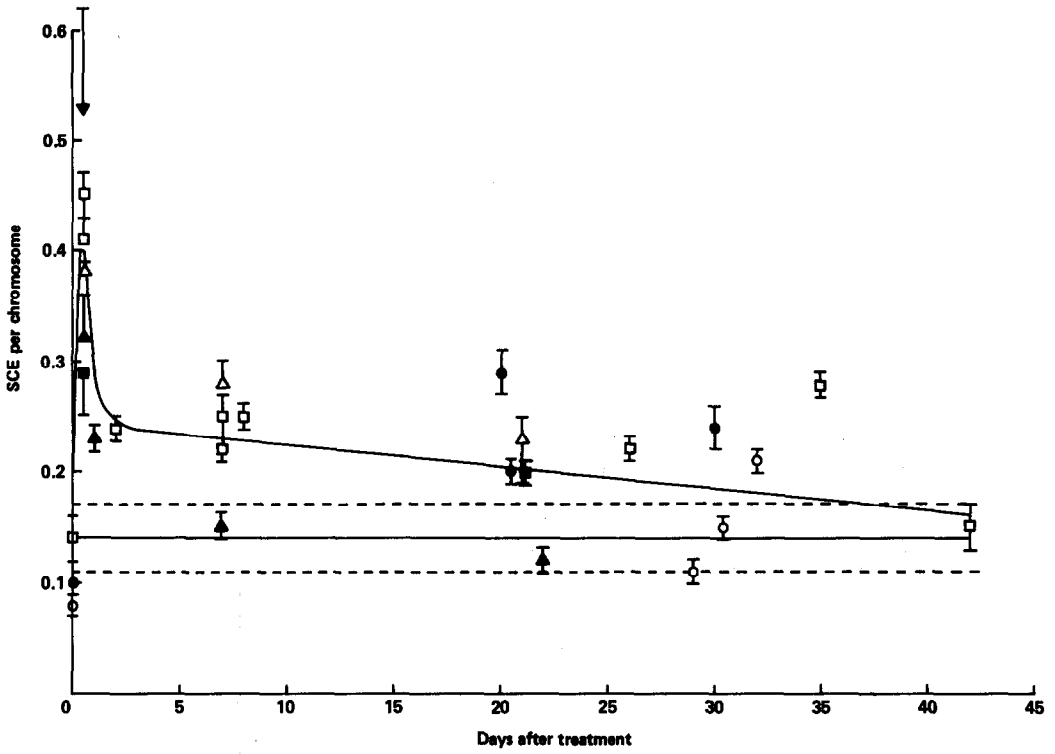

Fig. 2. The frequency of SCE in lymphocytes of women with cancer of the breast. Blood was taken at various times after injections of cyclophosphamide, usually during the first few cycles. Control value (± 1 S.D.) for subjects with cancer who had been irradiated. Best fit of line estimated. Results ± 1 S.E. ○ *7/78, 1.00 g CPM + others including doxorubicin;* ● *12/78, 0.5 g CPM + others including doxorubicin;* □ *19/78, 0.5 g CPM + others;* ▼ *5/79, 1.0 g CPM + doxorubicin;* ■ *8/79, 1.0 g CPM + others including doxorubicin;* △ *41/79, 0.5 g CPM + others;* ▲ *51/79, 0.5 g CPM + others. The other drugs included vincristine, methotrexate and 5-fluorouracil.*

and 21 days after administration of cyclophosphamide (Fig. 3). A dose of 15 mg/kg increased the pre-treatment frequency of SCE 2-fold immediately after treatment, by 6.7 times 4 or 20 hr after treatment and 1.3 times 21 days later. By contrast, an oral dose of 50 mg given to patients twice a day (approximately 0.85 mg/kg) raised the level of SCE about 2.9 times, an even higher increase per unit dose. After 21 days only the larger doses were associated with an increase in SCEs of twice or more relative to pre-treatment.

Some patients received exactly the same regimen for at least 4 courses, and a similar interval elapsed between treatment and collection of blood (Fig. 4). The pre-injection value of between 0.17 and 0.30 SCEs per chromosome was higher after several courses than before the first course (0.08–0.15 SCEs per chromosome). Lymphocytes tended to accumulate SCEs and after several courses a plateau in response was reached. Thus the effectiveness of cyclophosphamide in inducing SCEs decreased after several therapeutic cycles.

DISCUSSION

The analysis of SCE in peripheral blood lymphocytes specifically and accurately reflects the effects of cyclophosphamide *in vivo*. Even though the frequency of SCE varies with time after treatment with this drug, the changes are predictable and related to the dose. It is convenient to distinguish the SCEs into two subpopulations, one of which is frequent and disappears within about a day after treatment and the other of which is rarer but more persistent.

Blood transfusion with packed red cells was correlated with an increase in the frequency of SCE even though chemotherapy had not been administered to one of the patients. This is the reverse of what would be expected if the 'foreign' cells were diluting the numbers of damaged cells in the blood. Effects such as the preservation or storage of the donor's blood may have affected the frequency of SCE. Since very few measurements were carried out after transfusion the validity of the effect was not established.

In this study cyclophosphamide has usually been given as one of a combination of drugs. Although methotrexate, vincristine and 5-fluorouracil do not induce SCEs in peripheral blood lymphocytes, doxorubicin (adriamycin) and CCNU (lomustine) do so [11, 15]. However, the number of SCEs caused by doxorubicin appears to be relatively small. Shortly after therapy CCNU

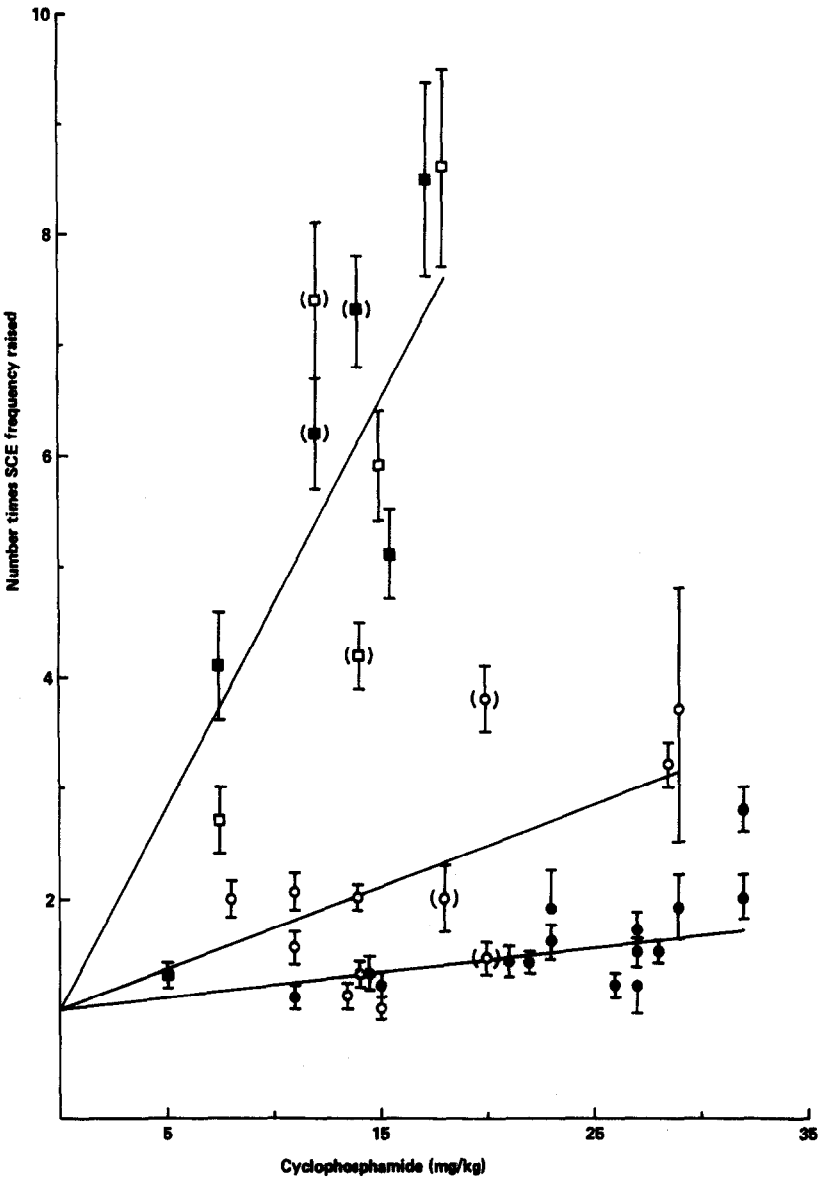

Fig. 3. The response of patients' lymphocytes at various times after injection of cyclophosphamide. ○ *Blood taken within* 5 min *after CPM and saline;* (○) *blood taken within* 5 min *after CPM and doxorubicin;* □ *blood taken* 4 hr *after CPM;* (□) *blood taken* 4 hr *after CPM and CCNU;* ■ *blood taken* 20 hr *after CPM;* (■) *blood taken* 20 hr *after CPM and CCNU;* ● *blood taken* 21 days (± 1 day) *after CPM. Other drugs, such as methotrexate, were also given during the course. Results* ± 1 *S.D.*

does not appear to be contributing to the SCEs induced by the cyclophosphamide. Since agents do not act more that additively in the production of SCEs [14] the effects of drugs other than cyclophosphamide have not been considered, though it is realized that they might have changed the time–effect relationship. The same arguments apply to the effects of radiotherapy, and most patients had previously received this form of treatment.

An increased number of SCEs, which was variable between subjects, occurred immediately after injection. It is unlikely that sufficient cyclophosphamide was transferred from the cannula to cause this damage during subsequent culture, especially when a saline infusion had intervened. Possibly the lymphocytes activated some of the drug. Alternatively, the length of time between administration and taking blood could have been sufficient for liver-mediated metabolism to have commenced.

The majority of SCEs counted shortly after treatment may have been caused by the metabolites of cyclophosphamide that were present in the plasma of the patients when blood was taken for analysis. There are three reasons for suggesting this. Firstly, the concentration of cyclophosphamide and its metabolites in the plasma closely parallels the frequency of SCEs shortly after therapy. The half-life for cyclophosphamide in

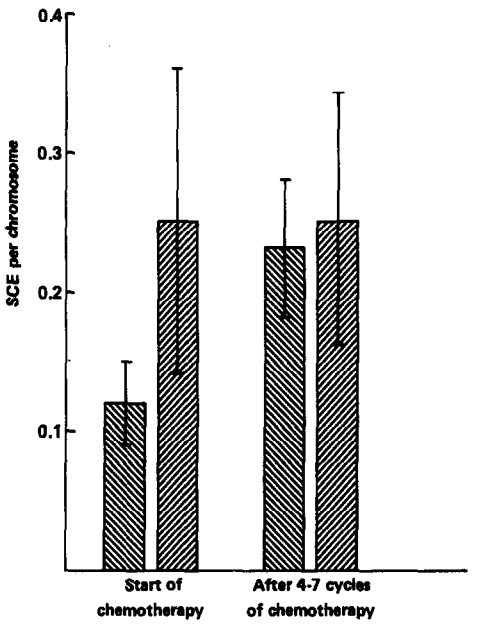

Fig. 4. The response of lymphocytes from patients 7/78, 16/78, 17/78, 7/79 and 18/79 to between 0.75 and 1.7 g cyclophosphamide. ▨ Before injection of CPM; ▩ 20–30 days after injection. Results ± 1 S.D.

the plasma is about 7.5 hr [16, 17]. Several metabolites reach their peak plasma concentrations between 4 and 6 hr after injection [18]. The half-life for a proportion of the metabolites is about 22 hr [16]. Secondly, there is considerable variability in the deposition of cyclophosphamide in different patients [16] which may be mirrored by differences in the frequency of SCE. Finally, plasma derived from patients on the same day after they had received various cytotoxic drugs increased the frequency of SCE in normal lymphocytes in proportion to the amount of plasma used [19].

On a dose per kg body weight basis, small daily doses of cyclophosphamide were more effective in inducing SCEs than large single doses. Possibly continuous exposure to the drug stimulates the activity of enzymes in the liver. Consistently high plasma levels of metabolites may be maintained in patients receiving daily doses, compared with those treated once a month.

It is conceivable that those SCEs which remain for one day or more after treatment reflect the true persistence of this type of damage and represent inherent lesions in the DNA induced in the peripheral blood lymphocytes shortly after treatment. Three weeks after the injection of cyclophosphamide the relationship between dose and SCE was maintained. This indicates a persisting causal relationship. Du Frain *et al.* [20] also concluded that, 24 hr after treating rabbits with cyclophosphamide, the induced SCEs were not caused by an ambient active mutagen during the culture of the lymphocytes.

The pattern of appearance and decline in the number of SCEs in the lymphocytes of patients treated with drugs such as mitomycin C, CCNU or melphalan appears to be specific to the agent administered [15, 21]. It has been suggested that drugs produce dissimilar types of lesions in the DNA, which cellular repair mechanisms have different capacities to remove [15]. However, a particular combination of agents is usually used to combat each type of malignancy. Thus the disease and its severity may also modify the response.

The same regimen was usually less effective in inducing SCE than it was at the beginning. The half-life of cyclophosphamide may be shorter in patients who have received it for a long period compared with at the start of chemotherapy [17]. The altered responses may therefore reflect changes in the pharmacokinetics of the drug. Alternatively, if SCEs represent the outcome of a repair mechanism [22], this trend indicates that the cells of patients with cancer that are exposed to cytotoxic drugs on many occasions become less able to respond to the insult. If so, SCEs could be used to predict the effects of continuing chemotherapy, so that treatment could be tailored to the needs of individual patients.

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