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Original Paper

Haematological and Non-haematological Toxicity After 5-Fluorouracil and Leucovorin in Patients with Advanced Colorectal Cancer is Significantly Associated with Gender, Increasing Age and Cycle Number

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5-Fluorouracil (5-FU) has been widely used for over 30 years. Recently, investigators have described interactions between toxicity with 5-FU and age and gender. Pharmacokinetics of infusional 5-FU are known to be gender dependent, with drug clearance being lower in females. The full impact of age and gender on both toxicity and response has not been fully explored and is worthy of further investigation. 439 patients were entered into a phase III trial comparing a novel thymidylate synthase (TS) inhibitor Tomudex™ (raltitrexed, formerly ZD1694) with 5-FU and leucovorin (LV) for the treatment of advanced colorectal cancer. Approximately 20–24% of patients in each treatment group were aged 70 years or older and 41% of the patients were female. In a multiple regression analysis, female patients receiving 5-FU + LV experienced significantly more grade 3/4 leucopenia, whilst those receiving raltitrexed had more rises in transaminase levels. Grade 3/4 leucopenia and mucositis were significantly correlated with age (especially > 70 years) only in patients receiving 5-FU + LV. Patients receiving 5-FU + LV were significantly more at risk of experiencing grade 3/4 haematological and non-haematological toxicity in the first three cycles than patients receiving raltitrexed. Female gender and increased age predict for increased grade 3/4 toxicity in patients receiving modulated 5-FU. Further studies with modulated 5-FU which utilise a modified dose reduction schema for female patients, or patients aged 70 years or over, may be appropriate. © **1998 Elsevier Science Ltd. All rights reserved.**

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INTRODUCTION

CANCER IS a disease of the elderly; with the continuing ageing of Western populations, the median age of patients treated for cancer is likely to increase further. However, despite the frequency of malignancies in patients considered elderly (i.e. over 70 years), systematic studies addressing age-related dose–response and toxicity issues are limited. This lack of specific clinical data may be compounded by the reluctance of physicians to treat elderly patients with advanced cancer, or to enter them into clinical trials, due to concerns for potential negative effects of drug toxicity on quality of life (QoL) and general well being.

Previous studies have demonstrated that dose intensity is an important predictor of outcome for a variety of different tumour types and that patients treated with inadequate doses of chemotherapy are relatively disadvantaged [1–3]. Increasingly, therefore, older patients of good performance status are being treated with full doses of chemotherapy and many clinical trials no longer have an upper age limit. Knowledge and understanding of the age-related kinetics and toxicity of

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commonly used chemotherapeutic agents is thus becoming increasingly important.

A number of published studies have demonstrated that the elderly may tolerate certain chemotherapeutic agents (such as anthracyclines, vinca alkaloids and nitrosoureas) less well than younger patients [4, 5]. A recently published retrospective study demonstrated age dependent effects for 5-fluorouracil (5-FU), but in addition demonstrated gender dependent toxicity in a smaller cohort of patients [6], although none of the 5-FU regimens used in the study underlying this analysis are commonly used at present. The pharmacokinetics of 5-FU when given as an infusion are known to be gender dependent, with drug clearance being lower in females [7]. The mechanism of this is unclear, but the authors postulated a role for gender-related differences in dihydropyrimidine dehydrogenase (DPD) activity.

During the analysis of a randomised phase III trial comparing a novel thymidylate synthase (TS) inhibitor Tomudex™ (raltitrexed) with 5-FU plus leucovorin (LV) for the treatment of advanced colorectal cancer [8], it was noted that in patients receiving 5-FU, age, gender and cycle number appeared to influence severe haematological and gastrointestinal toxicity, an effect that was not seen in the group treated with raltitrexed. We present here the results of the formal analyses of these data.

PATIENTS AND METHODS

Patients and treatment

Patients in Europe, Australia and South Africa were entered into a randomised clinical trial comparing the efficacy and safety of raltitrexed with 5-FU + LV. Entry criteria included the presence of histologically confirmed advanced colorectal cancer, the absence of prior chemotherapy for advanced disease, or adjuvant therapy within 12 months of randomisation, the absence of severe intercurrent medical conditions, performance status ≤ 2, and acceptable haematology (white cell count (WBC) $\geq 4\,10^9$/l, neutrophil count (PMN) $\geq 1.5\,10^9$/l, platelet count $\geq 100\times10^9$/l) and biochemistry (creatinine $< 1.5\times$upper limit of normal range (ULNR), (transaminases $< 2.5\times$ULNR (unless due to documented hepatic metastases) and bilirubin $< 1.5\times$ULNR. All patients gave informed consent; institutional review board approval was obtained at all centres and the study was performed according to the principles of the Declaration of Helsinki [9].

Methods and assessments

Patients were randomised to receive either raltitrexed 3.0 mg/m^2 given as a short intravenous (i.v.) infusion every 3 weeks, or 5-FU 425 mg/m^2 and LV 20 mg/m^2 each as a bolus injection, for 5 days every 4–5 weeks (the so-called 'Mayo regimen' [10]). Treatment was continued until there was evidence of tumour progression.

Patients were assessed weekly for toxicity and haematological changes, and every 3 weeks for biochemical changes. Tumour size was estimated every 3 months and responses were recorded using UICC criteria [11].

Toxicity and laboratory results were graded using standard WHO criteria [12]. All data were source data verified, and data management and analysis was performed by the Biometrics Unit at Zeneca Pharmaceuticals. Creatinine clearance was calculated using the Cockcroft–Gault formula [13].

Statistical analyses

For the purposes of statistical analysis, the age variable was redefined as < 60, 60–69 and ≥ 70 years to produce groups of roughly similar size. As raltitrexed is cleared via the kidney, creatinine clearance was considered to be a key variable that may affect relative toxicity, particularly given the fact that the toxicity of raltitrexed is greater in patients with renal dysfunction (data not shown). Creatinine clearance was dichotomised as 'up to and including 65 ml/min' and 'more than 65 ml/min', based on clinical interpretations of values indicating normal and impaired renal function. Lean body mass (LBM) using total body weight (TBW) and height (HT) was calculated for males [(LBM = 1.10TBW-120(TBW/HT)^2] and females [1.07TBW-148(TBW/HT)^2].

Univariate analyses were carried out comparing the incidence of grade 3/4 toxicity in relation to age group, gender and creatinine clearance separately. For comparisons between dichotomised groups, i.e. gender and creatinine clearance, the continuity corrected chi-square test was used, and for comparing age groups, logistic regression analysis was used.

In addition to univariate analyses, multiple regression analyses [14], were carried out using logistic regression models which included as independent variables all three variables (age group, gender and creatinine clearance) simultaneously. The objective of multiple regression analysis was to estimate the effect of each variable in isolation by adjusting for the simultaneous effect of the other two, thus diminishing the possible confounding effects that variables may exert on each other. Interaction terms were not included in models in order to avoid over complexity and because such interactions are biologically implausible.

For comparisons of raltitrexed and 5-FU + LV toxicity by cycle, the continuity corrected chi-square test was used at each cycle separately. The objectives of the analysis of factors predicting 5-FU + LV toxicity arose from previously published analyses, so it was not considered necessary to adjust *P* values for multiple comparisons. All tests were, therefore, carried out at the 5% level of statistical significance.

All comparisons between treatments, except for patient characteristics (Table 1), were carried out according to the treatment received irrespective of the treatment randomly allocated, because this was considered to be important in the interpretation of safety data. Patient characteristics were

Table 1. *Raltitrexed versus* 5*-fluorouracil plus leucovorin (*5*-FU + LV)*: demographic characteristics of patients*

	Raltitrexed $n = 223$ (%)	5-FU + LV $n = 216$ (%)
Mean age (years)	61	61
Male	133 (60)	127 (59)
Measurable disease	215 (96)	203 (94)
Prior adjuvant therapy	12 (5)	10 (5)
Disease site		
Liver	173 (78)	165 (76)
Lung	56 (25)	62 (29)
Nodal	45 (20)	41 (19)
Adverse factors†	78 (35)	80 (37)

*Treatment groups as randomised. †ECOG performance status (PS) = 2, liver metastases > 5×5 cm, or > 50 cm^2, (PS 2 = ambulatory and capable of all self-care, but unable to carry out any work. Up and about more than 50% of waking hours).

Table 2. Raltitrexed versus 5-fluorouracil plus leucovorin (5-FU + LV): gender, age and creatinine clearance

	5-FU + LV $n = 212$ (%)		Raltitrexed $n = 222$ (%)	
	Male $n = 125$	Female $n = 87$	Male $n = 132$	Female $n = 90$
Creatinine clearance (ml/min)				
≤ 65	33 (26)	40 (46)	34 (26)	42 (47)
> 65	92 (74)	47 (54)	96 (73)	48 (53)
Age (years)				
< 65	51 (41)	34 (39)	55 (42)	36 (40)
60–69	46 (37)	34 (39)	50 (38)	32 (36)
≥ 70	28 (22)	19 (22)	27 (20)	22 (24)

compared between groups formed by random allocation of treatment.

RESULTS

The study period extended from November 1993 to September 1994. A total of 439 patients were entered into the trial, 216 patients received 5-FU + LV and 223 received raltitrexed. Patient characteristics are presented in Table 1 and the distributions were similar between the two treatment groups as randomised.

The median follow-up was 18 months. The overall objective response rate was 19% (43/223) for patients randomised to raltitrexed and 17% (36/216) in patients randomised to 5-FU + LV (odds ratio 1.2, 95%, confidence interval (CI) 0.73–1.97, $P = 0.480$). There were no statistically significant differences in time to progression or survival between the two groups [8].

Approximately 20–24% of patients in each group were aged 70 years or older, and the proportion of males to females in this group was similar to that in other age groups (Table 2). Female patients were more likely to have a lower calculated creatinine clearance at entry and there were almost equal numbers of females in the two treatment groups (Table 2). In addition, all patients met the entry criteria requiring serum creatinine to be within 1.5×ULNR. Dose reductions for raltitrexed and 5-FU + LV were based on predefined criteria pertaining to WHO grading of haematological (neutrophil and platelet counts) and non-haematological (mucositis, diarrhoea and rash) effects seen with the preceding course of treatment.

The most common grade 3/4 toxicities reported were leucopenia, mucositis, diarrhoea and nausea and vomiting for patients receiving 5-FU + LV, and leucopenia, diarrhoea, nausea and vomiting and reversible increases in transaminases in patients receiving raltitrexed (Table 3). Although asthenia has been described in patients receiving raltitrexed, it occurred uncommonly, with a frequency of severe asthenia of 5.9% in the raltitrexed group. In contrast, severe asthenia occurred in 1.9% of patients receiving 5-FU + LV. Because of the number of adverse events in Table 3, the nominal significance level of each test was adjusted using Holm's procedure [15]. Statistically significant differences between the two treatment groups were noted for leucopenia and mucositis, which were more severe in the 5-FU + LV group and anaemia or reversible asymptomatic transaminase increases, which were more common in the raltitrexed group. There were a total of 312 deaths in the entire study, although these were equally distributed (164 in the raltitrexed treatment group and 148 in the 5-FU + LV treatment group).

In the multiple regression analysis, female patients receiving 5-FU + LV had significantly more grade 3/4 leucopenia ($P < 0.001$) (Table 4), and female patients receiving raltitrexed had significantly more increases in transaminase levels ($P < 0.001$) (Table 5). Univariate analysis of toxicity and LBM (data not shown) revealed a statistically significant relationship between low LBM and grade 3/4 leucopenia for patients receiving 5-FU + LV ($P = 0.009$). There was a (non-significant) trend for the association of mucositis with low LBM for modulated 5-FU ($P = 0.07$) and elevations in transaminases in the raltitrexed group ($P = 0.08$). However, on multivariate analysis, the effect of LBM on grade 3/4 leucopenia was not apparent, suggesting that the univariate effect was due to gender and to the lower LBM in females compared with males.

Grade 3/4 leucopenia and mucositis were significantly correlated with age only in patients receiving 5-FU + LV, especially in patients aged 70 years or more (Table 4). There was no protocol requirement for the use of ice-chips. Interestingly, increased age was associated with significantly less nausea and vomiting for the 5-FU + LV group, the explanation for which is uncertain. There does not appear to have been any difference in the use of prophylactic anti-emetics amongst older patients.

The occurrence of grade 314 haematological (leucocyte, platelet, haemoglobin and neutrophils) and non-haematological (diarrhoea, mucositis, rash) toxicity by cycle was also examined and showed that patients receiving 5-FU + LV

Table 3. Raltitrexed versus 5-fluorouracil plus leucovorin (5-FU + LV): incidence of grade 3/4 toxicity

	Raltitrexed $n = 222$ (%)	5-FU + LV $n = 212$ (%)	P value
Adverse event			
Anaemia	9	2	< 0.001
Asthenia*	6	2	ns
Leucopenia	14	30	< 0.001
Thrombocytopenia	4	1	ns
Transaminase increase	10	0	< 0.001
Diarrhoea	14	14	ns
Constipation	3	3	ns
Nausea/vomiting†	13	9	ns
Mucositis	2	22	< 0.001
Pain	5	7	ns
Fever	3	2	ns
Infection	5	5	ns

*Intensity severe (event not WHO graded). †Prophylaxis not recommended in protocol. ns, not significant.

Table 4. *5-Fluorouracil plus leucovorin (5-FU + LV) group: incidence of WHO grade* 3/4 *toxicity: influence of gender, creatine clearance (CrCl) and age*

	Female $n = 87$ (%)	Male $n = 125$ (%)	CrCl ≤ 65 ml/min $n = 73$ (%)	CrCl > 65 ml/min $n = 139$ (%)	Age < 60 years $n = 85$ (%)	Age 60–69 years $n = 80$ (%)	Age ≥ 70 years $n = 47$ (%)
Adverse event							
Anaemia	0	4	0	4	5	1	0
Asthenia (severe)	2	2	3	1	1	1	4
Leucopenia	39*†	23*†	32	29	21*‡	32*‡	40*‡
Thrombocytopenia	0	1	0	1	1	0	0
Transaminase increase	0	0	0	0	0	0	0
Diarrhoea	17	11	12	14	19	13	6
Constipation	2	3	3	3	6	0	2
Nausea/vomiting	9	10	10	9	17*§	5*§	4*§
Mucositis	28	18	27	19	11*‖	26*‖	36*‖
Pain	8	6	8	7	11	4	6
Fever	2	2	4	1	1	3	4
Infection	6	5	7	4	5	4	9

*$P < 0.05$ on multivariate analysis; †$P < 0.001$ on univariate analysis; ‡$P = 0.02$ on univariate analysis; §$P = 0.01$ on univariate analysis; ‖$P = 0.0006$ on univariate analysis.

Table 5. *Raltitrexed group: incidence of WHO grade* 3/4 *toxicity: influence of gender, creatinine clearance (CrCl)* and age*

	Female $n = 90$ (%)	Male $n = 132$ (%)	CrCl ≤ 65 ml/min $n = 76$ (%)	CrCl > 65 ml/min $n = 144$ (%)	Age < 60 years $n = 91$ (%)	Age 60–69 years $n = 82$ (%)	Age ≥ 70 years $n = 49$ (%)
Adverse event							
Anaemia	6	11	9	8	10	7	10
Asthenia (severe)	4	7	5	6	8	6	2
Leucopenia	14	14	16	13	13	11	22
Thrombocytopenia	3	5	4	3	2	4	8
Transaminase increase	16†‡	7†‡	15	8	11	13	4
Diarrhoea	10	17	16	12	12	13	20
Constipation	4	2	3	3	4	2	0
Nausea/vomiting	14	12	17	11	12	12	16
Mucositis	3	2	4	1	2	1	4
Pain	6	5	7	4	6	4	6
Fever	2	3	5	1	3	1	4
Infection	6	5	7	4	0	6	14

*Creatinine clearance could not be calculated for 2 patients. †$P < 0.05$ on multivariate analysis; ‡$P < 0.001$ on univariate analysis.

were significantly more at risk of experiencing this toxicity in the first three cycles than patients receiving raltitrexed (Table 6).

DISCUSSION

Increasingly, elderly patients are receiving cytotoxic chemotherapy, due in part to the increasing age of the population, but also to increasing acceptance of the benefits of chemotherapy for older patients. Although optimal dose intensity is recognised as an important predictor of response and prognosis, recent publications suggest that factors such as age and gender may significantly predict for increased toxicity.

Table 6. *Raltitrexed versus* 5*-fluorouracil plus leucovorin (5-FU + LV): percentage of patients at each cycle with WHO grade* 3/4 *haematological* and/or non-haematological toxicity*†

Cycle number	Raltitrexed (%)	5-FU + LV (%)	*P*
1	6	39	< 0.0001
2	8	27	< 0.0001
3	11	21	0.018
4	11	16	0.32
5	11	12	1.0
6	6	16	0.07

*Leucocyte, neutrophil and platelet count. †Diarrhoea for raltitrexed, diarrhoea, mucositis and rash for 5-FU + LV.

Whilst this study confirms that 5-FU + LV therapy is significantly less well tolerated in older patients, especially those aged 70 years or over, these effects were not seen in patients receiving raltitrexed. Pharmacokinetic studies have not demonstrated altered handling of 5-FU in the elderly and, thus, the mechanisms of increased toxicity may be related to other factors, such as diminished organ function [16], or the presence of other medical conditions. A significant impact of gender on haematological and non-haematological toxicity described by Stein and co-workers [6] has been confirmed in this study. Altered pharmacokinetics in female patients have been described by Milano and colleagues [7], and are likely to be responsible for the increased toxicity seen in females. Although these authors postulated that gender-related differences in DPD activity may explain their findings, it is interesting to note the significant relationship with LBM found in this study. These associations were not confirmed on multiple regression analysis and, thus, differences in LBM could not

be shown to explain the gender-based differences in the incidence of grade 3/4 leucopenia in this study. The significant impact of age and gender on haematological and non-haematological toxicity seen in patients receiving modulated 5-FU was only seen for grade 3/4 hepatotoxicity in patients receiving raltitrexed.

The demonstration of drug specific (modulated 5-FU) associations of haematological and gastrointestinal toxicity with age and gender in this comparative study suggests that the demonstrated effects are not merely a reflection of the altered handling of chemotherapeutic agents, in general, in older patients or women, but rather are specific to modulated 5-FU. Interestingly, the increased toxicity noted in female patients receiving modulated 5-FU did not translate into an advantage in terms of objective response (data not shown). There was no gender-related impact on time to progression or survival, and the potential interaction between toxicity and response rate remains unclear.

In conclusion, female gender and increased age predict for increased grade 3/4 toxicity in patients receiving modulated 5-FU. Further studies which utilise an appropriate dose reduction schema for female patients, or for patients aged 70 years or over, are appropriate and relevant stratification for gender may be useful in randomised trials. Alternatively, the use of drugs not associated with these effects is indicated.

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