A phase II randomized study of Combined Infusional Leucovorin Sodium and 5-FU versus the Leucovorin Calcium followed by 5-FU both in combination with Irinotecan or Oxaliplatin in patients with metastatic colorectal cancer

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Abstract

Background : **Leucovorin Sodium (LV/Na) has a high solubility, and is stable when given with continuous infusion of 5-FU. It could maintain significant plasma concentration of 5, 10-meTHF during the whole 5-FU perfusion with the potential of increasing 5-FU cytotoxicity. We conducted a randomized phase II clinical trial on leucovorin calcium (LV/Ca) and LV/Na in metastatic colorectal cancer patients (mCRC). Main objectives were to assess efficacy and safety.**

Patients and methods : **Fifty seven patients with mCRC and no previous chemotherapy for metastatic disease were randomized to receive LV/Na or LV/Ca with irinotecan or oxaliplatine combined with infusional 5-FU. LV/Na was defined as warranting further evaluation in phase III if true overall response rate (ORR) > 35% ($\alpha = 5\%$, $\beta = 10\%$ in case of true ORR > 55%, 51 evaluable patients planned/arm).**

Results : **Results for LV/Ca and LV/Na arm respectively were : observed ORR, 55% (significantly higher than 35%, p = 0.02) and 61% (p = 0.004). Median overall survival durations were 11.9 months and 22.9 months (p = 0.02) and PFS 8.0 vs. 11.5 months (ns). Grade ≥ 3 events were 64% and 46% (p = 0.28).**

Conclusion : **Both LV/Na and LV/Ca disclosed an ORR > 35% with comparable safety.** (Acta gastroenterol. belg., **2012**, 75, **14-21**).

Key words : colorectal cancer, chemotherapy, leucovorin continuous infusion.

Introduction

5-fluorouracil (5-FU) was synthesized in 1957 (1). Still widely used in many cancers, it remains pivotal for colorectal cancer (CRC) treatment. 5-FU acts as an antimetabolite and is active only during the S-phase of the cellular cycle. It interferes with the metabolism of uracil by substituting the hydrogen atom in position 5 with a fluorine atom and utilizes the same transport system and activation pathway as uracil and thus may alter the cellular function and result in cell death. Although delivering LV together with 5-FU was appealing, this approach was hampered by the incompatibility of LV/Ca with 5-FU containing solutions (2). New production techniques allow now the manufacturing of the pure sodium salt of leucovorin, disodium leucovorin (LV/Na, Vorina, Teva Belgium, Wilrijk, Belgium) that contains the same active ingredient, folinic acid, than LV/Ca the only difference being the pharmaceutical form : Na instead of Ca. When compared to LV/Ca, LV/Na has a higher solubility, enabling highly concentrated solutions and good compatibility with 5-FU containing solutions (3,4).

The present study was designed to assess, in terms of objective response rate (ORR), whether the combined LV/Na and 5-FU, in combination with standard Irinotecan or Oxaliplatin, has a sufficient activity to be further compared in a phase III superiority trial to the standard sequential administration of leucovorin calcium (LV/CA) and 5-FU and to explore safety and tolerability of this combined infusional approach.

Material and Methods

End-points and design

The primary endpoint for efficacy was the best objective response (OR) (using RECIST criteria version 1.0) (5) during the treatment period. Secondary endpoints included : safety [NCI Common Toxicity Criteria for Adverse Events (CTCAE, version 3.0) (6)], progression free survival (PFS), time to treatment failure (TTF) and overall survival (OS).

The study was a multicenter randomized Phase II with a 1:1 randomization ratio.

Eligibility Criteria

Eligibility criteria were histologically proven adenocarcinoma of the colon or rectum, progressive or histologically proven non-resectable metastases at presentation, measurable disease, no central nervous system metastasis, no exclusive bone metastases, no second malignancy (except adequately treated in situ carcinoma of the cervix or nonmelanomic skin cancer), absence of any uncontrolled medical condition, age between 18 and 75 years old, World Health Organization (WHO) performance

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status 0 to 2, no previous therapy for metastatic disease, no previous adjuvant therapy if completed less than 6 months before inclusion, clinical evaluation 3 weeks or less prior to randomization, including : leukocytes ≥ 3,000/mm^3, platelets ≥ 100,000/mm^3, adequate renal function : creatinine clearance ≥ 51 mL/min, total bilirubin ≤ 2 upper normal limit (ULN), ASAT/ALAT ≤ 2.5 ULN or ≤ 5 ULN in case of liver metastasis.

Human investigations were performed after approval by each institutional Ethic Committees. Written informed consent was obtained according to ICH/GCP, and national/local regulations.

Randomization

Randomization was performed centrally (through a secure web-based system) using the minimization algorithm which allows to stratify for several factors which were : the Institution, previous adjuvant therapy (Yes *vs* No), standard drug for combination (Oxaliplatin or Irinotecan), the risk groups (poor, intermediate, and good) as determined by Köhne *et al*. (8) by means of the concentration of alkaline phosphatase (≤ or > 300 U/l)) and three other variables (*i.e*. the WHO performance status (0-1 *vs* 2), the white blood cell count (> or ≤ 10*10^9/L), and the number of metastatic sites (1 *vs* > 1)).

Chemotherapy

Arm 1 (LV/CA) : irinotecan 180 mg/m^2 IV over 30 to 90 minutes or Oxaliplatin 100 mg/m^2 IV over 120 minutes, LV/CA 400 mg/m^2 over 2 hours, followed by 5-FU bolus 400 mg/m^2, followed by continuous infusion of 5-Fu 3000 mg/m^2 IV over 46 hours. **Arm 2** (LV/Na) : irinotecan 180 mg/m^2 IV over 30 to 90 minutes or Oxaliplatin 100 mg/m^2 IV over 120 minutes, LV/Na 400 mg/m^2 plus 5-FU 3000 mg/m^2, IV continuous infusion over 46 hours. In arm 2, for the first 10 patients, 5-FU dosage in first cycle administration was 2400 mg/m^2, and was raised to 2700 mg/m^2 and to 3000 mg/m^2 if no grade ≥ 2 non-haematological toxicity and no grade ≥ 3 hematological toxicity occurred.

Patients had to be treated until progression of the disease or unbearable toxicity.

Study Parameters

The pre-treatment evaluation consists of : history, physical examination, ECG, chest-X-ray, abdominal ultrasound, CT-scans, MRI was recommended in case of pelvic mass, routine blood tests, routine serum biochemistry, WHO performance status, measurements of measurable lesions according to RECIST criteria version 1.0.

Hematological toxicity was evaluated weekly during the first 8 weeks and then prior to next cycle. The following evaluations were performed before every cycle of treatment : history and physical examination, review of adverse events and concomitant medications, WHO performance status, routine blood tests and routine biochemistry to assess toxicity.

The following evaluation were performed every 8 weeks : evaluation of tumor response for all measurable lesions by chest-X-ray, MRI in case of pelvic mass, ultrasound or CT-scan whatever was appropriate.

After the end of treatment, further follow-up and treatment was done according to the hospital's routine practice. The date of progression, death or last contact was collected for all patients

Measurement of efficacy

Response

Responses were assessed, according to RECIST criteria version 1.0, by the investigators and reviewed by an independent panel of radiologists at the end of the study.

Analysis

The ORR was defined as the sum of the percentages of patients achieving a complete response (CRR) or a partial response (PRR) (ORR = CRR + PRR) as best response during the treatment period. Patients with stable disease, immediate progression, early death or unassessable were considered as failing to respond to treatment and classified in the same category (failure)

Overall Survival (OS) was defined as the time interval between the date of randomization and the date of death or date of last contact.

PFS was defined as the time interval between date of randomization and the date of first documentation of progression or death whichever occurs first. Patients still alive with no evidence of disease progression at the time of their last visit were censored at the time of the last visit. TTF was defined as the time interval between the date of randomization and the date of the first of the following events : documented progression, death or any other event leading to unplanned treatment discontinuation.

We carried out an ITT analysis for response and time-to-event variables (all randomized patients were included in the analysis in the arm they were allocated) ; safety analysis was based on patients who started treatment.

Statistical Considerations

The study used a Fleming's one-stage design (9). Sample size was a priori estimated considering the following expectations for the experimental treatment : a response rate < 35% was judged unacceptable, requiring treatment rejection at the end of the trial. A response rate of 55% or more, if true, had to be detected with a power of 90% and lead to the conclusion that the experimental treatment deserves further testing in a phase III trial formally designed with a comparative purpose. The probability of type I error (α) – falsely selecting the regimen as active – was set to 5%. Fifty one patients were required in arm 2 and, therefore, 102 for the whole study.

The ORR and their 95% confidence intervals are provided for both treatment groups. Testing the null

hypothesis that the true ORR is less than 35% in the experimental arm was the primary analysis.. Further to that test, exploratory inferential comparisons were performed between both treatment groups. Secondary efficacy endpoints were analyzed and reported, per treatment group, using Kaplan-Meier plots, logrank tests and estimates of median of the time to event distributions. In addition, treatment differences were described using estimated hazard ratios derived from Cox Proportional Hazard models.

Due to the exploratory nature of the comparisons, no adjustment for multiplicity was done. A p value less than 5% was considered as statistically significant.

Results

Administrative data

The study was opened on October 10 2005 and was previously submitted to health authorities in accordance with Directive 2001/20/EC and was given the following EudraCT n° : 2004-004901-12. Fifty seven patients were accrued out of 102 planned 29 in the control arm and 28 in the experimental arm. Study was closed on October 28, 2008 due to a poor accrual. All patients were eligible and the analysis was done on 57 patients for all endpoints except for safety where we excluded one patient (in the control arm) that never started treatment. As not treated, this patient was also excluded when administered doses of chemotherapy drugs are reported.

Pretreatment patient characteristics

Patient characteristics are displayed in Table 1. The variables listed are distributed without significant differences between the two treatment arms. Most of the patients were colon cancers. None had received adjuvant chemotherapy. Although almost all had a WHO PS of 0-1, about two third disclosed extensive disease as evidenced by ≥ 2 metastatic sites, > 20 times normal CEA levels, increased LDH and alkaline phosphatase.

Chemotherapy

Oxaliplatin and irinotecan were evenly distributed among treatments arms. Median dose of oxaliplatin was 900 mg/m^2 (range 280-1200) and 800 mg/m^2 (range 400-1200) in the LV/Ca and LV/Na arm, respectively. The median dose of irinotecan was 2295 mg/m^2 (100-3600) and 2160 mg/m^2 (500-8055) respectively. The main reasons for treatment discontinuation were progressive disease 29.2 % vs. 25.9%, surgery for resectable disease, 12.5% vs. 14.8%, toxicity, 20.8% vs. 22.2% and patient convenience 16.7% vs. 18.5% for LV/Ca and LV/Na, respectively.

Toxicity

LV/Na based chemotherapy was well tolerated. None of the 10 patients who started 5-FU at 2400 mg/m^2/2 days, had significant side effect and all could pursue their treatment at the target dose of 3000 mg/m^2/2 days. Eighteen patients in the LV/Ca arm and 13 in the LV/Na arm had at least one grade 3-4 adverse event (AE). The number of patients with more than one grade 3-4 AE was not higher in the LV/Na arm (Table 2).

Nausea, diarrhea and fatigue were the most frequent side effects related to both treatment arms and the majority was graded 1-2. Neutropenia was the most frequently observed grade 3-4 AE and occurred in 14.3% and 17.9% of cases in the LV/Ca and LV/Na arm, respectively (Table 3). The rates of patients having developed at least one grade 3 or 4 event were respectively 13/28 (46%, exact 95% CI : 28%-66%) in the leucovorin Na arm and 18/28 in the leucovorin Ca arm (64%, exact 95% CI : 44%-81%) (p = 0.28).

Objective response rate

Table 4 shows the distribution of best response for all randomized patients (n = 57) and table 5 for a subset of patients (n = 41) for whom a review of response by independent experts was done. Reasons for not performing the review were essentially the unavailability of images as well as the absence of measurable or cancer lesions on baseline scan.

Reason for classifying patients as “other failure” was :

In LV/Na arm : treatment stops due to AE and no post-treatment imaging

In LV/Ca : too long delay between chemotherapy cycles, treatment stop due to SAE, progressive disease documented clinically only, and death due to lung embolism.

If we consider all randomized patients (intent to treat approach), the ORR is respectively 16/29 or 55% (95% CI : 36% to 74%) for the LV/Ca arm and 17/28 or 61% (95% CI : 41% to 79%) for the LV/Na arm according to the assessment done by the investigators. The hypothesis that ORR $\leq$ 35% is rejected for both treatment arms with p value of 0.02 for the LV/Ca arm and of 0.004 for LV/Na arm.

Scans were available for review by an independent expert panel in 46 patients out of the 51 patients for whom the investigators assessed response on the basis of radiological images. In 5 out of these 46 patients, no measurable lesion was retrieved (3 patients) or even no cancer lesion was found (angiomas in 1 patient, biliary cysts in 1 patient). We therefore looked at concordance between the two assessments on 41 patients.

In terms of objective response, there were 4 responses that were not confirmed (out of 27), 3 in the control arm and 1 in the experimental arm. On the other hand, 4 patients initially assessed as failures were reviewed as responders by the independent panel, all in the experimental arm. Concordance as measured by the Kappa coefficient was only 0.55 (95% CI : 0.27-0.82) and can be qualified as moderate according to the Landis and Koch scale.

Table 1. — **Patient characteristics (n = 57)**

Baseline Characteristics	Leucovorin Na	Leucovorin Ca	Total %
No. of patients	28	29	100.0
Gender			
Male	18	16	59.7
Female	10	13	40.4
> Age, years			
Median	69,1	65.3	
Range	51.4-80.6	41,4-85.0	
> WHO PS Grade			
0	14	18	56.1
1	13	10	22.8
2	1	1	3.5
> Primary site			
Colon	24	23	82.5
Rectum	4	6	17.5
> Metastatic site (s)			
Liver	27	27	94.7
Lung	10	10	35.1
Other	13	13	45.6
> No. of sites			
1	10	11	36.8
> 1	18	18	63.2
> CEA			
Normal	5	3	14.0
1-20 × normal	10	7	29.8
> 20 × normal	10	16	45.6
Unknown	3	3	10.5
> Alkaline phosphatase			
Normal	13	10	35.7
Increased	15	18	59.6
> LDH			
Normal	13	10	40.4
Increased	15	19	59.6
> Adjuvant chemotherapy			
Yes	0	0	0.0
No	25	26	89.5
Missing	3	3	10.5
> Chemotherapy including :			
oxaliplatin	16	16	56.1
irinotecan	12	12	42.1
not tretated	0	1	1.8

Follow-up

Median length of follow-up estimated by the reverse Kaplan-Meier method was 21.2 months. Follow-up was complete for survival in 30 patients (17 in the control arm and 13 in the experimental arm). For progression free survival, progression documentation was obtained in 21/29 in the LV/Ca arm and 22/28 in the LV/Na arm.

Survival

Overall survival was 11.9 months (95% CI, 9.1-19.8) and 22.9 months (95% CI, 16.6-not reached) forLV/Ca and the LV/Na respectively (p = 0.02) (Fig. 1) Progression free survival (Fig. 2), time to treatment failure (not shown) were not significantly different with medians of 8.0 vs. 11.5 months and 6.8 vs. 6. 7 months respectively.

Table 2. — **Number of patients with grades 3-4 Adverse Events (n = 56°)**

EVALUATION	leucovorin Na (n = 28)	leucovorin Ca (n = 28)
Number of grade 3-4 AE per patient		
0	15 (53.6)	10 (35.7)
1	5 (17.9)	9 (32.1)
2	3 (10.7)	3 (10.7)
> 2	5 (17.8)	6 (21.4)
Number (%) of patients with at least one grade 3-4 AE	13 (46.4)	18 (64.3)

° One patient was excluded because never started treatment.

Table 3. — **Treatment related adverse events frequency, all grades (n = 56°)**

	leucovorin Ca (n = 28)		leucovorin Na (n = 28)	
Preferred term	grade 1-2	grade 3-4	grade 1-2	grade 3-4
Nausea	15 (53.5)	2 (7.1)	12 (42.9)	
Diarrhoea	12 (42.9)	1 (3.6)	8 (28.6)	2 (7.1)
Fatigue	11 (39.2)	1 (3.6)	8 (28.5)	
Vomiting	10 (35.7)	1 (3.6)	3 (10.7)	1 (3.6)
Neutropenia	4 (14.3)	4 (14.3)	1 (3.6)	5 (17.9)
Mucositis	8 (28.5)		7 (25.0)	
Anaemia	5 (17.9)		9 (32.1)	
Anorexia	2 (7.1)	1 (3.6)	7 (25.00)	1 (3.6)
Thrombocytopenia	6 (21.4)	1 (3.6)	3 (10.7)	
Febrile neutropenia		1 (3.6)		1 (3.6)
Pneumonia				1 (3.6)
Septic shock		1 (3.6)		

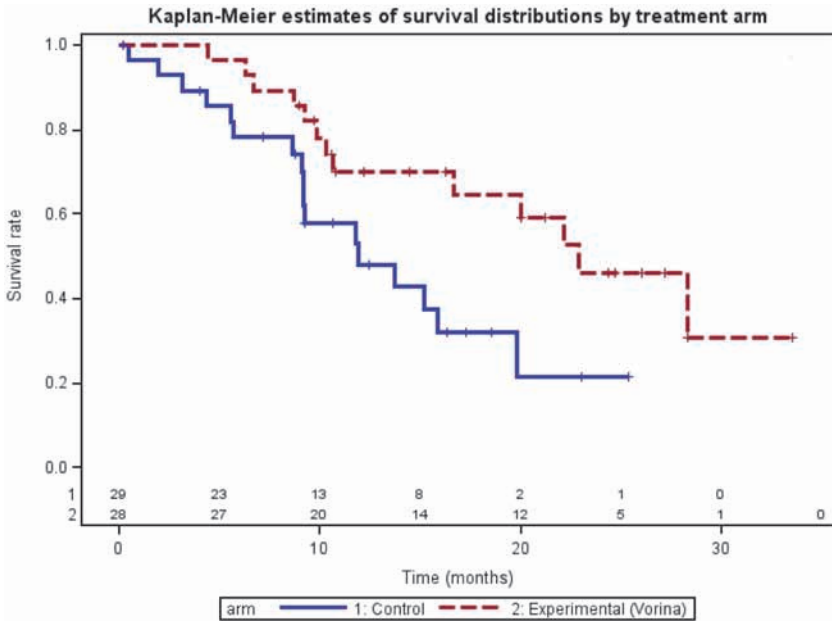
° One patient was excluded because treatment never started.

Table 4. — **ORR according to investigators (n = 57)**

	Leucovorin Na	Leucovorin Ca
Response	n = 28 (%)	n = 29 (%)
CR	2 (7.1%)	0 (0.0%)
PR	15 (53.6%)	16 (55.2%)
Stable disease	8 (28.6%)	6 (20.7%)
Progressive disease	2 (7.1%)	2 (6.9%)
Other failure	1 (3.6%)	5 (17.2%)

Table 5. — **Concordance between response as assessed by the investigators and the independent experts**

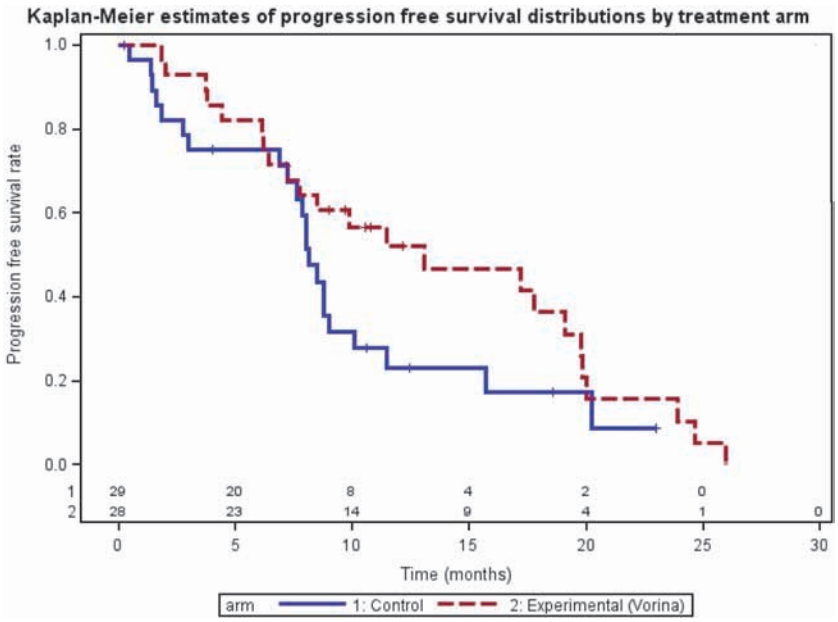
Leucovorin CA arm (n = 19)				
Investigators ▼ Reviewers►	CR	PR	SD	PD
CR	0	0	0	0
PR	0	12	0	0
SD	0	2	2	2
PD	0	1	0	0
Leucovorin NA arm (n = 22)				
Investigators ▼ Reviewers►	CR	PR	SD	PD
CR	1	0	0	0
PR	0	11	4	0
SD	0	1	2	2
PD	0	0	1	0



	Number of subjects	Events	Median survival	95% CI for median survival
LV/Ca	29	17 (59%)	11,9 months	9,1-19,8
LV/Na	28	13 (46%)	22,9 months	16,7-NR

Logrank test : p = 0.02, HR = 0.43 (95% CI : 0.20-0.91) using control arm as reference arm.

Fig. 1. — Overall survival (n = 57)



	Number of subjects	Events	Median survival	95% CI for median survival
LV/Ca	29	22 (76%)	8,1 months	7,3-9,0
LV/Na	28	23 (82%)	11,5 months	7,3-19,1

Logrank test : p = 0.12, HR = 0.62 (5% CI : 0.33-1.14) using control arm as reference arm.

Fig. 2. — Progression free survival (n = 57)

Discussion

Our study was positive with an ORR of 55% and 61% for the LV/Ca and LV/Na arm respectively). Safety was comparable. Due to a poor accrual, only fifty seven patients were accrued out of the 102 expected. The major reason was the lack of interest in running a 'leucovorin' study at a time when targeted therapies were accessible. In addition 1 patient never started treatment and 6 had no post treatment evaluation due to the occurrence of events requiring stopping treatment and were considered as failures (1 in the LV/Na arm, 5 in the LV/Ca arm). Moreover the imagery in 5 cases could not be retrieved for external review and further 5 patients were considered by the external experts as having no measurable disease (3) angiomas (1) or biliary cysts (1) leaving us with only 41 cases evaluated for response by the experts.

Reflecting that situation, concordance between independent reviewers and investigators is moderate. However, although 4 patients initially assessed as responders were not confirmed, 4 others initially assessed as failures in the LV/Na arm, were reviewed as responders by the independent panel. Therefore we think that the ORR of 55% (95% CI 36%-74%) and 61% (95% CI 41%-79%) for LV/Ca and LV/Na respectively based on the evaluation given by the investigators, including all patients, conservatively reflects the respective activity of the treatments. The Fleming's one-stage design used for the study does not give the power for an adequate comparison between groups (9). The P-values for ORR > 35% were significant for both LV/Ca ($p = .02$) and LV/Na ($p = .004$) and makes our study positive according to planning despite reduced sample size and support the testing of LV/Na in phase III.

Another limitation of our study is the observed poor overall survival in the control arm. The estimated median survival, is 11.9 months and 22.9 months in the LV/Ca and LV/Na arms respectively using the ITT population (all randomized patients). These results are difficult to interpret but the rather unfavorable survival distribution in the control arm may simply be due to random error or to the accrual in the study of patients with poor prognosis although the survival distribution in the experimental arm is in the range of published folfox/folfiri overall survival results (10). No methodological issues could be firmly identified. The study is a randomized phase II and the patient's baseline characteristics were evenly distributed between treatment arms (Table 1) as they should do due to the randomization procedure. They confirm however that our whole population had a poor prognostic features with 64% of the patients having ≥ 2 metastases, increase in alkaline phosphatase and LDH and 57% a CEA > 20 times normal values. Moreover none of the patients had received adjuvant chemotherapy suggesting that they had liver metastases concomitant to the diagnosis of their colon cancer. Imbalance in second line chemotherapy and liver metastases resection could also account for the difference in survival. The protocol did not plan to collect information on second line chemotherapy nor imposed a schedule for follow-up that was left to the discretion of each investigator after failure of the study allocated treatment and our rate of censored observations is quite high for a poor prognosis population of patients. Four patients in the LV/Na arm (14.8%) and 3 in the LV/Ca (12.5%) had liver metastases resection. These rates are similar to what is reported in other series (10,11).

The lack of difference in PFS between the two arms raises further doubts about the validity of the survival advantage observed in the LV/Na arm. We know PFS is more difficult to document than overall survival and due to the unblinded allocation of treatment, follow-up could have been less accurate in the control arm. However could one conceive such a clear cut advantage of OS taking place only after progression of the disease ? It is likely that the observed survival difference is due to an unidentified bias linked to possible variations in second line treatment or in follow-up. The possible subsequent imbalance in patients management during follow-up between groups that were comparable at baseline thanks to randomization may have a confounding effect on results.

The toxicity observed in the 5-FU/LV/Ca arm is comparable to what was seen with folfox 7 (2-hour LV/Ca perfusion before 46-hour 5-FU continuous infusion) (12) suggesting that the toxicities of the same chemotherapy with LV/Na is certainly not increased with, for grade 3-4 toxicities, 17.9% neutropenia, 4% thrombocytopenia, 7.1% nausea and 3.6% diarrhea. Moreover, numerically, more patients in the LV/Na arm have no grade 3-4 adverse events (53.5% vs. 35.7%) and less patients have more than 2 grade 3-4 adverse events (17.8% vs. 21.4%) as compared to the LV/Ca arm (Table 2 and 3).

The present study shows that LV/Na can safely be combined with 5-FU/oxaliplatin or irinotecan and provides a response rate in the range of what is observed with LV/Ca. According to our design, LV/Na deserves then further investigation in a phase III trial. Considering the cost of developing the new biologics it seems worthwhile testing continuous infusion of leucovorin in a randomized phase III study.

Disclosures

Diagnosis/ Hoffmann-La Roche / TEVA / Merck KGaA / Merck & Co, USA / Helsinn / Taiho Pharma USA / Sirtex Medical / Ely Lilly / Debiopharm.

Y. Humblet is a consultant for and/or receives honorarium from Roche, Merck KGa-Serono and Amgen.

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Marianne Paesmans is a consultant for TEVA and received honoraria.

A. Vandebroek ; I. Deleu ; P. Vergauwe ; H. Rezaei Kalantari ; G. D'Haens ; A. Efira have no potential conflict of interest to disclose.

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