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Therapeutic Drug Monitoring as a tool to optimize 5-FU based chemotherapy in gastro-intestinal cancer patients of more than 75 years

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ABSTRACT:

Aims

Most clinical trials exclude elderly people, leading to a limited understanding of the benefit/risk ratio in this population. In despite existing data regarding the oncological management of elderly receiving 5-FU-based regimen, our objective was to investigate 5-FU exposure/toxicity relationship in patients ≥ 75 years and compare the effectiveness of 5-FU Therapeutic Drug Monitoring between elderly and younger patients.

Methods

154 patients (31 of whom are over 75) with gastrointestinal cancers, who were to receive 5-FU-based regimens were included in our study. At cycle 1, the 5-FU dose was calculated using patient's Body Surface Area, then a blood sample was drawn to measure 5-FU concentration and 5-FU dose was adjusted at the subsequent cycles based on cycle 1 concentration. Assessments of toxicity was performed at the beginning of every cycle.

Results

71% of elderly patients required dose adjustments after cycle 1, compared to 50% for younger. Percentage of patients within 5-FU AUC range at cycle 2 was 64% and 68% for respectively elderly and younger patients. The proportion of elderly patients experiencing severe toxicities fell from 15% at cycle 1 to only 5% at cycle 3.

Conclusion

PK-guided 5-FU dosing algorithm, leading to an improved tolerability while remaining within therapeutic concentration range, is even more valuable for patients over 75 years old than in younger.

BACKGROUND

Since its original synthesis in the late 1950s, fluorouracil (5-FU) continues to be widely used in the treatment of many cancers, including oesophagus, stomach, pancreas and colorectal cancer (CRC) both as adjuvant therapy of early stage or in advanced setting (1, 2). Over the last decades, knowledge improvement in 5-FU pharmacokinetics (PK) and mechanism of action led to development of new treatments, based on the addition of the biomodulating agent folinic acid, association with other cytotoxic drugs, or modification of 5-FU administration schedule. Originally, 5-FU alone was delivered as a bolus. Nowadays, administration by continuous intravenous infusion is used. Indeed, it was shown that this delivery method increased 5-FU exposure duration, leading to an improved cytotoxic activity and clinical effectiveness, while limiting toxicity (3-5). Despite these therapeutic progresses, 5-FU is often the source of severe treatment-related toxicities requiring hospitalization and leading to death in 0.5% to 2% of cases (6-8).

The most well-known biochemical cause of intolerance to fluoropyrimidines is deficiency of dihydropyrimidine dehydrogenase (DPD) (9, 10). DPD is defined as the first and rate-limiting enzyme in the catabolic pathway of 5-FU, responsible for more than 80% of 5-FU elimination (11, 12). Partial or complete deficiency in the DPD enzyme has been observed in 3-5% and 0.1% of the general population, respectively (13-15). DPD deficient patients experience excessive and severe toxicity in the form of neutropenia, diarrhoea, mucositis and hand-foot syndrome. Overall, DPD deficiency is observed in 39-61% of patients developing severe toxicity (8, 16). In all patients, DPD deficiency is confirmed by sequence analysis of *DPYD*, the gene encoding DPD, used as predictor of fluoropyrimidines-related toxicity when a pathological mutation is found. To date, more than 30 sequence variations in the DPD gene

have been identified, with the most well-established variant being *DYPD*2A* (17, 18). To improve efficacy and reduce toxicity, previous investigations focused on the relationship between 5-FU plasma concentration and DPD activity to determine individual dose adjustment in patients presenting DPD gene mutation (19-22). However, 5-FU pharmacokinetic variability is affected by many others factors such as sex, disease status, nutritional condition, organ function, co-medication, explaining frequent over and under-exposure even though 5-FU dosage adjustment by *DYPD* genotype.

5-FU dosing is traditionally calculated according to Body Surface Area (BSA). Recent data confirm the lack of scientific rational for 5-FU BSA-based dosing (23). As previously demonstrated, there is no potential correlation between BSA and 5-FU plasma clearance (24), possibly explaining the large 5-FU interindividual concentrations variation in patients treated with standard schedule based on BSA. Because 5-FU is characterized by a strong toxicity-exposure relationship and a narrow therapeutic window, the use of therapeutic drug monitoring (TDM) approaches are greatly supported (25, 26). Some studies have demonstrated successful strategies to monitor 5-FU blood concentrations and adjust individual doses based on systemic exposure (27-30). Area Under the Curve (AUC) of 5-FU concentrations is considered to be the most relevant pharmacokinetic parameter associated to 5-FU-related efficacy and toxicity. Because of its intrinsic variability, it is generally considered that an AUC range of 20 - 30 mg.h/L is required for successful therapy (31, 32). In our centre, we have chosen to use an algorithm based on Gamelin's paper (29). Due to the precision of 5-FU measurements, a small dose modification (i.e., $\pm 5\%$) would not have a clinical or biological incidence, we extended the Gamelin's range of target AUC to 18-28 mg.h/L, to start dose adjustment at $\pm 10\%$.

Currently, 60% of malignant disease occurs in persons over 65 years and more than half of these patients are over 70 years old. However, most clinical trials exclude elderly people by design. Consequently, limited data are available to explore the risks and benefits of specific cancer-treatment regimens in this population. Commonly, chemotherapy doses are empirically reduced in elderly patients, mainly to prevent serious side effects. In therapeutic trials and randomized studies, 5-FU adjuvant monotherapy has shown comparable benefits and similar toxicity rates for patients aged 65 and over as for younger one's (33, 34). Nevertheless, this knowledge is based on clinical trials which, by definition, select patients less likely to have comorbidities or functional impairments when compared to the general elder population.

To date, no initial 5-FU dose reduction is recommended for elderly patients but, in clinical practice, empirical dose reductions or shorter chemotherapy regimens are often prescribed in elderly patients because of the hypothetical risk of toxicity. Thus, the main objective of the present study is to investigate exposure/toxicity relationship of 5-FU-based regimens in individuals aged ≥ 75 years old. The secondary objective of this work is to compare the effectiveness of 5-FU TDM between elderly and younger patients.

Patients & Methods

Population

This retrospective analysis was carried out in a database covering all patients diagnosed with gastrointestinal cancer who received a 46h continuous 5-FU infusion from April 2014 to February 2016 in the Dijon's Cancer Centre (Burgundy, France). The therapeutic follow-up in our centre includes a blood sample analysis to determine 5-FU exposure during the three 1st chemotherapy cycles. Patients eligible for this study were treated with specific digestive

cancer-treatment regimens by 5-FU infusion alone or associated with other cytotoxic ± biotherapy for adjuvant or advanced therapy purposes. The data routinely collected include gender, age, birth date, weight, height, Charlson Comorbidity Index (CCI) (elderly only), primary tumor, type of treatment (metastatic or adjuvant), treatment line, chemotherapy regimen, date of cycle, 5-FU bolus dose, 5-FU infusion dose, 5-FU concentration measured, AUC calculated, proposed dose for the next cycle and toxicities. Individuals were classified into two groups based on age: young group rounded up patients < 75 years old and elderly included all patients ≥ 75.

Study design and chemotherapy regimen adjustment

At cycle 1 (C1), patients received folinic acid (400 mg/m²) by i.v. infusion over 2 h followed by a 5-FU bolus (400 mg/m²) and immediately after by 46h continuous 5-FU infusion (2400 mg/m²) administered via a battery-operated pump. Patients could receive other cytotoxic drugs and/or biotherapy before 5-FU regimen. Area Under the Curve (AUC) of 5-FU infusion was calculated by multiplying the 5-FU steady-state concentration by the infusion duration (46h). At the cycle 2 (C2), the dose of 5-FU infusion was determined according to an algorithm derived from Gamelin's one, targeting AUC range of 18-28 mg.h/L. The same methodology was applied at C2 to ensure correct exposure and perform dose adaptation at the cycle 3 (C3) if necessary. Doses were to remain constant during the subsequent cycles, except in case of severe toxicity. Clinicians were free to individually adapt any other drugs doses included in the protocol.

Blood sampling and plasma concentration determination

To limit within-day variability of DPD activity (35), blood samples were taken between 8 and 10 a.m. the day following the beginning of 5-FU infusion. Samples were immediately

centrifuged and plasma kept frozen at -20°C until analysed. Plasma 5-FU concentrations were determined by liquid chromatography. Chloro-uracil was used as internal standard. 5-FU was extracted from the plasma with isopropanol-ethyl acetate (15/85 v:v) in the presence of 200 mg ammonium sulfate to precipitate proteins. The organic phase was dried at 50°C under nitrogen dioxide and reconstituted with 200 μL mobile phase before injection. Mobile phase consisted of methanol/water (5/95 v:v). UV detection was performed at 265 nm. This method was fully validated for routine measurement of 5-FU with a lower limit of quantification of 30 $\mu\text{g/L}$.

Toxicities classification

All toxicities graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) (Version 4.0) and were clinically or biologically evaluated before each cycle with particular attention to diarrhoea, neutropenia, mucositis and hand-foot syndrome. Severe toxicity was defined as grade 3 or grade 4 toxicity.

Statistical methods

The distribution of patients' characteristics was expressed as percentages or mean values presented as mean $\pm$ SD (range) or median (range) if deemed appropriate. The statistical analysis was conducted in patients older than 75 years or younger than 75 years. Univariate analyses were performed. Quantitative data were analysed using Student's test or non-parametric Mann-Whitney test for small sample size (effective < 30). Percentages were compared using two proportions comparison test, Pearson's Chi-squared test for multiple samples or Fisher's exact test for small sample size. The level of statistical significance was set at $p = 0.05$. Most analyses were performed using SIGMAPLOT[®] software (Version 11.0, SYSTAT Software, Inc).

RESULTS

Patient characteristics

A total of 154 patients with gastro-intestinal cancer were enrolled in our study. Thirty-one of them were above 75 years or older and were included in the elderly group, whereas 123 were included in the younger adult group. Demographic data and study treatment details are listed in Table 1.

The most frequent cancer which affects each group was the CRC (74% in elderly and 56.9% in younger adult). Pancreas was the 2nd most frequent site (25.2%) in young adults, while there was as much pancreatic cancer as stomach or oesophagus cancer (6.5%) in the elderly. The proportion of patients treated in adjuvant and in metastatic settings was similar in both groups. However, when only FOLFIRINOX regimens were considered, there was significantly more treated patients (38.2%) in younger adults than in elderly (9.7%). Overall, elderly patients received 28.2% less irinotecan-based chemotherapy than younger patients. It should be noted that 67.7% of elderly were treated as first line therapy.

Interindividual variability in 5-FU pharmacokinetics

Figure 1 represents plasma 5-FU concentrations as a function of 5-FU-infusion dose (calculated according to BSA) for each patient at C1. No proportionality between dose and concentration was found. A considerable difference in blood concentrations was observed for a same 5-FU dose in both groups. For example, in elderly, steady-state 5-FU concentrations were ranging from 110 µg/L to 706 µg/L for an identical total dose of 4 000 mg. Similar conclusions could be drawn from younger patients but this variability seems to be much more substantial among elderly. Consequently, the goal of 5-FU BSA dosing (i.e., bring all patients in the same target exposure) is not reached and those results confirm the

need to use another dose adjustment method than calculating 5-FU dose according to BSA and more for elderly patients.

To confirm that 75 years old was a valuable cut-off, proportions of patients inside or outside the 5-FU AUC range were compared for 4 age ranges (Table 2). AUC distribution was not significantly different between the three youngest groups ($p = 0.920$). To note, there was no patient over-exposed in the group of 55 years or less. On the contrary, the proportion of patients ≥ 75 years well-exposed did not reach 30% (i.e., 24% less compared with 65-75 years group ($p = 0.039$)). Applying a cut-off value of 70 years would lead to no significant difference in terms of proportion of patient well-exposed between 65-70 years (50%) and 70 years or more (40%) groups ($p = 0.386$). Same results were observed for under-exposed patients (36% vs 42%, $p = 0.583$) and over-exposed patients (14% vs 18%, $p = 0.611$) respectively. Thus, a cut-off value of 75 years was considered as pertinent.

Impact of 5-FU TDM and individual dose adjustment to reach target AUC range

At C1 (i.e., with dose adapted according to BSA), the mean initial exposure for elderly patients was 21.2 ± 10.1 mg.h/L and 20.2 ± 6.2 mg.h/L for younger adults with a 47% and 30% coefficient of variation (%CV) in each of the groups. Mean doses administered to elderly ($4\,239 \pm 418$ mg) were not significantly different from the ones administered to younger adults ($4\,234 \pm 536$ mg) ($p = 0.951$), which might explain why no difference was found between AUC in the two groups ($p = 0.598$). However, when AUC %CV are compared between C1 and C3 (i.e. after 2 cycles of TDM) an important decrease (- 20%) is observed for elderly. This decrease is reflected a lesser AUC variability among individuals.

As shown in Figure 2, at C1, where the initial 5-FU infusion doses were calculated based on BSA, only 29% of elderly presented an AUC within the therapeutic range, whereas 50% of younger adults were within this same range ($p = 0.049$). Of the 13 elderly patients who were under-dosed at cycle 1, 11 of them (85%) had a dose adjustment at cycle 2 with an average increase of 963 mg (23%) compared to the mean initial dose of 5-FU. Interestingly, after 5-FU PK-guided dosing adjustment, the percentage of elderly patients below the target AUC decreased from 46% (13 of 28) to 25% (7 of 28) between cycle 1 and cycle 2 while the percentage of elderly within the therapeutic range significantly increased from 29% (8 of 28) at cycle 1 to 64% (18 of 28) at cycle 2 ($p = 0.011$). Similarly, the proportion of younger patients who had an AUC within the therapeutic range progressed from 50% to 68% between the two 1st cycles ($p = 0.008$). At cycle 2 and 3, there was no statistically significant difference concerning proportion of under-, well- or over-exposed patients in both groups.

Relation between 5-FU exposure and toxicity

Main adverse events in the two groups were analysed at C1. All grades diarrhoea, hand-foot syndrome, and neutropenia tend to be more frequent in the younger group as compared to elderly, however differences were not statistically significant. The incidence of severe mucositis (grade ≥ 3) was relatively low in both groups, but elderly tended to be more exposed than younger patients (7.1% vs 2.7% respectively, $p = 0.024$).

At C1, 10% of elderly below or within AUC range declared severe toxicity compared to 29% of those above AUC target. The only grade III/IV toxicities, below or within therapeutic AUC range, declared among elderly patients were mucositis. Elderly patients above the AUC range presented mostly neutropenia and diarrhoeas. The incidence of serious toxicities for

patients below or within AUC range did not differ significantly between the 2 groups ($p = 0.905$); as for patients above AUC range ($p = 0.683$).

All cycles combined, lower AUC values were observed for elderly presenting no toxicity or at least non-severe (grade I/II) (21.3 ± 7.7 mg.h/L) compared to those presenting severe toxicity (grade III/IV) (28.2 ± 7.7 mg.h/L) (Figure 3). Conversely, all cycles and grade combined, patients over-dosed presented almost twice as much toxicities than patients under or well-dosed (67.3% vs 35.8% respectively, $p < 0.0001$).

Impact of 5-FU PK-guided dosing adjustment to reduce toxicity

At C1 and C3, lower incidence of grade III/IV toxic effects was observed for both groups (Figure 4). Decrease of severe toxicities was even more important for elderly (15% vs 5% respectively). Of note, no grade IV toxicities at cycles 2 and 3 was observed in the elderly group when compared to 1 during C1.

Seven young patients benefited from 5-FU bolus dose reduction between C1 and C2, due to grade IV toxicities or hospitalisation during the inter-cycle. Among them, four patients had also a 5-FU infusion reduction, while only 1 of them was over-exposed. Three elderly had bolus dose reduction due to grade III adverse events ($N = 2$) and/or over-exposure ($N = 2$). The 2 patients who had AUC above target benefited from an infusion dose reduction too. Overall, all cycles combined, 3 out of 5 elderly and 5 out of 13 young patients presented toxicities in spite of the absence of 5-FU bolus dose (mostly grade I/II for the 2 groups) and in the absence of an over-exposure for most of them.

Relationship between Charlson Comorbidity Index (CCI) and PK or toxicity

Comorbidity was calculated for all elderly patients; CCI ranged from 5 to 13, with 28 patients having a CCI ≥ 6 . All cycles combined, no correlation between comorbidity score and 5-FU AUC value was observed ($r^2 = 0.0204$). Similarly, the median of CCI was not statistically different between the no toxicity or at least non-severe group compared to the severe toxicity one ($p = 0.057$).

DISCUSSION

Considering population aging and the increasing proportion of elderly patients treated for cancer in general and particularly for gastrointestinal cancer, it is important to evaluate the impact of cytotoxic agents, such as 5-FU, in this population. Such studies could have a major impact in the improvement of elderly patient management in current practice. Indeed, aging can alter physiological functions and biological characteristics which could change the pharmacokinetics of drugs, modify the plasma concentrations, and consequently, affect the tolerability and effectiveness of the chemotherapy. Even if, Etienne's PK analysis (36) revealed that age, as model covariate, had a negative impact on 5-FU clearance, other publications founded no significant influence of age on liver DPD activity (37-39). Furthermore, Duffour's paper (40), which compared 5-FU pharmacokinetic parameters between two groups (age $<$ or ≥ 65 years) receiving LV5FU2 regimen, indicated that mean clearance in elderly patients did not differ from younger people. Because no initial 5-fluorouracil dose reduction is recommended for patients with altered renal or hepatic function, elderly patients should be treated as younger patients. However, in current clinical practice, empirical dose reductions or shorter chemotherapy regimens are often prescribed in elderly patients, mainly due to fear of severe toxicity.

Although 5-FU dosing is traditionally calculated according to BSA, a number of studies have been conducted to evaluate an appropriate dose adjustment algorithm and to demonstrate the advantage of 5-FU PK-guided dosing to reduce toxicity and enhance therapeutic outcomes. Nevertheless, those studies mainly concerned young patients (< 65 years old) and very few data exist for elderly ones. In our study, we have chosen to divide our population by age range and evaluate 5-FU AUC range for each group to find optimal cut-off value of age. Our data suggest that 75 years as a cut-off is better than the more frequently used 65 or 70 years. Indeed, we demonstrated that 5-FU BSA-dosing in 75 years or more patients is even less suitable than in young people (only 29% of well-exposed with dose adapted according to BSA), leading to a non-optimal treatment in this frail population. In this paper, we show that 5-FU PK-guided dosing may help to reduce toxicity from cycle 1 to subsequent cycle in elderly patients, and this, while increasing the dose in under-dosed patients.

At the 1st cycle, mean 5-FU doses administered to elderly ($4\,239 \pm 418$ mg) were not different from those administered to young people ($4\,234 \pm 536$ mg). However, a difference in terms of 5-FU combination was observed. Indeed, elderly received nearly 30% less irinotecan-based chemotherapy (the cornerstone of 1st line metastatic CRC) than younger patients, while 48% of them received a 1st line metastatic chemotherapy. This observation suggests that oncologists, in our Cancer Centre, tend to favour less aggressive regimens more than 5-FU dose reduction in elderly patients.

As a reminder, for this study, individual doses adjustment was based on systemic exposure measured at the previous cycle. A range of 18-28 mg.h/L, based on Gamelin's algorithm (29), was used as target AUC. Upon BSA-based dosing at cycle 1, only 29% of older than 75 years group had a 5-FU AUC within the target range while 50% of young patients were within this

therapeutic range. This result leads to an important variability in 5-FU steady-state concentrations, ranging, for example, from 110 µg/L to 706 µg/L for an identical total dose of 4 000 mg for elderly. In comparison, PK-guided 5-FU dosing performed at cycle 2 resulted in significantly higher proportion of elderly achieving the target AUC (64%), with, in particular, a considerable average increase of the dose (963 mg) among old patients under-dosed at C1. Indeed, almost half of elderly (46%) were under-exposed at cycle 1 versus 25% at cycle 2. In our study, dose adaptation upon over- or under-exposure was not mandatory; in some situations, for clinical reasons, some practitioners have decided not to follow our recommendations of 5-FU dosing adjustment. For the second cycle, 25% of elderly under-dosed did not had a dose increase as proposed, which could partly explain why still 25% of elderly patients are below the therapeutic range at cycle 2. However, this observation is not different for younger patients. In fact, the proportion of under-, over- and well-exposed patients were identical between young and elderly patients at cycle 2 and 3. The study of Wilhelm et al. (41), which enrolled 33 patients < 65 years and 42 patients ≥ 65 years with CRC receiving the weekly regimens of AIO (folinate, 5-FU), FUFOX (oxaliplatin, folinate, 5-FU) or the biweekly regimen of modified FOLFOX-6, resulted in 64% of all patients under the therapeutic range, 33% of them well-dosed and 3% who were over-exposed at the cycle 1. In Saam's paper (42), 5-FU AUC were monitored during 4 cycles in 64 colorectal cancer patients receiving any regimen in which 5-FU was administered over a period of 44-48 hours. If necessary, a 5-FU PK-guided adjustment was performed after receiving the first 5-FU BSA-based dose. The first measurement indicated that 68% of patients were under-exposed, 13% were in therapeutic range and 19% had an AUC over the superior target level. According to our investigations and the results presented in the studies previously referred, we demonstrate that the vast majority of patients is not in the expected therapeutic range after

317 receiving standard 5-FU BSA-based dose. The high interindividual variability following dose
318 adaptation testifies of a very limited interest of the 5-FU BSA-based dosing. Upon 5-FU PK-
319 guided dose adjustment in subsequent cycles, a significant decrease of this variability was
320 observed.

321 Reports concerning tolerance of 5-FU-based chemotherapy in elderly patients are
322 conflicting: some publications describe an increase rates of stomatitis, nausea, diarrhoea,
323 leukopenia, or neutropenia (43-45), whereas no excess toxicity have been observed in others
324 reports (33, 34, 46). In our investigation, after receiving a standard 5-FU BSA-based dose, the
325 frequency of diarrhoea, hand-foot syndrome, and neutropenia was statistically similar
326 between young and old patients. However, elderly tended to be more susceptible to severe
327 mucositis than younger patients; the use of dental prosthesis and fixed implant, often linked
328 to advanced age, is frequently responsible for inflammation of the oral mucosa (47, 48) and
329 could partly explain this higher proportion of elderly who presented serious mouth ulcers
330 compared with young people. Diarrhoea and neutropenia were mostly severe toxicities
331 observed among elderly over-dosed; this observation is not surprising given that numerous
332 publication demonstrate the link between cytotoxic concentrations and the severity of
333 neutropenia or diarrhoea (49, 50). Generally, we observed that grade III/IV toxicities were
334 associated with a higher AUC than grade I/II. Conversely, almost twice as much toxicities
335 were observed among patients over-exposed than patients under or well-exposed. As
336 expected, the 5-FU PK-guided dose adjustment reduced the risk of adverse events,
337 particularly severe toxicities. Lower incidence of grade III/IV were observed for the two
338 groups between cycle 1 and 3 and no grade IV toxicity was reported at cycle 2 and 3 among
339 elderly.

340 In Sargent's paper (33), a pooled analysis of 3 351 patients from 7 randomized phase 3 trials
341 was performed. Patients who received 5-FU alone as adjuvant treatment were grouped into
342 10-year age ranges categories of equal size including a group over 70 years old. Study
343 reported that treatment among elderly had the same benefit/risk ratio as for younger
344 patient groups, with no statistically significant increase in toxicity. However, most of the
345 time, clinical trials exclude elderly or include only highly selected old patients. For elderly
346 patients with good Performance Status (PS) and low Charlson Comorbidity Index (CCI), there
347 is evidence showing both efficacy and acceptable toxicity of chemotherapy (55), but what
348 about the influence of these two parameters on toxicity in older people more frail? In our
349 study, all elderly treated by 5-FU for a gastro-intestinal cancer during the study period were
350 included. Consequently, we believe that our study population is representative of the
351 general elderly population, contrarily to what is observed in a clinical trial. For analyses, PS
352 was not available for all the patients, but we were able to calculate CCI for elderly; 90% of
353 them having a $CCI \geq 6$, which associated with a very important 10-year mortality rate (56). As
354 Jehn's results (57), in our study using 5-FU, the presence of comorbidity did not confer
355 increased risk of toxicity or superior AUC values. Thus, we may consider that 5-FU PK-dosing
356 can improve the exposition and tolerability of 5-FU in elderly, regardless of clinical condition.

357 The main limitation of this work is that, at the time of the study, DPD genotyping or
358 phenotyping were not available in our institution. Thus, dose adaptation at cycle 1 based on
359 these criteria was not possible. Nowadays, a pre-therapeutic screening of DPD activity by
360 pharmacogenetics is systematically performed. Patients with no DPD deficiency receive full
361 dose, while the dose is decreased, as early as the first cycle, in agreement with *DPYD*
362 variants for patients presenting a DPD deficiency. During the following cycles, the dose is
363 adjusted according to AUC and toxicity.

364

365 We demonstrated, throughout this work, the importance of considering interindividual
366 variability of 5-FU exposure. However, efficacy of 5-FU is not only dependent on 5-FU
367 metabolism but also by the use of folinic acid in association which acts as co-activator of the
368 thymidylate synthetase, the main target of 5-FU. Even if folinic acid is associated to a limited
369 degree with clinical outcomes (58), it should be emphasized that its interindividual variability
370 was not considered for this study.

371 Generally, clinical trials exclude elderly or include highly selected old patients in terms of
372 performance status (PS) and co-morbidity. In our study, all elderly treated by 5-FU for a
373 gastrointestinal cancer during the study period were included. Consequently, despite a small
374 number of patients ($n = 31$), we believe that they represent the general elderly population.

375

376 CONCLUSION

377 Overall, our analysis confirms that BSA-based dosing explains high 5-FU concentration
378 variability among patients. The difficulty to predict 5-FU plasma levels for a given dose
379 frequently led to ineffective concentrations or severe toxicities. PK-guided 5-FU dosing
380 algorithm allowed 5-FU dose adaptation, leading to an improved tolerability while remaining
381 within therapeutic concentration range. This tool, previously described as effective in the
382 general population or young patients, is even more valuable for patients over 75 years old.

383 ADDITIONAL INFORMATION

384 Ethics approval

385 All patients routinely underwent a blood analysis in order to evaluate their 5-FU exposure
386 during the 3 first cycles. Consequently, no informed consent was required. However, data
387 used in this manuscript were recorded in such a manner that subjects could not be
388 identified. Patient confidentiality was maintained and the protocol for data collection and
389 analysis followed guidelines and were approved by our Institutional Review Board.

390 **Availability of data**

391 Data are available upon request to the corresponding author.

392 **Conflict of interest**

393 The authors declare no conflict of interest.

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395 None

396 **Author contributions**

397 Study conception and design: FG, LBL, AS

398 Acquisition of data: JV, VQ, SM, FG, LBL

399 Analysis and interpretation of data: PM, KM, FG, LBL, AS

400 Drafting of manuscript: PM, AS

401 Critical revision: PM, FG, LBL, AS

402 Final approval: PM, KM, JV, VQ, SM, FG, LBL, AS

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FIGURE LEGENDS

Figure 1 - Relationship between 5-FU plasma concentration and 5-FU continuous infusion dose at cycle 1 in elderly and young patients.

Figure 2 - Percentage of patients with 5-FU AUC values below, within or above the therapeutic range at C1 and C2

Figure 3 - Distribution of elderly patients presenting no toxicity or at least non-severe compared to those presenting severe toxicity according to AUC values

598 **Figure 4** - Percentage of elderly and young patients developing severe adverse events at C1 and C3

600 **Table 1** - Initial patients characteristics and treatment regimens (NS : non significant)

VARIABLE	YOUNG (n=123)	ELDERLY (n=31)	P- VALUE
GENDER , n (%)			
Male	69 (56.1)	20 (64.5)	NS
Female	54 (43.9)	11 (35.5)	NS
AGE , years, median (range)	64 (27-74)	79 (75-87)	< 0.001
WEIGHT , kg, mean $\pm$ SD (range)	69.5 $\pm$ 15.1 (35-115)	70.9 $\pm$ 13.3 (47-109)	NS
HEIGHT , cm, mean $\pm$ SD (range)	168.9 $\pm$ 8.4 (150-186)	166.4 $\pm$ 8.2 (150-182)	NS
CHARLSON COMORBODITY INDEX , median (range)	/	10 (5-13)	/
LOCATION OF CANCER , n (%)			
Colorectal	70 (56.9)	23 (74.0)	NS
Pancreas	31 (25.2)	2 (6.5)	0.026
Esophagus	9 (7.3)	2 (6.5)	NS
Stomach	8 (6.5)	2 (6.5)	NS
Others	5 (4.1)	2 (6.5)	NS
TYPE OF CHEMOTHERAPY , n (%)			
Metastatic	105 (85.4)	23 (74.2)	NS
Adjuvant	18 (14.6)	8 (25.8)	NS
PROTOCOL OF CHEMOTHERAPY , n (%)			
Simplified Folfox-6	44 (35.8)	17 (54.8)	NS
Folfirinox	47 (38.2)	3 (9.7)	0.002
Folfiri	22 (17.9)	6 (19.4)	NS
Lv5fu2	4 (3.3)	4 (12.9)	NS
Folfiri-3	6 (4.8)	1 (3.2)	NS
BIOTHERAPY , n (%)			
Yes	59 (48.0)	15 (48.4)	NS
No	64 (52.0)	16 (51.6)	NS
LINE OF TREATMENT , n (%)			
1st Line	58 (47.2)	21 (67.7)	0.043
2nd Line	39 (31.7)	6 (19.4)	NS
3rd Line or More	26 (21.1)	4 (12.9)	NS

601

602

603 **Table 2** - Percentage of patients with 5-FU AUC values below, within and above the therapeutic range
604 by age groups

AUC RANGE	< 55 YR	55 – 65 YR	65-75 YR	≥ 75 YR
<i>Below (%)</i>	53	39	36	46
<i>Within (%)</i>	47	51	53	29
<i>Above (%)</i>	0	10	11	25

605

Figure 1

Figure 2

Figure 3

Figure 4