Individual patient data meta-analyses evaluating the link between dihydropyrimidine deshydrogenase (DPD) genotype and/or phenotype and severe fluoropyrimidine-related toxicity

FUSAFE-MA

Project initiated by the DPD working group of

Groupe de Pharmacologie Clinique Oncologique (GPCO)-Unicancer and

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Table of Contents

I. RATIONALE

5-Fluorouracil (5FU) and its oral prodrug, capecitabine, are the cornerstone of chemotherapies of various solid tumors including digestive, breast and head and neck cancers. However, 15% to 30% of patients receiving a fluoropyrimidine-based treatment will develop severe grade 3-4 toxicity (4-5% will develop grade 4 toxicity), mainly digestive and hematological toxicity, that may affect the quality of life of patients, can cause life-threatening and entail extra costs. The pattern and/or severity of toxicity may depend on the nature of the drug itself, on the fluoropyrimidine dose-intensity, administration schedule (continuous or bolus, fluroropyrimidine alone or in association), patient gender (more frequent in women) and performance status (more frequent if poor performance status) (MAGIC 1998). Numerous studies have demonstrated that a deficiency in dihydropyrimidine dehydrogenase (DPD), the key enzyme of 5FU catabolism, confers a significant risk of major toxicity for patients receiving a fluoropyrimidine (*Fleming 1992, van Kuilenburg 2004, Deenen 2011, Caudle 2013).* Identification of at-risk patients is thus of major concern. DPD is encoded by the *DPYD* gene. So far, more than 100 *DPYD* gene variants have been reported, each at very low allele frequency, with only a few having a functional impact (*Offer 2013*). There is a strong consensus to consider that three *DPYD* deficient variants, namely*2A (IVS14+1G>A, 1905+1G>A, rs 3918290),*13 (1679T>G, rs 55886062) and 2846A>T (rs 67376798, no * assigned), are significantly related to fluoropyrimidine toxicity (*Caudle 2013,* site PharmGKb). DPD deficiency can also be identified by direct measurement of DPD enzyme activity in blood mononuclear cells (*Fleming 1992, Etienne 1994*), or can be indirectly evaluated by measuring concentrations of uracil (U, physiologic DPD substrate) and dihydrouracil (UH2, physiologic DPD product) in plasma or urine (*Boisdron-Celle 2007, Wettergren 2012*). Recently, Carlsson et al. (2014) have shown that physiological UH2/U ratio in saliva may be a reliable predictive marker of severe toxicities in colorectal cancer patients treated with FOLFOX regimen. Alternatively, the Uracil Breath Test, a non- invasive test based upon the ingestion of ^{13}C-uracil, has been developed and successfully tested by the Diasio group in the U.S. (*Ezzeldin, 2009*). Also, determination of uracil PK parameters after oral intake of exogenous U has been proposed to estimate DPD activity (*Van Staveren 2011*).

Numerous reviews have been published on DPD deficiency approaches for identifying patients at-risk for 5FU-related toxicity. Also, recommendations have been made, including the recent guidelines from the Clinical Pharmacogenetic Implementation Consortium, CPIC (*Caudle 2013*). However, literature data scarcely report sensitivity/specificity and predictive values of DPD genotyping/phenotyping approaches. In the recommendation paper of the CPIC (*Caudle 2013*), the reported sensitivity and specificity were based on only 2 prospective studies: from one analysis of the 3 relevant *DPYD* variants in 487 patients (*Morel 2006*), and from another study considering only the *2A variant in 683 patients (*Schwab 2008*). So far, no meta-analysis has been reported for phenotyping approaches, and only two meta-analyses, on summary data, have been published on the genotyping approach (*Terrazzino 2013, Rosmarin 2014*). However the former meta-analysis only considered the *DPYD* *2A and the 2846A>T variants separately: the pooled sensitivity for prediction of overall grade 3-4 toxicity was 5.2 % for *DPYD* *2A and 5.4 % for variant 2846A>T (*Terrazzino 2013*). The second meta-analysis, including data from the QUASAR2 study, did not provide knowledge on the combined *DPYD* *2A plus 2846A>T approach (*Rosmarin 2014*). The scarcity of *DPYD* deficient mutations explain the very low sensitivity of genotyping approaches, especially concerning the performance of a single *DPYD* consensual variant (less than 2% of carriers) for predicting grade 3-4 early toxicity that occurs in 15-30% of patients. In fact such figures will give a maximum theoretical sensitivity as low as 13% (2/15). One could thus expect to improve the sensitivity by considering more severe life-threatening grade 4 and grade 5 (lethal) toxicity, which is the more relevant to prevent, along with an expanded number of relevant *DPYD* mutations, i.e. considering at least the 2 or 3 main deficient *DYPD* variants (*2A,*13, 2846A>T), that are carried by approximately 2 to 4% of the

Caucasian population. However, such a grade 4-5 toxicity endpoint, occurring in around 4-5% of patients, requires large number of patients not always achieved in published studies. Sensitivity may also be improved by combining genotyping with the phenotyping approach. There is thus a need for conducting a meta-analysis project aimed at evaluating the impact of DPD genotyping (considering the 3 relevant *DPYD* mutations together) and/or DPD phenotyping (whatever the approach considered), on the risk of developing severe fluorouracil-related toxicity.

We thus planned to conduct meta-analysis (MA) on individual patient data (IPD), as usually performed in MA of randomized trials in oncology (*EBCTCG 1992, MAGIC 1998, NSCLC-CG 1995, MARCH 2006*), based on a systematic review of the literature data. Both published and unpublished studies will be considered since there is evidence that both investigators and journal editors are more likely to publish trials with positive results (*Begg 1994*). Detailed information on patient characteristics, treatment, toxicity and prognostic information, will be collected for all patients in each study since such a methodology permits a more reliable and flexible approach and a more sensitive analysis. In particular, the use of individual patient data will allow studying better the impact on toxicity of the three (or two) main *DPYD* variants all together, as well as their combination with a phenotyping approach, so as to identify the most powerful screening approach. Moreover, it will be possible to compute sensitivity, specificity and predictive (negative and positive) values, as well as number of patients needed to screen (*Rembold 1998*).

In practice, the project will include three meta-analyses. Firstly, we will conduct two individual patient data meta-analyses, one on the association of the combined three (or two) more frequent variants on toxicity (Genotype IPD MA), and a second one on the association between deficient phenotype (whatever the test) and toxicity (Phenotype IPD MA). Then, a third individual-patient data meta-analysis (Genotype/Phenotype IPD MA) of prospective studies that consider the analysis of two or three main *DPYD* variants along with a DPD phenotyping approach will be conducted. For these analyses, eligible studies not yet published but currently available will be included.

II. OBJECTIVES

The aim of the present meta-analysis project is to evaluate the impact of pre-treatment DPD genotyping and/or phenotyping on the risk of developing severe fluorouracil-related toxicity in cancer patients, in order to compare the predictive values, sensitivity and specificity of DPD genotyping alone *versus* DPD phenotyping alone *versus* combined genotyping plus phenotyping approach.

<u>The detailed steps of this project are the following:</u>

1) A systematic review of the literature data reporting on the association between severe fluoropyrimidine-related toxicities and the presence of *DPYD**2A, *13 or 2846A>T variant, and/or the presence of a deficient DPD phenotype, whatever the phenotyping approach (enzyme activity, uracil concentration, UH2/uracil ratio …).

2) Collection of not yet published studies evaluating the relationship between severe fluoropyrimidine-related toxicities and the presence of DPYD*2A, *13 or 2846A>T variant, and/or the presence of a deficient DPD phenotype, whatever the phenotyping approach.

3) Collection and checking of individual data corresponding to the above selected studies. Such a methodology will allow duplicate patients to be identified and excluded from the meta-analyses.

4) Making of three individual patient data meta-analysis in order to estimate the association between the risk of early severe fluoropyrimidine-related toxicities and i) the presence of at least two of the three main DPYD variant, ii) the presence of a DPD-deficient phenotype, iii) the presence of either a DPYD variant or a DPD-deficient phenotype.

5) We will estimate the clinical utility of each of the three above analyses by assessing their sensitivity, specificity, positive and negative values, along with odds ratio of the association between abnormal genotype and/or phenotype and early severe toxicity, and number of patients needed to screen, so as to compare these 3 approaches and provide future recommendations on the more efficient strategy.

III. STUDY SELECTION CRITERIA

A. INCLUSION CRITERIA

- Studies with unbiased patient recruitment and prospective collection of toxicity: randomized clinical trials or ancillary studies from randomized trials, non-randomized prospective studies or retrospective studies with consecutive patients' recruitment (cohort studies).

- Studies including patients receiving 5FU or capecitabine based treatment for solid tumor, irrespective of administration schedule, chemotherapy protocol, treatment line, tumor localization or tumor stage.

- Studies with non-ambiguous information on the chemotherapy protocol.

- Studies with toxicity evaluated at least at cycle 1, based on CTCAE or WHO criteria.

- Studies with at least 50 assessable patients.

- For the genotype meta-analysis, studies including only Caucasian patients, or for mixed population studies including at least 50 Caucasians with available ethnic group information at the patient level.

- For the genotype meta-analysis, studies reporting non-biased data on at least *DPYD* variants *2A and 2846A>T, possibly associated with *13.

- For the phenotype meta-analysis, studies including patients of any origin, reporting non-biased data on a quantitative pre-treatment DPD phenotyping approach, whatever the methodological approach.

B. EXCLUSION CRITERIA

- Patients receiving UFT-based therapy.

- Patients with non-solid tumor.

- Patients with fluoropyrimidine dose adjustment at cycle 1 based on DPD genotyping and/or phenotyping.

- Patients with fluoropyrimidine dose adjustment at cycle 1 based on 5FU pharmacokinetics.

- Studies with biased/opportunistic recruitment such as selection according to fluoropyrimidine-related toxicity or presence of a DPYD variant.

- Studies with retrospective collection of data on toxicity.

- For the genotype meta-analysis, studies including only one (or no) of the 3 DPYD variants of interest.

- For the genotype meta-analysis, less than 50 Caucasian patients in the study.

IV. LITERATURE SEARCH STRATEGY

The three electronic databases were used: NCBI PubMed, Web of science and Scopus. The search started on January 1990. It was conducted without language restriction. In addition, this search was completed by electronic/hand search of the proceedings of major cancer meeting (ASCO, AACR, ESMO, ECCO, SABCS) since 2000. The search was performed on May 2014 and will be updated during meta-analysis progress (end of 2015, and of 2016 if needed). Research equations were:

1) [DPD OR DPYD OR dihydropyrimidine dehydrogenase]

2) [Fluorouracil OR FU OR 5-fluorouracil OR 5FU OR 5-FU OR capecitabine OR fluoropyrimidine]

3) {[genotype OR *2A OR IVS14 OR rs 3918290 OR*13 OR 1679T>G OR rs 55886062 OR 2846A>T OR rs 67376798]

4) [phenotype OR activity OR uracil OR dihydrouracil OR UH2]}.

Search will combine 1) and 2), with or without 3) or 4).

Information on relevant unpublished studies will be obtained from international experts in the field, i.e. from investigators already identified through the above systematic review. To this end, a specific question is included in the *Study Information Form* (Appendix 2) to be completed by investigators.

V. RESULTS OF THE SYSTEMATIC REVIEW

A. CONDUCT OF THE REVIEW

The above-detailed literature search performed at Gustave Roussy allowed 1700 references to be identified (including abstracts of major international cancer meetings). Their review was then conducted by the 14 biologists of the DPD working group of GPCO-Unicancer and RNPGx. The selection process was as follows:

- Firstly, the DPD working group shared the first selection based on reading summaries, in line with above defined inclusion/exclusion criteria. For summaries with relevant information not available, the references were kept for second step comprehensive analysis. This first step allows a total of 56 references to be pre-selected for second step.

- Second step consisted of a thoroughly analysis of full corresponding papers, each paper being analyzed by 2 biologists (independently). Each reader filled a specific data collection form and ruled on the eligibility of the study for genotype MA and/or phenotype MA. The joint-leader/clinical coordinator synthesized all analyses and asked for a third reviewer in case of discrepancies. When completed, the final results were sent to the DPD working group.

- Finally, the eligibility of papers was validated by both the joint-leader/clinical coordinator and joint-leader/statistician.

B. DESCRIPTION OF SELECTED STUDIES (APPENDIX 1)

This review allowed 24 published eligible studies to be identified, including 2 French studies from one of us presented at ASCO 2015. In addition, we had identified one French non-published study conducted on 173 patients having both DPD phenotyping (plasma U and UH2) and genotyping (the 3 consensual variants).Thus, in total, 25 studies have been selected, accounting for a total of 10 344 patients. The number of eligible studies and eligible patients for the 3 meta-analyses is as follows:

- 17 studies for Genotype MA, totaling 9 661 eligible patients with *DPYD* *2A and 2846A>T, and 7 546 patients with both *2A, 2846A>T and *13.

- 14 studies for Phenotype MA, totaling 1 918 eligible patients.

- 6 studies for combined Phenotype + Genotype MA, totaling 1 235 eligible patients with DPD phenotype + *DPYD* *2A and 2846A>T.

VI. CRITERIA OF EVALUATION

A. ENDPOINTS

The **main endpoint** will be the presence or absence of early hematological or digestive grade 4-5 toxicity (NCI-CTCAE or equivalent).

Secondary endpoints will be:
- grade 3-4-5 early hematological or digestive toxicities.
- grade 3-4-5 early hematological toxicities.
- grade 3-4-5 early digestive toxicities.

Exploratory endpoints will be:
- grade 3-4-5 early global toxicity (with all toxicities).
- grade 3-4-5 early cutaneous toxicity (including hand-foot syndrome) with the hypothesis that this toxicity is not driven by DPD deficiency.
- grade 3-4-5 early cardio-toxicity with the hypothesis that this toxicity is not driven by DPD deficiency.
- grade 3-4-5 early toxicity for each toxicity type (other than cardiac and cutaneous).
- grade 3-4-5 overall toxicity (on the entire treatment) for each toxicity type.
- grade 4-5 early global toxicity (with all toxicities).
- grade 4-5 early toxicity for each toxicity type.
- grade 4-5 overall toxicity (on the entire treatment) for each toxicity type.

For the two last endpoints, the observed number of events will be taken into account to decide to perform or not the analysis for the less frequent toxicities.
Early toxicity is defined as the maximum toxicity grade occurring within cycle 1 to 3 (or within cycle 1 to 2, or within cycle 1 only, depending on available data).
Overall toxicity is defined as the maximum toxicity grade occurring within the entire treatment duration.
Main and secondary endpoints are focussed on toxicities usually related to fluoropyrimines in DPD-deficient patients, i.e. hematological and digestive toxicity.
Digestive toxicity will include diarrhea, nausea, vomiting, mucositis, stomatitis. Hematological toxicity will include anemia, leucopenia, neutropenia, febrile neutropenia, thrombocytopenia. Other toxicities will be considered as exploratory endpoints, in particular severe infection, neurological toxicity, cutaneous toxicity, hand-foot syndrome, asthenia and cardio-toxicity.

B. COVARIATES

Collection of covariates (patient and treatment characteristics) is important to adjust (multivariate analyses) on confounding factors of the association between DPD genotype/phenotype and toxicity. It is also important to test (through interaction) whether (and how) these covariates influence the relationship(s) between DPD phenotype/genotype and toxicity. The following covariates will be taken into account:

- Sex, age, performance status, renal function (in particular for capecitabine), before starting the considered fluoropyrimidine treatment.
- Race/ethnic group (if assessable),
- Type of cancer: breast, colorectal (along with localization), pancreas, stomach, head and neck (along with localization), other solid tumor,
- Cancer stage (early, locally advanced, advanced, or TNM or AJCC staging),
- Patients naïve or not of previous fluoropyrimidine prior to the start of treatment considered for the study (yes/no),
- Line of treatment (for advanced stage),
- Type of fluoropyrimidine (5FU, capecitabine),
- Modalities of 5FU administration (bolus vs. continuous infusion vs. both),
- Fluoropyrimidine dose received at cycle 1,
- Associated drugs (nature of associated drug(s) along with regimen),
- Nature of the DPD phenotyping approach.

VII. DATA COLLECTION AND QUALITY CONTROLS

For each pre-selected study, the main investigator will be asked to provide the data described below. The list of data to be collected corresponds to the ideal situation and we are indeed aware that part of these data may not be available in some studies.

A. DATA TO BE COLLECTED FOR EACH STUDY (APPENDIX 2):

- Study publication(s) (if not already identified), study name and protocol,
- Information on possible patient overlaps across different publications from the same team,
- Recruitment type,
- Design of the study (nested in a clinical trial, prospective cohort, retrospective cohort…), including selection process, and blinding of the biologist to clinical results,
- Period of patient recruitment,
- Inclusion criteria, including request for informed consent,
- Criteria for assessing toxicity, performance status, and tumor staging,
- Frequency and duration of follow-up for toxicity evaluation,
- Criteria for fluoropyrimidine dose adjustment, if available,
- Presence of an associated fluoropyrimidine pharmacokinetic study,
- Nature of DPD phenotyping along with analytical method, pre-analytical requirements (if any) and quality criteria (intra- and inter-assay variability if available)
- DNA source and genotyping analytical method used.

B. DATA TO BE COLLECTED FOR EACH PATIENT (APPENDIX 3)

- Patient age and performance status (before starting the considered fluoropyrimidine treatment).
- Fluoropyrimidine dosage at cycle 1 (mg/m² or mg along with BSA),
- Total number of cycles administered,
- Renal function before starting the considered fluoropyrimidine treatment (clearance of creatinin with method used, or creatin with normal value if possible or inclusion criteria on renal function),
- For each toxicity type, the toxicity grade at cycle 1, cycle 2 and cycle 3 separately if available, as well as for all subsequent cycles if available. The list of toxicities to be collected will include if possible: diarrhea, nausea, vomiting, mucositis, stomatitis, anemia, leucopenia, neutropenia, febrile

neutropenia, thrombocytopenia, infection, cutaneous toxicity, hand-foot syndrome, neuropathy, asthenia, cardiotoxicity, and any other toxicity available.
- True value of DPD phenotype along with unit.
- For DPD phenotype, time of day for biological sampling, and time delay between biological sampling and start of treatment.
- Patient DPYD genotype (*2A, *13, and 2846A>T separately), along with additional polymorphisms, if any.

Depending on the homogeneity/heterogeneity of studies and for facilitating the task of investigators, following variables with a single value defined as such in the study protocol may be provided at the study level. However, if at least one patient has a different value for a given variable, the specific value for that particular patient must be given in the corresponding data file (Appendix 3):

- Sex,
- Race/ethnic group,
- Patients naïve or not of previous fluoropyrimidine prior to the start of treatment considered for the study,
- Tumor type (breast, colorectal, pancreas, stomach, head and neck, other solid tumor),
- Tumor localization for colorectal cancer (right colon, left colon, rectum) and head and neck cancer (oral cavity, nasopharynx, oropharynx, hypopharynx, larynx, other),
- Tumor stage (early, locally advanced, advanced, or TNM or AJCC staging),
- Treatment line (neoadjuvant, adjuvant, and for advanced stage first line, second line …),
- Fluoropyrimidine drug (5FU, capecitabine),
- 5FU administration route (bolus vs. continuous vs. both),
- Associated drugs: folinic acid, oxaliplatin, irinotecan, cetuximab, bevacizumab….
- Chemotherapy regimen (name, for instance FOLFOX …)
- Any other covariates that the investigators fell important to take into consideration to study the association between DPD and toxicity.

All data will be checked for internal consistency and consistency with study protocol and published report. Extreme values will be checked with the investigator. Each study will be analysed individually, and the resulting analyses, and study data, will be sent to the investigator for verification. Data format and coding are proposed to investigators in **Appendix 3**. STREGA (Little, 2009) will be used to build quality criteria that will be evaluated on all data available (publication, protocol, individual patient data). In line with STREGA recommendations (2009), special attention will be paid to the quality of data, including a detailed analysis of possible source of bias, relevant co-variables to include in the final model, as well as analytical/methodological quality criteria such as Hardy-Weinberg equilibrium for DPYD genotypes.

VIII. STATISTICAL ANALYSES

Due to uncertainties to get complete individual data, statistical analysis plan will be finalized once individual data have been collected. At present time, we plan to do firstly a “prognostic” meta-analysis that offers large flexibility, in particular to take into account covariates and to select the best model. Then, we will study the diagnostic value of the corresponding area under receiver operating characteristic (ROC) curves.

A. STATISTICAL POWER CONSIDERATIONS

Considering a 4% risk of grade 4-5 toxicities and 4% of patients with mutations, a one-side type I error of 5%, inclusion of 854 patients will allow to detect an odds ratio of 5 with a power of 80% using continuity corrected Chi^2 test. With a risk of toxicities of 20% (grade 3-4-5), 326 patients are needed to have the same power. Considering a risk of 2% of mutation (other parameters as in the first computation), the figure is 1 614 patients. All in all, considering the expected number of patients ranging from 1 235 to 9 661 depending on the nature of MA, this study will guarantee adequate statistical power.

B. ANALYSIS OF THE ASSOCIATION BETWEEN DPD DEFICIENCY (PHENOTYPE AND/OR GENOTYPE) AND TOXICITY

Genotypes will be considered as a two-category variable, corresponding to the presence or not of at least one variant among the two (or three) consensual variants. For genotyping/phenotyping meta-analysis, we will define a deficient patient in case of deficient DPYD variant and/or deficient phenotype (so as to increase sensitivity).

For phenotype analysis, whatever the nature of the phenotype, individual true values (continuous variables) will be firstly considered. If we recover enough covariate data, we will build a logistic model with stratification by study and adjustment on covariates (one-step approach) that will allow a pool ROC curve to be obtained (preferred option). The second option will consist to build ROC curve for each study and then pool them (two-step approach in case of missing covariates).

Regarding performance comparison between genotype, phenotype and combined approach, we propose to perform the genotype and phenotype analyses on the larger sets of available data in order to find out the best model. Then, we will apply this model to the subgroup of studies with both genotype and phenotype, and we will compare the performance of the model in this subgroup and in the overall population.

The association between deficient DPD genotype (or phenotype) and toxicity will be assessed using logistic model stratified on study and adjusted on covariates using fixed effect model. The measure of interest will be the odds ratio (OR), expressed with 95% confidence intervals (CIs). We will estimate the between-study heterogeneity using the Cochran's Q test (significant for $p < 0.10$). We will also report the I^2 index which quantifies heterogeneity irrespective of the number of studies (Higgins 2002). In case of heterogeneity, the random-effects model (method) that incorporates the between-study heterogeneity and allows for a different effect in each population (Der Simonian-Laird 1986) will be considered (Zintzaras 2008), but if necessary more complex models such as hierarchical model will be applied. The contribution of each genotype on the relation between their combination and toxicity will be evaluated by the study of the marginal gain of adding new SNP to the one with the largest association. The overall OR of the genotype MA and phenotype MA will be compared by interaction test (indirect comparison adjusted on covariates). Based on the multivariate logistic models stratified on studies, prognostic score will be built and the quality of their prediction will be assessed using ROC curves. One-step (preferred option) or two-step approach will be used according to the availability of covariates. The performance of the model will be studied using: 1) Discriminant capacity using area under receiver operating characteristic (ROC) curves, 2) Calibration performance. In case of acceptable performance, for example AUC significantly higher than 0.5, the model will be evaluated using an external-internal cross validation (Royston, 2004).

C. DIAGNOSTIC ACCURACY

Based on the above mentioned ROC curves, we will also compute sensitivity, specificity, positive and negative predictive value of the models with the best prediction performance for DPD genotyping, DPD phenotyping and phenotyping/genotyping approach. Sensitivity is defined as the proportion of patients

found to be positive for DPD deficiency among those experiencing toxicity, whereas specificity is defined as the proportion of patients without DPD deficiency among those without toxicity. For approach combining genotype and phenotype, both simultaneous (positivity of either one of the two approaches) and sequential (look to the result of one test only in patient positive for the second one) approaches will be studied. In addition, the number of patients needed to be screened to spare one life-threatening toxicity will be computed.

D. PUBLICATION BIAS

The presence of publication bias will be assessed using three tests: the Egger regression asymmetry test for funnel plot (Egger 1997), the Begg–Mazumdar adjusted rank correlation test (1994), and the Harbord's test (similar to Egger's test), which uses a modified linear regression method to reduce the false-positive rate (Harbor 2006). P-values <0.10 will be considered to indicate statistically significant publication bias.

E. SUBGROUP AND SUBSET ANALYSES

Impact of relevant covariates, linked to the treatment (drug, administration route, dose, associated drugs …) or to the patient (sex, age, performance status, tumor type and stage …), on the association between genotype/phenotype and toxicity will be studied. It is in particular expected that associated drug will have major impact on toxicity. Odds ratios of subgroups (defined by patient characteristics, e.g. sex) or of subsets (defined study characteristics, e.g. prospective vs. retrospective or phenotype methods) will be compared by interaction test. Fisher et al (2011) methods will be used for subgroup analysis.
Concerning the variability in the quality of phenotyping assays, we plan to collect information on the analytical variability and build a quality criterion (via classification in 2 or 3 levels) in order to perform subgroup analysis according to this quality criteria. The decision to perform or not such analysis should be taken before any analysis.

F. SENSITIVITY ANALYSES

To assess the robustness of our findings and explore possible reasons for heterogeneity, sensitivity analyses will be performed on subgroups of studies (or patients) selected according to one of the following inclusion criteria:

- True prospective studies,
- Higher quality studies, including analytical quality of phenotyping (to be defined and blindly selected),
- Sample size ≥200,
- 5FU-based regimens
- Colorectal cancer.

Depending of the distribution by categories of a given covariate, for instance breast as localization of cancer, a study of the interaction between the covariate and the genotype on toxicity or an analysis after exclusion of the category that represent a small proportion of the overall population may be proposed.

IX. WORKING PARTIES

In order to complete the meta-analyses successfully, three groups with specific functions have been created: The Secretariat, The Advisory Board and The FUSAFE-MA Collaborative Group.

The Secretariat is in charge of the coordination of the meta-analyses. It is responsible for completing the study register and for inviting investigators to provide patient data. The Secretariat is also in charge of

checking, processing and analyzing the data. Finally, the Secretariat is responsible for preparing reports, publications and works in very close collaboration with the Advisory Board.

The Advisory Board will include international experts in the field of pharmacogenetic and oncology, especially involved in the pharmacogenetic of fluoropyrimidine, as well as experts in meta-analysis. The Advisory Board will support the Secretariat with biological, medical and methodological expertise, help determine studies relevant to the overview, promote contact between investigators and collaborators, discuss and improve the MA protocol, discuss the results of the meta-analysis, and comment and approve the manuscript(s) arising from this project. The list of its members is the following:

- Pr Robert Diasio, Director of the Mayo Clinic Cancer Center, Rochester, USA
- Pr Qian Shi, Statistician at the Mayo Clinic College of Medicine, Rochester, USA
- Pr Howard McLeod, Medical Director, Moffitt Cancer Center, Tampa, USA
- Pr André van Kuilenburg, Clinical Biochemical Geneticist at Academic Medical Center, Amsterdam, The Netherlands
- Pr Gilles Chatellier, Statisticien, HEGP, Paris, France.

The FUSAFE-MA Collaborative group will include the investigators responsible for the studies included in the meta-analyses. The members of the Secretariat and the Advisory Board will also be included in this group. The investigators will be responsible for providing the Secretariat with patient data and for discussing the reports prepared by the Secretariat and the Advisory Board. All the Collaborative group members will comment and approve the manuscript(s) arising from this project before it submission.

X. PRACTICAL CONSIDERATION

The Secretariat, located in the Meta-Analysis Unit of the Biostatistics Department at Gustave Roussy, will be responsible for liaising with investigators. The main database will be run by the Secretariat. All data, updating and correction should be sent there. All supplied data will remain confidential and used exclusively for the meta-analyses. A meeting of all group members will be organized by the Secretariat to discuss the preliminary results, discuss the way to publish this project and the future of the collaboration.

XI. PUBLICATION POLICY

Present rules apply for the FUSAFE-MA project herein described. Any publication arising from the FUSAFE-MA project will be made in the name of the **FUSAFE-MA Collaborative Group.** The name of each involved study investigator (at least one per study) as well as the members of the Secretariat and Advisory Board will be included in the list of investigator of the Collaborative Group given in appendix of the publication. If compatible with the journal rules, the preferred option will be to include the maximum number of authorized authors, so as to include at least one investigator by study and members of the Secretariat and Advisory Board. During the investigator meeting, we will define more detailed publication policy of the present project, as well as decide on publication policy for future unplanned analyses.

Acknowledgments
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XII. REFERENCES

References of preselected studies are indicated in bold.

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XIII. APPENDIX

APPENDIX 1: DESCRIPTION OF STUDIES PRE-SELECTED FOR THE META-ANALYSES

Table 1: Studies preselected for the *DPYD* genotype meta-analysis (at least *2A and 2846A>T ± *13)

Ref number	Ref	Team (Country)	Study type	Patient Number	Fluoropyrimidine Schedule	Cancer type	Analyzed SNP (DNA source)	Clinical end-point (toxicity scale)
11	**Deenen MJ** (Clin Cancer Res 2011)	Amsterdam (Netherlands)	Randomized trial CAIRO2	568 (out of 736)	**Capecitabine** (+oxaliplatin + bevacizumab ±cetuximab)	Colorectal metastatic	8 SNP including *2A and 2846 (blood)	85% G3-4 toxicity 24% G3-4 diarrhea (CTCAE v3)
6	**Boisdron-Celle M** (ASCO 2013 #3601)	Angers (France)	Prospective cohort (B arm, non-adjusted 5FU dose)	385 (out of 410)	**5FU**-based	Colorectal (51 % adjuvant setting, 49% metastatic)	Various SNP including *2A, *13 and 2846 (blood)	Cycles 1-2-3: 1.6% G3-4-5 toxicity 0.25% lethal toxicity
5	**Boisdron-Celle M** (Cancer Letter 2007)	Angers (France)	Prospective cohort	252	**LV5FU2 or FUFOL** 5FU dose adjustment on PK from cycle 2	Colorectal	Various SNP including *2A and 2846 (blood)	Cycles 1-2: 6.3% G3-4 toxicity 3.8% G4 toxicity 0.8% lethal toxicity (CTCAE)
8	**Capitain O** (Pharmacogenomics J 2008)	Angers (France)	Retrospective	76	**LV5FU2** or **FUFOL** 5FU dose adjustment on PK from cycle 2	Colorectal	Various SNP including *2A, *13 and 2846 (blood)	Cycle 1: 6.6% G3 toxicity G3, 3.9% G4 toxicity (WHO)
35	**Morel A** (Mol Cancer Ther 2006)	Angers (France)	Prospective cohort	487	**5FU-based** CT (5 different regimens) 5FU dose adjustment on PK from cycle 2	Colorectal, stomach, breast, head & neck (FU naive)	9 SNP including *2A, *13 and 2846 (blood)	Cycles 1-2: 4.9% G3 toxicity, 4.1% G4 toxicity 4% G3-4 diarrhea 4% G3-4 neutropenia (CTCAE)
20	**Froelich T** (Int J Cancer 2015)	Berne (Switzerland)	2 prospective cohorts	485	79% **5FU** 21% **capecitabine** (5 schedules)	85% gastro-intestinal, most of them colorectal	Exome sequencing + jonctions	14% G3-4 toxicity (CTCAE v3)
42	**Schwab M** (J Clin Oncol 2008)	Hambug (Germany)	Retrospective consecutive	656 (out of 683)	**5FU** + folinic acid or levamisole	Gastrointestinal, breast and others	Variant *2A and 2846	Cycles 1-2-3: 16.1% G3-4 toxicity: 8.6% diarrhea, 7.6% mucositis, 4.7% leucopenia (WHO)
40	**Rosmarin D** (Gut 2015)	International	Randomized trial QUASAR2	940 (out of 1892)	**Capecitabine** ± bevacizumab	Colorectal (adjuvant)	239 SNP including *2A and 2846 (possibly *13)	Cycles 1 to 8: 34% G3-4 toxicity: 10% diarrhea, 2% neutropenia, 1% vomiting,1% mucositis (CTCAE v3)

Ref number	Ref	Team (Country)	Study type	Patient Number	Fluoropyrimidine Schedule	Cancer type	Analyzed SNP (DNA source)	Clinical end-point (toxicity scale)
-	**BIOCOLON study** (unpublished)	Limoges (France)	Prospective	173	**5FU**-FA or **capecitabine** combined with irinotecan or oxaliplatin	Colorectal (adjuvant, 1st line)	4 SNP including *2A, *13 and 2846 (blood)	Cycle 1: 43% G3-4 hematotoxiciy + diarrhea + mucositis
31	**Loganayagam A** (Br J Cancer 2013)	London (UK)	Retrospective (a priori non selected on toxicity)	430	**5FU** (43%) **Capecitabine** (57%) most of them combined with oxaliplatin	Gastro-intestinal (85% colorectal) 48% adjuvant, 4% neo-adjuvant, 48% palliative	9 SNP including *2A, *13 and 2846 (blood)	Cycle 1: 16% G3-4 diarrhea, 4% G3-4 mucositis, 10% G3-4 neutropenia (CTCAE v3)
29	**Lee A, Diasio RB** (J Natl Cancer Inst 2014)	Mayo Clinic Rochester (USA)	Randomized trial NCCTG N0147	2594	**5FU** (FOLFOX or FOLFIRI ± Cetuximab)	Colon stage III	Variants *2A, *13 and 2846 (blood)	All cycles: 33% G3-4 toxicity (CTCAE v3)
23	**Gross A** (PLOS One 2008)	Munich (Germany)	1 prospective cohort + 2 retrospective cohorts	128	**5FU**-based CT or **Capecitabine** (various regimens)	Gastrointestinal, Breast	Variants *2A and 2846 (blood)	Cycles 1-2-3: 30% G3-4 toxicity, 1.6% lethal toxicity (CTCAE v3)
34	**Milano G** (SABCS 2013)	Nice (France)	Prospective	281	**Capecitabine** (88% monotherapy, 22% with target therapy)	Breast metastatic	4 SNP including *2A, *13 and 2846 (blood)	Cycles 1-2: 19.6% G3-4 toxicity, 12.2% G3-4 hematological or diarrhea (CTCAE v3)
28	**Kristensen MH** (J International Med Research 2010)	Naestved (Denmark)	Sequential recruitment	68	75% **5FU**-FA ± oxaliplatin, 25% Capecitabine	Colorectal	Variants *2A, *13 and 2846 (blood)	Cycles 1-2: 13% grade 3-4 (CTCAE v3)
26	**Jennings BA** (PLOS One 2013)	Norwich (UK)	Prospective	254	37% **5FU** (12% alone, 23% in association), 63% **Capecitabine** (23% alone, 40% in association)	Colorectal	4 SNP including *2A and 2846 (blood)	Toxicity recorded for 12 weeks: 17% G3-4 toxicity (CTCAE v4)
4	**Boige V** (ASCO 2015)	Villejuif (France)	Randomized trial FFCD 2000-05 (out of 410)	339	**5FU** (FOLFOX or FOLFIRI)	Colorectal	25 SNP including *2A, *13 and 2846 (blood)	All cycles: 50% G3-4-5 toxicity (40% hematologic, 13 to 22% gastrointestinal)
4	**Boige V** (ASCO 2015)	Villejuif (France)	Randomized trial PETTAC-8	1545 (out of 2559)	**5FU** (FOLFOX ±Cetuximab)	Colorectal	25 SNP including *2A, *13 and 2846 (blood)	All cycles (CTCAE v3)

CT = Chemotherapy, 5FU = Fluorouracil, PK= pharmacokinetics, SNP= Single Nucleotide Polymorphism, variant *2A=IVS14+1G>A, variant *13=1679T>G.

Table 2: Studies preselected for the DPD phenotype meta-analysis (U and/or UH2/U in plasma or urine, or DPD activity)

Ref number	Ref	Team (Country)	Study type	Patient number	Fluoropyrimidine Schedule	Cancer type	Phenotype approach	Clinical end-point (toxicity scale)
6	**Boisdron-Celle M** (ASCO 2013 #3601)	Angers (France)	Prospective cohort (B arm, non-adjusted 5FU dose)	385 (out of 410)	**5FU**-based	Colorectal (51 % adjuvant setting, 49% metastatic)	U and UH2/U plasma	Cycles 1-2-3: 1.6% G3-4-5 toxicity, 0.25% lethal toxicity
5	**Boisdron-Celle M** (Cancer Letter 2007)	Angers (France)	Prospective cohort	252	**LV5FU2 or FUFOL** 5FU dose adjustment on PK from cycle 2	Colorectal	U and UH2/U plasma	Cycles 1-2: 6.3% G3-4 toxicity, 3.8% G4 toxicity 0.8% lethal toxicity (CTCAE)
8	**Capitain O** (Pharmacogenomics J 2008)	Angers (France)	Retrospective	76	**LV5FU2** or **FUFOL** 5FU dose adjustment on PK from cycle 2	Colorectal metastatic	U and UH2/U plasma	Cycle 1: 6.6% G3 toxicity G3, 3.9% G4 toxicity (WHO)
21	**Gamelin E** (J Clin Oncol 1999)	Angers (France)	Prospective cohort	81	**5FU** + FA	Colorectal	U and UH2/U plasma	All cycles. Toxicity grade and kind not described (WHO)
7	**Budai B** (Pharmacogenetics and Genomics 2012)	Budapest (Hungary)	Prospective cohort	85	**FOLFIRI** + bévacizumab	Colorectal metastatic	PBMC-DPD activity	14% neutropenia G3-4, 6% diarrhea G3-4, 5% vomiting G3-4 (CTCAE v3)
48	**Zhang X** (Int J Med Sci 2013)	Changchun (China)	Cohort study	60	**5FU** (FOLFOX)	Colorectal	UH2/U plasma	15% hematotox G3-4, 22% digestive toxicity G3-4 (WHO)
46	**Vokes EE** (J Clin Oncol 1996)	Chicago (USA)	Prospective cohort	59	**5FU**-AF-Cisplatine-Interferon	Head and neck	PBMC-DPD activity	Cycle 1: 24% neutropenia G3, 3% neutropenia G4, 7% diarrhea G3, 6% vomiting G3, 22% mucositis G3, 11% mucositis G4, 5% lethal toxicity
9	**Carlsson G** (Cancer Chem Pharm 2014)	Göteborg (Sweden)	Prospective	73	**5FU**-FA ± oxaliplatin	Colorectal (adjuvant)	U and UH2/U saliva	All cycles: 50% neutropenia G3-4, 26% nausea G3-4, 5,5% vomiting G3-4, 15,3% diarrhea G3-4, 1,4% mucositis G3-4 (CTCAE v4)

Ref number	Ref	Team (Country)	Study type	Patient number	Fluoropyrimidine Schedule	Cancer type	Phenotype approach	Clinical end-point (toxicity scale)
47	**Wettergren Y** (Cancer 2012)	Göteborg (Sweden)	Prospective	143	**5FU**-FA ± oxaliplatin	Colorectal (adjuvant)	U and UH2/U urinary	Toxicity score (CTCAE v4)
-	**BIOCOLON study** (unpublished)	Limoges (France)	Prospective	173	**5FU**-FA or **capecitabine** combined with irinotecan or oxaliplatin	Colorectal (adjuvant, first line)	U and UH2/U plasma	Cycle 1: 43% G3-4 hematotoxiciy/ diarrhea/mucositis
22	**Garg MB** (Br J Cancer 2012)	Newcastle (Australia)	Prospective	67	**5FU** + FA (Mayo and weekly schedules)	Colorectal	U and UH2/U plasma	Cycle 1 Mayo: 2% leucopenia G4, 32% neutropenia G4, Cycle 1 Weekly: 6% diarrhea G3
28	**Kristensen MH** (J International Med Research 2010)	Naestved (Denmark)	Sequential recruitment	68	75% **5FU**-FA ± oxaliplatin, 25% Capecitabine	Colorectal	U and UH2/U plasma	Cycles 1-2: 13% grade 3-4 (CTCAE v3)
34	**Milano G** (SABCS 2013)	Nice (France)	Prospective	286	**Capecitabine** (88% monotherapy, 22% with target therapy)	Breast metastatic	U and UH2/U plasma	Cycles 1-2: 19.6% G3-4 toxicity, 12.2% G3-4 hematological or diarrhea (CTCAE v3)
13	**Di Paolo** (Ann Oncol 2001)	Pise (Italy)	Prospective	110	**5FU** + FA	Colorectal	PBMC-DPD activity	Cycle 1: 4.5% toxicity G3-4 (WHO)

DPD= dihydropyrimidine deshydrogenase, FA = Folinic acid, 5FU= 5-Fluorouracil, PBMC = Peripheral blood mononuclear cell, U = Uracil, UH2 = dihydrouracil, WHO = World Health Organization.

Table 3: Prospective studies combining genotype and phenotype approaches

(See Table 2 and 3 for abbreviations)

Ref number	Ref	Team (country)	Study type	Patient number	Fluoropyrimidine Schedule	Cancer type	Analyzed SNP (DNA source)	Phenotype approach	Clinical endpoint (toxicity scale)
6	**Boisdron-Celle M** (ASCO 2013 #3601)	Angers (France)	Prospective cohort (B arm, non-adjusted 5FU dose)	385 (out of 410)	**5FU**-based	Colorectal (51 % adjuvant setting, 49% metastatic)	Various SNP including *2A, *13 and 2846 (blood)	U and UH2/U plasma	Cycles 1-2-3: 1.6% G3-4-5 toxicity, 0.25% lethal toxicity
5	**Boisdron-Celle M** (Cancer Letter 2007)	Angers (France)	Prospective cohort	252	**LV5FU2 or FUFOL** 5FU dose adjustment on PK from cycle 2	Colorectal	Various SNP including *2A and 2846 (blood)	U and UH2/U plasma	Cycles 1-2: 6.3% G3-4 toxicity, 3.8% G4 toxicity 0.8% lethal toxicity (CTCAE)
8	**Capitain O** (Pharmacogenomics J 2008)	Angers (France)	Retrospective	76	**LV5FU2** or **FUFOL** 5FU dose adjustment on PK from cycle 2	Colorectal metastatic	Various SNP including *2A, *13 and 2846 (blood)	U and UH2/U plasma	Cycle 1: 6.6% G3 toxicity G3, 3.9% G4 toxicity (WHO)
-	**BIOCOLON study** (unpublished)	Limoges (France)	Prospective	173	**5FU**-FA or **capecitabine** combined with irinotecan or oxaliplatin	Colorectal (adjuvant, first line)	4 SNP including *2A, *13 and 2846 (blood)	U and UH2/U plasma	Cycle 1: 43% G3-4 hematotoxiciy/diarrhea/ mucositis
28	**Kristensen MH** (J International Med Research 2010)	Naestved (Denmark)	Sequential recruitment	68	75% **5FU**-FA ± oxaliplatin, 25% Capecitabine	Colorectal	Variants *2A, *13 and 2846 (blood)	U and UH2/U plasma	Cycles 1-2: 13% grade 3-4 (CTCAE v3)
34	**Milano G** (SABCS 2013)	Nice (France)	Prospective	281	**Capecitabine** (88% monotherapy, 22% with target therapy)	Breast metastatic	4 SNP including *2A, *13 and 2846 (blood)	U and UH2/U plasma	Cycles 1-2: 19.6% G3-4 toxicity, 12.2% G3-4 hematological or diarrhea (CTCAE v3)

Individual patient data meta-analyses evaluating the link between dihydropyrimidine deshydrogenase (DPD) genotype and/or phenotype and severe fluoropyrimidine toxicity

Reference of your study (1st author+ year + complete reference of the journal (vol/N°/page) or Ref in protocol): ……
……
Study name / Protocol number (if any): …… / ……
First name/Last name of Investigator: …… /……
Address: ……
……
Telephone: …… Fax: …… Email: …… @……
Is the above-cited investigator, the appropriate contact person for the collection of study data? ☐ Yes ☐ No
If different, please indicate the statistician or other appropriate person to contact:
First name/Last name: …… /……
Address: ……
……
Telephone: …… Fax: …… Email: …… @……

Information from your publication met the following criteria and led us to select it as potentially eligible for the Meta-analysis:
- *Unbiased patient recruitment with prospective collection of toxicity and more than 50 assessable patients:* ☐ Yes ☐ No
- *Patients with solid tumor receiving 5FU or capecitabine, whatever the administration route and regimen, with non-ambiguous information on the chemotherapy protocol:* ☐ Yes ☐ No
- *Patients without fluoropyrimidine dose adjustment from the first cycle based on 5FU pharmacokinetics, or based on DPD phenotyping and/or genotyping:* ☐ Yes ☐ No
- *Toxicity documented at least at cycle 1 (based on CTCAE or WHO criteria):* ☐ Yes ☐ No
- *Patients with available pre-treatment quantitative DPD phenotyping (whatever the approach) and/or Caucasian patients with at least known DPYD *2A and 2846A>T genotypes:* ☐ Yes ☐ No

If you answered no at least once, please specify: ……
……
……

Does the present patient cohort overlap with patients included in additional selected studies (Appendix1) by your team?
☐ No ☐ Yes, specify the reference …….. and how many patients overlapped ……..

Is a copy of your study protocol enclosed? ☐ Yes ☐ No

Are the information extracted from your publication, detailed in **Appendix I** of the MA protocol, correct? ☐ Yes ☐ No
If no, please make corrections: ……
……
……
……
……
……

Is the above-cited publication the most recent published paper relative to this study? ☐ Yes ☐ No
If no, please give the reference of the updated publication: ……
……

Did your team perform additional relevant studies not listed in Appendix I of the protocol? ☐ Yes ☐ No
If yes, please provide information (either reference of published study, or short description of unpublished study):
……
……
……

Are you aware of any other relevant studies not listed in Appendix I of the protocol ☐ Yes ☐ No
If yes, please provide information (reference of studies) ……
……
……

Regarding patients included in your study, do you have any additional biological data potentially relevant for fluoropyrimidine toxicity (for instance, other analyzed genes (TS, MTHFR…), pharmacokinetic data …)? ☐ Yes ☐ No
As a matter of principle, would you agree to share these additional data? ☐ Yes ☐ No ☐ Don't known
If yes, please outline the nature of additional biological data and indicate how many patients would be concerned:
……
……
……
We will contact you for further possible collaboration and regulatory agreement.

Regarding patients included in your study, do you have germinal DNA still available? ☐ Yes ☐ No
As a matter of principle, would you agree to share this DNA material for additional genetic analyses in the context of a future international study? ☐ Yes ☐ No ☐ Don't known
If yes, please indicate approximately how many patients would be concerned: ……
and if feasible please indicate the averaged quantity of DNA available per patient ……
We will contact you for further possible collaboration and regulatory agreement.

Regarding the use of individual patient data for future statistical methodological research, do you consent to the use of the anonymized study data that you supplied for future statistical methodological research? ☐ Yes ☐ No

If you agree to share your data and join the FUSAFE-MA collaborative group, all supplied data will remain the property of the trialist(s) who supply it. These data will remain confidential and will not be used or circulated in any way.
First name/last name: ……
Acting as: ……

☐ **Yes, I agree to join the FUSAFE-MA collaborative group and take part in the Meta-analysis.**
☐ No, I don't agree to join the FUSAFE-MA collaborative group.
Date: …… Signature: ……

Please return the completed signed form (pages 1 to 5)
by mail, fax (33 142 115 258) or Email (jean-pierre.pignon@gustaveroussy.fr) to:
Dr Jean-Pierre Pignon
Gustave Roussy Cancer Campus,
114 rue Edouard Vaillant,
94805 Villejuif cedex, France
(Tel 33 142 114 565)

Additional information to be completed regarding your study

Date of first patient inclusion (day/month/year): |___|___| / |___|___| / |___|___|___|___|
Date of last patient inclusion (day/month/year): |___|___| / |___|___| / |___|___|___|___|

Were the patient naïve of previous fluoropyrimidine treatment? ☐ Yes ☐ No ☐ Both ☐ Unknown
Was patient informed consent required? ☐ Yes ☐ No ☐ Unknown
Were the biologists blind to the clinical data? ☐ Yes ☐ No ☐ Unknown

What was/were the ethnic group/s of studied patients? ☐ Mainly Caucasian (>90%) ☐ Unknown
☐ Other ethnic group/s, please specify: ……..

Was the study part of a clinical trial?
☐ Yes, the study was: ☐ a randomized trial ☐ a non-randomized trial ☐ a subgroup from a trial
☐ No, the study was: ☐ a cohort study with consecutive patient recruitment ☐ a retrospective study
Whatever the answer, please give details (name of the trial, corresponding reference……): ……
……

- If your study was not a clinical trial, do you consider that patient recruitment was unbiased regarding fluoropyrimidine toxicity? ☐ Yes (unbiased, well perform case-control study and exposed/no exposed study are appropriate)
  ☐ No (patient recruitment potentially biased: enriched population with DPD deficiency or enriched population with toxicity, or phenotype/genotype analysis performed on a selected subgroup population ….)

- If the series of patients assessable for DPD phenotype/genotype is a result of subgroup selection, please outline the selection process criteria: ……
……

Were the data on toxicity collected? ☐ Prospectively ☐ Retrospectively

If answers to the following queries are not provided in the enclosed protocol or the publication, please complete:

Which TNM or staging classification was used? ……
Which Performance Status was used? ☐ ECOG ☐ Karnofsky ☐ Other, specify: ……

Are 5FU pharmacokinetics data available for at least some of the patients? ☐ No ☐ Yes
If Yes, please give details and indicate how many patients are concerned: ……
……

Fluoropyrimidine-dose adjustment

Were individual fluoropyrimidine doses adjusted based on a criterion related to DPD status (i.e. fluoropyrimidine pharmacokinetics, DPD phenotyping or *DPYD* genotyping)? ☐ No ☐ Yes
If yes, please specify the DPD-related criteria used for dose adjustment?
☐ fluoropyrimidine pharmacokinetics or test-dose pharmacokinetics (5FU, uracil)
☐ DPD phenotyping, please specify: ……
☐ DPD genotyping, please specify: ……
If yes, please specify at which cycles such dose-adjustments were performed:
☐ cycle 1 ☐ cycle 2 ☐ cycle 3 ☐ others, specify: ……

Fluoropyrimidine-related toxicity

Which criterion was used?

☐ NCI-CTCAE version: …… ☐ WHO version: …… ☐ Other, specify: ……

What was the frequency of toxicity evaluation (e.g. every 2 weeks)? ……

What are the toxicity cycles documented in the electronic database :

☐ cycle 1 alone ☐ cycle 2 alone ☐ cycle 3 alone ☐ cycles 1-2-3 together

☐ all cycles together

☐ each cycle separately (preferred format)

☐ other, specify:……

What is the format of the maximum toxicity grade(s) documented in your electronic database:

☐ each grade separately including zero (0-1-2-3-4-5, preferred format)

☐ only toxic grade separately (1-2-3-4-5)

☐ merged grade 0-1-2 *versus* 3-4

☐ other merged grade, specify: ……

What are the toxicities documented in your electronic database:

☐ global hematotoxicity ☐ anemia ☐ leucopenia ☐ neutropenia ☐ thrombopenia

☐ global digestive toxicity ☐ diarrhea ☐ nausea ☐ vomiting ☐ mucositis/stomatitis

☐ severe infection ☐ asthenia ☐ neurotoxicity ☐ cardiotoxicity

☐ cutaneous toxicity ☐ hand-foot syndrome ☐ others: ……..

DPD phenotyping

Are pre-treatment quantitative DPD phenotype data (true values) available? ☐ No ☐ Yes

Were these data collected: ☐ Prospectively ☐ Retrospectively

What is the nature of available DPD phenotyping:

☐ Plasma uracil, unit: …… ☐ Plasma UH2/U

☐ Urinary uracil, unit: …… ☐ Urinary UH2/U

☐ Salivary uracil, unit: …… ☐ Salivary UH2/U

☐ DPD enzyme activity in blood mononuclear cells, unit: ……

☐ Other, specify: …… unit: ……

Which analytical method was used? Please specify or give the corresponding reference: ……

……

……

Did you participate in inter-laboratory or external quality controls? ☐ Yes ☐ No ☐ Unknown

Did you systematically introduce internal quality controls in your assays? ☐ Yes ☐ No ☐ Unknown

Could you inform us of the variability of your assays? ☐ Yes ☐ No

If yes and easy to provide us these information, please state the variability (CV%) for each of the analysed markers:

Nature of biological marker…… Intra-assay variability |_____| % Inter-assay variability |_____| %

Nature of biological marker…… Intra-assay variability |_____| % Inter-assay variability |_____| %

Nature of biological marker…… Intra-assay variability |_____| % Inter-assay variability |_____| %

What was the time-interval in days (min-max) between biological sampling and the start of fluoropyrimidine chemotherapy?

Min |_____| day Max |_____| days ☐ Unknown

Was the sampling time (within the day) specified?

☐ No, there was no requirement for sampling time ☐ Unknown

☐ Yes, sampling time was performed between |_____| ..... (am/pm) and |_____| ..... (am/pm)

Did you have special pre-analytical requirements (sample handling)? ☐ Yes ☐ No ☐ Unknown

If yes, please specify (e.g. delay for sample handling, storage conditions …): ……

……

***DPYD* genotyping**

Were *DPYD* genotype data available in your study? ☐ No ☐ Yes,

Was DNA source collected: ☐ Prospectively ☐ Retrospectively ☐ Unknown

Were genotype data collected: ☐ Prospectively ☐ Retrospectively ☐ Unknown

What was the DNA source? ☐ Blood ☐ Other, specify: ……

What were the analysed *DPYD* variants: ☐ variant *2A (1905+1G>A, IVS14+1G>A, rs3918290)

☐ variant 2846A>T (D949V, rs67376798)

☐ variant *13 (1679T>G, I560S, rs55886062)

☐ Others, specify: ……

……

……

Alternatively, specify if full exome *DPYD* sequencing (or full *DPYD* sequencing) was performed in at least a number of patients:

……

……

Did you perform haplotype analyses? ☐ Yes ☐ No

Which analytical method(s) did you use? Please specify or give the corresponding reference: ……

……

Did you participate in inter-laboratory or external quality controls for at least one *DPYD* variant? ☐ Yes ☐ No

Regarding the above-requested individual data

Do you think that some of these data will never be available? ☐ No ☐ Yes

If yes, please specify which one: ……

……

……

Do you consider that additional covariate(s) not included in the list of *Data to be collected* (chapter VII MA protocol, pages 9-10), would be relevant to consider in the context of the present study? ☐ No ☐ Yes, please specify:……

……

……

……

We thank you very much for your collaboration in the FUSAFE-MA project. Last step is to complete the individual database, if possible according to the suggested format presented in a file a part (Appendix 3).

APPENDIX 3: DATA TO BE COLLECTED AT PATIENT LEVEL

The preferred format for the information is described in the following pages. Following them will greatly facilitate the work for this project. However, if a different format is more convenient for you, this should cause no great difficulty as long as it is clearly specified.

The easiest way for us to receive the data is by e-mail. If you consider sending data via email, please encrypt the data and let us know the encryption key in a separate email, or protect them by a password. SAS database is the most convenient format for us, but we may handle other formats.

The guidelines consider all the information we may enter in the analyses, we are aware that you did not necessarily collect all listed variable. Please feel free to provide the data you collected in a format that is closest as possible as the format provided in the guidelines. In order to consider your data in the meta-analysis project, you must, at least, provide some toxicity and genotype and /or phenotype data.

The patient characteristics requested must be considered at study entrance. The treatment doses and regimens must be the treatment that patients actually received.

The preferred requirement for toxicity is toxicity cycle by cycle and type par type. Such data may be not available. Please provide the more detailed data available and describe the method use to report them in the database (cf. Toxicity data presentation suggestion).
If toxicity is not available cycle by cycle, specify the corresponding period (e.g. C1-C3 or any cycle), and provide, if possible, which cycle corresponds to the maximum grade observed for the first time.

Variable	Format/Coding	specify
Study identifier	Character - Width 15	
Patient Identifier	Character (No name) - Width 15	
Patient characteristics at entrance		
Entrance date$^{\mu}$	Date - dd/mm/yyyy - Width 10	
Date of birth$^{\mu}$	Date - dd/mm/yyyy - Width 10	
or Age (years)$^{\mu}$	Numeric width 3	
Sex*	Numeric 1=male, 2=female, 9=unknown - Width 1	
Ethnic group*	Numeric (1=Caucasian, 2=Asian, 3=Subsharian African/Afro-American, 4=Other) - width 1	other specify
Weight kg$^{\mu}$	Numeric - width 3	
Height cm$^{\mu}$	Numeric - width 3	
or BMI kg/m$^{2\mu}$	Numeric - width 2	
and BSA m$^{2\mu}$	Numeric - width 2	
Cancer localization*	Numeric (right colon = 1, left colon =2, colon ns=3, rectum= 4, Colorectal ns=5, gastric=6, head & neck =7, breast=8, other =9) - width 1	other specify
Stage*$^{\mu}$	Numeric (1=localized, 2= locally advanced, 3=advanced/metastatic, or better TNM or AJC staging) - width 1	other specify
Tstage /N stage /M stage$^{\mu}$	Numeric (TNM: 0 to 6, 9=unknown x 3)- Width 3	other scale specify
Performance Status (Karnofsky)$^{\mu}$	Numeric (999=unknown) - Width 3	
Performance Status (WHO/ECOG) $^{\mu}$	Numeric (9=unknown) - Width 1	
Creatinine clearance computing method	Character - width 20	
Creatinine clearance (ml/min)$^{\mu}$	Numeric - width 3	other unit specify
Creatinine (micromol/L) in blood$^{\mu}$	Numeric - width 4	other unit specify
See last page for footnotes		

Variable	Format/Coding	specify
DPYD genotype (if relevant)		
type of sample*	1=blood; 2 tumor; 3=other	
*2A	Numeric - width 1 (0=wild type, 1=heterozygote mutation, 2 = homozygote mutation)	other specify
*13	Numeric - width 1 (0=wild type, 1= heterozygote mutation, 2 = homozygote mutation)	other specify
2846A>T	Numeric - width 1 (0=wild type, 1=heterozygote mutation, 2 = homozygote mutation)	other specify
other DPYD polymorphism 1	Numeric - width 1 (0=wild type, 1=heterozygote mutation, 2 = homozygote mutation)	other specify
…..	….	
other DPYD polymorphism N	Numeric - width 1 (0=wild type, 1=heterozygote mutation, 2 = homozygote mutation)	specify the name of the SNP
DPD phenotype (if relevant)		
Tissue collected*	Numeric (1=blood, 2=urine, 3=saliva 4=other) - width 1	other specify
Time of day of sample collection*	Date - hh:mm - width 5	
Nature of DPD test	Numeric (1= DPD activity, 2=uracil alone, 3=UH2/U, 4=Other) - width 1	other specify
Nature of second DPD test, if any	Numeric (1= DPD activity, 2=uracil alone, 3=UH2/U, 4=Other) - width 1	other specify
DPD test results 1[$]	Numeric - width 8	specify unit
DPD test results 2[$]	Numeric - width 8	specify unit
DPD test results 3[²]	Numeric - width 8	specify unit
DPD test results 4[²]	Numeric - width 8	specify unit
See last page for footnotes		

Variable	Format/Coding	specify
Treatment characteristics		
Date of first fluoro-pyrimidine administration in the current study	date - dd/mm/yyyy - Width10	
Type of fluoropyrimidine*	Numeric (1=5FU, 2=capecitabine) - width 1	
Modality of fluoro-pyrimidine administration*	Numeric (1=bolus, 2= continuous infusion, 3=both, 4=per os) - width 1	
Line of treatment	Numeric (1=first line for advanced disease, 2=second line for advanced disease, 3=third line for advanced disease, 4=neoadjuvant/induction, 5=adjuvant/consolidation, 6= concomitant to radiotherapy) - width 1	
Fluoropyrimidine naive*$^{\mu}$	Numeric (1=Yes , 0= No) - width 1	
Combined drugs (regimen)*	Numeric (1= FUFOL, 2= LV5FU2, 3=FOLFOX, 4=FOLFIRI, 5=XELOX, 6=XELIRI, 7=FOLFIRINOX, 8=other specify) - width 1	
Combined drug 1 at cycle 1*	Numeric (1=irinotecan, 2=oxaliplatin, 3=cisplatin, 4=carboplatin, 5=other) - width 1,	other specify
Combined drug 2 at cycle 1*	Numeric (1=irinotecan, 2=oxaliplatin, 3=cisplatin, 4=carboplatin, 5=other) - width 1,	other specify
Combined drug 3 at cycle 1 (target therapy or other)*	Numeric (1=bevacizumab, 2=cetuximab, 3=panitumumab, 4=interferon, 5=levamisol, 6=other) - width 1	other specify
Folinic Acid	Numeric (1=Yes , 0= No) - width 1	
Received fluoropyrimidine dose mg/m² at cycle 1*	Numeric - width 8	other unit specify
Received fluoropyrimidine dose mg/m² at cycle 1*$^{,\%}$	Numeric - width 8	other unit specify
Number of fluoropyrimidine cycles received	Numeric - Width 2	

Variable	Format/Coding	specify
Toxicity (list of items to be collected)		
Cycle	Numeric - width 1	
Toxicity type[§]	Numeric (code given below) - width2	
Criterion for toxicity grading*	Numeric (1=WHO, 2= CTAE, 3=other) - width 1	If other specify
Maximum toxicity grade by cycle if available - see below the format	Numeric - width 1	

[μ] At the start of the treatment considered in the study

* for variable with the same value for all patients within a given study, information may be provided in the Study Form description instead of the database.

[$] For UH2/U (or other ratio), please provide when available both UH2 and U data, if UH2/U in urine please provide creatinine value in urine (with unit).

[%] In case of both 5FU bolus and continuous, report here the bolus value and above the continuous value.

[§]1=diarrhea,2= nausea,3= vomiting,4= mucositis,5= stomatitis, 6=anemia,7= leucopenia,8= neutropenia, 9=febrile neutropenia,10=thrombocytopenia, 11=severe infection,12= neuropathy, 13=cutaneous toxicity,14= hand-foot syndrome, 15=asthenia, 16=cardio-toxicity, 17=other. or 20=hematology, 21= cutaneous, 22=digestive, 23=any type

Toxicity data presentation suggestion

Patient id	Toxicity type	cycle	grade
1	1	1	x
1	2	1	x
1	3	1	x
1	…	…	x
2	1	1	x
…	…	…	x
N	…	…	x

If toxicity not available cycle by cycle, specify the corresponding period (e.g., C1-C3 or any cycle), and provide if possible the cycle number in which the maximum grade was observed for the first time.