Synopsis

Title	**Evaluating fruquintinib in combination with tislelizumab in metastatic colorectal cancer**
Short title	AIO QUINTIS trial
Trial type	prospective, randomized, open-label, multicenter phase II
Investigational medicinal product	Fruquintinib in combination with tislelizumab
Objectives / endpoints	**Primary Objective** To evaluate the efficacy of fruquintinib in combination with the PD-1 inhibitor tislelizumab in metastatic colorectal cancer. Corresponding primary endpoint • Overall survival rate at 12 months (OS@12) defined as proportion of subjects alive at 12 months after randomization **Secondary Objectives** a) To further determine the efficacy of fruquintinib in combination with the PD-1 inhibitor tislelizumab in metastatic colorectal cancer. Corresponding endpoints • Overall survival (OS) • Progression-free survival (PFS) acc. RECIST v1.1 • Objective response rate (ORR), defined as proportion of patients achieving complete or partial response (CR/PR) acc. RECIST v1.1 • Disease control rate (DCR), defined as proportion of patients achieving CR or PR or stable disease (SD) acc. RECIST v1.1 • • Duration of response (DoR) b) To evaluate the safety and tolerability of fruquintinib in combination with the PD-1 inhibitor tislelizumab in metastatic colorectal cancer Corresponding endpoint • Assessment of safety of the treatment as determined by the incidence, nature, causality, frequency, timing and severity of adverse events using NCI CTCAE 5.0 c) To assess quality of life (QoL) data of patients treated with bemarituzumab in combination with SOC treatment. Corresponding endpoints • Assessment of QoL during treatment and follow-up using EORTC QLQ C30 and EQ-5D-5L questionnaires **Exploratory Objectives** To correlate analysis between selected molecular serum parameters and clinical data to identify molecular biomarkers predictive for tumor response and survival.

Trial design	Participants eligible for this trial will be randomized 2:1 into one of the two arms stratified by • previous anti-angiogenic therapy ≥ or <12 months in total, • BRAF/RAS mutation status (mutation vs. wildtype) and • history of liver metastases (never vs. prior but treated) **Arm A (experimental arm)** • Fruquintinib (orally, 5 mg once a day, at day 1-21 of each 28-days cycle [Q4W]) plus • Tislelizumab (i.v., 200 mg, Q3W) **Arm B (control arm)** • Trifluridin/tipiracil (orally, 35 mg/m^2 twice a day, day 1-5 and day 8-12, Q4W) plus • Bevacizumab (i.v., 15 mg/kg, Q2W) The treatment will be performed until disease progression, unacceptable toxicity, patients' request or end of protocol defined treatment time (maximum of 9 months). All patients will be followed up for 18 months after last patient in or until death, withdrawal of consent or loss to follow-up, whatever occurs first.
Rationale	Clinical trial Patients with mCRC who have progressed on/after or are intolerant to fluoropyrimidines, oxaliplatin, irinotecan, anti-angiogenic and anti-EGFR therapies have limited therapeutic options with a dismal prognosis and a median overall survival (mOS) about 6 months with single agent regorafenib or trifluridin/tipiracil (Grothey, Van Cutsem et al. 2013, Mayer, Van Cutsem et al. 2015). Recent developments established the combination of trifluridin/tipiracil and bevacizumab in the 3rd line setting with a mOS of 10.8 months (hazard ratio for death, 0.61; 95% confidence interval [CI], 0.49 to 0.77; P<0.001 compared to single agent trifluridin/tipiracil) (Prager, Taieb et al. 2023). Furthermore, fruquintinib, a highly selective and potent oral inhibitor of vascular endothelial growth factor receptors (VEGFRs) 1, 2, and 3 showed significantly improved mOS of 7.4 months compared to 4.8 months with placebo in refractory colorectal cancer after median 4 lines of treatment (Dasari, Lonardi et al. 2023). Combination regimen with PD-1/L1 and/or CTLA4 inhibitors and/or TKIs/antiangiogenics in an all comer microsatellite stable (MSS) metastatic colorectal cancer (MCRC) population has not shown significant benefit in several randomized trials Atezolizumab/Bevacizumab (MODUL), Pembrolizumab/Lenvatinib (LEAP 17), Durvalumab/Tremelimumab (CCTG CO.26) (Chen, Jonker et al. 2020, Tabernero, Grothey et al. 2022, Kawazoe, Xu et al. 2023). Post hoc analyses revealed the existence of liver metastases as negative predictor

for treatment benefit (hazard ratio for overall survival of 0.65 in case of absence of liver metastases, compared to 0.78-0.91 if present). Beside defining an immunotherapy sensitive subgrooup in mCRC, no liver metastases (NLM) is a positive prognostic factor in the advanced setting (e.g. mOS of 12.1 for NLM vs. 6.4 months with liver metastases in patients treated with single agent fruquintinib). The combination of fruquintinib and PD-1 inhibitors has shown good tolerability and promising efficacy (Sun, Huang et al. 2021, Gou, Qian et al. 2022).

The studies mentioned above provide a strong rationale to conduct a randomized study comparing the efficacy and safety fruquintinb in combination with tislelizumab vs. trifluridin/tipiracil and bevacizumab in patients with refractory mCRC without active liver metastases (NLM) to improve overall survival and prevent resistance.

<u>Translational part</u>

For our translational analysis, we'll focus on three key areas crucial for understanding responses to chemo-immunotherapies. Central to this will be the examination of circulating tumor DNA (ctDNA), which involves identifying mutations present in both blood samples and tumor tissues through sequencing or PCR techniques. Notably, ctDNA has shown strong correlations with survival rates across various solid cancers, including colorectal cancer (Tie et al., NEJM, 2022). Our second area of translational investigation delves into the intricate interplay among diet, the microbiota, and microbiota-derived metabolites. Emerging evidence highlights the profound impact of the microbiota composition on immunotherapy response across various solid cancers (Routy et al., Science, 2018). Remarkably, studies in melanoma have demonstrated that manipulating the microbiota can effectively restore sensitivity to immunotherapies in patients receiving PD-1 inhibitors (Davar et al. and Baruch et al., Science, 2021). It's been observed, by us and others, that the microbiota's role partly hinges on metabolite production (Spencer et al., Science, 2022; Tintelnot et al., Nature, 2023). Notably, the diet serves as a crucial source of substrates for microbiota-derived metabolites. Thus, analyzing the diet, microbiota, and microbiota-derived metabolites will deepen our understanding of how the microbiota influences immunotherapy response. Our third focus in translational investigation revolves around how the peripheral and intratumoral immune cell compartment is modulated during therapy. We aim to examine the T cell receptor (TCR) repertoire within the tumor microenvironment (TME) and peripheral blood at baseline and potentially also throughout therapy. Notably, we and others have shown the role of TCR clonality during checkpoint inhibitor therapy in peripheral blood to predict response (Tintelnot et al. Frontiers Oncology 2022, Paschold et al. JITC 2023). Additionally, the modulation of peripheral immune dynamics by chemokines and cytokines serves as strong predictors for immunotherapy response. For instance, elevated levels of IL6 have been associated with decreased peripheral lymphocytes and increased neutrophils, leading to poorer immunotherapy response in gastroesophageal adenocarcinoma (Tintelnot and Stein et al. ASCO 2023)

Inclusion criteria	1. Patient* provide signed informed consent form. 2. Patient is ≥ 18 years at the time of given informed consent. 3. Patient has been diagnosed with histologically or cytologically proven microsatellite stable (MSS)/proficient mismatch repair (pMMR) metastatic adenocarcinoma of the colon or rectum, which is not amenable to potentially curative resection. 4. Patient without liver metastases (NLM) defined as subjects without active liver metastases at screening as determined on baseline imaging of the liver as performed by CT scan with contrast or MRI. Definitively treated liver metastases (which includes surgical resection, microwave or radiofrequency ablation, or stereotactic body radiation therapy, but not yttrium-90 or chemoembolization alone) that were treated at least 6 months prior to enrollment with no evidence of radiologic progression on subsequent imaging are considered to be non-active liver metastases. 5. Patient received previous treatment, which includes a 5-FU, oxaliplatin, irinotecan, bevacizumab and if indicated EGFR inhibitors, in the advanced setting or the patient has been intolerable or ineligible to those treatments. 6. Patient has an ECOG performance status ≤ 1. 7. Patient has a life expectancy > 12 weeks. 8. Patient has adequate hematological, hepatic and renal function. a. Absolute number of neutrophils (ANC) ≥ 1.5 x 10^9/L b. Platelets ≥ 100 x 10^9/L c. Hemoglobin ≥ 9 g/dL (5.58 mmol/L) d. Total bilirubin ≤ 1.5 times the upper limit of normal (ULN) (or < 2 x ULN in case of liver involvement or Gilbert's disease) e. AST (SGOT) and ALT (SGPT) ≤ 2.5 x ULN, AP ≤ 5 x ULN f. Serum creatinine ≤ 1.5 x ULN or creatinine clearance (measured by 24 h urine) ≥ 30 mL/min (i.e., if serum creatinine level is > 1.5 x ULN, then a 24-hour urine test must be performed to check the creatinine clearance to be determined). g. Urinary protein ≤2+ on dipstick or routine urinalysis (UA; if urine dipstick or routine analysis is ≥3+, a 24-hour urine collection for protein must demonstrate <2000 mg of protein in 24 hours to allow participation in this protocol) 10. Adequate coagulation function as defined by International Normalized Ratio (INR) ≤ 1.5, and a partial thromboplastin time (PTT) ≤ 5 seconds above the ULN (unless receiving anticoagulation therapy). 11. Female patients of childbearing potential or male patients with female partners of childbearing potential must agree to remain abstinent (refrain from heterosexual intercourse) or use contraceptive methods that result in a failure rate of <1% per year during the treatment period and for at least 4 months after the last trial treatment if randomized to Arm A or at least 6 months if randomized to Arm B. Male patients with a pregnant partner must agree to remain abstinent or to use a condom for the duration of the pregnancy. Female patients of child-bearing potential must have a negative pregnancy test within the last 7 days prior to the start of trial therapy. 12. Patient is willing and able to comply with the protocol (including contraceptive

	measures) for the duration of the study including undergoing treatment and scheduled visits and examinations including follow up * There is no data that indicates a specific gender distribution. Therefore, patients are included regardless of their gender.
Exclusion criteria	1. Patient has known allergic / hypersensitive reactions to at least one of the treatment components 2. Patient has known presence of tumors other than adenocarcinomas (e.g., leiomyosarcoma, lymphoma) or a secondary tumor other than squamous or basal cell carcinomas of the skin or in situ carcinomas of the cervix which have been effectively treated. The sponsor decides to include patients who have received curative treatment and have been disease-free for at least 5 years. 3. Patient receives simultaneous, ongoing, systemic immunotherapy, chemotherapy, or hormone therapy not described in the trial protocol. 4. Prior therapy with fruquintinib, trifluridin/tipiracil or anti-PD-1 or anti-PD-L1 5. Patient receives current treatment with any anti-cancer therapy ≤ 2 weeks prior to study treatment start unless rapidly progressing disease is measured. 6. Patient receives simultaneous treatment with a different anti-cancer therapy other than that provided for in the trial (excluding palliative radiotherapy for symptom control). 7. Patient has known untreated or symptomatic CNS or leptomeningeal metastases. 8. Patient has impaired cardiac function or clinically significant cardiac disease including unstable angina within 6 months before the first dose of study treatment, acute myocardial infarction < 6 months prior to the first dose of study treatment, New York Heart Association (NYHA) class II–IV congestive heart failure, uncontrolled hypertension (defined as an average systolic blood pressure > 160 mmHg or diastolic > 100 mmHg despite optimal treatment, uncontrolled cardiac arrhythmias requiring antiarrhythmic therapy other than beta blockers or digoxin, active coronary artery disease or corrected QT interval (QTc) ≥ 470 9. Patient has a serious infection requiring oral or IV antibiotics within 14 days prior to trial enrollment. 10. Patient has history of uncontrolled infection with human deficiency virus (HIV) or chronic infection with hepatitis B or C virus (HBV, HCV) 11. Patient has chronic inflammatory bowel disease 12. Patient has evidence of bleeding diathesis 13. Patient has history of gastrointestinal perforation or fistulae in past 6 months or risk factors for perforation 14. Patient has grade 3-4 GI bleeding within 3 months prior to first dose of trial therapy 15. Use of strong inducers or inhibitors of CYP3A4 within 2 weeks (or 5 half-lives, whichever is longer) before the first dose of study drug 16. Patient had a major surgery within 2 weeks prior to first dose of trial therapy 17. Patient experienced severe, life-threatening, or recurrent (Grade 2 or higher) immune-mediated adverse events (AEs) or infusion-related reactions including those that led to permanent discontinuation while on treatment

	with immune-oncology agents 18. Patient received prior immunosuppressive therapy: immunosuppressive doses of systemic medications of > 10 mg/day of prednisone or equivalent must be discontinued ≥ 2 weeks before the first dose of study treatment. Short courses of high dose corticosteroids and/or continuous low dose of prednisone (< 10 mg/day) are permitted. In addition, inhaled, intranasal, intraocular, and/or joint injections of corticosteroids are allowed 19. Patients has evidence of any other serious concomitant or medical condition that, in the opinion of the investigator, presents a high risk of complications to the patient or reduces the likelihood of clinical effect. 20. Female patient is pregnant or breast feeding or planning to become pregnant within and up to 4 months after end of treatment (if randomized to Arm A) or up to 6 months after the end of treatment (if randomized to Arm B).
Sample size	The efficacy assumption of the reference therapy is based on the results of the SUNLIGHT study, showing a 1-year overall survival rate of about 60% for mCRC NLM patients treated with trifluridine/tipiracil plus bevacizumab. We envisage that the OS@12 for the experimental treatments could increase to 74%, which is of clinically relevant advantage and achievable. Hence, for the experimental treatment to be considered as a desirable candidate for further development, the OS@12 rate of 74% or more should be achieved, but if it is 60% or less the experimental treatment would be insufficient for further investigation. Based on the above conditions for the maximum and minimum required level of efficacy, a standard exact single-stage phase 2 phase II design for the sample size calculation is applied with the following parameters, P0= 0.60, P1= 0.74, a one-sided type I error of 0.05, and a power of 80%. A sample size of 74 patients is needed for the experimental arm. Survival after 12 months detected in XX or more out of these patients will allow to formally reject the hypothesis of insufficient efficacy. The present trial has a randomized, non-comparative design with an calibration arm, whereas a 2:1 randomization favors the experimental arm. Therefore, a total of 106 patients are needed (74 in Arm A and 36 in Arm B). Thus, overall, 110 patients will be enrolled.
Efficacy evaluations/ criteria	On study, tumor assessment (CT or MRI) will be performed at baseline and every 8 weeks (Q8W ± 7 days) during trial treament calculated from the date of treatment initiation according to the standard of care. Response criteria according to RECIST v1.1 will be used by the site for treatment decisions until evidence of tumor progression. Subjects who complete the maximum treatment as specified in the protocol or discontinue trial intervention for reasons other than PD will have post-treatment follow-up for disease status performed Q12W ± 14 days (which is in line with the routine follow up tumor assessment) until progression, initiation of another anti-cancer treatment, withdrawing consent, lost to follow-up, death or end of the

	trial, whichever occurs first. A change from CT into MRI in the follow-up period is possible at any time. All subjects will be followed for survival until death, withdrawing consent, lost to follow-up or end of the study, whichever occurs first.
Safety evaluation	Safety assessments including physical examinations, performance status (ECOG), clinical laboratory profile and continuous assessments of adverse events will be performed at every visit. Adverse events will be monitored throughout the trial and graded in severity according to the guidelines outlined in the NCI CTCAE v5.0. Each subject will be followed during the treatment and for 30 days after last administration of study medication for adverse event, and for 90 days after last administration for serious adverse events (SAE) adverse events of special interest (AESIs) monitoring. SAEs/AESIs which are characterized as related to the treatment will be collected even after expiration of the 90 days after the last administration of study medication.
Translational research sample collection	Tumor tissue as well as blood and stool samples will be collected for analysis in separate translational research (TR) project if patients give their consent. Archival tumor biopsy samples will be collected for concomitant translational research whenever possible. Re-biopsy should be performed at time of progression as far as considered feasible and safe for the patient. In addition, blood and stool samples will be collected based on the below mentioned schedules. We will gather 20ml blood samples in STRECK's cell-free DNA BCT tubes at three distinct time points for ctDNA, cytokine/chemokine analysis, microbiota-derived metabolites, and TCR sequencing. These time points include baseline, after 4-6 weeks, and upon progression or after 6 months of therapy. Additionally, we will procure baseline formalin-fixed paraffin-embedded tumor tissue to identify cancer-specific mutations. Microbiota analysis will involve the examination of stool samples collected at the baseline time point. Stool collection will be facilitated using OMNIgene self-collection kits (OM200) and a toilet accessory kit (OM-AC-1) at home. Dietary patterns will be assessed through questionnaires, which participants can complete either at study centers or at home. These assessments will occur at baseline, after 4-6 weeks, and upon progression or after 6 months of therapy.
Statistical Analysis	At the analysis of primary endpoint the XXX design decision rules will be used. The OS rate together with 95% Clopper- Pearson CIs will be provided. Missing data for the primary endpoint will be considered as a treatment failure. Categorical variables will be summarized by frequency distributions (number, percentages of subjects, and missing values) and will be compared using Fisher's exact test; continuous variables will be summarized by number of subjects (N), mean, standard deviation, median, first and third quartile, minimum, maximum, missing values and will be compared using Wilcoxon Rank Sum tests. Time-to-

	event endpoints will be summarized using Kaplan-Meier methods and displayed both graphically and in tabular form. The tables contain the number of events, median and 25% and 75% percentile of the survival, and the survival rates at certain times. Statistical analysis for the secondary, exploratory and safety endpoints will also be primarily descriptive in nature. If any additional p values are calculated (e.g., in subgroup comparisons), they are purely descriptive and will be presented explicitly without referring to hypotheses or a significance level. Unless otherwise specified, all statistical tests are two-sided. Missing values will not be imputed. Data will be analyzed as observed. For time-to-event endpoints, missing values are treated as non-informative censoring values. Safety: Frequency tables will be compiled with the number and percentage of patients experienced the AE by treatment and total. Additionally, AEs will be summarized by seriousness of AE, relationship to the trial drug, and grading for all NCI CTC categories. QoL will be summarized using descriptive statistics and change in XXX before and after treatment at respective time points by treatment and total at defined timepoints. All analyses will be done using SAS v9.4 or higher
Number of trial sites	This trial will be conducted at approximately 30 centers in Germany and Austria
Duration of the trial (planned)	The estimated trial duration is 48 months (18 months recruitment plus 18 months' time span from LPI to LPLV plus 12 months study set up and closure activities). The global end of this trial is defined as the date of database closure to ensure the collection of survival data of patients and the active involvement of sites in the data cleaning process (e.g., addition source data may be requested or an additional monitoring visit may be necessary). The Sponsor may decide to terminate the trial at any time (refer to protocol section 5.6.2 for criteria for early trial termination).

Schedule of assessments

Table 1 Schedule of assessments

			Screening	Treatment Arm A: Tislelizumab (Q3W) plus fruquintinib (Q4W) Arm B: Bevacizumab (Q2W) plus Trifluridin/tipiracil (Q4W)	End of treatment/ Safety FU[a]	Follow-up[b]
	≤ 28 days	≤14 days	≤7 days	Day 1 of every cycle	30 days (± 3 days) after last dose	Every 12 weeks ± 14/21 days
Arm A Tislelizumab (i.v., 200 mg)				X		
Arm A Fruquintinib (p.o., 5 mg, OD)				day 1-21 of every 28-day cycle		
Arm B Bevacizumab (i.v., 15 mg/kg)				X		
Arm B Trifluridin/tipiracil (p.o., 35 mg/m² BD)				day 1-5 and day 8-12 of every 28-day cycle		
Informed Consent	X[c]					
Review of Inclusion/Exclusion Criteria	X					
Demographics and Medical/Cancer History [1]	X					
Prior and Concomitant Medication Review [2]		X		X	X	
Review Adverse Events		X[d]		X	X	X[e,f]
Full Physical Examination [3]		X			X	
Directed Physical Examination [4]				X		
Height, Weight and Vital Signs [5]		X		X	X	
12-Lead Electrocardiogram [6]		X		whenever clinically indicated	X	
ECOG Performance Status		X		X	X	
Viral Testing (HBV, HCV, HIV)			X			
Pregnancy Test – Urine or Serum β-hCG [7]			X	Monthly or whenever clinically indicated for up to 6 months after last dose of study treatment		
Hematology [8]			X	X	X	
Comprehensive Serum Chemistry Panel [9]			X	X	X	
Urinalysis [10]			X	X	X	
Coagulation [aPTT and INR or PT]			X	Whenever clinically indicated		
TSH (free T3, free T4) [11]			X	Whenever clinically indicated		
Tumor imaging [12]	X			every 8 weeks (Q8W ± 7 days)	X	(X)
Quality of Life (XX)	X			Together with tumor assessment (Q8W ± 7 days)	X	
Optional: Sampling tumor tissue [13]	X			Optional: re-biopsy at the timepoint of disease progression		
Optional: Blood and stool sampling			X	4-6 weeks after treatment initiation and at time of disease progression		
Optional: Diet Assessment	X					
Post-study anticancer therapy status					X	X
Survival Status					X	X

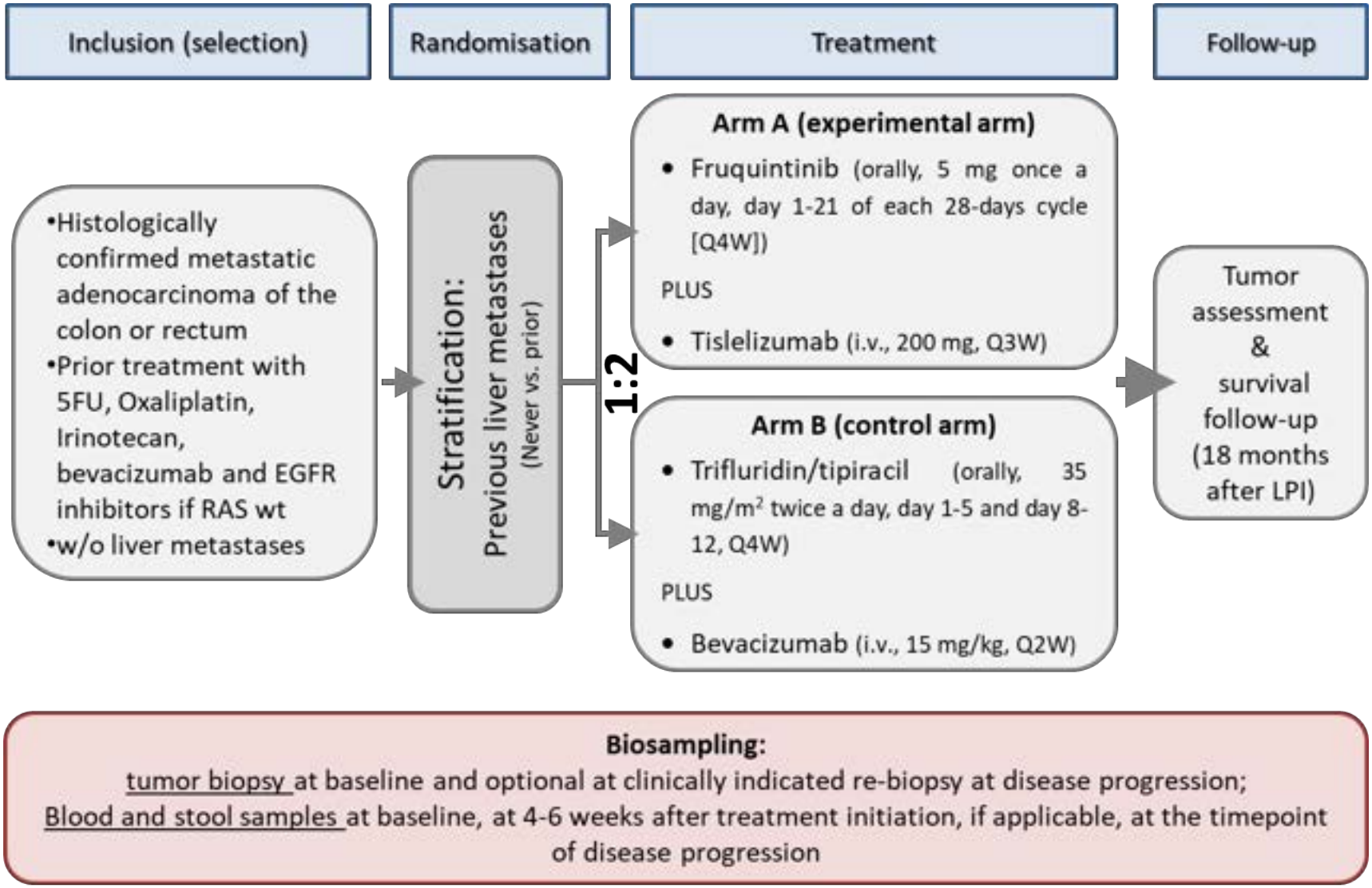
Note: All visits and procedures may be administered within a window of ± 3 days. The -28, -14 and -7 days screening timeframes refer to the maximum temporal distance between performance of the

specific assessment and enrollment and are not intended to define distinct screening visits. Unless specified otherwise, all study treatment visits must occur within ± 3 days from scheduled visit. Results of standard-of-care tests or examinations performed prior to obtaining informed consent and within 28 days prior to study entry may be used for screening assessments rather than repeating such tests. Screening local laboratory assessments obtained ≤ 7 days prior to the initiation of study treatment do not have to be repeated for visit 1 (baseline).

a. Patients will be asked to return to the clinic 30 days after the last dose of study treatment for an end-of-treatment/safety FU visit (or if not possible shortly before). If results of examinations are available which are not older than 14 days, examinations do not need to be repeated for EOT visit. Tumor imaging performed within 8 weeks prior to the treatment discontinuation visit do not need to be repeated for EOT visit.
b. Patients who discontinue study treatment will enter the follow-up phase with visits to be performed at least Q12W.
   Tumor status follow-up: For patients who discontinue study treatment in the absence of disease progression or who complete the maximum treatment duration specified in the protocol, tumor assessments should continue until disease progression OR until the patient dies, withdraws consent, is lost to follow-up, initiates subsequent anti-cancer therapy, or the study is terminated by the Sponsor, whichever occurs first. Sites should take every effort to keep following up patients for disease status according to the imaging schedule (i.e., Q12W ± 14 days) until disease progression has occurred, to the maximum extent feasible according to local standards.
   Survival follow-up: Patients who experienced progressive disease either during treatment or in the follow-up phase will be followed-up for survival status, protracted toxicities and post-study anti-cancer treatment documentation Q12W ± 21 days. Information can also be collected via telephone calls or patient medical records if the patient does not present regularly at the study site unless the patient requests to be withdrawn from follow-up. This request must be documented in the source documents and signed by the investigator. If the patient withdraws from the study, the study staff may use public information source (e.g., county records) to obtain information about the survival status only.
c. Written informed consent may be obtained up to 7 days before the 28-day screening period but must be obtained prior to performing any study-specific screening examination.
d. After informed consent has been obtained but prior to initiation of study drug, only SAEs caused by a protocol-mandated intervention need to be reported.
e. AEs will be reported starting with first administration of the trial treatment until 30 days after the last dose of trial treatment or until initiation of another anti-cancer therapy, whichever occurs first. SAEs and AESIs will be reported until 90 days after last dose of trial treatment. After this period, investigators should report any SAE/AESIs that is believed to be related to prior treatment with study drug. The investigator should follow each AE until the event has resolved to baseline grade or better, the event is assessed as stable by the investigator, new anti-cancer treatment is initiated, the patient is lost to follow-up, the patient withdraws consent, or it is determined that the study treatment or participation is not the cause of AEs. Every effort should be made to follow all SAEs considered to be related to study drug or study-related procedures until a final outcome can be reported.

1. Cancer history includes stage, date of diagnosis, etiology and prior anti-tumor treatment. Demographic information includes age and self-reported race/ethnicity and reproductive status should also be captured.
2. Concomitant medications include any prescription medications or over-the-counter medications. At screening, any medications the patient has used within the 14 days prior to initiation of study treatment should be documented. At subsequent visits, changes to current medications or medications used since the last documentation of medications will be recorded.
3. A complete/full physical examination at screening should include the evaluation of head, eye, ear, nose, and throat and cardiovascular, dermatologic, musculoskeletal, respiratory, gastrointestinal, genitourinary, and neurologic systems. Worsening of baseline abnormalities during trial participation should be recorded as adverse events at the timepoint of getting knowledge of worsening, as appropriate. To enable for judgement on worsening of abnormalities during trial participation, all abnormalities noticed during screening should be documented with a baseline grade.
4. A limited/directed physical examination will be performed at other visits to assess changes from baseline abnormalities and any new abnormalities and to evaluate patient reported symptoms. New or worsened abnormalities should be recorded as adverse events if appropriate.
5. Vital signs should include body weight, temperature, pulse and blood pressure. Height will be measured at screening only.
6. Patients should be resting and in a supine position for at least 10 minutes prior to each ECG collection. Any clinically significant abnormalities detected require triplicate ECG results.
7. WOCBP should only be included after a negative serum pregnancy test within 7 days before initiation of treatment. If applicable, this test should be repeated a maximum of 72 h before the first dose of medication administration. Following initiation of treatment, urine pregnancy testing is sufficient; if a urine pregnancy test is positive, it must be confirmed by a serum pregnancy test. During therapy and until 6 months after last dose of study treatment pregnancy testing will be performed monthly or whenever clinically indicated, in accordance with the CTFG guidance on contraception. Pregnancy test may occur on Day 1, but results must be available and reviewed by the treating physician or investigator prior to commencing an infusion.

8. Complete Blood Count, including hemoglobin, hematocrit, platelet count, red blood cell (RBC) count, absolute neutrophil count (ANC) and total white blood cell (WBC) count with differential: neutrophils, eosinophils, basophils, lymphocytes and monocytes. These assessments may be performed up to 3 days prior to the visit (entire study) in order to have the results available on the visit day (pre-dose).
9. Albumin, Alkaline phosphatase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), bicarbonate, bilirubin total/ bilirubin direct (If total bilirubin is elevated above the upper limit of normal), blood urea nitrogen (BUN) or urea, calcium, chloride, creatinine, glucose, lactate dehydrogenase (LDH), magnesium, phosphate, potassium, sodium, total protein, uric acid. Amylase and lipase collected only at screening and as clinically indicated. These assessments may be performed up to 3 days prior to the visit (entire study) in order to have the results available on the visit day (pre-dose).
10. Urinalysis by qualitative examination (stick) for: bilirubin, blood, glucose, ketones, nitrite or leukocytes or leukocyte esterase, pH, proteins, specific gravity. These assessments may be performed up to 3 days prior to the visit (entire study) in order to have the results available on the visit day (pre-dose).
11. Free T3 or free T4 will only be measured if TSH is abnormal or if there is clinical suspicious of an AE related to the endocrine system.
12. CT scan of chest, pelvis and upper abdomen including the entire liver and both adrenal glands (with contrast, unless contraindicated), or CT scan of chest and MRI of abdomen and pelvis to be performed according to standard of care. MRI of brain (with contrast, unless contraindicated) is recommended in subjects with suspected or known brain metastases, as per local standard. If patient presents with external imaging, investigator can decide to enroll patient based on external imaging if data was collected within the -28-day timeframe and if quality is sufficient. During the investigational therapy part of the trial, tumor imaging will be performed Q8W ± 7 days until first radiologic evidence of disease progression (PD). Subjects who discontinue study intervention for reasons other than PD will have post-treatment follow-up for disease status performed Q8W ± 7 days until disease progression or unless the patient dies, withdraws consent, is lost to follow-up, initiates subsequent anti-cancer therapy, or the study is terminated by the Sponsor, whichever occurs first. Tumor response will be evaluated by the investigator using RECIST v1.1 definition
13. Archival tumor samples will be collected for translational research if patients have given their consent (see section 6). If available, samples taken during a re-biopsy at the time of disease progression will also be collected. It will be at the discretion of the investigator to determine whether a re-biopsy at time of progression is feasible and safe for a patient. The decision to re-biopsy shall be based on clinical judgment and should follow local guidelines and standards. If a re-biopsy after progression under study treatment is performed, submission of this tumor material is also highly encouraged.


Inclusion (selection)
Randomisation
Treatment
Follow-up
•Histologically confirmed metastatic adenocarcinoma of the colon or rectum
•Prior treatment with 5FU, Oxaliplatin, Irinotecan, bevacizumab and EGFR inhibitors if RAS wt
•w/o liver metastases
Stratification:
Previous liver metastases
(Never vs. prior)
1:2
Arm A (experimental arm)
• Fruquintinib (orally, 5 mg once a day, day 1-21 of each 28-days cycle [Q4W])
PLUS
• Tislelizumab (i.v., 200 mg, Q3W)
Arm B (control arm)
• Trifluridin/tipiracil (orally, 35 mg/m² twice a day, day 1-5 and day 8-12, Q4W)
PLUS
• Bevacizumab (i.v., 15 mg/kg, Q2W)
Tumor assessment & survival follow-up (18 months after LPI)
Biosampling:
tumor biopsy at baseline and optional at clinically indicated re-biopsy at disease progression;
Blood and stool samples at baseline, at 4-6 weeks after treatment initiation, if applicable, at the timepoint of disease progression