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Progress Report

Pembrolizumab with Capox Bevacizumab in patients with microsatellite stable metastatic colorectal cancer and a high immune infiltrate: the FFCD 1703-POCHI trial

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Abstract

Pembrolizumab, a PD1 immune checkpoint inhibitor (ICI), was recently reported to be very effective in patients with microsatellite instable/deficient mismatch repair metastatic colorectal cancer (MSI/dMMR mCRC), unlike patients with microsatellite stable/proficient MMR (MSS/pMMR) mCRC, in whom ICIs are generally ineffective. However, about 15% of MSS/pMMR CRCs are highly infiltrated by tumor infiltrating lymphocytes. In addition, both oxaliplatin and bevacizumab have been shown to have immunomodulatory properties that may increase the efficacy of an ICI. We formulated the hypothesis that patients with MSS/pMMR mCRC with a high immune infiltrate can be sensitive to ICI plus oxalipatin and bevacizumab-based chemotherapy.

POCHI is a multicenter, open-label, single-arm phase II trial to evaluate efficacy of Pembrolizumab with Capox Bevacizumab as first-line treatment of MSS/pMMR mCRC with a high immune infiltrate for which we plan to enrol 55 patients. Primary endpoint is progression-free survival (PFS) at 10 months, which is expected greater than 50%, but a 70% rate is hoped for. Main secondary objectives are overall survival, secondary resection rate and depth of response. Patients must have been resected of their primary tumor so as to evaluate two different immune scores (Immunoscore® and TuLIS) and are eligible if one score is “high”. The first patient was included on April 20, 2021.

Key words

Clinical trial; Tumor-infiltrating lymphocytes; Colorectal cancer; Checkpoint blockade

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1. Background

About 85% of non-metastatic colorectal cancers (CRC) are related to chromosomal instability and have a proficient DNA mismatch repair system (pMMR). Other CRCs, i.e. 15%, are microsatellite unstable (MSI) with deficient DNA mismatch repair system (dMMR). They are characterised by high tumor mutational burden (TMB) and the generation of many neo-antigens, which result in a high anti-tumour immune response and a high peri- and/or intra-tumour lymphocyte infiltration (TIL) [1]. We recently showed, with a prospectively validated immune score called TuLIS (Tumor Lymphocyte Infiltration Score), that 22% of localised MSS/pMMR CRCs are also highly infiltrated by CD3+ lymphocytes [2]. This score has also been used to measure lymphocyte infiltration in hepatic metastases, particularly in patients treated with CAPOX/FOLFOX [3].

Pembrolizumab, an anti-PD1 (programmed death-1) monoclonal antibody, is an immune checkpoint inhibitor (ICI) of PD1/PD-L1 axis, recently approved in many cancers. Anti-PD1 antibodies have recently been reported as being very effective in patients with MSI/dMMR metastatic CRC (mCRC) [4,5]. The recently published phase III KEYNOTE-177 trial, showing a significant improvement of progression-free survival (PFS) in patients with MSI/dMMR tumors treated with pembrolizumab compared to those treated with chemotherapy as first-line treatment [5], raised pembrolizumab as the new standard of care in this setting. By contrast, MSS/pMMR mCRC has not benefited from monotherapy with anti-PD1 antibodies [4], and the results of early phase trials combining atezolizumab (anti-PD-L1) with fluoropyrimidine +/- oxaliplatin and bevacizumab in these patients have been disappointing [6–8]. However, 7% of MSS/pMMR CRCs are hypermutated [9], and it remains possible that the 20% of MSS/pMMR mCRCs presenting tumors highly infiltrated by CD3+ may be sensitive to ICI.

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Finally, immunogenic cell death induced by oxaliplatin, the regulation of myeloid derived suppressor cells (MDSC) reported with fluoropyrimidine and the modulation of regulatory T cells and PD1 expression on T lymphocytes reported with bevacizumab, may boost ICI efficacy [10–12].

The prognostic value of lymphocyte infiltrate has been demonstrated in CRC by several teams. However, no validated test is used in routine clinical practice. Previously, we described an automated and reproducible method for analysis of TIL [13] and validated it for clinical use [2]. Automated test (TuLIS) evaluating TIL are performed on virtual slides and has shown that, out of 1220 CRCs tested, 22% of MSS/pMMR tumours were highly infiltrated by CD3+ T cells. Patients presenting with a MSS/pMMR CRC with a high immune infiltrate had better PFS (HR=0.70; p=0.02). Another immunoscore® described by Galon et al. also has a high prognostic value in CRC and is based on CD3+ and CD8+ T cell infiltration in the center and periphery of the tumour [14–16], approximately 20% of tumours with a high immune infiltrate using this score have been found in patients with MSS/pMMR mCRC [2,15]. This immunoscore® has recently been shown to have a predictive value of treatment efficacy favoring 6 months of adjuvant FOLFOX in stage III CRC for patients with intermediate and high immunoscore, compared to 3 months of FOLFOX, from the International Duration Evaluation of Adjuvant Therapy (IDEA) France trial [16]. Interestingly, the immune infiltrate seemed heterogeneous between the primary site and the metastatic sites (synchronous or metachronous) in mCRC, with high heterogeneity between different metastases from the same patient, making it difficult to interpret a single metastasis biopsy [17,18].

The POCHI trial was designed to evaluate the efficacy of pembrolizumab in combination with CAPOX and bevacizumab in patients with MSS/pMMR mCRC with a high immune infiltrate diagnosed in the primary tumor.

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2. Study design

2.1 Population

The FFCD 1703-POCHI trial is a single arm, open-label, multicentre, phase II trial conducted in France, sponsored and coordinated by the *Fédération Francophone de Cancérologie Digestive* (FFCD), evaluating as first-line treatment of MSS/pMMR mCRC with a high immune infiltrate, pembrolizumab in combination with CAPOX and bevacizumab every three weeks. All French centres affiliated to the PRODIGE group (*Partenariat de Recherche en Oncologie DIGEstive*) could participate to the study.

Main inclusion criteria are patients ≥ 18 years old, with unresectable MSS/pMMR mCRC histologically proven, and a high immune response as defined by the 2 previously mentioned scores, assessed on the resected primary tumour (Table 1). Patients with synchronous or metachronous metastases and surgery of primary tumour are eligible. Patients with metachronous metastases could also be enrolled if they have a previous curative treatment of both primary tumour and metastatic disease and a recurrence more than 6 months after the end of this treatment. All patients must have been resected of their primary tumour and not have received any neo-adjuvant treatment that could have modified local immune infiltrates. Both MSI and MMR IHC status must have been determined before inclusion, either locally or in a centralised manner (Figure 1).

This study (clinicaltrials.gov NCT 04262687) is performed in accordance with the declaration of Helsinki and Good Clinical Practice Guidelines. A French ethics committee approved the study. All patients have to provide written informed consent before entering the study.

2.2 Immune scores

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The anti-tumour immune response will be evaluated with two different scores: Immunoscore® described by Galon et al. [14] and TuLIS score described by Emile et al. [2], on a surgical specimen of the primary tumour, containing at least 2 mm of tumour-free margin between the tumour and the non-tumour area, in centralised review by HalioDX (Marseille, France) and the pathology department of Ambroise Paré Hospital (Assistance Publique-Hôpitaux de Paris, France). For patient inclusion, one of these two scores must be considered as positive. Immunohistochemistry will be performed with anti-CD3 and anti-CD8 antibodies.

For TuLIS score, the intensity of the anti-tumour immune response will be determined based on the profile of the curve of variation of density of CD3 staining compared to the distance on either side of the tumour front [2,13]. For immunoscore®, the intensity of the anti-tumour immune response will be determined based on the profile of density of CD3 and CD8 staining in the center and periphery of the tumour [14,15]. Immunoscore® results were categorized using 5 sub-groups (IS-0, IS-1, IS-2, IS-3 or IS-4), as already published, and patients eligible in the study with high immune response were those with an Immunoscore® IS-3 or IS-4.

2.3 Inclusion, treatment and follow-up

Clinical examination and laboratory tests for inclusion should be performed within 2 weeks prior to inclusion, CT-scan should be performed within 3 weeks prior to inclusion (Table 2).

Eligible patients will be treated with pembrolizumab 200 mg at day 1 (IV infusion over 30 minutes) in combination with capecitabine 2000 mg/m²/day on days 1 to 14, oxaliplatin 130 mg/m² at day 1 (IV infusion over 2 hours) and bevacizumab 7.5 mg/kg at day 1 (IV infusion over 60 minutes). We chose capecitabine over 5-fluorouracil (5-FU) because of the rhythm of administration of pembrolizumab every 3 weeks, and a lack of arguments in

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the literature for lower efficacy of capecitabine associated with ICI, compared to 5-FU. Cycle 1 will be administered without pembrolizumab and without bevacizumab due to the time necessary to obtain the results of the immune scores (approximately 2 weeks), and not to impose a targeted therapy on patients who would ultimately not be eligible for the study. Treatment will be repeated every three weeks, up until disease progression, unacceptable toxicity, refusal by the patient, withdrawal of consent, pregnancy or decision by the investigator.

The treatment must be administered on the day 1 of each new cycle, provided that the following criteria are satisfied: neutrophil count > 1500/mm^3, platelets > 100,000/mm^3, gastro-intestinal toxicity < grade 1. If the CAPOX bevacizumab regimen must be delayed due to toxicity, pembrolizumab will also be delayed and restarted at the same time.

Pembrolizumab is provided in the setting of the protocol up to disease progression or up to the maximum treatment duration of pembrolizumab, which is 35 cycles.

2.4 Safety

Toxicities will all be evaluated according to National Cancer Institute – Common Terminology Criteria for Adverse Events (NCI-CTCAE v4.0) scale. Immune-related AEs (irAEs) may occur shortly after the first dose or several months after the last dose of pembrolizumab treatment and may affect more than one body system simultaneously. Based on irAE severity, pembrolizumab will be withheld or permanently discontinued and corticosteroids will be administered.

Two analyses of safety will be performed. The first analysis will be done when 5 patients have been treated for 3 months and the second analysis when 10 patients have been treated for 3 months. The review of safety data will be carried out by an Independent Data Monitoring Committee (IDMC).

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2.4 Endpoints and Assessments

The primary objective is to evaluate the efficacy of pembrolizumab in combination with CAPOX plus bevacizumab as first-line treatment of MSS/pMMR mCRC with a high immune infiltrate. Efficacy will be determined by analysis of the number of patients alive and without radiological and/or clinical progression at 10 months from the first chemotherapy course (according to the RECIST 1.1 criteria evaluated by the investigator).

Secondary objectives include overall survival (OS), serious adverse events evaluated according to NCI-CTCAE v4.0, secondary resection rate (R0 and R1), pathological response in case of secondary resection (TRG criteria) and outcome of tumour markers (CEA and CA19.9). Other secondary objectives, evaluated according to the investigator and centralised review according to the RECIST V1.1 criteria, will be: median PFS, time to radiological progression (TTP), time to objective response, best response during treatment, depth of response and early tumour shrinkage at 9 weeks; and, in those evaluated according to iRECIST criteria, the percentage of patients alive and without radiological progression at 10 months, median PFS, TTP and early tumour shrinkage at 9 weeks. Exploratory analyses of primary and secondary endpoints will also include investigation of prognostic and predictive values according to sub-classes of Immunoscore® and TuLIS score for all patients, and the correlation between the two immune scores.

Thoraco-abdominal and pelvic CT-scan will be performed within 3 weeks prior to inclusion and every 9 weeks during treatment and in case of discontinuation of treatment with no disease progression (Table 2). The CEA and CA19.9 levels will be assessed with the same schedule.

2.5 Statistical analysis plan

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The clinical hypotheses represent a percentage of patients alive and without radiological and/or clinical progression at 10 months greater than 50% (H1), but a 70% rate is expected. With an alpha risk of 5% (one-sided) and a power of 85%, using the exact binomial method, 50 patients are necessary. Taking into account the fact that 10% of patients will be not evaluable or lost to follow-up at 10 months, 55 patients will have to be included.

The conclusion of the study will be defined according to the following rule: based on the 50 evaluable patients, if 32 patients or more are still alive and without radiological and/or clinical progression at 10 months, then the treatment will be considered as effective.

Analysis of the primary endpoint, the secondary endpoints and safety criteria will be performed on the modified intention to treat population (mITT): all the patients included, whatever their eligibility and who have received at least one dose of treatment (whatever the dose and the treatment). The primary evaluation endpoint and secondary efficacy endpoints will also be evaluated on the per-protocol population (mITT patients without any major protocol deviation).

2.6 Ancillary study

The ancillary study on biological samples (blood and stool) and tumour samples (primary tumours) will consist of analysis of circulating tumor DNA (at cycle 1, cycle 3 and disease progression), circulating lymphocyte phenotyping (at cycle 1 and cycle 3), pharmacokinetics of pembrolizumab and bevacizumab, microbiome study (before study and at week 9), tumor mutational burden, other immune scores, immunohistochemistry of PD-L1 and PD-L2 and expression and/or amplification of PD-L1 and PD-L2 (Table 2).

The objective of these studies is to identify prognostic biomarkers and/or predictive biomarkers of response to treatment (OS, response rate and PFS). For microbiome study, some studies suggest that the gut microbiota might be involved in the efficacy and toxicity of

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chemotherapies and immunotherapies [19–21]. We aim to evaluate the influence of gut microbiota composition on toxicity and efficacy of bevacizumab and pembrolizumab combination. We are seeking to detect an association between TMB, a high immune response and response to treatment, as described in other tumours.

3. Discussion

POCHI is a single arm phase II trial, evaluating in patients with MSS/pMMR mCRC and a high immune infiltrate, the efficacy of pembrolizumab in combination with CAPOX bevacizumab in a first-line setting. The study is open in April 2021 in about 70 centers in France and the end of inclusion is scheduled for end of 2023. The first patient was included on April 20, 2021.

In the recently published NICHE trial [22], of the 15 patients with localized pMMR CCR treated with ipilimumab (anti-CTLA4) plus nivolumab (anti-PD1) in the neo-adjuvant setting, 3 had a major pathological response, including one with ≤ 10% residual viable tumour and 2 complete pathological responses, and one had a partial response with 50% residual viable tumor. The presence of T cells with co-expression of CD8 and PD-1 before starting treatment was a predictive biomarker of response in patients with pMMR tumor. In the same way, in the phase II CO.26 trial [23], among 166 patients with refractory pMMR/MSS mCRC, treatment with durvalumab (anti-PDL1) plus tremelimumab (anti-CTLA4) was associated with significantly longer OS (HR=0.66; p=0.02) compared to best supportive care alone, with the greatest OS benefit for patients with TMB of 28 or more mutations by megabase (21% of pMMR/MSS patients).

Nevertheless, at this stage, we do not have enough strong data to use ICI combination alone in MSS/pMMR mCRC with a high immune infiltrate. In addition, combination of

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CAPOX, bevacizumab and dual blockage with ICIs (anti-PD1 plus anti-CTLA4) seems to us to be toxic.

If we identify an immune score, which seems clinically relevant to predict sensitivity to ICI in MSS/pMMR mCRC, this will make it possible to plan a randomised phase III trial comparing chemotherapy and anti-angiogenic antibody versus chemotherapy and anti-angiogenic antibody plus ICI in this mCRC sub-group.

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Conflict of interest

CG has participated in consulting and/or advisory boards for Servier and Sanofi, and has received support for travel to meetings from Amgen. JFE, NL and CK are co-inventors of a patented method for evaluation of lymphocyte infiltration of tumor. JFE participated in compensated boards organized by HalioDX and MSD. AL has received honoraria as a speaker and/or in an advisory role from Amgen, Bayer, BMS, HalioDx, Merck, Pierre Fabre, Roche, Sanofi and Servier and has received support for travel to meetings from Bayer, BMS, Merck, MSD, Roche and Servier. PLP has received honoraria from MSD, Biocartis, Roche, Servier, Pierre Fabre, Lilly, MSD, AstraZeneca and Amgen. JMP has received honoraria as a speaker and/or in an advisory role from Merck, Amgen, Roche, MSD, Bayer, Servier, Pierre Favre and BMS and has received support for travel to meetings from Merck, Amgen, Roche, MSD, Bayer, Servier, Pierre Favre and BMS. TL has received honoraria as a speaker and/or in an advisory role from Amgen, Merck, AstraZeneca, Sanofi and Servier and has received support for travel to meetings from Amgen Ipsen and Servier. JT has received honoraria as a speaker and/or in an advisory role from Merck KGaA, Sanofi, Roche Genentech, MSD, Lilly, Astra Zeneca, Servier, Novartis, Pierre Fabre, HallioDx and Amgen. DT has received honoraria for education events and board from MSD. The other authors have no declared conflicts of interest.

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Figure Legends

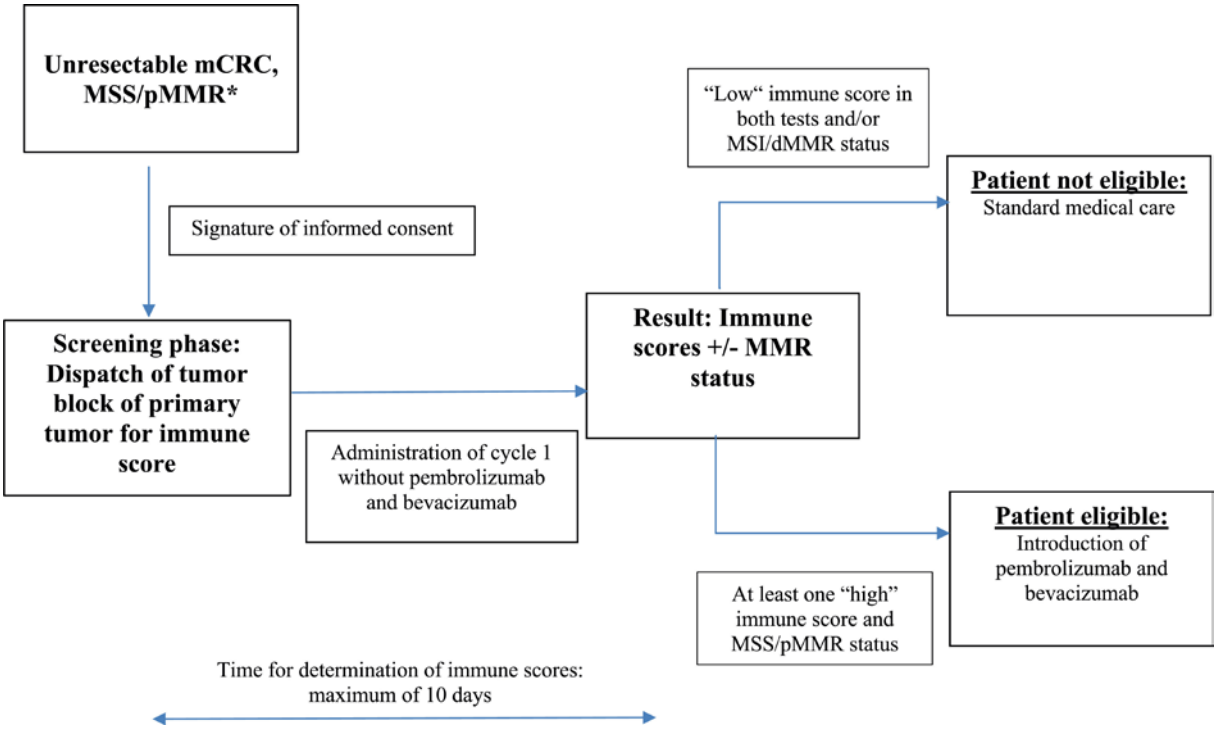


Figure 1. Study flowchart

* MSS and dMMR status are normally determined by the centre, if not available it is possible to request determination of the MMR status in centralised review at the same time as determination of the immune score.

mCRC: metastatic colorectal cancer ; MSS: microsatellite stability ; MSI: microsatellite instability ; pMMR: proficient mismatch repair ; dMMR: deficient mismatch repair

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Table 1
Main eligibility criteria.

Main inclusion criteria
- Age $\geq$ 18 years
- Both MSS and pMMR mCRC
- Patients with synchronous or metachronous metastases and surgery of primary tumour are eligible. Patients with metachronous metastases could also be enroled if they have a previous curative treatment of both primary tumour and metastatic disease and a recurrence more than 6 months after the end of this treatment.
- High immune response defined as the immune infiltration score obtained on the primary tumour (Immunoscore® and TuLIS)
- Unresectable cancer with at least one measurable metastatic target according to RECIST v1.1 criteria
- WHO PS $\leq$ 1
- Adequate organ function: absolute neutrophil count $\geq$ 1.5 $\times$ 10^9/L, haemoglobin $>$ 9 g/dL, platelets $\geq$ 100 $\times$ 10^9/L, AST/ALT $\leq$ 5 x ULN, total bilirubin $\leq$ 2 x ULN, Alkaline phosphatase $\leq$ 5 x ULN, creatinine clearance $\geq$ 50 mL/min (MDRD).
- Information provided to patient and signature of the informed consent form
Main exclusion criteria
- mCRC with microsatellite instability (dMMR and/or MSI)
- Patient eligible for curative treatment
- Patient with *RAS* wild-type tumour for whom a tumour shrinkage with an anti-EGFR is necessary to perhaps obtain a downstaging for a secondary surgery
- Patient with only primary tumour biopsies available or only a sample of a metastasis (no surgical resection of the primary tumour)
- Patient who have had neoadjuvant treatment (chemotherapy or radiotherapy) for the treatment of primary tumour can not be included
- Auto-immune disease which may worsen during treatment with an immune-stimulating agent
- Dihydropyrimidine dehydrogenase deficiency
- All uncontrolled progressive disorders during the last 6 months
- History of an inflammatory digestive disease, obstruction or sub-obstruction not resolved with symptomatic treatment

MSS: microsatellite stable; pMMR: proficient mismatch repair; mCRC: metastatic colorectal cancer; RECIST: response evaluation criteria in solid tumors; WHO PS: world health organization performance status; dMMR: deficient mismatch repair; MSI: microsatellite instability; ULN: upper limit of normal; MDRD: modification of diet in renal disease.

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Table 2
Main examination and follow-up schedule.

	Inclusion	During treatment		After end of treatment
	Within 2 weeks prior to inclusion	Before each course	Every 9 weeks	Every 8 to 12 weeks during 1 year or up until death
Clinical and biological informed consent	X			
CLINICAL EXAMINATION				
WHO performance status	X	X	X	
Evaluation of toxicities NCI-CTCAE version 4.0		X	X and 4 months after end of treatment	
BIOLOGICAL ASSESSMENT				
Laboratory assessment	X[a]	X[b]	X[a]	
CEA and CA19.9 markers	X		X	
DPD status	X			
PARACLINICAL TESTS				
ct-scan of thorax-abdomen-pelvis or mri	x[c]		x[d]	
despatch of tumour tissue blocks for determination of immune scores	x			
Screening for microsatellite instability (MSI and dMMR status)	X[e]			
Ancillary study				
Blood samples	X	X (before 3rd cycle)		X
Stools	X (before first course of treatment)	X (before 3rd cycle)		
Subsequent lines				
Dates of start and of end of treatment and type of treatment of subsequent lines of chemotherapies will be completed.				X

[a] CBC, platelets, PT, sodium, potassium, calcium, bilirubin (total and conjugated), GGT, ALT, AST, alkaline phosphatase, LDH, TSH, serum creatinine, creatinine clearance (MDRD), serum albumin and proteinuria on dipstick test (+ 24 h proteinuria if ≥ 2+), glycaemia.

[b] CBC, platelets, serum creatinine, creatinine clearance (MDRD), sodium, potassium, AST, ALT, GGT, phosphatase alkaline, total and conjugated serum bilirubin, and proteinuria on dipstick test (+ 24 h proteinuria if ≥ 2+).

[c] within 3 weeks prior to inclusion.

[d] to be performed until radiological progression.

[e] screening for microsatellite instability can be done locally or in centralised manner (MMR protein IHC and molecular microsatellite testing), but should not delay despatch of the tumour formalin fixed paraffin embedded (FFPE) block.WHO: World Health Organization; NCI-CTCAE: National Cancer Institute - Common Terminology Criteria for Adverse Events; CEA: carcino-embryonic antigen; DPD: dihydropyrimidine dehydrogenase; MRI: magnetic resonance imaging; MSI: microsatellite instability; dMMR: deficient mismatch repair.

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