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# Phase Ib/II trial evaluating the safety, tolerability and immunological activity of durvalumab (MEDI4736) (anti-PD-L1) plus tremelimumab (anti-CTLA-4) combined with FOLFOX in patients with metastatic colorectal cancer

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## ABSTRACT

**Background** 5-Fluorouracil plus irinotecan or oxaliplatin alone or in association with target therapy are standard first-line therapy for metastatic colorectal cancer (mCRC). Checkpoint inhibitors targeting PD-1/PD-L1 demonstrated efficacy on mCRC with microsatellite instability but remain ineffective alone in microsatellite stable tumour. 5-Fluorouracil and oxaliplatin were known to present immunogenic properties. Durvalumab (D) is a human monoclonal antibody (mAb) that inhibits binding of programmed cell death ligand 1 (PD-L1) to its receptor. Tremelimumab (T) is a mAb directed against the cytotoxic T-lymphocyte-associated protein 4 (CTLA-4). This study is designed to evaluate whether the addition of PD-L1 and CTLA-4 inhibition to oxaliplatin, fluorouracil and leucovorin (FOLFOX) increases treatment efficacy.

**Methods** This phase II study (ClinicalTrials.gov NCT03202758) will assess the efficacy and safety of FOLFOX/D/T association in patients with mCRC (n=48). Good performance status patients (Eastern Cooperative Oncology Group <2) with untreated, RAS mutational status mCRC will be eligible. Prior adjuvant therapy is allowed provided recurrence is >6 months postcompletion. There is a safety lead in nine patients receiving FOLFOX/D/T. Assuming no safety concerns the study will go on to include 39 additional patients. Patients will receive folinic acid (400 mg/m²)/5-fluorouracil (400 mg/m² as bolus followed by 2400 mg/m$^2$ as a 46-hour infusion)/oxaliplatin (85 mg/m$^2$) every 14 days with D (750 mg) D1 every 14 days and T (75 mg) D1 every 28 days. After six cycles of FOLFOX only D/T will continue until disease progression, death, intolerable toxicity, or patient/investigator decision to stop. Primary endpoint is safety and efficacy according to progression-free survival (PFS); secondary endpoints include overall response rate and quality of life. Hypothesis is that a PFS of 50% at 6 months is insufficient and a PFS of 70.7% is expected (with $\alpha$=10%, $\beta$=10%). Blood, plasma and tumour tissue will be collected and assessed for potential prognostic and predictive biomarkers.

## BACKGROUND

Colorectal cancer (CRC) is still one of the leading causes of cancer death worldwide. In France, approximately 40 500 new cases are diagnosed each year. With more than 17 500 deaths in France in 2011, CRC is responsible for more than 12% of all cancer deaths, the overwhelming number of deaths occurring in patients with metastatic disease. Metastatic disease treatment relies mainly on chemotherapy with a more palliative objective when metastases could not be removed. Approximately half of the patients with CRC will develop metastases, with a liver localisation in 50%–70% of cases; only 10%–20% will be accessible to curative resection. For the 80%–90% of the remaining cases, the prognosis is bad[1]; however, the median overall survival (OS) of patients with CRC increases with improvement of the chemotherapeutic protocol from 12 months with 5-fluorouracil (5-FU) monotherapy to around 30 months in recent clinical trials.[2–8]

The conventional treatment of non-resectable metastatic CRC is based on palliative systematic chemotherapy. Drugs having demonstrated an efficacy are fluoropyrimidines, irinotecan in monotherapy or in association with fluoropyrimidine, and oxaliplatin in association with fluoropyrimidines. More recently, targeted therapies strengthened the armamentarium. Indeed, bevacizumab presents an interest in association with fluoropyrimidines, oxaliplatin and irinotecan. Anti-epidermal growth factor receptor antibodies (panitumumab and cetuximab) present an efficacy in patients bearing

metastatic tumours without mutation of the KRAS and NRAS genes.[3 9 10]

In addition to chemotherapy, a promising approach of clinical development in CRC is immunotherapy. Many studies highlight the fact that CRC can be recognised by the immune system and it is well admitted that high CD8 T cell infiltrates are associated with better cancer prognosis in localised or metastatic CRC.[11 12] This discovery was linked to the development of recent immunotherapies in cancer treatment. Anti-PD-1/PD-L1 mAbs give antitumorous response in many different types of human cancer. These mAbs target PD-1$^{+}$ exhausted T cells that infiltrate tumours and restore their cytotoxic functions. As an antibody, which blocks the interaction between PD-L1 and its receptors, durvalumab, a human mAb of the IgG 1 kappa subclass, relieves PD-L1-dependent immunosuppressive effects on CD8 T cells, and therefore enhances the cytotoxic activity of antitumour CD8 T cells. This hypothesis is supported by emerging clinical data from durvalumab and other mAbs targeting the PD-L1/PD-1 pathway, which provide early evidence of clinical activity and a manageable safety profile.[13–16] Responses have been observed in patients with PD-L1-positive tumours and patients with PD-L1-negative tumours.

In addition to PD-1, exhausted T cells could express many other checkpoint inhibitor molecules.[17] Preclinical studies suggest that such checkpoint inhibitors have no redundant activity and the combination of mAb targeting multiple checkpoint is more effective than monotherapy.[18–20] Targeting PD-1 and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) by combining durvalumab with tremelimumab, an IgG 2 kappa isotype mAb directed against the CTLA-4, is interesting because the mechanisms of CTLA-4 and PD-1 are non-redundant checkpoint inhibitors, suggesting that targeting both pathways may have additive or synergistic activity.[21] In fact, combining anti-CTLA-4 and anti-PD-1 immunotherapy agents has been shown to result in improved response rates (RR) relative to monotherapy. For example, the concurrent administration of nivolumab and ipilimumab to patients with advanced melanoma induced higher objective response rates than those obtained with single-agent therapy.[22] Importantly, responses appeared to be deep and durable. Similar results have been observed in an ongoing study of durvalumab + tremelimumab in non-small cell lung carcinoma.[16]

In the field of CRC, RR to monotherapy against PD-1 seems very modest except in patients with colorectal tumour with mismatch repair deficiency (microsatellite instability, MSI).[23] Recent preclinical data suggest that combination of PD-1/PD-L1 inhibitor with immunogenic cell death inducer like radiotherapy or oxaliplatin could enhance the efficacy of such immunotherapy.[24 25]

5-FU plus oxaliplatin were known to present immunogenic properties. 5-FU could eliminate myeloid-derived suppressor cells and oxaliplatin could induce immunogenic cell death and increase the immunogenicity of microsatellite stable (MSS) tumours.[26–29] Dosset *et* al have shown that 5-FU plus oxaliplatin combination, among several chemotherapy regimens, is the better chemotherapy to induce PD-L1 expression and CD8 recruitment at tumour site. Interestingly, in two in vivo tumour models of MSS colon cancer in mice, we observed a synergic effect of using an anti-PD-L1 in combination with standard treatment of CRC (oxaliplatin, fluorouracil and leucovorin (FOLFOX)), while anti PD-L1 alone is not effective. In these models, the combination therapy cure is 40% whereas no cure is observed with FOLFOX or anti PD-L1 alone. These results suggest that the combination of chemotherapy with immunotherapy would act synergistically in patients with MSS CRC. Chemotherapy is administrated to enhance the efficacy of immune checkpoint and we could think that this immunogenic context will continue after stopping FOLFOX.[26 29]

We focus on CRC with RAS mutated status. In one report, RAS pathway activation is associated with elevated PD-L1 expression in human lung and colorectal tumours, which implies PD-1-PD-L1 blockade may prove more successful.[30] RAS mutational status may be one of predictive markers for the combination therapy with immune checkpoint blockade.

The objective of this study is to determine combination of FOLFOX plus durvalumab and tremelimumab could be effective in MSS tumour with RAS mutated status.

## METHODS

### Study objectives

Our multicentre phase I/II study aims to establish the safety and efficacy of durvalumab plus tremelimumab combined with FOLFOX in patients with metastatic CRC with MSS or MSI status of RAS mutated status. The study will be performed in two steps (figure 1):

Step 1 will assess the safety of the combination of durvalumab 750 mg every 2 weeks + tremelimumab 75 mg every 4 weeks + FOLFOX during the first two cycles of treatment.

Step 2 will assess the efficacy of the combination of durvalumab 750 mg every 2 weeks + tremelimumab 75 mg every 4 weeks + FOLFOX.

The trial is registered on the ClinicalTrials.gov database NCT03202758 (NCT).

### Study assessments and criteria for evaluation

#### Phase Ib primary objective (step 1)

To determine the safety of the combination of durvalumab (anti-PD-L1) + tremelimumab (anti-CTLA-4) + FOLFOX.

#### Phase II primary objective (step 2)

To determine the efficacy of the combination of durvalumab (anti-PD-L1) + tremelimumab (anti-CTLA-4) + FOLFOX in terms of progression-free survival (PFS).

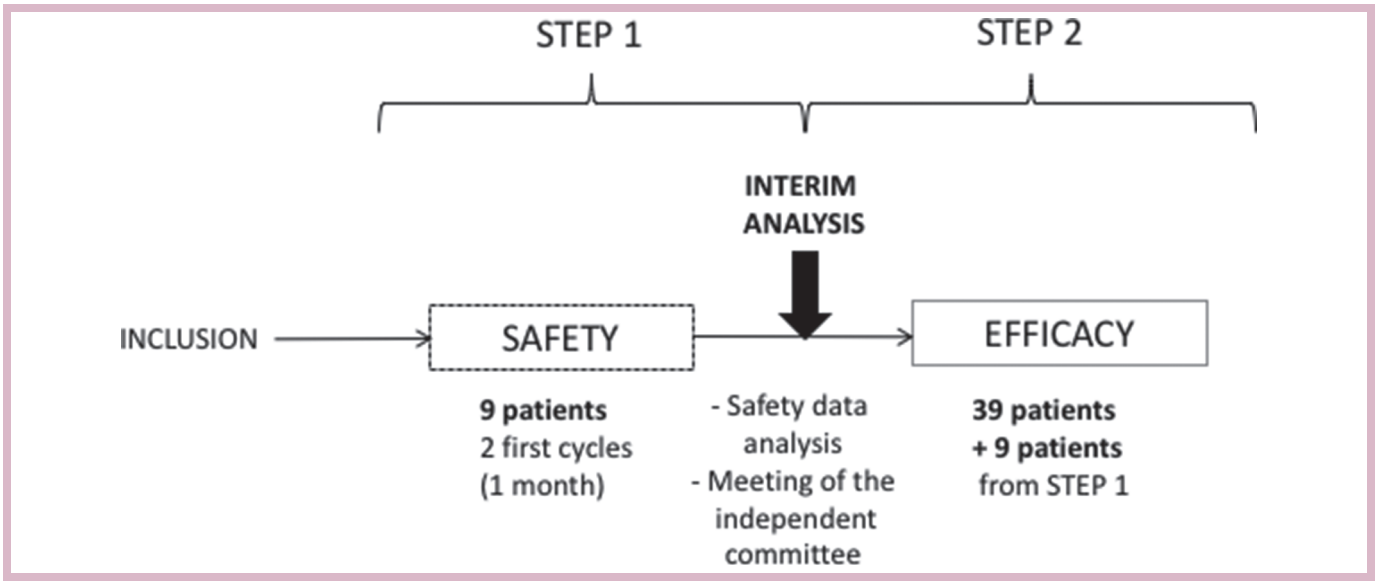


**Figure 1** Overview of the study design in two steps: Step 1 is designed to determine the safety in nine patients on first two cycles. After an interim analysis, step 2 is designed to assess the efficacy of this treatment on 39 supplementary patients.

### Phase II secondary objective

To determine the efficacy of the combination of durvalumab (anti-PD-L1) + tremelimumab (anti-CTLA-4) + FOLFOX in terms of response to treatment and OS.

### Exploratory studies

- To evaluate quality of life at each cycle.
- To determine the MSI status.
- To study the immune cell infiltration (CD3, CD8, Foxp3, CD163) into the tumour by histochemistry,
- To analyse PD-1, PD-L1, CTLA-4 expression by histochemistry.
- To determine presence of Th1, Th2, Th17, follicular helper T cells and exhausted T cells using spectral microscopy.
- To perform identification of tumour-specific mutations using exome analysis.
- To determine candidate of neoantigens and also prediction for proteasomal processing and HLA class I binding will be assessed using exome analysis.
- To analyse evolution of tumour transcriptome using RNA sequencing.
- Analyse peripheral T cell immune response before and after treatment start using telomerase immunomonitoring.
- To assess local immune response before and after therapy by histochemistry.
- To analyse cytokine production by T cells during therapy.

### Step 1: safety assessments

The primary endpoint is toxicity following National Cancer Institute Common Toxicity Criteria v4.0. Toxicity will be assessed on the first nine patients within two cycles (30 days) following the first administration of durvalumab + tremelimumab + FOLFOX and is defined as an adverse event (AE) that may be linked to one of the study drugs.

A monitoring during treatment will consist of:

- Clinical examination with analysis of intercurrent events, concomitant treatments, Eastern Cooperative Oncology Group Performance Status.
- Haematology: white cell, neutrophils, haemoglobin and platelet biochemistry.
- Toxicity/symptoms: evaluation of treatment-related toxicities.
- Tumour biopsy before and after 3 months of therapy will be performed for ancillary study of histology, exome and RNA sequencing.
- Blood collection will be performed for analysis of peripheral T cell functions.
- Plasma collection will be performed for cytokine analysis.

Safety data will also be completed during all the step 2 of the study.

### Step 2: efficacy assessments

Efficacy analyses will be performed in modified intent-to-treat (mITT) population, that is, all patients following the major inclusion criteria and with a 3-month evaluation. Analyses will be repeated in the ITT principle, that is, including all enrolled patients whatever eligibility criteria and treatment received by patients and in the per protocol population (patients who had received all the planned doses).

The primary objective will be evaluated at 6 months according to the Simon's design.

## Disease assessment

The response to immunotherapy may differ from the typical responses observed with cytotoxic chemotherapy including the following:

- Response to immunotherapy may be delayed.

- Response to immunotherapy may occur after progressive disease (PD) by conventional criteria.
- The appearance of new lesions may not represent PD with immunotherapy.
- Stable disease (SD), while on immunotherapy, may be durable and represent clinical benefit.

Based on the above-described unique response to immunotherapy and based on guidelines from regulatory agencies, for example, European Medicines Agency's 'Guideline on the evaluation of anti-cancer medicinal products in man' (EMA/CHMP/205/95/Rev.4) for immune-modulating anticancer compounds, the study may wish to implement the following in addition to standard Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 criteria:

- RECIST will be modified so that PD must be confirmed at the next scheduled visit, preferably, and no earlier than 4 weeks after the initial assessment of PD in the absence of clinically significant deterioration. Treatment with durvalumab + tremelimumab would continue between the initial assessment of progression and confirmation for progression.
- In addition, subjects may continue to receive durvalumab + tremelimumab beyond confirmed PD in the absence of clinically significant deterioration and if investigators consider that subjects continue to receive benefit from treatment.

Patients will undergo regular tumour assessments until documented disease progression as described by RECIST 1.1 criteria. CT scan was performed at the following time points: screening (baseline), cycle 6, cycle 12 and cycle 18. Response criteria are based on RECIST 1.1 and immune RECIST.

### RECIST 1.1

Complete response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduced its shortest axis to <10 mm.

Partial response (PR): A ≥30% decrease in the sum of diameters of target lesions, relative to the sum of diameters at baseline.

PD: A ≥20% increase in the sum of diameters of target lesions, relative to the smallest sum of diameters during the study. In addition to the relative increase of 20%, the sum must also have an absolute increase of ≥5 mm. The appearance of one or more new lesions is also considered as a progression.

SD: The tumour shrinkage is not sufficient to qualify for PR nor has the tumour size increase sufficiently to qualify for PD relative to the smallest sum of diameters during the study.

### IMMUNE RECIST

- Overall irCR: Complete disappearance of all lesions (whether measurable or not) and no new lesions. All measurable lymph nodes also must have a reduction in short axis to 10 mm or less.
- Overall irPR: Sum of the longest diameters of target and new measurable lesions decreases ≥30% (compared with baseline).
- Overall irSD: Sum of the longest diameters of target and new measurable lesions neither irCR, irPR (compared with baseline) nor irPD (compared with nadir).
- Overall irPD: Sum of the longest diameters of target and new measurable lesions increases ≥20% (compared with nadir), confirmed by a repeat, consecutive observation at least 4 weeks (normally it should be done at 6 weeks) from the date first documented

### Progression-free survival

PFS is defined as the time from enrolment until the date of objective disease progression or death (by any cause in the absence of progression). The date of PFS will be recorded by the investigator and defined according to local standard clinical practice and may involve any of objective radiological progression, symptomatic progression or death. PFS data will be collected at months 3 and 6.

### Overall survival

OS (months) is defined as the time from the date of enrolment until death due to any cause. Any patient not known to have died at the time of analysis will be censored based on the last recorded date on which the patient was known to be alive. OS data will be collected at 12 months.

Subjects who have disease control following completion of 12 months of treatment or subjects who are withdrawn from durvalumab + tremelimumab treatment for reasons other than confirmed PD will continue to have objective tumour assessments.

#### Study population

The study population consists of patients with metastatic CRC in first line of treatment. The inclusion and exclusion criteria are detailed in table 1. Eligible patients are informed about the study, and given an information leaflet.

#### Study procedures

The sequence of regimens is presented in figure 2.

#### Durvalumab + tremelimumab

Patient will receive 750 mg durvalumab via intravenous infusion every 2 weeks for up to eight doses/cycles and 75 mg tremelimumab via intravenous infusion every 4 weeks for up to four doses/cycles, and then continue 750 mg durvalumab every 2 weeks starting on week 16 for up to 8 months (18 doses). Dosing outside the window should be discussed with the study physician. Tremelimumab will be administered first. Durvalumab infusion will start approximately 1 hour after the end of tremelimumab infusion. The duration will be approximately 1 hour for each infusion. A 1-hour observation period is required after the first infusion of durvalumab and tremelimumab. If no clinically significant infusion

**Table 1** Inclusion and exclusion criteria of the trial inclusion criteria

| Inclusion criteria | Exclusion criteria |
|---|---|
| 1. Written informed consent and any locally required authorisation obtained from the subject prior to performing any protocol-related procedures, including screening evaluations | 1. Involvement in the planning and/or conduct of the study (applies to both AstraZeneca staff and/or staff at the study site). Previous enrolment in the present study. |
| 2. Male or female aged >18 years at time of study entry | 2 Participation in another clinical study with an investigational product during the last 4 weeks |
| 3. Performance status of 0 or 1 according to the Eastern Cooperative Oncology Group (ECOG) and WHO | 3. Any previous treatment with a PD-1 or PD-L1 inhibitor, including durvalumab or an anti-CTLA-4, including tremelimumab (unless prior PD-1/PD-L1 or CTLA-4 inhibition is a specific entry criterion) |
| 4. Histologically confirmed diagnoses of colorectal cancer with positive mutated KRas or NRas | 4. History of another malignancy within the 5 previous years with low potential risk for recurrence other than: |
| 5. Patients with metastatic disease | Adequately treated non-melanoma skin cancer or lentigo maligna without evidence of disease |
| 6. First-line metastatic therapy or first line after curative treatment of liver or lung metastases with curative intent associated or not with preoperative chemotherapy | Adequately treated carcinoma in situ without evidence of disease, for example, cervical cancer in situ |
| 7. Life expectancy of >12 weeks | 5. Receipt of the last dose of anticancer therapy 28 days prior to the first dose of study drug (14 days prior to the first dose of study drug for subjects who have received prior TKIs and within 6 weeks for nitrosourea or mitomycin C) |
| 8. Adequate normal organ and marrow function as defined below: | 6. Mean QT interval corrected for heart rate (QTc) ≥470 ms calculated from three ECGs using Fridericia's correction |
| Haemoglobin >9.0 g/dL | 7. Current or prior use of immunosuppressive medication within 28 days before the first dose of durvalumab or tremelimumab, with the exceptions of intranasal and inhaled corticosteroids or systemic corticosteroids at physiological doses, which are not to exceed 10 mg/day of prednisone, or an equivalent corticosteroid |
| Absolute neutrophil count (ANC) >1.5×$10^9$/L (>1500 per L) | 8. Any history of hypersensitivity to FOLFOX or their excipients |
| Platelet count >100×$10^9$/L (≥100 000 per $mm^3$) | 9. Any unresolved toxicity (CTCAE grade >1) from previous anticancer therapy. Subjects with irreversible toxicity that is not reasonably expected to be exacerbated by the investigational product may be included (eg, hearing loss, peripherally neuropathy). |
| Serum bilirubin ≤1.5 × institutional upper limit of normal (ULN) | 10. Active or prior documented autoimmune disease within the past 2 years. Note: Subjects with vitiligo, Grave's disease or psoriasis not requiring systemic treatment (within the past 2 years) are not excluded. |
| AST (SGOT)/ALT (SGPT) ≤2.5 × institutional upper limit of normal unless liver metastases are present, in which case it must be ≤5 × ULN | 11. Active or prior documented inflammatory bowel disease (eg, Crohn's disease, ulcerative colitis) |
| Albumin >30 g/L | 12. History of primary immunodeficiency |
| Creatinine <1.5 × institutional ULN | 13. History of organ transplant that requires use of immunosuppressive |
| Serum creatinine Clearance >40 mL/min by the Cockcroft-Gault formula or by 24-hour urine collection for determination of creatinine clearance | 14. History of allogeneic organ transplant |

Continued

**Table 1** Continued

| Inclusion criteria | Exclusion criteria |
|---|---|
| 9. Tumour evaluation (CT scan) in the previous 4 weeks with presence of at least one measurable lesion according to RECIST 1.1 criteria | 15. Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection; clinically significant cardiovascular disease including: myocardial infarction within 6 months, symptomatic congestive heart failure, uncontrolled hypertension, unstable angina pectoris, cardiac arrhythmia; history of Mobitz II second-degree or third-degree heart block without a permanent pacemaker in place, hypotension; rest limb claudication or ischaemia within 6 months; active peptic ulcer disease or gastritis, active bleeding diatheses including any subject known to have evidence of acute or chronic hepatitis B, hepatitis C or HIV, or psychiatric illness/social situations that would limit compliance with study requirements or compromise the ability of the subject to give written informed consent |
| 10. At least 4 weeks since the last chemotherapy, immunotherapy or other drug therapy and/or radiotherapy | 16. Ongoing treatment with CYP3A4 substrates or regularly taking of grapefruit juice |
| 11. Recovery to grade ≤1 from any adverse event (AE) derived from previous treatment according to the criteria of the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 4.0 | 17. Known history of active tuberculosis |
| 12. For principal study: willingness to provide consent for use of archived tissue with sufficient material available for analysis. For ancillary study: metastasis should be accessible to performed biopsy. | 18. History of leptomeningeal carcinomatosis |
| | 19. Brain metastases or spinal cord compression |
| 13. Female subjects must either be of non-reproductive potential (ie, postmenopausal by history: ≥60 years old and no menses for ≥1 year without an alternative medical cause; OR history of hysterectomy, OR history of bilateral tubal ligation, OR history of bilateral oophorectomy) or must have a negative serum pregnancy test upon study entry. | 20. Receipt of live attenuated vaccination within 30 days prior to study entry or within 30 days of receiving durvalumab or tremelimumab |
| 14. Patients must be affiliated to a social security system. | 21. Female subjects who are pregnant, breast feeding, or male or female patients of reproductive potential who are not employing an effective method of birth control |
| 15. Subject is willing and able to comply with the protocol for the duration of the study including undergoing treatment and scheduled visits and examinations including follow-up. | 22. Patients with any history of bleeding related to the current colorectal cancer |

ALT, alanine aminotransferase; AST, aspartate aminotransferase; FOLFOX, oxaliplatin, fluorouracil and leucovorin; RECIST, Response Evaluation Criteria in Solid Tumors; TKI, tyrosine kinase inhibitor.

reactions are observed during or after the first cycle, subsequent infusion observation periods can be at the investigator's discretion (suggested 30 min after each durvalumab and tremelimumab infusion).

### Fixed dosing for durvalumab and tremelimumab

A population pharmacokinetic model was developed for durvalumab using monotherapy data from a phase 1 study.[31] Population PK analysis indicated only minor impact of body weight (WT) on PK of durvalumab (coefficient of ≤0.5). The impact of body WT-based (10 mg/kg every 2 weeks) and fixed dosing (750 mg every 2 weeks) of durvalumab was evaluated by comparing predicted steady-state PK concentrations (5th, median and 95th percentiles) using the population PK model. A fixed dose of 750 mg was selected to approximate 10 mg/kg (based on median body WT of ~75 kg). A total of 1000 patients were simulated using body WT distribution of 40–120 kg.

Similarly, a population PK model was developed for tremelimumab using data from phase 1 through phase 3.[32] Population PK model indicated minor impact of body WT on PK of tremelimumab (coefficient of ≤0.5). The WT-based (1 mg/kg every 4 weeks) and fixed dosing (75 mg/kg every 4 weeks; based on median body WT of ~75 kg) regimens were compared using predicted PK concentrations (5th, median and 95th percentiles) using population PK model in a simulated population of 1000 patients with body WT distribution of 40–120 kg. Similar to durvalumab, simulations indicated that both body WT-based and fixed dosing regimens of tremelimumab yield similar median steady-state PK concentrations with

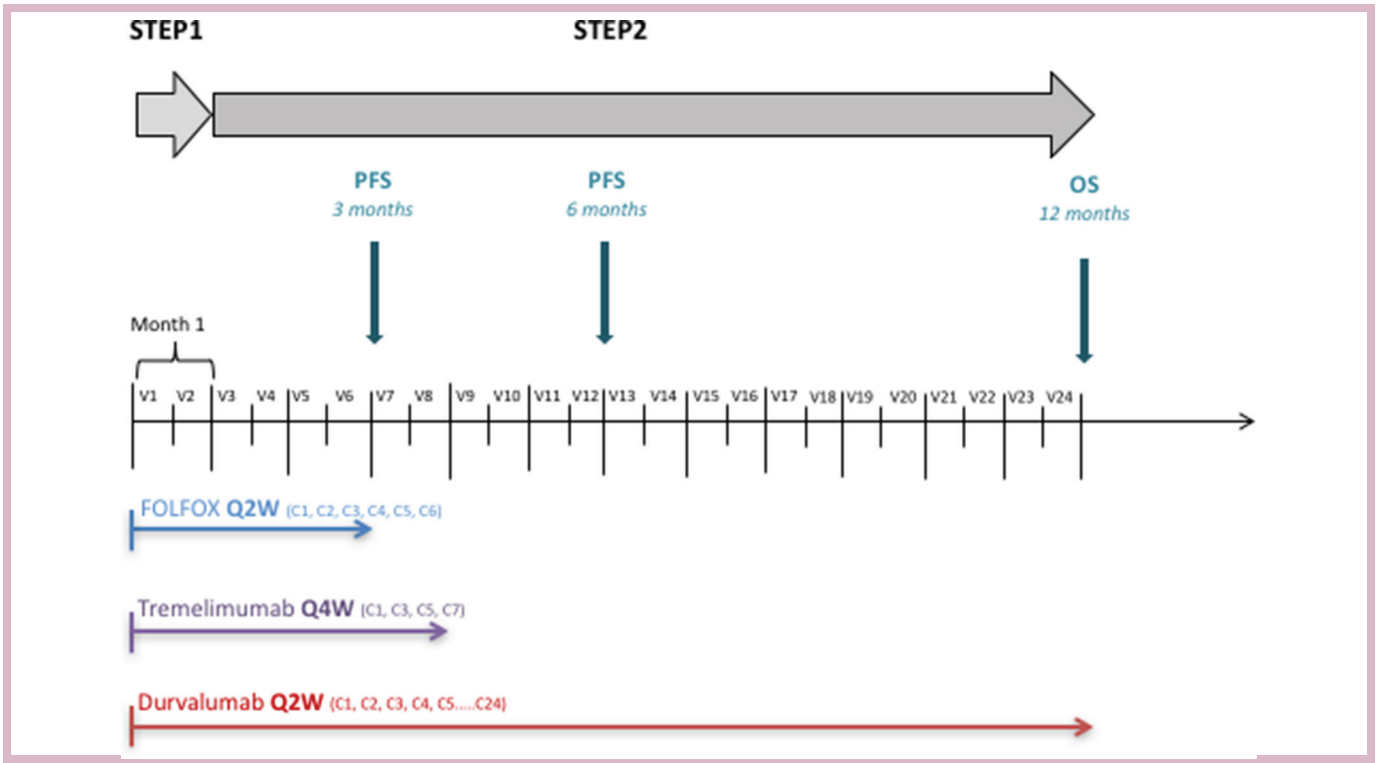


**Figure 2** Overview of regimen's protocol. C, course of treatment; OS, overall survival; PFS, progression-free survival; V, visit.

slightly less between-subject variability with fixed dosing regimen.

A fixed dosing approach is preferred by the prescribing community due to ease of use and reduced dosing errors. Given expectation of similar pharmacokinetic exposure and variability, we considered it feasible to switch to fixed dosing regimens. Based on average body WT of 75 kg, a fixed dose of 750 mg every 2 weeks MEDI4736 (equivalent to 10 mg/kg every 2 weeks), 1500 mg every 4 weeks durvalumab (equivalent to 20 mg/kg every 4 weeks) and 75 mg every 4 weeks tremelimumab (equivalent to 1 mg/kg every 4 weeks) is included in the current study.

### Oxaliplatin, fluorouracil and leucovorin

Subjects will be administered with FOLFOX in line with normal clinical practice, with a dose and schedule of oxaliplatin, 85 mg/m$^2$ administered as intravenous infusion over 2 hours in 250 mL dextrose 5% or sterile water for injection concurrently (via a Y-connector) with Leucovorin, 400 mg/m$^2$ (400 mg/m$^2$ for form DL (dextro-levogyre) or 200 mg/m$^2$ for form L(levogyre)) administered as intravenous infusion over 2 hours, in 250 mL dextrose 5%, or sterile water for injection followed by 5-FU, 400 mg/m$^2$ administered as a bolus injection (intravenous push administered manually in approximately 2 min), followed by 5-FU, 2400 mg/m$^2$ administered as an intravenous infusion over 46 hours. Oxaliplatin should always be administered before fluoropyrimidines.

Dose of oxaliplatin, 5-FU and LV will be administered on the basis of milligrams of each drug per square metre of body surface area (BSA) as measured at baseline (mg/m$^2$). The dose of oxaliplatin administered should be as close as possible to the calculated dose and will be limited to a maximum BSA of 2.0 m$^2$. Though the WT of the patient may change throughout the study, BSA will be assumed to stay close to that measured at baseline, that is, no dose adjustments for changes in body WT will be done unless WT loss alone is considered to be an AE of grade 2 or more. Cycle length is 2 weeks comprising approximately 46 hours of infusion and 12 days of rest. It is expected that subjects will receive six cycles of FOLFOX (3 months).

### Statistical consideration

Safety analyses will be performed on the safety-evaluable population, defined as all subjects treated with at least one dose of investigational product.

*Toxicities and grades will be described at each cycle.

*The following data will be given:

- The number and percentage of patients with at least one AE.
- The number and percentage of patients with at least one grade 3 or 4 AE.
- The number and percentage of patients with at least one serious AE.
- The number and percentage of patients with at least one AE leading to treatment premature stop.
- Time until grade 3–4 toxicity will be determined using the Kaplan-Meier method.

Patients without toxicities will be censored.

Efficacy analyses will be performed in mITT population, that is, all patients following the major inclusion

criteria and with a 3-month evaluation. Analyses will be repeated in the ITT principle, that is, including all enrolled patients whatever eligibility criteria and treatment received by patients and in the per protocol population (patients who had received all the planned doses).

The primary objective will be evaluated at 3 months. According to the Simon's design, at 3 months, on the first evaluable 43 patients (mITT population): if 26 or more patients respond to treatment, the treatment will be considered of interest for evaluation in a phase III trial.

RRs will be evaluated using RECIST criteria. Response will be considered for patient with CR, PR and SD. OS and PFS will be estimated using Kaplan-Meier method. Median survivals will be reported with 95% CIs. Median follow-up will be estimated using the reverse Kaplan-Meier method. All statistical analyses will be performed with Stata V.11 or SAS V.9.3.

The efficacy will be determined using a Simon 2 step phase II design. The hypotheses are the following:

1. A PFS of 3 months is not considered of interest.

2. A PFS of 6 months is expected. This is equivalent to assume that a PFS of 50% at 3 months is insufficient and a PFS of 70.7% is expected. With $\alpha$=10%, $\beta$=10% (90% power), 10% of non-evaluable patients, 48 patients are needed including patients.

## DISCUSSION

Immune checkpoint inhibitors have recently changed the management of several types of cancer. In CRC, results remain modest except in MSI tumour. Preclinical data have shown that some chemotherapies such as oxaliplatin and 5-FU could generate an increase of immunogenicity of tumour and improve the efficacy of immune checkpoint inhibitors. In the light of these results, immunogenic chemotherapy in combination with immune checkpoint would act synergistically and might be a promising treatment for metastatic CRC. Furthermore, blood, plasma and tumour tissue will be collected and assessed for potential prognostic and predictive biomarkers.

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**Contributors** NI and FG designed the study. NI, AH, SZ, JFG, AH and FG will include and follow patients. AB and ER will perform statistical analyses and figures. FG supervised the study. JDF and FG wrote the manuscript. All coauthors read and approved the final manuscript.

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**Competing interests** None declared.

**Patient consent** Obtained.

**Ethics approval** Comité de Protection des Personnes Tours–Région Centre–Ouest

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