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Phase II study on first-line treatment of *NIV*olumab in combination with folfoxiri/ bevacizumab in patients with Advanced *COloR*ectal cancer RAS or BRAF mutated – NIVACOR trial (GOIRC-03-2018)

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Abstract

Background: FOLFOXIRI (fluorouracil, leucovorin, oxaliplatin, and irinotecan) plus bevacizumab has shown to be one of the therapeutic regimens in first line with the highest activity in patients (pts.) with metastatic colorectal cancer (mCRC) unselected for biomolecular alterations. Generally, tumors co-opt the programmed death-1/ligand 1 (PD-1/PD-L1) signaling pathway as one key mechanism to evade immune surveillance. As today, anti-PD-1 monoclonal antibodies are FDA approved only for DNA mismatch repair deficient/microsatellite instability-high (MMRd/MSI-H), which represent only about 5% among all mCRC. Nowadays, there are no data demonstrating anti PD-1 activity in proficient and stable disease (MMRp/MSS). A different target in mCRC is also the Vascular Endothelial Growth Factor A (VEGF-A), which acts on endothelial cells to stimulate angiogenesis. VEGF-A inhibition with bevacizumab has shown to increase the immune cell infiltration, providing a solid rationale for combining VEGF targeted agents with immune checkpoint inhibitors. Based on these evidences, we explore the combination of triplet chemotherapy (FOLFOXIRI) with bevacizumab and nivolumab in pts. with mCRC *RAS/BRAF* mutant regardless of microsatellite status.

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Methods/design: This is a prospective, open-label, multicentric phase II trial where pts. with mCRC *RAS/BRAF* mutated, in first line will receive nivolumab in combination with FOLFOXIRI/bevacizumab every 2 weeks for 8 cycles followed by maintenance with bevacizumab plus nivolumab every 2 weeks. Bevacizumab will be administered intravenously at dose of 5 mg/kg every 2 weeks and nivolumab intravenously as a flat dose of 240 mg every 2 weeks. The primary endpoint is the overall response rate (ORR). This study hypothesis is that the treatment is able to improve the ORR from 66 to 80%. Secondary endpoints include OS, safety, time to progression, duration of response. Collateral translational studies evaluate the i) tumor mutational burden, and ii) genetic alterations by circulating free DNA (cfDNA) obtained from plasma samples. The trial is open to enrollment, 9 of planned 70 pts. have been enrolled.

Trial registration: NIVACOR is registered at ClinicalTrials.gov: NCT04072198, August 28, 2019.

Keywords: Metastatic colorectal Cancer, First line therapy, Nivolumab, FOLFOXIRI Bevacizumab

Background

The colorectal cancer (CRC) is diagnosed at advanced stages in almost 50%, and in this setting the 5-year survival rate is approximately of the 12% [1]. CRC is a heterogeneous tumor consisting of multiple genetic, genomic and epigenetic alterations, and this entails the stratification of the patients into different subgroups susceptible to different treatments. In 2015, a large-scale consortium reported four consensus molecular subtypes (CMS) of CRC described in MSI Immune (CMS1), Canonical (CMS2), Metabolic (CMS3), and Mesenchymal (CMS4) [2, 3], each with specific biomolecular and prognostic features.

In metastatic CRC (mCRC) the *RAS* gene (*KRAS, NRAS*) is mutated approximately in 50–55%. Currently, detection of *RAS* mutations is the only predictive marker of response to the anti-*EGFR* antibodies, cetuximab and panitumumab [4, 5].

The second potential biomarker in mCRC is *BRAF*, mutated in 5–11% of cases [6]. The *BRAFV600E* point mutation is the most common alteration and believed to be mutually exclusive with *KRAS* exon 2 mutations [7]. Accordingly, several clinical trials have highlighted the negative prognostic role of the *BRAF* mutation associated with high mortality [8].

In patients who harbor *RAS/BRAF* mutant tumors, the addition of anti-vascular growth factor (VEGF) antibody to cytotoxic drugs based on fluorouracil/levofolinate/irinotecan or oxaliplatin, has become one of the standard treatments in first-line of mCRC [9].

Several randomized studies, have proved that the triplet of chemotherapy with fluorouracil/levofolinate/irinotecan/oxaliplatin (FOLFOXIRI) combined to bevacizumab is more effective than doublet of chemotherapy plus bevacizumab, and this combination was well tolerated as first-line treatment in selected fit patients [10, 11]. In the TRIBE study [9], a phase III study, in first-line setting the treatment with FOLFOXIRI plus bevacizumab improved the primary endpoint, progression-free survival (PFS), compared with FOLFIRI (fluorouracil, leucovorin, and irinotecan) plus bevacizumab (HR 0.75; 95% CI 0.62–0.90;

$p = 0.003$). A significant improvement and depth of tumor response associated with early tumor shrinkage assessed by Response Evaluation Criteria In Solid Tumors (RECIST) version 1.0, was also reported in experimental arm (FOLFOXIRI plus bevacizumab). Moreover, an advantage in terms of median overall survival (mOS) in FOLFOXIRI plus bevacizumab arm was revealed (29.8 months vs. 25.8 months; HR 0.80, 95% CI 0.65–0.98; $p = 0.03$). The molecular sub-analysis of the TRIBE study showed a better mOS in *RAS* and *BRAF* wild-type subgroups compared to *RAS* mutated and *BRAF* mutated subgroups (37.1 months vs. 25.6 months vs. 13.4 months), respectively [12].

In the VOLFI study [13], a phase II, patients affected by *RAS* wild type mCRC treated in first line with modified-FOLFOXIRI (m-FOLFOXIRI) plus an anti-EGFR antibody, panitumumab, presented a significantly improved the ORR (87.3%) compared to control arm (60.6%) both investigator and centrally assessment (95% CI, 1.61–12.38; $p = .004$). No difference in PFS was found (9.7 months in both arms, HR 1.071; 95%-CI 0.689–1.665, $p = 0.76$), but a strong trend about enhanced mOS in the experimental arm has been reported (35.7 months vs. 29.8 months; HR: 0.67; 95%-CI 0.41–1.11, $p = 0.12$) [14].

Currently, a further tumor feature being studied and of the great interest is the description of immune landscape of the microenvironment in mCRC, especially concern to microsatellite status. Most of tumors (85–90%) had a low-to-moderate mutation load and two main groups of CRCs were recognized: proficient in terms of mismatch repair mechanisms (MMRp) of DNA and microsatellite-stable (MSS). The minority are highly mutated with deficient mismatch-repair mechanisms (MMRd) relating to a microsatellite-instable phenotype (MSI, more accurately MSI-high [MSI-H]). This classification systems, MMRp/MSS vs. MMRd/MSI afford a way to stratify patients concerning to immunotherapy response [15, 16]. Nowadays, the immunotherapy approach with anti-PD1/PD-L1 antibodies for mCRC has demonstrated efficacy only in MMRd/MSI tumor subgroups but no in MMRp/MSS tumors. Several phase I-

II-III clinical trials have been conducted (Table 1) and others are still ongoing (Table 2) to establish the immunotherapeutic efficacy alone or in combination with other drugs, especially with chemotherapy.

Preclinical studies, have shown the close connection between tumor cells and the tumor microenvironment (TME) status, especially the surrounding milieu composed by the stroma, tumor-infiltrating lymphocytes, and lymphatic and vascular layers. In this context, endothelial cells play a key role in the extravasation of immune cells, influencing the arrangement of the tumor environment [38]. It is known that extremely inflamed tumors reflect poor tumor angiogenesis; however, highly vascularized tumors may conversely entail tumors with deprived immune infiltration. One of the biomolecules responsible for affecting the hematopoietic progenitor cell differentiation to dendritic cells (DCs) is the tumor-derived VEGF. DCs are the most efficient antigen presenting cells due to the peptide presentation of tumor antigens on the major histocompatibility complex (MHC) I and II molecules, eliciting T-cells by B7 molecule expression, against cancer antigens [39, 40]. Active extravasation of leukocytes in the tumor stroma requires a series of events starting from the rolling, firm adhesion of leukocytes on endothelial cells, and leading to wandering into the interstitial areas. VEGF plays an essential role in this process as the blood vessels could present an obstacle to extravasation of immune cells in the interstitial space [41–43].

Additionally, VEGF inhibition by bevacizumab, involves a normalization of tumor vascularization rises the permeability to immune cell infiltration.

Given the strong preclinical rationale for combining VEGF inhibitors with immune checkpoint regulators, an increasing number of clinical trials are underway in several solid tumors including urothelial carcinoma [44, 45], metastatic renal cell carcinoma (mRCC) [46–48], and non-small cell lung cancer (NSCLC) [49–51], aiming to evaluate the anti-angiogenesis agents reinforce the benefit and durable responses afforded by anti- cytotoxic T-lymphocyte associated protein-4 (CTLA4) and the PD-1/ PD-L1 agents.

It is essential to restore an immunological environment to sensitize mCRC to immune checkpoint inhibitors, to combine them with treatments that stimulate T-cells as chemotherapy, although the molecular mechanisms of sensitization are still not clear. Preclinical models suggest that some chemotherapies can improve the immunotherapy efficacy [52, 53]. The association of fluorouracil and oxaliplatin with immune checkpoint inhibitors in vivo could deplete Myeloid-derived Suppressor Cells (MDSCs) [54], and trigger an immunogenic arrangement of tumor cell death [55]. Dosset et al. [56], have investigated in two mouse models the use of FOLFOX in association with anti-PD-1 therapy. The combination induced a strong expression of PD-1 on CD8$^+$ TILs, and the IFN-γ secreted by FOLFOX-induced CD8$^+$ T cells leads PD-L1

Table 1 Clinical Trials in mCRC of immune-checkpoint inhibitors as single agents or in combination

Drugs	Trial/Phase	Setting	Population	ORR n/N (%)	DCR n/N (%)	PFS (mo = months)	OS (mo = months)
Pembrolizumab [17]	NCT01876511 Phase II	≥ 2 L	MSI-H/ dMMR	21/41 (52)	33/40 (82)	2-year = 59% mPFS NR	2-year = 85% mOS NR
Pembrolizumab [18, 19]	KEYNOTE-164 Phase II	≥ 2 L	MSI-H/ dMMR	21/63 (33)	36/63 (57)	12-mo = 41% mPFS 4.1 mo (2.1 – NR)	12-mo = 76% mOS NR (19.2 – NR)
		≥ 3 L	MSI-H/ dMMR	17/61 (28)	31/61 (51)	12-mo = 34% mPFS 2.3 mo (2.1–8.1)	12-mo = 72% mOS NR
Pembrolizumab + mFOLFOX6 [20]	NCT02375672 Phase II	1 L	MSI- unselected	12/30 (40)	23/30 (77)	PFS not reported mPFS 16.9 mo (7.4, 16.9)	OS not reported mOS 8.8 mo (18.3-NE)
Nivolumab [21]	CheckMate 142 Phase II	≥ 2 L	MSI-H/ dMMR	23/74 (31)	51/74 (69)	12-mo = 50% mPFS 14.3 mo (4.3, NE)	12-mo = 73% mOS, NR (18.0, NE)
Nivolumab + low dose Ipilimumab [22]	ChackMate 142 Phase II	≥ 2 L	MSI-H/ dMMR	65/119 (55)	95/119 (80)	12-mo = 71% mPFS NR	12-mo = 85% mOS NR
Atezolizumab + bevacizumab and fluoropyrimidine [23]	NCT02291289 Phase II	1 L (maintenance)	MSI- unselected	Not reported	Not reported	mPFS 7.2 mo	mOS 22.1 mo
Atezolizumab + FOLFOX + bevacizumab [24]	NCT01633970 Phase Ib	≥ 2 L	Oxaliplatin naïve	9/25 (31)	Not reported	Not reported	Not reported
Atezolizumab + bevacizumab [25]	NCT01633970 Phase I	≥ 2 L	MSI-H/ dMMR	4/10 (40)	9/10 (90)	mPFS NR (1.5– 21.9)	mOS NR (2.6– 23.7)

*ORR Overall response rate, PFS Progression free survival, OS Overall survival, NE Not estimable, NR Not reached, m Median

Table 2 Clinical Trials ongoing in mCRC of immune-checkpoint inhibitors as single agents or in combination with chemotherapy

Drugs	Trial	Setting	MSI/MSS status population	Primary Endpoint
Nivolumab + standard therapy vs standard therapy [26]	CheckMate 9X8 NCT03414983 Phase II/III	1 L	unselected	PFS
Nivolumab alone Nivolumab in combination with other drugs [27]	CheckMate 142 NCT02060188 Phase II	1 L	MSI/MSS	ORR by investigators
Nivolumab + Ipilimumab + Temozolomide [28]	NCT03832621 Phase II	1 L	MSS MGMT silenced	8-months PFS
Nivolumab Nivolumab + Ipilimumab or standard therapy [29]	NCT04008030 Phase III	1 L	MSI-H/MMRd	PFS
Pembrolizumab vs standard therapy [30]	KEYNOTE-177 NCT02563002 Phase III	1 L	MSI-H/MMRd	PFS, OS
Pembrolizumab + pemetrexed and oxaliplatin [31]	NCT03626992 Phase Ib	2 L+	MSS	ORR
Atezolizumab vs atezolizumab + FOLFOX/bevacizumab vs FOLFOX/ bevacizumab [32]	COMMIT GI004/S1610 NCT02997228 Phase III	1 L	MMRd	PFS
Avelumab [33]	NCT03150706 Phase II	2 L+	MSI-H/MMRd POLE	ORR
Avelumab vs standard chemotherapy +/− targeted therapy [34]	NCT03186326 Phase II	2 L	MSI	PFS by central review
Durvalumab [35]	NCT02227667 Phase II	3 L+	MSI-I/MMRd	best response rate
Durvalumab [36]	NCT03435107 Phase II	2 L	MSI/MMRd POLE	ORR
Durvalumab plus tremelimumab + FOLFOX [37]	NCT03202758 Phase Ib/II	1 L	unselected	safety, PFS

*MGMT, O6-methylguanine-DNA methyltransferase; POLE, DNA polymerase epsilon, catalytic subunit

expression on tumor cells and this mechanism is considered as an adaptive immune resistance system to FOL-FOX. In neoadjuvant setting, mCRC patients treated with FOLFOX showed an increased CD8$^+$ cell infiltrate and tumor PD-L1 expression. Another chemotherapeutic drug, trifluridine/tipiracil (FTD/TPI), an antimetabolite agent used to treat chemo-refractory mCRC, induced immunogenic arrangement of tumor cell death in vitro in MSS CT26 mouse colon carcinoma cell line, as well as in various human MSS colorectal cancer cell lines [57]. In vivo, the combination of FTD/TPI with oxaliplatin was able to induce immunogenic arrangement of tumor cell death, but not the single agents. Furthermore, the combination abolished type-2 tumor-associated macrophages (TAM2), resulting in higher cytotoxic CD8$^+$ T-cell infiltration and activation. This effect was associated with tumor cells PD-L1 expression and PD-1 induction in CD8$^+$ T cells, resulting in T-cell exhaustion.

Based on these preclinical and clinical data, there is sufficient evidence to explore the combination of chemotherapy with immunotherapy and antiangiogenetic inhibitors in pts. with mCRC *RAS/ BRAF* mutated.

Methods
Protocol overview/study treatment
This is a prospective, open-label, multicentric phase II trial in which pts. with *RAS* or *BRAF* mutated will receive nivolumab in combination with FOLFOXIRI/bevacizumab as first line treatment. Study screening will take place within 28 days prior to initiation of study treatment. At screening, every patient must have local *RAS/ BRAF* known status. A centralized review of *RAS/BRAF* status will be performed.

Eligible pts. will be enrolled and begin treatment with FOLFOXIRI/bevacizumab plus nivolumab every 2 weeks for 8 cycles followed by maintenance with bevacizumab plus nivolumab every 2 weeks until disease progression, unacceptable toxicity or patient/physician decision. Bevacizumab will be administered intravenously at dose of 5 mg/kg every 2 weeks. Nivolumab will be administered intravenously at flat dose of 240 mg every 2 weeks.

FOLFOXIRI will be administered as $165 \, mg/m^2$ intravenous infusion of irinotecan for 60 min, followed by an $85 \, mg/m^2$ intravenous infusion of oxaliplatin given concurrently with leucovorin at a dose of $200 \, mg/m^2$ for 120 min, followed by a $3200 \, mg/m^2$ continuous infusion of fluorouracil for 48 h (Fig. 1).

During the protocol's treatment, pts. will be followed for safety based on Adverse Event (AE) assessments including vital signs, physical findings and clinical laboratory test results.

In order to guarantee the safety of pts., the enrolment will be stopped when the 10th patient will start treatment. An Independent Monitoring Committee will evaluate the safety data of these pts. and will decide if the study should be completed, amended or closed.

The efficacy will be evaluated by the investigator according to RECIST 1.1 criteria every 8 weeks during treatment, and then every 3 months for 3 years.

During the study baseline tumor blocks will be centrally analyzed to determinate MSI/MSS and PD-L1 status, inflammatory infiltrate through evaluation of high peri- and/or intra-tumor lymphocyte infiltration (TIL) using $CD3^+$ and $CD8^+$ TILs, tumor-associated macrophages (TAMs), tumor-associated neutrophils (TANs), and regulatory T cells (Tregs) well as the expression of marker of autophagy.

Following discontinuation of the treatment, safety assessments will be conducted 30 days after the last drug administration or until initiation of other anti-cancer therapy. Thereafter, pts. will be followed for disease progression (unless this has already occurred), serious AEs, anticancer therapy and survival. Follow-up will continue for up to 3 years.

A blood sample will be collected at baseline, prior to cycle 5, at the end of chemotherapy and at disease progression. Quality of life will be assessed at baseline, every 4 weeks during treatment and study discontinuation visit.

A list of participating centers is provided in Table 3.

Inclusion criteria

For inclusion in the study, all of the following inclusion criteria must be fulfilled: (i) histopathological confirmed colon adenocarcinoma; (ii) initially unresectable metastatic colorectal cancer not previously treated with chemotherapy for metastatic disease; (iii) assessment of *RAS* and *BRAF* status of the primary and/or secondary colon cancer on biopsies (mutant); (iv) age ≥ 18 years and ≤ 75 years; (v) ECOG performance status 0–1; (vi) if dihydropyridine dehydrogenase (DPD) status is known it must be wild type; (vii) laboratory data including: white blood cell count $\geq 3 \times 10^9/L$ with neutrophils $\geq 1.5 \times 10^9/L$, platelet count $\geq 100 \times 10^9/L$, hemoglobin ≥9 g/dL (5,6 mmol/l), total bilirubin ≤1.5 x ULN (upper limit of normal), ASAT and ALAT ≤2.5 x ULN, alkaline phosphatase ≤1.5 x ULN, serum creatinine ≤1.5 x ULN; (viii) signed written informed consent obtained prior to any study specific screening procedures.

Exclusion criteria

Patients are not eligible for this study if any of the following exclusion criteria apply: (i) prior chemotherapy, excluded pts. treated in neo/adjuvant setting at least 12 months before diagnosis of metastatic disease; (ii) radiotherapy to any site within 4 weeks before the study; (iii) evidence of bleeding diathesis or coagulopathy; (iv) uncontrolled hypertension and prior history of hypertensive crisis or hypertensive encephalopathy (v) systemic corticosteroids within 2 weeks of the first dose of nivolumab; (vi) diagnosis of immunodeficiency or is receiving systemic steroid therapy within 14 days prior to the first dose of trial treatment; (vii) active and untreated brain (CNS) metastases and/or carcinomatous meningitis or subjects with previously treated brain metastases may participate provided they are not using steroids for at least 7
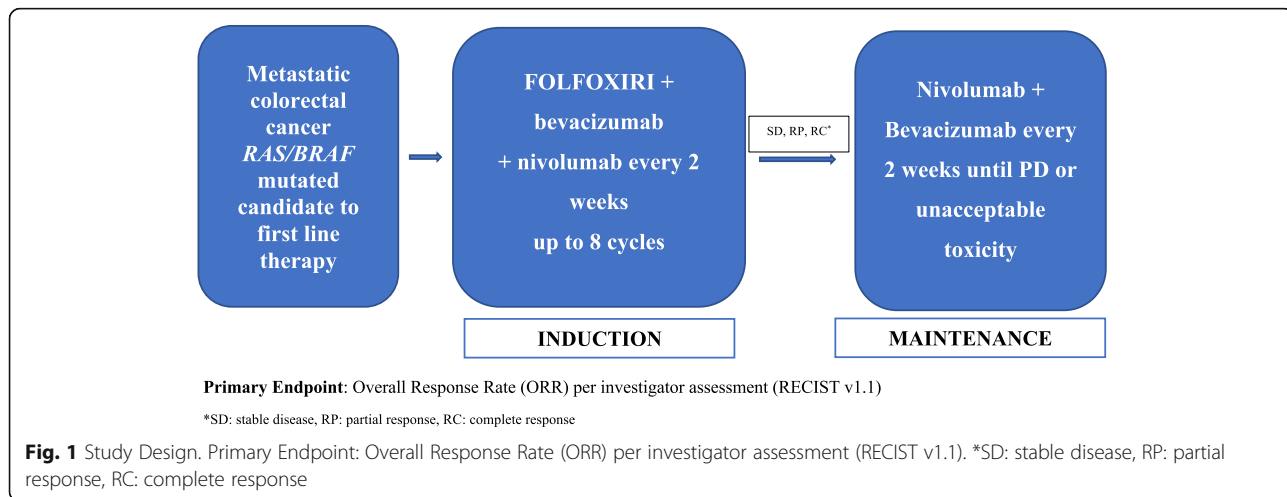

Fig. 1 Study Design. Primary Endpoint: Overall Response Rate (ORR) per investigator assessment (RECIST v1.1). *SD: stable disease, RP: partial response, RC: complete response

Table 3 Participating Centers

Principal Investigator	Site	City
Carmine Pinto	Azienda USL – IRCCS Reggio Emilia	Reggio Emilia
Francesca Bergamo	Istituto Oncologico Veneto	Padova
Evaristo Maiello	Casa Sollievo della Sofferenza	San Giovanni Rotondo
Alberto Zaniboni	Fondazione Poliambulanza	Brescia
Lorenzo Antonuzzo	Azienda Ospedaliera Careggi	Firenze
Guglielmo Nasti	Istituto Nazionale Tumori di Napoli – IRCCS Pascale	Napoli
Giuseppe Tonini	Policlinico Universitario Campus Biomedico	Roma
Roberto Bordonaro	ARNAS Garibaldi – Azienda Ospedaliera di Rilievo Nazionale e di Alta Specializzazione Garibaldi	Catania

days prior to trial treatment; (viii) evidence of interstitial lung disease, active non-infectious pneumonitis, or a history of grade 3 or greater pneumonitis; (ix) live vaccine within 30 days prior to the first dose of trial treatment; (x) significant vascular disease (e.g. aortic aneurysm requiring surgical repair or recent arterial thrombosis) within 6 months of study enrollment; (xi) history of abdominal fistula, gastrointestinal (GI) perforation, intra-abdominal abscess or active GI bleeding within 6 months prior to the first study treatment; (xii) pregnancy (absence to be confirmed by ß-hCG test) or breast-feeding period; (xiii) any significant disease which, in the investigator's opinion, would exclude the patient from the study.

Study endpoints

The present trial will determine if adding nivolumab to the first line therapy with FOLFOXIRI/bevacizumab is efficient in terms of response rate in mCRC *RAS/BRAF* mutated. To evaluate the Overall Response Rate (ORR), defined as complete response (CR), partial response (PR), and stable disease (SD), we will use RECIST version 1.1 criteria.

Secondary endpoints are the following: (i) safety assessment of the combination treatment with FOLFOXIRI/bevacizumab plus nivolumab graded by National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) v. 4.03; (ii) OS defined as the time from beginning of the study-drug administration to the date of death from any cause; (iii) Time To Progression (TTP) defined as the time from beginning of the study-drug administration and the first date of documented progression, based on investigator assessment as per RECIST 1.1 criteria, or death due to any cause, whichever occurs first; (iv) the duration of response defined as the time between the first evidence of response (SD/PR/CR) and the date of documented progression or death due to any cause; (v) the quality of life of pts. determinate with the EORTC QLQ-C30 that consists of 30 questions that assess five aspects of patient functions (physical, emotional, role, cognitive, and social), three symptom scales (fatigue, nausea and vomiting, pain), global health and/or quality of life, and six single items (dyspnea, insomnia, appetite loss, constipation, diarrhea, financial difficulties) with a recall period of the previous week. Scale scores can be obtained for the multi-item scales.

The collateral study includes the TMB, MSI status and the role of genetic and molecular pattern analysis in relation to patient's outcome. Formalin-fixed and paraffin-embedded (FFPE) tumor samples will be collected before starting fist-line therapy (at baseline), as primary and/or metastatic tumor tissue blocks or as 15 5-μm unstained slides. The neoplastic cell content of each tumor sample will be assessed and in those cases with neoplastic cells < 50% a macro-dissection of the specimen will be performed, if possible. For all the pts. enrolled, venous blood will be obtained by standard phlebotomy technique from a peripheral access point or from a central line, by trained personnel. Blood samples will be collected at different points: at baseline, prior to 5 cycle, at the end of chemotherapy and at disease progression.

Data collection and follow up

Study drug administration occurs on Day 1 (± 3 days) of each cycle. Each cycle is 14 days. Cycle 1 should occur within 3 days from registration of pts. All procedures during the study treatment must occur within 3 days prior to the administration, except for radiological assessment required for baseline within 28 days prior to initiation of the study treatment. The following assessments will be performed prior to each cycle every 2 weeks. All radiological assessments will be performed each 8 weeks (± 1 week), regardless of the treatment cycle. CEA will be testing every 8 weeks with radiological assessments. The end of the study treatment visit should occur within 30 days after last dose of study treatment is administered. The post-treatment follow-up visits will occur every 3 months (± 14 days) for 3 years (Table 4).

Statistical analysis and sample size

The primary objective of this study is to assess the ORR, defined as the best response recorded on the ITT population according to RECIST v1.1. In the TRIBE study, ORR

Table 4 Study assessments

Procedures	Screening (−28 days)	Cycle 1,3,5,7[a] (+ 3 days)	Cycle 2,4,6,8 [a] (+ 3 days)	Maintenance [a] (+ 3 days)	End of treatment [a]	Post-treatment Follow up [a]
Signed informed consent	X					
Medical history and baseline conditions	X					
Physical examination	X	X	X	X	X	
Parameters[b]	X	X	X	X	X	
Hematology and serum chemistry[c]	X	X	X	X	X	
Protein dipstick	X	X	X	X	X	
Adverse Events	X	X	X	X	X	
Radiological assessment[e], CEA[c]	X	X		X	X	
QLQ-C30 questionnaire[f]	X		X	X	X	
Blood sample[g]	X				X	
FOLFOXIRI administration		X	X			
Nivolumab and Bevacizumab administration		X	X	X		
Survival follow-up						X

[a]Each cycle is 14 days. Study drugs administration occurs on day1 (+/− 3 days) of each cycle. All clinical and laboratory assessments must occur within 3 days prior the administration. The end of treatment should occur within 30 days after last dose of study treatment. The post-treatment follow-up visit occur every 3 months (+/− 14 days) for 3 years

[b]Vital signs will include: weight, respiratory rate, pulse rate, temperature and systolic and diastolic blood pressure. At baseline height and BSA

[c]Hematology analysis (within 7 days before Cycle 1) consist of: hemoglobin, WBC and platelet count, BUN, creatinine, glucose, total bilirubin, sodium, potassium, calcium, AST, ALT, alkaline phosphatase, LDH, albumin. CEA will be tested every 8 weeks with radiological assessment. Amylase, lipase, TSH, FT3, FT4, will be done on cycle 2,4,6,8

[d]If proteinuria is 2+, should undergo a 24-h urine collection and must demonstrate 1 g of protein/24 h

[e]Radiological assessment will be performed within 28 days prior to start of study treatment and every 8 weeks (± 1 week), regardless cycle of treatment; in details, during chemotherapy phase prior to cycle 5, at the end of chemotherapy (cycle 8)

[f]QLQ-C30 will be completed at baseline, at cycles 4 and 8 of chemotherapy phase, every 4 cycles thereafter and at end of treatment visit

[g]Blood sample will be collected at baseline, prior to cycle 5, at the end of chemotherapy and at time of progression

for pts. *RAS/BRAF* mutated treated in first line with FOL-FOXIRI and bevacizumab regimen was 66% [11]. Our hypothesis is that FOLFOXIRI and bevacizumab regimen plus nivolumab is able to improve the ORR from 66 to 80%. An ORR of 80% is considered enough valuable to pursue this combination in a phase III trial.

The sample size was calculated using the A'Hern [19] modification of the original Fleming [20] one-stage design. Calculations were performed by the use of PASS Professional v.11.0.10 software [21].

The study requires 64 subjects to decide whether the proportion responding, P, is less than or equal to 0,66 or greater than or equal to 0,80. If the number of responses is 49 or more, the Hypothesis that $P \leq 0,66$ is rejected with a target error rate of 0,05 and an actual error rate of 0,046. If the number of responses is 48 or less, the hypothesis that $P \geq 0,800$ is rejected with a target error rate of 0,200 and an actual error rate of 0,197. A total 70 pts. will to be enrolled assuming 10% pts. discontinuation rate due to non-compliance or toxicity.

Preliminary safety evaluation
An Independent Monitoring Committee (IDMC) will review safety data 28 days after the inclusion of the 10th

patient. Safety data, including demographics, adverse events, serious adverse events, and relevant laboratory data, will be reviewed.

The IDMC will provide a recommendation as to whether the study may continue, whether amendment(s) to the protocol should be implemented, or whether the study should be stopped. The final decision will rest with the Sponsor.

Coordination
Azienda Unità Sanitaria Locale di Reggio Emilia – IRCCS is responsible for the coordination and management of the study on behalf of Gruppo Oncologico Italiano Ricerca Clinica (G.O.I.R.C.) Cooperative Group.

Discussion
The binding of PD-L1 to PD-1 plays a central role in T-cell tolerance by hindering naive and effector T-cell responses. Clinical experience with checkpoint inhibitors has shown that tumors co-opt the PD-L1/PD-1 signaling pathway as one key mechanism to escape immune damage. Nivolumab, an anti-PD-1 monoclonal antibody may block tumor growth in different ways by targeting certain cells.

It's well known that chemotherapy makes the cancer more immunogenic, and more suitable for immunotherapy.

Moreover, angiogenetic inhibitors could promote enhanced tumor T-cell infiltration causing in a reprogramming of the tumor microenvironment from immune-suppressive to immune-permissive status. Novel anti-PD-L1 drugs reinforce the action of the antiangiogenetic drugs when administered in combination.

Encouraging early indicators of efficacy have been detected with combination strategies using immune-checkpoint inhibitors and biological targeted therapies, such as axitinib in combination with pembrolizumab [46], and nivolumab in combination with sunitinib or pazopanib [47] in mRCC. In another phase 1b study in mRCC, investigating the combination of bevacizumab and an anti -PD-L1, atezolizumab, increased intratumoral CD8+ T-cells and macrophages compared to bevacizumab alone, leading to an increase of MHC I expression, as well as Th-1 and T effector gene signatures in post treatment biopsies assessment [48]. Atezolizumab plus bevacizumab were examined in phase I, II and III studies. The safety of this combination resulted acceptable and AEs leading to treatment interruption were very low. In a phase III study, 40% of pts. treated with atezolizumab plus bevacizumab and 54% of pts. with sunitinib had grade 3–4 AEs; 12 and 8% of all-grade AEs led to discontinuation of treatment, respectively [48, 58, 59].

Recent findings for enhancement in PFS using bevacizumab and atezolizumab in combination with carboplatin/paclitaxel in front-line lung cancer is a promising strategy, indorsing clinically meaningful and durable benefit for patients [49–51].

In a clinical trial conducted in melanoma pts., has been explored the combination of bevacizumab with anti-CTLA-4 inhibitor, ipilimumab, revealed widespread morphological modifications in CD31+ endothelial cells and an extensive tumor penetration of immune cells post-treatment including CD8+ cells and CD163+ macrophages in comparison to ipilimumab treatment alone, thus demonstrating that the combination of anti-VEGF and anti-CTLA-4 inhibitors has the ability to promote immune cell access in the TME [60].

A recent phase III study revealed that in pts. with NSCLC, atezolizumab in addition to bevacizumab plus carboplatin and paclitaxel (ABCP) in 692 pts. with advanced non-squamous NSCLC improve OS (19.2 months vs. 14.7 months; HR 0.78; 95% CI, 0.64 to 0.96; $p = 0.02$) [51]. The safety profile of ABCP was consistent with safety profiles of each drugs and AEs occurred in 94.4% vs. 95.4% in ABCP and BCP control group, respectively. The most common grade 3 or 4 AEs were febrile neutropenia, and hypertension, and related serious AEs were noticed in 25.4 and 19.3% in the ABCP and BCP groups, respectively. The immune-related AEs (irAEs) grade 1 or 2 occurred in 77.4% of the ABCP group, and the

treatment-related deaths occurred in 2.8% of the ABCP group [51].

In mCRC, a phase Ib study examined the safety and efficacy of atezolizumab plus bevacizumab (Arm A) with the dosage of atezolizumab 20 mg/kg q3w and bevacizumab 15 mg/kg q3w versus atezolizumab plus bevacizumab and mFOLFOX6 (Arm B) with atezolizumab 14 mg/kg q2w, bevacizumab 10 mg/kg q2w, and mFOLFOX6 at standard doses. The safety profile in Arm A showed a 64% of grade 3–4 AEs, while in Arm B, 73% pts. had grade 3–4 AEs, especially hematological toxicity. The irAEs grade 3 and 4 were 7 and 20%, respectively. The authors concluded that the addition of atezolizumab plus bevacizumab with or without FOLFOX was well tolerated without unexpected toxicities [61]. Efficacy data are not yet available.

In a phase II study, in 30 mCRC pts., pembrolizumab combined with mFOLFOX6 in first line treatment showed an acceptable toxicity thought suggesting a trend towards an increase of neutropenia; in the initial cohort grade 3 and 4 neutropenia was described but after dose reduction of mFOLFOX6, rate of grade 3 and 4 toxicity was 36.7 and 13.2% with FOLFOX/pembrolizumab and pembrolizumab alone respectively. Best response was partial response in 15 pts. with 100% of disease control rate (DCR) at 8 weeks. After 2 months of therapy, one patient with MMRd had surgical resection accounting complete pathological response. Moreover, the mPFS has not been reached [62].

In conclusion, we assume that there are sufficient evidences to support the combination of treatments with triplet chemotherapy (FOLFOXIRI), antibody anti-VEGF (bevacizumab), and immunotherapy (nivolumab, anti PD-1 antibody) in pts. with mCRC *RAS/BRAF* mutated, regardless to MMR status.

Availability of data and materials
Not applicable.

Ethics approval and consent to participate
This study is conducted in agreement with either the Declaration of Helsinki or the laws and regulations of the country, whichever provides the greatest protection of the patient. The protocol has been written, and the study is conducted according to the ICH Harmonized Tripartite Guideline for Good Clinical Practice. The study (Protocol version 2.0, January 14th 2019) was approved for all participating centers by AIFA, the Italian health authority (Agenzia Italiana del Farmaco) on February 8th 2019 and registered on August 28th 2019 at Clinicaltrials.gov (NCT04072198). IEC(s)/IRB(s) approved the submitted documents for each center. Company QBE Insurance was appointed by Gruppo Oncologico Italiano Ricerca Clinica (G.O.I.R.C.) for an insurance policy to provide patients for reimbursement to any injury associated with the study. Changes to eligibility criteria, outcomes, analysis or other important protocol modifications will be notified to the IEC/IRB for approval and will be forwarded to the Sponsor. Informed consent to study procedures before enrollment in the study was signed by all candidates; moreover, those will be informed about the study purpose, the activities involved, the expected duration, the potential risks and benefits by the investigators (or legally authorized representative).

Consent for publication
Not applicable.

Competing interests
The authors declare no conflict of interest.

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References
1. National Cancer Institute Surveillance, Epidemiology, and end results program. SEER Stat Fact Sheets: Colon and Rectum Cancer. https://seer.cancer.gov/statfacts/html/colorect.html. Accessed 3 Mar 2016.
2. Guinney J, Dienstmann R, Wang X, et al. The consensus molecular subtypes of colorectal cancer. Nat Med. 2015;21(11):1350–6.
3. Dienstmann R, Vermeulen L, Guinney J, et al. Consensus molecular subtypes and the evolution of precision medicine in colorectal cancer. Nat Rev Cancer. 2017;17:79–92.
4. Fiala O, Buchler T, Mohelnikova-Duchonova B, et al. G12V and G12A KRAS mutations are associated with poor outcome in patients with metastatic colorectal cancer treated with bevacizumab. Tumour Biol. 2016;37:6823–30.
5. Sorich MJ, Wiese MD, Rowland A, et al. Extended *RAS* mutations and anti-EGFR monoclonal antibody survival benefit in metastatic colorectal cancer: a meta-analysis of randomized, controlled trials. Ann Oncol. 2015;26(1):13–21.
6. Davies H, Bignell GR, Cox C, et al. Mutations of the BRAF gene in human cancer. Nature. 2002;417:949–54.
7. Prahallad A, Sun C, Huang S, et al. Unresponsiveness of colon cancer to BRAF(V600E) inhibition through feedback activation of EGFR. Nature. 2012;483(7387):100–3.
8. Bokemeyer C, Van Cutsem E, Rougier P, et al. Addition of cetuximab to chemotherapy as first-line treatment for KRAS wild-type metastatic colorectal cancer: pooled analysis of the CRYSTAL and OPUS randomised clinical trials. Eur J Cancer. 2012;48(10):1466–75.
9. Loupakis F, Cremolini C, Masi G, et al. Initial therapy with FOLFOXIRI and bevacizumab for metastatic colorectal Cancer. N Engl J Med. 2014;371:1609–18.
10. Hurwitz H, Tan BR, Reeves JA, et al. Updated efficacy, safety, and biomarker analyses of STEAM, a randomized, open-label, phase II trial of sequential (s) and concurrent (c) FOLFOXIRI-bevacizumab (BV) vs FOLFOX-BV for first-line (1L) treatment (tx) of patients with metastatic colorectal cancer (mCRC). J Clin Oncol. 2017;35:657.
11. Schmoll HJ, Meinert FM, Cygon F, et al. "CHARTA": FOLFOX/bevacizumab vs. FOLFOXIRI/ bevacizumab in advanced colorectal cancer—Final results, prognostic and potentially predictive factors from the randomized Phase II trial of the AIO. J Clin Oncol. 2017;35(15 suppl):3533.
12. Cremolini C, Loupakis F, Antoniotti C, et al. FOLFOXIRI plus bevacizumab versus FOLFIRI plus bevacizumab as first-line treatment of patients with metastatic colorectal cancer: updated overall survival and molecular subgroup analyses of the open-label, phase 3 TRIBE study. Lancet Oncol. 2015;16(13):1306–15.
13. Modest DP, Martens UM, Riera-Knorrenschild J, et al. FOLFOXIRI Plus Panitumumab As First-Line Treatment of RAS Wild-Type Metastatic Colorectal Cancer: The Randomized, Open-Label, Phase II VOLFI Study (AIO KRK0109). J Clin Oncol. 2019;37(35):3401-11. https://doi.org/10.1200/JCO.19.01340.
14. Geissler M, Tannapfel A, Reinacher-Schick A, et al. Final results of the randomized phase II VOLFI trial (AIO- KRK0109): mFOLFOXIRI 1 Panitumumab versus FOLFOXIRI as first- line treatment in patients with RAS wild-type metastatic colorectal cancer (mCRC). Abstract PD-030. Ann Oncol. 2019:iv119. https://doi.org/10.1093/annonc/mdz156.
15. Mlecnik B, Bindea GG, Angell HK, et al. Integrative analyses of colorectal cancer show immunoscore is a stronger predictor of patient survival than microsatellite instability. Immunity. 2016;44(3):698–711.
16. Kim JM, Chen DS. Immune escape to PD-L1/PD-1 blockade: seven steps to success (or failure). Ann Oncol. 2016;27:1492–504.
17. Le DT, Durham JN, Smith KN, et al. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. Science. 2017;357:409–13.
18. Le DT, Kavan P, Kim TW et al. KEYNOTE-164: Pembrolizumab for patients with advanced microsatellite instability high (MSI-H) colorectal cancer. Presented at: American Society of Clinical Oncology; June 1–5, 2018; Chicago, IL. 2018; abstract 3514.
19. Diaz L, Marabelle A, Kim TW et al. Efficacy of pembrolizumab in phase 2 KEYNOTE-164 and KEYNOTE-158 studies of microsatellite instability high cancers. Presented at: European Society for Medical Oncology; September 8–12, 2017; Madrid, Spain. 2017; abstract 386P.
20. Shahda S, Noonan AM, Bekaii-Saab TS, et al. A phase II study of pembrolizumab in combination with mFOLFOX6 for patients with advanced colorectal. Presented at: American Society of Clinical Oncology; June 2–6, 2017; Chicago. IL 2017; abstract 3541.
21. Overman MJ, McDermott R, Leach JL, et al. Nivolumab in patients with metastatic DNA mismatch repair-deficient or microsatellite instability high colorectal cancer (CheckMate 142): an open-label, multicentre, phase 2 study. Lancet Oncol. 2017;18:1182–91.
22. Overman MJ, Lonardi S, Wong KYM, et al. Durable clinical benefit with nivolumab plus ipilimumab in DNA mismatch repair-deficient/ microsatellite instability-high metastatic colorectal cancer. J Clin Oncol. 2018;36:773–9.
23. Grothey A, Tabernero J, Arnold D et al. Fluoropyrimidine (FP) and bevacizumab + atezolizumab as first-line treatment for BRAFwt metastatic colorectal cancer: Findings from the MODUL trial of biomarker-driven maintenance. Presented at: European Society for Medical Oncology; October 19–23, 2018; Munich, Germany. 2018; abstract LBA19.
24. Hochster HS, Bendell JC, Cleary JM et al. Efficacy and safety of atezolizumab (atezo) and bevacizumab (bev) in a phase Ib study of microsatellite

instability (MSI)-high metastatic colorectal cancer (mCRC). Presented at: American Society of Clinical Oncology Gastrointestinal Cancers Symposium; January 19–21, 2017; San Francisco, CA. 2017; abstract 673.

25. Bendell JC, Powderly JD, Lieu CH et al. Safety and efficacy of MPDL320A (anti-PDL1) in combination with bevacizumab (bev)and/or FOLFOX in patients (pts) with metastatic colorectal cancer (mCRC). Presented at: American Society of Clinical Oncology; May 29 June, 2015; Chicago, IL 2015; abstract 704.

26. An Investigational immunotherapy study of Nivolumab with standard of care therapy vs standard of care therapy for first line treatment of colorectal cancer that has spread (CheckMate 9X8). https://clinicaltrials.gov/ct2/show/NCT03414983.

27. An Investigational Immuno-therapy Study of Nivolumab, and Nivolumab in Combination With Other Anti-cancer Drugs, in Colon Cancer That Has Come Back or Has Spread (CheckMate142). https://clinicaltrials.gov/ct2/show/NCT02060188.

28. NIVOLUMAB Plus IPILIMUMAB and TEMOZOLOMIDE in microsatellite stable, MGMT Silenced Metastatic Colorectal Cancer (MAYA) https://clinicaltrials.gov/ct2/show/NCT03832621.

29. A Study of Nivolumab, Nivolumab Plus Ipilimumab, or Investigator's Choice Chemotherapy for the Treatment of Patients With Deficient Mismatch Repair (dMMR)/Microsatellite Instability High (MSI-H) Metastatic Colorectal Cancer (mCRC) (CheckMate 8HW). https://clinicaltrials.gov/ct2/show/NCT04008030.

30. Study of pembrolizumab (MK-3475) vs standard therapy in partecipants with microsatellite instability high (MSI-H) or mismatch repair deficient (dMMR) stage IV colorectal carcinoma (MK-3475-177/KEY-NOTE-177). https://clinicaltrials.gov/ct2/show/NCT02563002.

31. Study of Pembrolizumab With Pemetrexed and Oxaliplatin in Chemo-Refractory Metastatic Colorectal Cancer Patients. NCT03626922. https://clinicaltrials.gov/ct2/show/NCT03626922.

32. Combination Chemotherapy, Bevacizumab, and/or Atezolizumab in Treating Patients With Deficient DNA Mismatch Repair Metastatic Colorectal Cancer, the COMMIT Study. https://clinicaltrials.gov/ct2/show/NCT02997228.

33. Avelumab for MSI-H or POLE Mutated Metastatic Colorectal Cancer. https://clinicaltrials.gov/ct2/show/NCT03150706.

34. Standard Chemotherapy vs Immunotherapie in 2nd Line Treatment of MSI Colorectal Mestastatic Cancer (SAMCO). https://clinicaltrials.gov/ct2/show/NCT03186326.

35. Evaluate the Efficacy of MEDI4736 in Immunological Subsets of Advanced Colorectal Cancer. https://clinicaltrials.gov/ct2/show/NCT02227667.

36. Durvalumab for MSI-H or POLE Mutated Metastatic Colorectal Cancer. https://clinicaltrials.gov/ct2/show/NCT03435107.

37. Fumet JD, Isambert N, Hervieu A, et al. 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. ESMO Open. 2018;3:e000375. https://doi.org/10.1136/esmoopen-2018-000375.

38. Oyama T, Ran S, Ishida T, et al. Vascular endothelial growth factor affects dendritic cell maturation through the inhibition of nuclear factor-kappa B activation in hemopoietic progenitor cells. J Immunol. 1998;160:1224–32.

39. Gabrilovich D, Ishida T, Oyama T, Ran S, et al. Vascular endothelial growth factor inhibits the development of dendritic cells and dramatically affects the differentiation of multiple hematopoietic lineages in vivo. Blood. 1998; 92:4150–66.

40. Huang Y, Yuan J, Righi E, et al. Vascular normalizing doses of antiangiogenic treatment reprogram the immunosuppressive tumor microenvironment and enhance immunotherapy, Proc. Natl Acad Sci U S A. 2012;109:17561–6.

41. Becht E, de Reynies A, Giraldo NA, Pilati C, et al. Immune and stromal classification of colorectal cancer is associated with molecular subtypes and relevant for precision immunotherapy. Clin Cancer Res. 2016;22:4057–66.

42. Tian L, Goldstein A, Wang H, Ching Lo H, et al. Mutual regulation of tumor vessel normalization and immunostimulatory reprogramming. Nature. 2017; 544(7649):250-4. https://doi.org/10.1038/nature21724.

43. Borgstrom P, Hughes GK, Hansell P, et al. Leukocyte adhesion in angiogenic blood vessels role of E-selectin, P-selectin, and beta2 integrin in lymphotoxin-mediated leukocyte recruitment in tumor microvessels. J Clin Invest. 1997;99(9):2246–53. https://doi.org/10.1172/JCI119399.

44. Balar AV, Galsky MD, Rosenberg JE, Powles T, et al. Atezolizumab as first-line treatment in cisplatin-ineligible patients with locally advanced and metastatic urothelial carcinoma: a single-arm, multicentre, phase 2 trial. Lancet. 2017;389(10064):67–76.

45. Rosenberg JE, Hoffman-Censits J, Powles T, et al. Atezolizumab in patients with locally advanced and metastatic urothelial carcinoma who have progressed following treatment with platinum-based chemotherapy: a single-arm, multicentre, phase 2 trial. Lancet. 2016;387(10031):1909-20.

46. Atkins MB, Plimack ER, Puzanov I, et al. Axitinib in combination with pembrolizumab in patients (pts) with advanced renal cell carcinoma (aRCC): preliminary safety and efficacy results. Annals of Oncology. 2016;27(6):266-95. https://doi.org/10.1093/annonc/mdw373.

47. Amin AERP, R. Infante J, Ernstoff SM, et al. Nivolumab (anti-PD-1; BMS-936558, ONO-4538) in combination with sunitinib or pazopanib in patients (pts) with metastatic renal cell carcinoma (mRCC). J Clin Oncol. 2014;32(15_suppl):5010. https://doi.org/10.1200/jco.2014.32.15_suppl.5010.

48. Wallin JC, Bendell R, Funke M, et al. Atezolizumab in combination with bevacizumab enhances antigen-specific T-cell migration in metastatic renal cell carcinoma. Nat Commun. 2016;7:12624. https://doi.org/10.1038/ncomms12624.

49. Fehrenbacher L, Spira A, Ballinger M, et al. Atezolizumab versus docetaxel for patients with previously treated non-small cell lung cancer (POPLAR): a multicentre, open-label, phase 2 randomised controlled trial. Lancet. 2016; 387(10030):1837-46. https://doi.org/10.1016/S0140-6736(16)00587-0.

50. Reck MS, Cappuzzo F, Orlandi F, et al. Primary PFS and safety analyses of a randomized Phase III study of carboplatin+paclitaxel +/− bevacizumab, with or without Atezolizumab in 1L non squamous metastatic NSCLC (IMpower150). ESMO Immuno Oncology Congress. 2017; Abstract 204. https://oncologypro.esmo.org/meeting-resources/esmo-immuno-oncology-congress-2017/Primary-PFS-and-safety-analyses-of-a-randomized-Phase-III-study-of-carboplatin-paclitaxel-bevacizumab-with-or-without-atezolizumab-in-1L-non-squamous-metastatic-NSCLC-IMpower150.

51. Socinski MA, Jotte RM, Cappuzzo F, et al. Atezolizumab for first-line treatment of metastatic nonsquamous NSCLC. N Engl J Med. 2018;378:2288-301. https://doi.org/10.1056/NEJMoa1716948.

52. Galluzzi L, Buque A, Kepp O, et al. Immunological effects of conventional chemotherapy and targeted anticancer agents. Cancer Cell. 2015;28(6):690-714. https://doi.org/10.1016/j.ccell.2015.10.012.

53. Pfirschke C, Engblom C, Rickelt S, et al. Immunogenic chemotherapy sensitizes tumors to checkpoint blockade therapy. Immunity. 2016;44(2):343-54. https://doi.org/10.1016/j.immuni.2015.11.024.

54. Vincent J, Mignot G, Chalmin F, et al. F. 5-fluorouracil selectively kills tumor-associated myeloid-derived suppressor cells resulting in enhanced T cell-dependent antitumor immunity. Cancer Res. 2010;70(8):3052-61. https://doi.org/10.1158/0008-5472.CAN-09-3690.

55. Tesniere A, Schlemmer F, Boige V, et al. Immunogenic death of colon cancer cells treated with oxaliplatin. Oncogene. 2010;29(4):482-91. https://doi.org/10.1038/onc.2009.356.

56. Dosset M, Vargas TR, Lagrange A, et al. PD-1/PD-L1 pathway: an adaptive immune resistance mechanism to immunogenic chemotherapy in colorectal cancer. ONCOIMMUNOLOGY. 2018;7(6):e1433981. https://doi.org/10.1080/2162402X.2018.1433981.

57. Limagne E, Thibaudin M, Nuttin L, et al. Trifluridine/Tipiracil plus Oxaliplatin improves PD-1 blockade in colorectal Cancer by inducing immunogenic Cella death and depleting macrophages. Cancer Immunol Res. 2019;7:1958–69. https://doi.org/10.1158/2326-6066.CIR-19-0228.

58. McDermott DF, Huseni MA, Atkins MB, et al. Clinical activity and molecular correlates of response to atezolizumab alone or in combination with bevacizumab versus sunitinib in renal cell carcinoma. Nat Med. 2018;24(6):749–57.

59. Motzer RJ, et al. IMmotion151: a randomized phase III study of atezolizumab plus bevacizumab vs. sunitinib in untreated metastatic renal cell carcinoma (mRCC). J Clin Oncol. 2018;36(6):578.

60. Hodi FS, Lawrance D, Lezcano C, et al. Bevacizumab plus Ipilimumab in patients with metastatic melanoma. Cancer Immunol Res. 2014;2(7):632–42.

61. Bendell JC, Powderly JD, Lieu CH, et al. Safety and efficacy of MPDL3280A (anti-PDL1) in combination with bevacizumab (bev) and/or FOLFOX in patients (pts) with metastatic colorectal cancer (mCRC). J Clin Oncol. 2015; 33(Suppl. S3):704.

62. Shahda S, Noonan AM, Bekaii-Saab TS, et al. A phase II study of pembrolizumab in combination with mFOLFOX6 for patients with advanced colorectal cancer. J Clin Oncol. 2017;35(15):3541.

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