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TITLE:

PICCASSO – A phase I trial of combined **P**D-1 **i**nhibition (Pembrolizumab) and **CC**R5 inhibition (Maraviroc) for the treatment of refr**a**ctory microsatellite stable (M**SS**) metastatic c**o**lorectal cancer (mCRC)

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1.0 TRIAL SUMMARY

Abbreviated Title	PICCASSO – A phase I trial of combined PD-1 inhibition (Pembrolizumab) and CCR5 inhibition (Maraviroc) for the treatment of refractory microsatellite stable (MSS) metastatic colorectal cancer (mCRC)
Trial Phase	Phase I
Clinical Indication	Refractory microsatellite stable (MSS) metastatic colorectal cancer
Trial Type	Interventional
Type of control	No treatment control
Route of administration	Intravenous (pembrolizumab); peroral (maraviroc)
Trial Blinding	Unblinded open-label
Treatment Groups	Pembrolizumab 200 mg administered i.v. on day 1 every three weeks (d1, qd22) *plus* Maraviroc 2 x 300mg administered perorally on day 1 to 21, every three weeks (d1-21; qd22)
Number of trial subjects	Approximately 20 subjects will be enrolled
Estimated enrollment period	18 months
Estimated duration of trial	24-44 months from FPI until LPO
Duration of Participation	Each subject will participate in the trial from the time the subject signs the Informed Consent Form (ICF) through the final contact. Eligible subjects will receive pembrolizumab beginning on Day 1 of each 3-week dosing cycle (d1, qd22) together with maraviroc administered perorally on day 1 to 21 of each cycle (d1-21; qd22). Treatment with pembrolizumab / maraviroc combination will continue until progressive disease (PD), unacceptable adverse events (AEs), intercurrent illness that prevents further administration of treatment, investigator's decision to withdraw the subject, subject withdraws consent, pregnancy of the subject, noncompliance with trial treatment or procedure requirements, administrative reasons requiring cessation of treatment, or completion of treatment per protocol. Subjects with a treatment response or stable disease after completion of the first treatment phase of eight cycles (core treatment period) will be offered, at the discretion of the investigator, participation in a maintenance phase consisting of up to 24 additional treatment cycles of pembrolizumab monotherapy (total treatment duration up to 24 months). Subjects who discontinue for reasons other than PD will have post-treatment follow-up for disease status until PD, initiating a non-study cancer treatment, withdrawing consent, or becoming lost to follow-up. All subjects will be followed for overall survival (OS) until death, withdrawal of consent, loss to follow-up, or the end of the study. After the end of treatment, each subject will be followed for 30 days for AE monitoring. Serious adverse events (SAEs) and AEs of special interest (AESIs) will be collected for 90 days after the end of treatment or for 30 days after the end of treatment if the subject initiates new anticancer therapy, whichever is earlier.
Estimated average length of treatment per patient	4 months

2.0 TRIAL DESIGN

2.1 Trial Design

This is a monocentric, single arm, prospective, open-label trial of a combination treatment consisting of pembrolizumab and maraviroc in previously treated subjects who have refractory microsatellite stable (MSS) metastatic colorectal cancer (mCRC).

Subjects will be required to have at least one measureable lesion by Response Evaluation Criteria in Solid Tumors (RECIST 1.1) for response assessment as well as at least one metastatic lesion accessible for repetitive biopsies (see Section 5.1 "Entry Criteria" for details). Additionally, subjects have to have been previously treated with systemic therapy against mCRC, which must include a fluoropyrimidine, oxaliplatin, irinotecan, an antiangiogenic monoclonal antibody (e.g. bevacizumab, aflibercept, ramucirumab), an EGFR inhibitor (in case of RAS/BRAF wildtype tumors) and optionally regorafenib or TAS 102.
Subjects who have withdrawn from standard treatment due to unacceptable toxicity warranting discontinuation of treatment and precluding retreatment with the same agent before progression of disease will also be eligible. Approximately 20 subjects will be allocated in this study to receive pembrolizumab / maraviroc combination treatment.

The primary objective of this phase I trial is to investigate the feasibility, safety and toxicity of pembrolizumab combined with maraviroc in patients with MSS mCRC. The most important secondary objectives are DCR and ORR of pembrolizumab / maraviroc combination treatment. Imaging assessments will be performed using RECIST 1.1 for determining assessment of response. On study imaging assessments will be performed every 9 weeks (Q9W) calculated from the date of first study drug administration independent of treatment delays. RECIST 1.1 will be used by the site for treatment decisions until first radiologic evidence of PD. Following the first evidence of radiologic PD, treatment decisions may be made by the adaption of RECIST 1.1 as described in Section **Fehler! Verweisquelle konnte nicht gefunden werden.** termed immune-related RECIST (irRECIST) to accommodate for the tumor response patterns seen with pembrolizumab treatment (e.g., tumor flare). For a clinically stable subject with first radiologic evidence of PD it is at the discretion of the site investigator to continue treating the subject with pembrolizumab / maraviroc combination until PD is confirmed at least 4 weeks from the date of the first tumor imaging suggesting PD. If radiologic PD is confirmed by the subsequent tumor imaging the subject should be discontinued from treatment unless, in the opinion of the investigator, the subject is achieving a clinically meaningful benefit; an exception to continue treatment may be considered following consultation with the Sponsor.
Subjects will continue to be treated with pembrolizumab until PD, unacceptable AEs, intercurrent illness that prevents further administration of treatment, investigator's decision to withdraw the subject, subject withdraws consent, pregnancy of the subject, noncompliance with trial treatment or procedure requirements, administrative reasons, or the subject has received all trial treatments per protocol.

Subjects who discontinue treatment for reasons other than PD will have post-treatment follow-up for disease status until PD, initiating a non-study cancer treatment, withdrawing consent, or

becoming lost to follow-up. All subjects will be followed for OS until death, withdrawal of consent, loss to follow-up, or the end of the study – whichever comes first.

Adverse events will be monitored throughout the trial and graded in severity according to the guidelines outlined in the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 (Section **Fehler! Verweisquelle konnte nicht gefunden werden.**) After the end of treatment, each subject will be followed for 30 days for AE monitoring. SAEs and AESIs will be collected for 90 days after the end of treatment or 30 days after the end of treatment if the subject initiates new anticancer therapy, whichever is earlier.

The Continuous Toxicity Monitoring Board (CTMB) will regularly monitor all SAE/SUSAR that will arise throughout the trial. Data for the CTMB will be collected and prepared by the CRO. The CTMB may meet via telephone conference call or via email exchange in regular intervals. Depending on the toxicity results, the CTMB will decide about continuation, necessary modifications, suspension or early termination of the trial. The CRO will be informed in writing.

Details of the frequency of the toxicity assessment by the CTMB and the planned toxicity analyses for the CTMB meetings are described in the CTMB Charter.There is no full interim analysis planned for this study, due to the small sample size and the relatively short recruitment period. However, single objectives may be analyzed as soon as sufficient events are available for analysis as detailed in the SAP.

This study will be conducted in conformance with Good Clinical Practices.

Specific procedures to be performed during the trial, as well as their prescribed times and associated visit windows, are outlined in the Trial Flow Chart – Section 6.0. Details of each procedure are provided in Section **Fehler! Verweisquelle konnte nicht gefunden werden.** – Trial Procedures.

2.2 Trial Diagram

The trial design is depicted in Figure 1 below.

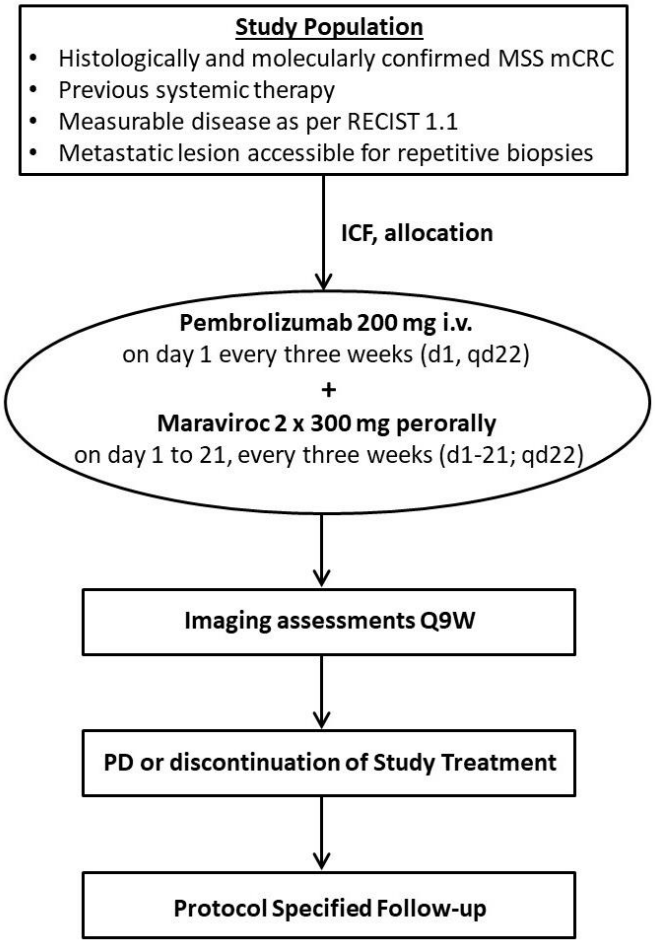

Figure 1 – Trial Diagram

3.0 OBJECTIVE(S) & HYPOTHESIS(ES)

3.1 Primary Objective(s) & Hypothesis(es)

(1) **Objective:** To evaluate the feasibility, safety and toxicity of pembrolizumab combined with maraviroc in patients with MSS mCRC.

Hypothesis: The combination of pembrolizumab with maraviroc is feasible and safe. This will be displayed by a feasibility rate of >70% during the core treatment period, i.e. the first 8 cycles of study treatment (feasibility rate = 1 – severe toxicity/withdrawal rate; for exact definition see **Fehler! Verweisquelle konnte nicht gefunden werden.** "Primary Endpoint" and 7.2 "Justification of sample size").

3.2 Secondary Objective(s) & Hypothesis(es)

(1) **Objective**: To assess the DCR (defined as percentage of patients displaying CR/PR or SD as best response) of the pembrolizumab / maraviroc combination in patients with MSS mCRC according to either the RECIST criteria (version 1.1) or the irRECIST criteria.

Hypothesis: The DCR in subjects with MSS mCRC is between 30% and 35%.

(2) **Objective**: To assess the ORR of the pembrolizumab / maraviroc combination in patients with MSS mCRC according to the RECIST criteria (version 1.1) and the irRECIST criteria (irORR).

(3) **Objective:** To assess individual progression-free (PFS) and overall survival (OS) as well as immune related (ir) progression-free survival (irPFS).

3.3 Exploratory Objective(s)

(1) **Objective:** To assess the immune response in whole blood and tumor tissue.

(2) **Objective:** To identify biomarkers that correlate with clinical response and/or clinical outcome.

4.0 BACKGROUND & RATIONALE

4.1 Background

4.1.1 Metastatic Colorectal Cancer

Globally, CRC is the third most commonly diagnosed cancer in males and the second in females, with a total of 1.4 million new cases and almost 694,000 deaths estimated to have occurred in 2012 [1]. The liver is the most common metastatic site, whereby only in a subgroup of patients characterized by a limited number of liver metastases surgery can lead to a cure in 20-30% of patients [2].

Despite advances in the development of targeted therapies such as monoclonal antibodies, systemic chemotherapy is still the backbone in the treatment of metastatic disease. With modern chemotherapeutical regimens overall survival in recent phase III trials reaches about 28-30 months [3]. However, in patients being refractory to one or two chemotherapeutical regimens such as Folfox or Folfiri, treatment options are still very limited and an objective tumor regression is rarely observed. Although approved for the treatment in this patient population, targeted agents such as regorafenib only show a modest efficacy by prolonging overall survival for further 1.4 months [4].
Immunotherapy with checkpoint blockade by anti PD-1 / PD-L1 antibodies such as pembrolizumab only showed efficacy in the subgroup of microsatellite instable tumors, whereas for the vast majority of colorectal cancer patients with MSS tumors no clinical activity

was observed [5]. Therefore, the overall prognosis of patients with MSS mCRC who have progressed on all standard therapies is very poor.

4.1.2 The PD-1 Inhibitor Pembrolizumab

Pembrolizumab (MK-3475) is a potent and highly selective humanized monoclonal antibody (mAb) of the IgG4/kappa isotype designed to directly block the interaction between PD-1 and its ligands, PD-L1 and PD-L2. Pembrolizumab has recently been approved in the United States for the treatment of patients with unresectable or metastatic melanoma and disease progression following ipilimumab and, if BRAF V600 mutation positive, a BRAF inhibitor.
The importance of intact immune surveillance in controlling outgrowth of neoplastic transformation has been known for decades. Accumulating evidence shows a correlation between tumor-infiltrating lymphocytes (TILs) in cancer tissue and favorable prognosis in various malignancies. In particular, the presence of CD8+ T-cells and the ratio of CD8+ effector T-cells / FoxP3+ regulatory T-cells seems to correlate with improved prognosis and long-term survival in many solid tumors.

The PD-1 receptor-ligand interaction is a major pathway hijacked by tumors to suppress immune control. The normal function of PD-1, expressed on the cell surface of activated T-cells under healthy conditions, is to down-modulate unwanted or excessive immune responses, including autoimmune reactions. PD-1 (encoded by the gene Pdcd1) is an Ig superfamily member related to CD28 and CTLA-4 which has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD-L1 and/or PD-L2). The structure of murine PD-1 has been resolved. PD-1 and family members are type I transmembrane glycoproteins containing an Ig Variable-type (V-type) domain responsible for ligand binding and a cytoplasmic tail which is responsible for the binding of signaling molecules. The cytoplasmic tail of PD-1 contains 2 tyrosine-based signaling motifs, an immunoreceptor tyrosine-based inhibition motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM). Following T-cell stimulation, PD-1 recruits the tyrosine phosphatases SHP-1 and SHP-2 to the ITSM motif within its cytoplasmic tail, leading to the dephosphorylation of effector molecules such as CD3ζ, PKCθ and ZAP70 which are involved in the CD3 T-cell signaling cascade. The mechanism by which PD-1 down modulates T-cell responses is similar to, but distinct from that of CTLA-4 as both molecules regulate an overlapping set of signaling proteins. PD-1 was shown to be expressed on activated lymphocytes including peripheral CD4+ and CD8+ T-cells, B-cells, T regs and Natural Killer cells. Expression has also been shown during thymic development on CD4-CD8- (double negative) T-cells as well as subsets of macrophages and dendritic cells. The ligands for PD-1 (PD-L1 and PD-L2) are constitutively expressed or can be induced in a variety of cell types, including non-hematopoietic tissues as well as in various tumors. Both ligands are type I transmembrane receptors containing both IgV- and IgC-like domains in the extracellular region and contain short cytoplasmic regions with no known signaling motifs. Binding of either PD-1 ligand to PD-1 inhibits T-cell activation triggered through the T-cell receptor. PD-L1 is expressed at low levels on various non-hematopoietic tissues, most notably on vascular endothelium, whereas PD-L2 protein is only detectably expressed on antigen-presenting cells found in lymphoid tissue or chronic inflammatory environments. PD-L2 is thought to control immune T-cell activation in lymphoid organs, whereas PD-L1 serves to dampen unwarranted T-cell function in peripheral tissues. Although healthy organs express little (if any) PD-L1, a variety

of cancers were demonstrated to express abundant levels of this T-cell inhibitor. PD-1 has been suggested to regulate tumor-specific T-cell expansion in subjects with melanoma (MEL). This suggests that the PD-1/PD-L1 pathway plays a critical role in tumor immune evasion and should be considered as an attractive target for therapeutic intervention.

Refer to the Investigator's Brochure for Preclinical and Clinical data.

A recent phase 2 study evaluated the activity of pembrolizumab in 41 patients with progressive metastatic carcinoma with or without mismatch-repair deficiency. Pembrolizumab was administered intravenously at a dose of 10 mg per kilogram of body weight every 14 days. The immune-related objective response rate was 40% (4 of 10 patients) for mismatch repair-deficient and 0% (0 of 18 patients) for mismatch repair-proficient mCRC. Similar between-group distributions of PFS were seen. Patients with mismatch repair-deficient non-colorectal cancer had responses similar to those of patients with mismatch repair-deficient colorectal cancer [5].

4.1.3 Chemokines in Malignancy

Chemokines and their receptors are key regulators of immune activities. Their role in malignancy is diverse and at times conflicting. The family of chemokines consists of about 50 low molecular weight proteins, sharing the main function of regulating leucocyte trafficking in physiological and pathological conditions. They bind to receptors that are expressed predominantly by leucocytes. Functionally, chemokines can be divided in homeostatic and inflammatory, with the first one being constitutively expressed in lymphoid organs and the last one being expressed in infected/damaged tissue.

Chemokines are key players in cancer-related inflammation. Chemokine ligands and receptors are downstream of genetic events that cause neoplastic transformation and are abundantly expressed in chronic inflammatory conditions which predispose to cancer. Components of the chemokine system affect multiple pathways of tumor progression including: leukocyte recruitment, neo-angiogenesis, tumor cell proliferation and survival, as well as invasion and metastasis [6] with not only leucocytes but also different types of stromal cells and malignant cells being involved.
Under specific conditions chemokines can initiate recruitment of leucocytes to tumors, thereby exerting anti-tumor effects. On the contrary, a large number of chemokines have powerful malignancy promoting effects. Thus, it is the composition of chemokines in the tumor and/or its direct vicinity that has a strong impact on the immunological tumor milieu which can exert either synergistic or antagonistic effects on tumor growth.

4.1.4 The CCL-CCR 5 Axis in Cancer

CCR5 is a receptor that was discovered in the analysis of CCL5, a chemokine that is produced with a time lag after activation of T cells and which also binds to other cytokine/chemokine receptors, like CCR3 and CCR1. CCR5 is mainly found on Th1 cells, CD8+ T-cells, macrophages and monocytes, but several studies showed that it is also found on tumor cells, endothelial cells and MSCs.

The ligand for CCR5, CCL5 is expressed by leucocytes, fibroblasts, endothelial cells and MSCs at the tumor sites, and also directly by cancer cells with differences being observed between different tumor entities [7].
Cancer cells secrete CCL5 or induce fibroblasts to secrete CCL5 which act in a paracrine or autocrine fashion on CCR5-positive tumor cells to sustain their proliferation, to recruit immunosuppressive cells (T-reg cells, monocytes), to induce osteoclasts activation and bone metastasis, to induce neoangiogenesis, and to guide tumor cells to disseminate to distant organs [7]. Further, CCL5 inhibits anti cancerous function of CD8+ CTL [8].

Upon activation of the CCR5-CCL5 axis, leucocytes are skewed into the pro-malignancy phenotype: elevated level of TAMs, MDSCs and Treg reside the tumor, accompanied by a reduced level of CD8+ CTLs [7]. Also, a change in the subtype of macrophages, which are functionally plastic cells, from a tumor-inhibiting type 1 (M1) into a tumor growth promoting type 2 (M2) can be observed.
Conversely, CCR 5 blockade repolarizes M2 macrophages towards M1, thereby leading to a tumor growth inhibiting immune-milieu [9]. Interestingly, inhibition of CCL5-dependent monocyte recruitment during the early phase of metastasis by a CCL5 receptor antagonist strongly reduced tumor cell survival and attenuated metastasis.
These findings suggest that blockage of the CCL-CCR5 axis could help rendering the immunosuppressive tumor environment towards a more immunogenic phenotype, thus making the tumor more accessible towards new immunotherapeutic approaches like immune checkpoint inhibition by PD-1 / PD-L1 antibodies.

4.1.5 The CCR5 Inhibitor Maraviroc

Initially, interest for CCR5 was sparked after it was identified as a co-receptor for the entry of HI virus into the CD4+ T helper cell. It was reasoned that blockade of this receptor should hinder entry of HIV into the patients' cells. An array of CCR5 antagonists was developed with one of the resulting agents used in clinical routine today being maraviroc.

Maraviroc is a small molecule inhibitor that selectively binds to CCR5, blocking the binding of CCL5 to CCR5 and thus entry of HIV into the cell. Experimental work has elicited some of the relevant effects of CCR5 blockade on the cells. Interestingly, it has been shown that the intracellular signaling effects of CCL5 can be completely shut off with maraviroc even in very low doses. Maraviroc binds to the CCR5 and binds the receptor for over 130h. This leads to an increased receptor expression on the cells for a short time period, which is – however – completely covered by the maraviroc binding time.

In sum, Maraviroc did not affect CCR5 cell surface levels or associated intracellular signaling, confirming it as a functional antagonist of CCR5. Maraviroc has no detectable *in vitro* cytotoxicity and is highly selective for CCR5, as confirmed against a wide range of receptors and enzymes. These features led to a quick introduction of this orally available drug into clinical testing during which it was successfully approved for the treatment of HIV patients with a HI virus with CCR5-tropism. The treatment of healthy volunteers and HIV infected patients revealed a low rate of side effects, the latter being not above Placebo level. No grade 3 or 4 toxicities were seen with standard dosing (150-600 mg/bid). Even under long term treatment conditions, the side effects did not increase although it was originally anticipated that HIV infected patients on long term treatment with CCR5 antagonists would develop

immune system abnormalities, due to the ubiquitous presence of CCR5 on different immune cells. This absence of immune-pathologies despite CCR5 blockade is explained through the availability of alternative receptors (CCR3, CCR1, and/or other unknown receptors) that can compensate for the functions of CCR5 [10].
Taken together, maraviroc has a very favorable safety profile when used in standard dosage (150-600 mg/bid).

Detailed background information on maraviroc is available in the maraviroc Investigator's Brochure (IB)/approved labeling.

4.1.6 Phase I Trial on Maraviroc Monotherapy in Colorectal Cancer

A phase I trial ("MARACON") using maraviroc as a monotherapy in refractory colorectal cancer has recently been finished [16]. In the MARACON trial, 12 patients suffering advanced stage colorectal cancer and displaying liver metastases after extensive pre-treatment have been administered maraviroc at a dosage of 300 mg/bid for a maximum of sixty days.
A pre-treatment biopsy was taken for the evaluation of CCR5 expression on tumor cells. Within the first four weeks of treatment, a second biopsy was performed to assess a potential tissue level response.

Clinical data showed no unexpected toxicity, especially no unexpected immune-related adverse events or AEs of infectious origins. Disease stabilization was observed in single patients [16].

4.2 Rationale

4.2.1 Rationale for the Trial and Selected Subject Population

In mCRC, after failure of standard cytotoxic chemotherapy only limited efficacy can be observed from targeted therapies such as regorafenib treatment. A tumor response in this setting is only rarely achieved.
Furthermore, new immunotherapeutic approaches like PD-1 blockage is barely effective in microsatellite stable colorectal cancer with clinical responses almost exclusively observed in microsatellite unstable tumors [5]. Thus, for the vast majority (85-95%) of CRC patients in advanced stages no effective immune therapy is available.
The limited efficacy of PD-1 blockage in MSS CRC may be based on the immunosuppressive environment keeping immune cells, i.e. mainly T cells, at the invasive margin of the tumor. By modulating the tumor environment via CCR5 blockage, CRC tumors may get more accessible for T cells and – combined with activation of these T cells – an effective anti-tumoral effect could be induced.
Therefore, the combination of CCR5 blockage with a PD-1 blocking antibody may represent a promising therapy option in MSS mCRC, which is the largest mCRC group.

This rationale leads us to conduct a phase I trial combining intravenous administration of pembrolizumab with peroral application of maraviroc in patients with refractory MSS mCRC to investigate the feasibility, safety, and toxicity of this new therapeutic approach. If treated patients display immune and clinical responses, this proof-of-concept (POC) data will build

the basis to evaluate the safety and efficacy of pembrolizumab / maraviroc combinations in larger sets of patients.

Such a combination therapy could represent a valuable treatment option for patients with MSS mCRC – a group of patients with an unfavorable prognosis and that cannot yet benefit from the new class of immunotherapeutic PD-1 / PD-L1 inhibitors up to now. Thus, a combination therapy like the one investigated in this trial could result in a more favorable prognosis for these patients.

5.0 METHODOLOGY

5.1 Entry Criteria

5.1.1 Diagnosis/Condition for Entry into the Trial

Patients with MSS mCRC which are refractory towards standard therapy or are intolerable towards standard therapy and who fulfill all other eligibility criteria (see 5.1.2 and 5.1.3) will be enrolled in the trial and treated with pembrolizumab combined with maraviroc until progressive disease (PD), unacceptable adverse events (AEs), intercurrent illness that prevents further administration of treatment, investigator's decision to withdraw the subject, subject withdraws consent, pregnancy of the subject, noncompliance with trial treatment or procedure requirements, treatment completed per protocol, or administrative reasons requiring discontinuation of treatment.

5.1.2 Subject Inclusion Criteria

In order to be eligible for participation in this trial, the subject must meet the following inclusion criteria:

1. Histologically confirmed metastatic colorectal cancer. Microsatellite stability (MSS) is confirmed by PCR or immunohistochemistry.

2. Patient failed standard therapy or is intolerable towards standard therapy which must include a fluoropyrimidine, oxaliplatin, irinotecan, an antiangiogenic monoclonal antibody (e.g. bevacizumab, aflibercept, ramucirumab), an EGFR inhibitor in case of RAS/BRAF wildtype tumors and optional regorafenib or TAS 102

3. Measurable disease as per RECIST 1.1

4. Metastatic lesion accessible for repetitive biopsies and patient willing to provide tissue from newly obtained biopsies. Patients without accessible lesions might be enrolled after discussion with the principle investigator.

5. ECOG performance status 0 or 1

6. Adequate hematological, hepatic and renal function parameters:
    - Leucocytes> 3.000/µl
    - Hemoglobin >9 g/dl

- Thrombocytes > 100.000/µl
- Serum creatinine ≤ 1.5 x upper limit of normal (ULN) or
  GFR ≥60 mL/min for subject with creatinine levels > 1.5 x institutional ULN
- Serum total bilirubin ≤ 1.5 x upper limit of normal or direct bilirubin ≤ ULN for subjects with total bilirubin levels > 1.5 ULN
- AST and ALT ≤ 2.5 x upper limit of normal (or ≤ 5 x if liver metastases are present)
- Albumin ≥ 2.5 mg/dL

7. Adequate coagulation functions as defined by International Normalized Ratio (INR) ≤ 1.5, and a partial thromboplastin time (PTT) ≤ 5 seconds above the ULN (unless receiving anticoagulation therapy). Patients receiving warfarin/phenprocoumon must be switched to low molecular weight heparin and have achieved stable coagulation profile.

8. Female and male patients' ≥ 18 years. Patients in reproductive age must be willing to use adequate contraception during the study and 4 months after the end of the study (appropriate contraception is defined as surgical sterilization (e.g., bilateral tubal ligation, vasectomy), hormonal contraception (implantable, patch, oral), and double-barrier methods (any double combination of: IUD, female condom with spermicidal gel, diaphragm, sponge, cervical cap)). Abstinence (relative to heterosexual activity) can be used as the sole method of contraception if it is consistently employed as the subject's preferred and usual lifestyle and if considered acceptable by local regulatory agencies and ERCs/IRBs. Periodic abstinence (e.g., calendar, ovulation, sympto-thermal, post-ovulation methods, etc.) and withdrawal are not acceptable methods of contraception. Female patients with childbearing potential need to have a negative pregnancy test within 7 days before study start.

9. Patient able and willing to provide written informed consent and to comply with the study protocol and with the planned surgical procedures.

5.1.3 Subject Exclusion Criteria

The subject must be excluded from participating in the trial if the subject meets one or more of the following exclusion criteria:

1. Female and male patients' < 18 years.

2. Inability to understand the aims of the study and/or protocol procedures

3. Hypersensitivity towards pembrolizumab, maraviroc, or any ingredients of the formulations administered

4. History of severe allergic, anaphylactic, or other hypersensitivity reactions to chimeric or humanized antibodies

5. Any other concurrent antineoplastic treatment including irradiation (local radiation of single non-target lesions for palliation only allowed)

6. Active autoimmune disease requiring immunosuppressive therapy

7. Any condition requiring continuous systemic treatment with either corticosteroids (>10 mg daily prednisone equivalents) or other immunosuppressive medications within 2 weeks prior to first dose of study treatment. Inhaled or topical steroids and physiological replacement doses of up to 10 mg daily prednisone equivalent are permitted in the absence of active autoimmune disease.

8. Secondary malignant disease during the last 5 years (exceptions include basal cell carcinoma of the skin, squamous cell carcinoma of the skin, or in situ cervical cancer that has undergone potentially curative therapy).

9. Clinical relevant comorbidity also including significant psychiatric disease

10. Clinically significant active coronary heart disease, cardiomyopathy or congestive heart failure, NYHA III-IV

11. Cardiocirculatory insufficiency with hypotension (systolic blood pressure <100 mmHg)

12. Cirrhosis of the liver (Child > Grade A), pronounced alcohol abuse with anticipated detoxification, severe pulmonary infection with considerable reduction of pulmonary function

13. Prior allogeneic bone marrow transplantation

14. Prior treatment with anti-PD-1, anti-PD-L1, or anti-PD-L2 therapeutic antibody

15. Administration of a live, attenuated vaccine within four weeks prior to start of maintenance treatment or anticipation that such a live attenuated vaccine will be required during the remainder of the study

    Note: Seasonal influenza vaccines for injection are generally inactivated flu vaccines and are allowed; however intranasal influenza vaccines (e.g., Flu-Mist®) are live attenuated vaccines, and are not allowed.

16. Chronic intake of drugs that lead to known interference with Maraviroc metabolism through strong Cytochrome P450 3A4 (CYP3A4) interaction: e.g. Rifampicin, Rifabutin, Clarithromycin, Telithromycin, Ketoconazole, Itraconazole, Fluconazole, Hypericum perforatum (St. John's Worth /Johanniskraut) or any strong CYP3A4 inducing or inhibiting drug (See Section 5.5.2)

17. Positive test for human immunodeficiency virus (HIV) or HIV infection

18. Active hepatitis B (defined as having a positive hepatitis B surface antigen [HBsAg] test) or hepatitis C. Note: Patients with past hepatitis B virus (HBV) infection or resolved HBV infection (defined as having a negative HBsAg test and a positive antibody to hepatitis B core antigen antibody test) are eligible

19. Known active or latent tuberculosis

20. Known clinically active brain metastases, defined as untreated symptomatic, or requiring therapy with steroids or anticonvulsants to control associated symptoms.

    Subjects with treated brain metastases that are no longer symptomatic and require no treatment with steroids may be included in the study if they have recovered from the acute toxic effect of radiotherapy and have no evidence of disease progression on imaging studies (MRI/CT scan).

21. On-treatment participation in another clinical study in the period 30 days prior to start of study treatment and during the study

22. Patients in a closed institution according to an authority or court decision (AMG § 40, Abs. 1 No. 4)

23. Pregnancy or lactation

24. Known history of, or any evidence of active, non-infectious pneumonitis or interstitial lung disease.

25. Active infection requiring systemic therapy.

5.2 Trial Treatments

The treatment to be used in this trial is outlined below in Table 1

Table 1 Trial Treatment

Drug	Dose/Potency	Dose Frequency	Route of Administration	Regimen	Use
Pembrolizumab (MK-3475)	200 mg	D1, Q3W (d1, qd22)	i.v. infusion	Day 1 of each 3 week cycle	experimental
Maraviroc	300 mg	b.i.d. every day	Perorally	Continuous treatment, starting on d1	experimental

6.0 TRIAL FLOW CHART

6.1 Study Flow Chart

Trial Period:	Screening Phase	Treatment Cycles[a]								End of Treatment	Post-Treatment		
Treatment Cycle/Title:	Screening (Visit 1)	1	2	3	4	To be repeated for additional cycles* 5	6	7	8	Discon	Safety Follow-up	Follow Up Visits[b]	Survival Follow-Up[a]
		←---------- Core treatment period (cycles 1 – 8) ----------→								At time of Discon	30 days post discon	Every 12 weeks post discon	Every 12 weeks
Scheduling Window (Days)[b]:	-28 to -1		± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 7	± 7	± 7
Administrative Procedures													
Informed Consent	X												
Inclusion/Exclusion Criteria	X												
Demographics and Medical History	X												
Prior and Concomitant Medication Review	X	X	X	X	X	X	X	X	X	X	X		
Clinical Procedures/Assessments													
Review Adverse Events	X	X*	X	X	X	X	X	X	X	X	X		
Full Physical Examination	X									X			
Directed Physical Examination		X	X	X	X	X	X	X	X				
Height, Weight, and Vital Signs (T,P,RR,BP)[c]	X	X	X	X	X	X	X	X	X	X			
12-Lead Electrocardiogram	X	X[q]	X[q]	X[q]	X[q]	X[q]	X[q]	X[q]	X[q]	X[q]			
ECOG Performance Status[d]	X	X	X	X	X	X	X	X	X	X			
MSS Testing[n]	X												
Maraviroc Administration		continuously[o]											
Pembrolizumab Administration		X	X	X	X	X	X	X	X				
Post-study anticancer therapy status											X	X	X
Survival Status													X

Trial Period:	Screening Phase	Treatment Cycles[a]								End of Treatment	Post-Treatment		
Treatment Cycle/Title:	Screening (Visit 1)	1	2	3	4	To be repeated for additional cycles* 5	6	7	8	Discon	Safety Follow-up	Follow Up Visits[b]	Survival Follow-Up[a]
		←---------- Core treatment period (cycles 1 – 8) ----------→								At time of Discon	30 days post discon	Every 12 weeks post discon	Every 12 weeks
Scheduling Window (Days)[b]:	-28 to -1		± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 7	± 7	± 7
HIV status[p]	X												
Hepatitis B/C status[p]	X												
Laboratory Procedures/Assessments: analysis performed by LOCAL laboratory													
Pregnancy Test – Urine or Serum β-HCG[e]	X	X	X	X	X	X	X	X	X				
PT/INR and aPTT	X												
CBC with Differential[f,d]	X	(X[d])	X	X	X	X	X	X	X	X	X		
Comprehensive Serum Chemistry Panel[f,d]	X	(X[d])	X	X	X	X	X	X	X	X	X		
Urinalysis[d]	X	(X[d])	X		X		X		X		X		
T3, FT4 and TSH[f]	X		X		X		X		X	X	X		
Serum Tumor Markers[g]	X	(X[d])	X	X	X	X	X	X	X	X			
Efficacy Measurements													
Tumor Imaging	X[h]	←---------- X[i] ----------→								X[j]		X[k]	
Tumor Biopsies/Archival Tissue Collection/Correlative Studies Blood													
Biopsy Collection	X[l]		X[l]										
Biomarker Studies Blood Collection	X[m]	X	X	X	X	X	X	X	X	X			

*** Maintenance phase: up to 24 additional cycles of pembrolizumab monotherapy after core treatment period – maraviroc administration will be discontinued**

a. For subjects that experience PD or start a new anti-cancer therapy contacts should be made by telephone Q12W to assess for survival status.

b. The window for each visit is ± 3 days unless otherwise noted.

c. Height will be measured at Visit 1 only.

d. ECOG Performance Status and Laboratory tests for screening are to be performed within 10 days prior to the first dose of trial treatment. Laboratory tests should be repeated in Cycle 1 if older than 10 days.

Trial Period:	Screening Phase	Treatment Cycles[a]								End of Treatment	Post-Treatment		
Treatment Cycle/Title:	Screening (Visit 1)	1	2	3	4	To be repeated for additional cycles* 5	6	7	8	Discon	Safety Follow-up	Follow Up Visits[b]	Survival Follow-Up[a]
		←---------- Core treatment period ----------→ (cycles 1 – 8)								At time of Discon	30 days post discon	Every 12 weeks post discon	Every 12 weeks
Scheduling Window (Days)[b]:	-28 to -1		± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 7	± 7	± 7

e. For women of reproductive potential, a serum or urine pregnancy test should be performed during screening and within 72 hours prior to each application of pembrolizumab.

f. After Cycle 1, lab samples can be collected up to 72 hours prior to the scheduled time point. Thyroid function tests to be performed every other cycle. CBC and Chemistry to be performed every cycle.

g. Serum tumor markers (CEA, CA19-9) should be collected at screening (baseline) and every Cycle until treatment discontinuation.

h. Screening tumor imaging will be performed within 28 days (+/- 3 days) prior to patient enrollment.

i. The first on-study imaging time point will be performed at 9 weeks (63 days ± 7 days) calculated from the date of first study treatment administration and will continue to be performed Q9W (63 days ± 7 days), or earlier if clinically indicated.

j. In subjects who discontinue study therapy without confirmed PD per irRECIST, tumor imaging should be performed at the time of treatment discontinuation (± 4 weeks). If previous tumor imaging was obtained within 4 weeks prior to the date of discontinuation, then additional tumor imaging at treatment discontinuation is not required.

k. In subjects who discontinue study therapy without confirmed PD per irRECIST, tumor imaging should be performed every 12 weeks ± 7 days in the follow-up period until the start of a further tumorspecific treatment.

l. For each patient, repeated biopsies of the same metastatic lesion will be taken. Patients without accessible lesions might be enrolled after discussion with the principle investigator. The first biopsy will be taken before start of therapy. A subsequent biopsy will be taken at the beginning of cycle 2 (week 4 +/-14 days) if considered feasible and safe for the patient.

m. Whole blood samples are taken before start of therapy and before every subsequent administration of pembrolizumab. Further information on the types and volumes of blood samples collected will be available in the Biomarker Research Manual.

n. A tissue sample for MSS testing should be collected per local assay requirements.

o. NOTE: In core treatment period only – maraviroc administration will not be continued in additional cycles (pembrolizomab monotherapy maintenance)

p. If HIV and hepatitis status are uncertain, undefined or unobtainable from medical records HIV and hepatitis B and C serologies must be performed.

q if clinically indicated

In addition to the assessments performed during visits at the day of study treatment administration (d1 of each cycle), safety and toxicity will be assessed on day 8 of the first cycle. Safety and toxicity assessments will be performed via structured patient interviews, directed physical examination and the laboratory peripheral blood and urine tests defined above.

7.0 STATISTICAL ANALYSIS PLAN

7.1 Statistical Analysis Plan Summary

A statistical analysis plan (SAP) will be drafted to provide details of the methods of analysis to address all study objectives. The SAP may be amended during the course of the study, but will be finalized before the cut-off date for any analysis. Due to the explorative nature of this trial and the small number of patients only descriptive statistics will be performed (e.g. describing the distribution of the baseline demographic data with predefined subgroups).

7.2 Justification of sample size

The main objective of this phase I study is to assess, whether the experimental regimen at the chosen dosage shows a promising feasibility profile in the treatment of refractory MSS mCRC. The feasibility rate, i.e. the rate of patients receiving the protocol treatment according to the planned schedule during the core treatment phase (i.e. the first 8 treatment cycles) without occurrence of at least one of the following events, is chosen as primary endpoint:

- Study treatment-related Grade ≥ 3 immune-related abnormalities
- Study treatment-related Grade ≥ 4 AEs of any aetiology
- Any toxic event leading to the premature withdrawal of protocol treatment

Conventional empirical phase I study designs in clinical oncology assume, that an antineoplastic treatment is not feasible, if an unacceptable toxicity occurs in more than 1 out of 3 or 4 patients; however, the occurrence of dose limiting toxicities (DLT) in 1/6 is accepted [11, 12, 13, 14]. This leads to the conclusion that the limit of acceptance is considered to be around 20%.

A one-stage design for pilot studies according to Fleming [15] will be applied. In summary, the trial design is based on the following assumptions:

- The experimental therapy would be rated as unacceptable, if the actual feasibility rate (= 1 – severe toxicity/withdrawal rate) was only 70 % or lower.
- On the other hand, the therapy regimen would be considered to be a promising candidate for further development, if the true feasibility rate amounted to 90% or more.
- Probability to accept the experimental therapy as well tolerable, in spite of a true feasibility rate of < 70% (i.e. severe toxicity/withdrawal rate > 30%): 10% (type I error)
- Probability to reject the experimental therapy as not sufficiently feasible (<70%), although the true feasibility rate is promising (> 90%): 20% (type II error, corresponding to a power of 80%).

According to these parameters n = 18 patients evaluable for feasibility have to be recruited into the trial. In order to allow for some non-informative drop-outs, a total number of 20 patients should be recruited. The final conclusion of the trial will depend on the definite feasibility rate (and its confidence interval), the secondary efficacy endpoints as well as the complete information on type, frequency and severity of toxicities.

The precision of the estimation of the feasibility rate is provided by confidence intervals in the following table, for different actual feasibility rate findings:

Feasibility rate	exact 90% confidence interval
14/20 (70%)	49 ... 86 %
16/20 (80%)	60 ... 93 %
18/20 (90%)	72 ... 98 %