The CARACAS STUDY

Randomized phase 2 trial of cetuximab and avelumab or avelumab alone for unresectable, locally advanced or metastatic squamous cell anal carcinoma (SCCAC) progressed after at least one line of systemic treatment

EUDRACT 2018-000737-12

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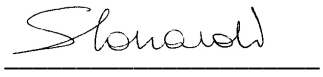
Protocol Signature Page (Sponsor)

I have read and understood the contents of the clinical protocol for Clinical Study CARACAS v 2.4 dated 23rd March 2018 and agree to meet all obligations of Fondazione GONO as detailed in all applicable regulations and guidelines. In addition, I will ensure that the investigators are informed of all relevant informations becoming available during the conduction of this study.

Coordinator's signature

Dr. Sara Lonardi



Date

16th April 2018

Lonardi S, *et al. J Immunother Cancer* 2021; 9:e002996. doi: 10.1136/jitc-2021-002996

Protocol Acceptance Form

TITLE	Randomized phase 2 trial of cetuximab and avelumab or avelumab alone for unresectable, locally advanced or metastatic squamous cell anal carcinoma (SCCAC) progressed after at least one line of systemic treatment
PROTOCOL NUMBER:	**CARACAS**
VERSION NUMBER	2.4
EUDRACT NUMBER	2018-000737-12
IND NUMBER	NA
TEST PRODUCTS	avelumab, cetuximab
MEDICAL MONITOR	Dr. Sara Lonardi
SPONSOR	Fondazione GONO
DATE FINAL	23rd March 2018

I confirm that l have read and understood the clinical trial protocol and will undertake my work according to the provisions stipulated in the protocol and in ethical principles found in the latest version of the Declaration of Helsinki, International Conference on Harmonisation (ICH) good clinical practice (GCP) guidelines and applicable legal requirements

l agree to conduct the study in accordance with the current protocol

_____
Principal Investigator's name (print)

_____ _____
Principal Investigator's signature Date

Participating Centres

About 25 Italian Oncology Units will participate to the trial.

Table of Contents

1. List of Abbreviations and Acronyms

5-FU	5-fluorouracil
ADCC	Antibody dependent cytotoxicity
ADR	Adverse Drug Reaction
APTT	Activated partial Thromboplastin Time
CNS	Central Nervous System
CT	Computed Tomography
EC	Ethics Committee
eCRF	Electronic case record forms
ECOG PS	Eastern Cooperative Oncology Group – Performance Status
EGF	Epithelial growth factor
EGFR	Epithelial growth factor receptor
GCP	Good Clinical Practice
GONO	Gruppo Oncologico Nord-Ovest
MRI	Magnetic Resonance Imaging
HNSCC	Head and Neck Squamous Cell Carcinoma
HPV	Human papilloma virus
INR	International normalized ratio
ITT	Intention to treat
IV	Intravenous
NCI-CTCAE	Cancer Institute-Common Terminology Criteria for Adverse Event
NSAIDs	Nonsteroidal anti-inflammatory drugs
ORR	Objective response rate
OS	Overall survival
PD	Progression of disease
PD-1	Programmed death protein 1
PD-L1	Programmed death ligand 1
PFS	Progression free survival
QoL	Quality of life
RECIST	Response Evaluation Criteria In Solid Tumors

SCCAC	Squamous cell anal carcinoma
SMC	Safety Monitoring Committee
SP	Safety population
T_{reg}	Regulatory T cells
UNL	Upper-normal limits

2. Introduction

2.1. Squamous cell anal carcinoma epidemiology and aetiology

Squamous cell anal carcinoma (SCCAC) is a rare malignancy that accounts for around 2% of all gastrointestinal tumours (Siegel 2016). Around 27,000 new cases of SCCAC are diagnosed per year worldwide (Van K Morris, Salem 2017). For the majority of SCCAC, etiology is related to viral infection thus its incidence has increased with the increase of viral infections (Casadei Gardini 2014). A high percentage (around 88%) of patients affected with SCCAC are positive to human papilloma virus (HPV) infection (Prigge 2017, Glynne-Jones 2016). The most common HPV-induced genetic modifications are mutation of the *PIK3CA* gene and increased gene methylation (Prigge 2017). Moreover, HPV-associated tumours are associated with induced immunological alterations such as immunosuppression through programmed death ligand 1 (PD-L1) overexpression (Prigge 2014). Another viral infection related to SCCAC aetiology is human immunodeficiency virus (HIV) infection (Glynne-Jones 2016).

Around 25% of the patients diagnosed with locoregional disease and treated with chemo-radiation relapse or develop distant metastases. The median overall survival for metastatic SCCAC is around 15 to 20 months (Van K Morris, Rao 2017). Historically, the conduction of clinical trials in metastatic SCCAC has been a tough challenge due to its relatively low incidence and to low expectations with traditional approaches.

2.2. Standard treatments for Squamous cell anal carcinoma

The standard first-line treatment for localised SCCAC is a combination of chemotherapy with 5-fluorouracil (5-FU) and mitomycin and radiotherapy resulting in a 5 year disease-free survival of 60 % (Ott 2017). After progression or development of distant metastases, the standard treatment is chemotherapy with 5-FU in association with cisplatin resulting in a 5-years disease-free survival of about 32 % (Faivre 1999). In a recent case series of patients affected by SCCAC, 34.4% objective response rate (ORR) was demonstrated with 5-FU and cisplatin as first-line treatment, while the 5-years disease-free survival rate was 15 % in the overall population (Sclafani 2017).

At present, no standard second-line treatment exists for advanced SCCAC. Slightly improved results in ORR have been reported in some small case series of patients treated with paclitaxel, alone or in combination with carboplatin (Abbas 2011, Kim 2014). Thus, there is an unmet need to identify more effective therapeutic strategies for the management of this malignancy.

2.3. Immune checkpoint inhibitors

In the last years, a number of biologics have been developed for the treatment of various solid tumours with promising results (Procaccio 2017). Recent insights on tumour pathogenesis and molecular landscape allowed investigation of new classes of drugs able to interfere with several mechanisms responsible of tumour development and progression.

Amongst the hallmarks of cancer is the acquired capability of avoiding immune destruction (Hannan 2011). T-cell mediated responses rely on a fine balance between inhibitory and co-stimulatory signals, named immune checkpoints. Cancer cells have the ability to disregulate the expression of such molecules thus generating a cellular environment in which cancer cells proliferation is sustained and immuno-tolerance is induced (Pardoll 2012). Immune checkpoints inhibitors constitute a promising therapeutic option for advanced solid tumours. Those molecules have been designed in order to enhance the endogenous anticancer activity of lymphocytes rather than directly target tumour cells (Pardoll 2012). The cytotoxic T-lymphocyte-associated antigen 4 and the Programmed death protein 1 (PD-1) have been extensively studied for the development of these new biologics. The latter, PD-1, is expressed on regulatory T cells (T_{reg}) upon T cells activation to limit inflammatory responses and avoid autoimmunity (Pardoll 2012). Additionally, PD-1 is expressed on B cells and natural killer cells, thus its blockade might directly enhance B cells mediated antibodies production (Pardoll 2012). The receptor binds two ligands named PD-1 Ligand 1 (PD-L1) and PD-1 Ligand 2 (PD-L2), whose expression in physiologic conditions is induced in the tissue by inflammatory signals to limit tissue damaging. PD-1 ligands are commonly upregulated on the surface of various tumors to inhibit anti-tumour responses (Jomrich 2016). Tumour induced immuno-tolerance through PD-1 ligand overexpression relies on two mechanisms. The first, named innate immune resistance, is the expression of PD-L1 due to constitutive oncogenic signalling pathways similarly to what has been described for HPV and SCCAC (Pardoll 2012, Prigge 2014). According to the second, named adaptive immune resistance,

PD-L1 expression is induced in response to interferon production by tumour-specific lymphocytes during inflammation.

Anti-PD1 and anti- PDL1 treatments are under investigation in SCCAC.

Amongst the anti PD-1 antibodies recently developed are nivolumab and pembrolizumab. The first completed phase II trial with nivolumab in metastatic, refractory SCCAC has been recently published. In this trial, nivolumab in monotherapy proved to be effective in terms of response rate, showing an acceptable toxicity profile (Morris 2017). This study has disclosed new opportunities in the treatment of SCCAC, suggesting that immunotherapy can represent a promising approach for the cure of this disease. Moreover, this was the first trial in which an immune checkpoint inhibitor has been proven to be active in an immuno-compromised population such as HIV-positive patients (Morris 2017). Interestingly, treatment response correlation with tumour $CD3^+$ and $CD8^+$ T cells infiltration was observed (Morris 2017). Pembrolizumab was studied in patients affected by anal cancer in the KEYNOTE-028 trial. Patients were eligible only if they had PD-L1 expressing tumor. Pembrolizumab showed activity in terms of disease control and a manageable toxicity profile (Ott 2017).

A recently developed PD-L1 antibody is avelumab. Avelumab has been approved for the treatment of metastatic Merkel cell carcinoma and recently investigated as second line treatment for metastatic or recurrent non small-cell lung cancer (JAVELIN Solid Tumor trial). In this trial, the drug showed acceptable safety profile and activity (Gulley 2017). Many trials are currently ongoing with avelumab alone or in combination for the treatment of different solid tumours. In the AVETUX-CRC trial avelumab is administered in combination with FOLFOX and cetuximab in patients with previously untreated metastatic colorectal cancer (https://clinicaltrials.gov). There are no data yet available and no trials currently ongoing for patients affected by SCCAC.

2.4. Anti-EGFRs for the treatment of advanced SCCAC

Another hallmark of cancer is uncontrolled cell division and invasion (Hannan 2000, Hannan 2011). The epidermal growth factor (EGF) pathway is one of the many signalling pathways altered during neoplastic transformation (Seshacharyulu 2012). In physiological conditions, EGF binding to its receptor (i.e. epidermal growth factor receptor (EGFR)) activates a complex downstream signalling cascade resulting in cell proliferation. Aberrant activation of EFGR is a feature typical of many

tumours (Seshacharyulu 2012). Cetuximab, is an anti-EGFR monoclonal antibody. Cetuximab binds to the EGFR with greater affinity than its endogenous ligand, thus blocking its activation and promoting receptor internalisation (Garg 2017).

The efficacy of cetuximab in metastatic head and neck squamous tumours (HNSCC) is well established. In this setting, cetuximab remarkably improved progression free survival (PFS) and overall survival (OS) when administered in association with cisplatin (Vermorken 2008). EGFR is broadly expressed in SCCAC cells. Moreover, the HPV-Associated E5 protein was shown to be involved in amplification of its mitogenic signalling (Garg 2017). SCCAC rarely harbour RAS mutations, which are an established determinant of resistance in to anti-EGFRs antibodies in colorectal cancer: in a case series of 50 patients, none of them was KRAS or NRAS mutated (Casadei Gardini 2014, Capelli 2016); in another report of 84 patients, only 4 (5%) resulted KRAS mutated (Martin 2014). This observation, together with the need for drugs that could improve outcomes in both first and subsequent lines, led to test the efficacy of cetuximab in patients affected by SCCAC, with promising results in association with irinotecan in pretreated patients (Lukan 2009). In addition, cetuximab has been recently tested in stage I-III SCCAC in association with cisplatin plus 5-FU and radiotherapy, both in HIV negative and positive patients: the regimens tested showed interesting activity in local control of disease, even if neither of these studies reached their pre-specified endpoints (Garg 2017, Sparano 2017).

2.5 AntiEGFRs and Immune Checkpoints inhibitors combination

Some *in vitro* and *in vivo* studies demonstrated that cetuximab exerts antibody dependent citotoxicity (ADCC) on tumor cells. This effect is mediated by natural killer (NK) cells. PD-1 expression on NK cells is not clearly defined, but has been associated in some models with a special constitutive hyperactivated phenotype of NK cells that turns into inactivity only after ligation by programmed death ligand-1 (PD-L1). The direct consequence of this hypothesis would be an inactivation of NK cells in presence of tumor cells expressing PD-L1, thus decreasing the ADCC induced by cetuximab. Interestingly, a correlation between PD-1 expression and other markers of activation of NK cells was observed in 500 HNSCC patients' tumors (Choncha-Beenavente 2015). Additionally, there is a correlation between PD-L1 expression on HNSCC cells and EGFR stimulation, and between the former and interferon γ (IFNγ) secreted by activated NK

cells and lymphocytes T. These findings open interesting scenarios particularly in the perspective of association therapies of anti EGFR and anti PD1 (Choncha-Benavente 2016).

3. Study Rationale

- The standard first line treatment in SCCAC is the association of 5-FU with cisplatin reaching a percentage of survival at 5 years of about 32% (Faivre 1999); in a recent case series of patients affected by SCCAC, the combination of 5-FU and cisplatin as first line treatment produced 34.4% objective response rate (ORR) and a 5 years survival rate of 15% (Sclafani 2017);
- No standard second line treatment exists for SCCAC;
- Cetuximab in association with irinotecan has demonstrated promising results in pretreated patients affected by SCCAC (Lukan 2009). In addition, it was recently tested in stage I-III SCCAC in association with cisplatin plus 5-FU and radiotherapy. Despite not reaching their pre-specified endpoints both studies reported an interesting activity in local control of disease, leading to hypothesize that cetuximab warrant further investigation in new strategies (Garg 2017, Sparano 2017);
- Anti-PD1 treatments such as nivolumab and pembrolizumab showed promising activity in metastatic refractory SCCAC in terms of response rate and disease control with acceptable toxicity profiles (Morris 2017, Ott 2017);
- The induction of immunogenic cell death was recently shown for cetuximab-based regimens (Pozzi 2016) and PD-L1 blockade should lead to NK cells activation enhancing cetuximab ADCC (Concha-Benavente 2015, Concha-Benavente 2016).

On the basis of these considerations, we designed the present randomized phase II trial of avelumab alone or avelumab plus cetuximab for previously treated unresectable locally advanced or metastatic SCCAC.

4. Study Design

The CARACAS study is a randomized prospective phase II for patients affected by unresectable locally advanced or metastatic SCCAC progressed after standard treatments.

Patients will be randomised to receive:

- ARM A: avelumab 10 mg/kg ev day 1
- ARM B: avelumab 10 mg/kg ev plus cetuximab 500 mg/m^2 day 1

In each arm the treatment will be repeated every 14 days, until progression of disease (PD), refuse or unacceptable toxicity.

4.1. Safety run-in phase

The enrollment of new patients will be temporary interrupted after the first 6 patients will be randomly assigned to arm B. When 6 patients enrolled in arm B receive 2 cycles of study treatment, a Safety Monitoring Committee (SMC) will complete a safety evaluation.
The enrollment will then resume only if the study treatment combination is judged feasible and no major safety concerns arise.
The study design is displayed below.

Figure 1: Study Scheme

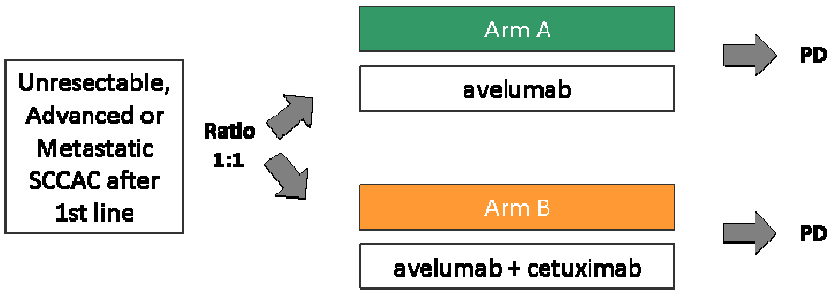
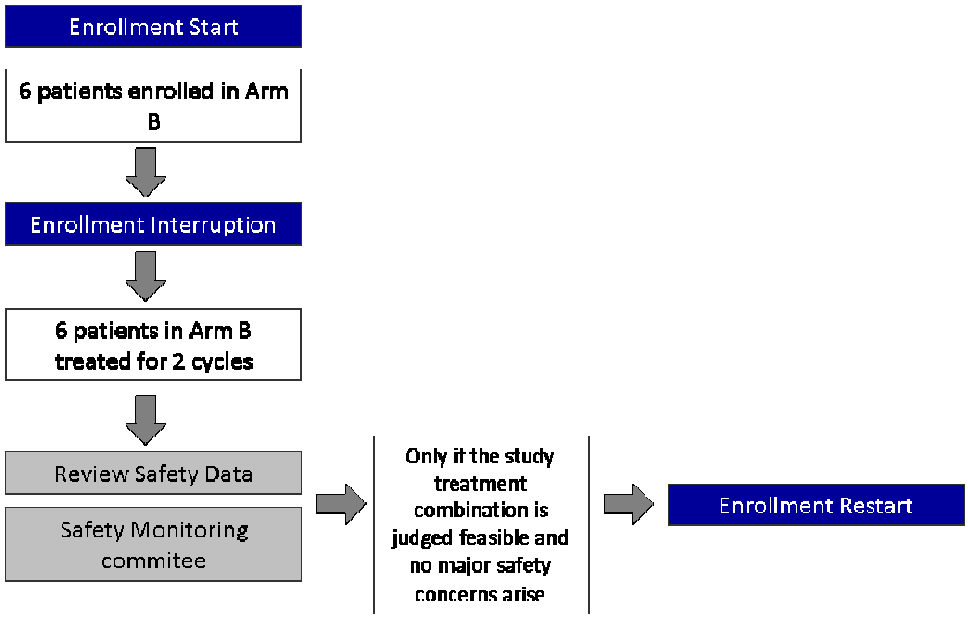


Figure 2: Safety run-in Phase Scheme



4.2 Treatment beyond progression

Accumulating evidence indicates a minority of subjects treated with immunotherapy may derive clinical benefit despite initial evidence of PD.

Subjects will be permitted to continue treatment beyond initial RECIST 1.1 defined, investigator-assessed, PD as long as they meet the following criteria:

1) Investigator-assessed clinical benefit, and do not have rapid disease progression

2) Tolerance of study drug

3) Stable performance status

4) Treatment beyond progression will not delay an imminent intervention to prevent serious complications of disease progression (eg, CNS metastases)

5) Subject provides written informed consent prior to receiving any additional avelumab treatment, or avelumab in combination with cetuximab treatment, using an ICF describing any reasonably foreseeable risks or discomforts, or other alternative treatment options.

The assessment of clinical benefit should take into account whether the subject is clinically deteriorating and unlikely to receive further benefit from continued treatment.

All decisions to continue treatment beyond initial progression must be discussed with the PI of the Study and documented in the study records. Subjects will be re-consented with an ICF describing any reasonably foreseeable risks or discomforts.

Subjects should discontinue study therapy upon further evidence of further progression, defined as an additional 10% or greater increase in tumor burden volume from time of initial progression (including all target lesions and new measurable lesions).

New lesions are considered measurable at the time of initial progression if the longest diameter is at least 10 mm (except for pathological lymph nodes, which must have a short axis of at least 15 mm). Any new lesion considered non-measurable at the time of initial progression may become measurable and therefore included in the tumor burden measurement if the longest diameter increases to at least 10 mm (except for pathological lymph nodes, which must have an increase in short axis to at least 15 mm).

For statistical analyses that include the investigator-assessed progression date, subjects who continue treatment beyond initial investigator-assessed, RECIST 1.1-defined progression will be considered to have investigator-assessed progressive disease at the time of the initial progression event.

5. Safety Monitoring Committee

A SMC, including three academic experts not directly involved in the trial conduction, will early evaluate the safety of the study treatment.

Unblinded safety data will be firstly reviewed by the SMC when the first 6 patients in arm B will receive at least 2 cycles of the study treatment. The SMC 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.

In addition, on a periodic basis, approximately every 3 months from the date of the first-patient-in, the SMC will review safety data, including demographics, adverse events, serious adverse events, adverse events of special interest and relevant laboratory data.

6. Statistical design

6.1. Primary Objective

To evaluate the activity of avelumab alone or in combination with cetuximab in patients with advanced SCCAC.

6.2. Primary Endpoint

The primary endpoint is Objective Response Rate (ORR). ORR is defined as the percentage of patients, relative to the total of enrolled subjects, achieving a complete (CR) or partial (PR) response, according to Response Evaluation Criteria In Solid Tumors (RECIST) v1.1 criteria. The determination of the radiological response will be based on the investigator's reported evaluation. Radiological responses will be evaluated every 8 weeks starting from cycle 1 day 1 of treatment until disease progression, withdrawal of consent or death for any reason, whichever occurs first.

6.3. Secondary Endpoints

- To estimate Progression-Free Survival (PFS),
- To estimate Overall survival (OS);
- To describe the safety profile of the monotherapy and of the combination;
- To collect archival tumor specimens, blood samples, newly obtained tumor specimens (optional) for translational analyses

6.4. Secondary Endpoints Definition

PFS is defined as the time from study enrollment to the first documentation of objective disease progression or death due to any cause, whichever occurs first. Documentation of progressive disease is defined as per RECIST 1.1 criteria, based on investigator's assessment. PFS will be censored at the time of the last evaluable tumour assessment documenting absence of

progressive disease for patients who are alive and progression free at the time of the analysis. Alive patients having no tumour assessments after baseline will have time to event censored on the date of study enrollment.

- OS is defined as the time from study enrollment to the date of death due to any cause. For patients still alive at the time of analysis, the OS time will be censored on the last date the patients were known to be alive.
- Overall Toxicity Rate is defined as the percentage of patients, out of those receiving at least one dose of treatment, experiencing any grade 3-4 adverse event, according to NCI CTCAE v4.03. Toxicity Rate is defined as the percentage of patients experiencing a specific adverse event of grade 3-4, according to NCI CTCAE v 4.03.

6.5. Study populations for primary and secondary analyses

Intention to treat population (ITT) The ITT population will include all randomized patients. The ITT population will be the population for evaluating all primary and secondary endpoints, with the exception of toxicity rate and overall toxicity rate.

Safety population (SP) The SP will include all patients who receive at least one dose of the study medication designated according to the randomization arm. The SP will be the population for evaluating treatment administration/compliance and safety.

6.6. Analyses of endpoints

Analysis of the primary endpoint

The primary analysis of ORR will be performed in the ITT population and calculated as the number of patients with a best response of CR or PR divided by the total number of enrolled patients. The corresponding exact 2-sided 95% confidence interval will be calculated using a method based on the binomial distribution. No formal statistical comparisons will be made between the arms.

Analyses of secondary endpoints

All analyses will be exploratory only and no formal statistical comparisons will be made between the arms.

Survival curves (PFS and OS) will be calculated on the ITT population according to Kaplan–Meier methods. The median event times and corresponding 2-sided 95% CI for the median will be provided.

Toxicity rates and overall toxicity rate will be calculated as the number of patients experiencing a specific adverse event of grade 3/4 or any adverse event of grade 3/4 divided by the total number of the patients included in the safety population The corresponding exact 2-sided 95% confidence interval will be calculated using a method based on the binomial distribution.

6.7. Statistical Considerations and sample size

A Simon's two-stage Mini-Max design (Simon, 1989) will be used for both arms in parallel.

The null hypothesis that the true response rate is 5 % [p0 = 0.05] will be tested against the one-sided alternative of a true response rate of 20% [p1 = 0.20]. Type I error rate is set at 0.05 and power at 80%.

For both arms, in the first stage, 13 patients will be accrued. If there will be no responses in these 13 patients, the study will be stopped. Otherwise, 14 additional patients will be accrued for a total of 27 in each arm. The null hypothesis will be rejected if 4 or more responses will be observed in 27 patients. No formal comparison between the results of the two treatment arms will be allowed.

7. Selection of Patients

7.1. Inclusion Criteria

- Histologically proven diagnosis of SCCAC;
- Progression on or after first line systemic therapy for surgically unresectable or metastatic disease. Systemic radiosensitizing chemotherapy with curative intent in limited-stage disease should be considered equal to a first line for a patient experiencing progression during or within 6 months of completion;
- Evaluable disease lesion according to RECIST v1.1 criteria;
- Availability of tumor sample (primary and/or metastatic sites);
- Age ≥ 18 years;
- Eastern Cooperative Oncology Group – Performance Status (ECOG PS) ≤ 2;
- Life expectancy of at least 12 weeks;
- Laboratory Requirements:
    - Neutrophils ≥ 1.5 x 10^9/L;
    - Platelets ≥ 100 x 10^9/L;
    - Hemoglobin ≥ 9 g/dL;
    - Total bilirubin ≤ 1.5 time the upper-normal limits (UNL) of the normal values and ASAT (SGOT) and/or ALAT (SGPT) ≤ 2.5 x UNL (or <5 x UNL in case of liver metastases);
    - Alkaline phosphatase ≤ 2.5 x UNL (or <5 x UNL in case of liver metastases);
    - Creatinine clearance ≥ 30 mL/min according to the Cockcroft-Gault formula (or local institutional standard method) or serum creatinine ≤1.5 x UNL;
- HIV-positive patients are eligible if their $CD4^+$ cell count amounts to 300 cells per µL or more; HIV viral load has to be undetectable, and they have to be compliant with antiretroviral treatment;
- Negative serum or urine pregnancy test at screening for women of childbearing potential. Female subjects, or male subjects with female partners of child-bearing potential must be willing to use highly effective contraception as approved by the investigator (i.e. barrier contraceptive measure or oral contraception, total abstinence) during the study and until 30 days after last study treatment;

- Written informed consent to the study procedures and to molecular analyses before patients registration;
- Will and ability to comply with the protocol.

7.2. Exclusion Criteria

- Previous therapy with any drug targeting T-cell co-regulatory proteins (i.e., immune checkpoint inhibitors);
- Concurrent anticancer treatment or use of any investigational drug within 28 days before the start of the trial treatment;
- Major surgical procedure, open biopsy, or significant traumatic injury occurred within 28 days prior to study treatment start, or anticipation of the need for major surgical procedure during the course of the study;
- History or evidence upon physical examination of CNS disease unless adequately treated. Patients with treated brain metastases are eligible if their lesions were stable and asymptomatic for at least 3 months;
- Neutrophils < 1.5 x 10^9/L; platelets < 100 x 10^9/L; hemoglobin < 9 g/dL;
- Active uncontrolled infections requiring systemic therapy or other clinically relevant concomitant illness contraindicating therapy administration or putting the patient at high risk for treatment-related toxicities;
- Hepatitis B virus (HBV) or hepatitis C virus (HCV) infection at screening (positive HBV surface antigen or HCV RNA if anti-HCV antibody screening test positive)
- Patients with active autoimmune disease or history of autoimmune disease that might deteriorate when receiving an immuno-stimulatory agent or might potentially affect vital organ function, or require use of immunosuppressive treatment including chronic prolonged systemic corticosteroids (defined as corticosteroid use for ≥ 1 month). Patients with diabetes type I, vitiligo, psoriasis, or hypo- or hyperthyroid diseases not requiring immunosuppressive treatment are eligible;
- Current use of immunosuppressive medication, EXCEPT for the following: a. intranasal, inhaled, topical steroids, or local steroid injection (e.g., intra-articular injection); b. Systemic

corticosteroids at physiologic doses ≤ 10 mg/day of prednisone or equivalent; c. Steroids as premedication for hypersensitivity reactions (e.g., CT scan premedication)

- Prior organ transplantation including allogenic stem-cell transplantation;
- Vaccination within 4 weeks of the first dose of avelumab and while on trials is prohibited except for administration of inactivated vaccines;
- Known severe hypersensitivity reactions to monoclonal antibodies, any history of anaphylaxis, or uncontrolled asthma;
- Clinically significant (i.e., active) cardiovascular disease: cerebral vascular accident/stroke (< 6 months prior to enrollment), myocardial infarction (< 6 months prior to enrollment), unstable angina, congestive heart failure (≥ New York Heart Association Classification Class II), or serious cardiac arrhythmia requiring medication;
- Other severe acute or chronic medical conditions including immune colitis, inflammatory bowel disease, immune pneumonia, pulmonary fibrosis or psychiatric conditions including recent (within the past year) or active suicidal ideation or behavior; or laboratory abnormalities that may increase the risk associated with study participation or study treatment administration or may interfere with the interpretation of study results and, in the judgment of the investigator, would make the patient inappropriate for entry into this study;
- Persisting toxicity related to prior therapy (NCI CTCAE v. 4.03 Grade > 1); however, alopecia, sensory neuropathy Grade ≤ 2, or other Grade ≤ 2 not constituting a safety risk based on investigator's judgment are acceptable;
- Other co-existing malignancies or malignancies diagnosed within the last 5 years with the exception of localized basal and squamous cell carcinoma of the skin or cervical cancer in situ;
- Pregnant or lactating women;
- Any psychological, familial, sociological or geographical condition potentially hampering compliance with the study protocol and follow-up schedule.

7.3. Discontinuation Criteria

A patient may be discontinued from the clinical trial at any time for any reason.

It is the right and the duty of the investigator to stop treatment in any case in which emerging effects are of unacceptable risk to the individual subject. In addition, patients have the right to voluntarily discontinue study treatment or withdraw from the study at any time for any reason. In instances where consent is withdrawn, the Investigator must clarify whether the patient is willing to continue to be followed (i.e. for survival).

Reasons for discontinuation of study treatment may include, but are not limited to, the following:

- Any medical condition that at the judgement of the Investigator or of the Sponsor may jeopardise patient's safety if he or she continues on study treatment;
- Major protocol violation (i.e. affecting the patients' safety);
- Investigator or Sponsor determines it is in the best interest of the patient;
- Patient's non-compliance to the protocol;
- Patient withdrawal of consent.

Reasons for withdrawal from the study may include, but are not limited to, the following:

- Patient withdrawal of consent;
- Patient lost to follow-up;
- Death.

7.4. Replacement of Subjects

A subject who discontinues from the trial will not be replaced.

Lonardi S, *et al. J Immunother Cancer* 2021; 9:e002996. doi: 10.1136/jitc-2021-002996

8. Enrollment and Study Timeline

A total of 54 patients (27 patients for each arm) will be accrued in the study.

The registration and randomization procedures will be centralized at Istituto Oncologico Veneto (IOV) IRCCS, Padova. Patients considered eligible and who have signed a written informed consent will be randomly assigned to one of the two treatment arms in a 1:1 ratio. The randomization will be performed by using an electronic WEB-based system. The randomization code will consist of a unique identification code. This code must be used on all further documentation and correspondence, including electronic case record forms (e-CRFs). e-CRFs fac-simile are provided as a separate addendum to this study protocol. It is responsibility of the principal investigator to ensure that each patient is eligible for the study before requesting randomization.

Study length is planned to be about 30 months, the enrollment is expected to be about 18 months, with a minimum period of follow-up of 12 months. The end of study is defined as the time when all randomized patients will have experienced evidence of disease progression or will be out of treatment as per protocol, toxicity or medical decision.

The planned study timeline is as follows:

- Submission date to health authority/ ethics: April 2018
- First Patient In: 1st September 2018
- Enrollment rate: 3 pts/months
- Last Patient In: 28th February 2020
- Last Patient Last Visit: 28th February 2021
- First data release on PFS: 30th April 2021
- Manuscript submission: 28th February 2022

Lonardi S, *et al. J Immunother Cancer* 2021; 9:e002996. doi: 10.1136/jitc-2021-002996

9. Study Treatment and procedures

9.1. Study Treatment

Eligible patients will be randomized to receive:

- **Arm A: avelumab 10 mg/kg iv day 1;**
  To be repeated every 2 weeks (14 days) until progression of disease, refuse or inacceptable toxicity.
- **Arm B**: cetuximab 500 mg/m2 plus avelumab 10 mg/kg iv day 1.
  To be repeated every 2 weeks (14 days) until progression of disease, refuse or inacceptable toxicity.

In order to mitigate infusion-related reactions, a premedication with an antihistamine and with paracetamol (acetaminophen) 30 to 60 minutes prior to the first 4 infusions of avelumab is mandatory (for example, 25-50 mg diphenhydramine and 500-650 mg paracetamol IV or oral). Premedication should be administered for subsequent avelumab infusions based upon clinical judgment and presence/severity of prior infusion reactions. This may be modified based on local treatment standards and guidelines, as appropriate.

Avelumab should be administered in a setting that allows for immediate access to an intensive care unit or equivalent environment and administration of therapy for anaphylaxis, such as the ability to implement immediate resuscitation measures. Steroids (dexamethasone 10 mg), epinephrine (1:1,000 dilution), allergy medications (IV antihistamines), bronchodilators, or equivalents, and oxygen should be available for immediate access.

Following avelumab infusions, patients must be observed for 30 minutes post-infusion for potential infusion-related reactions.

9.2. Study Procedures

Screening (within 28 days prior to first infusion)

- Obtained written informed consent;
- Verification of inclusion and exclusion criteria;
- Medical history and demographics;

- Physical examination (including height, weight, vital sign, blood pressure and heart rate);
- Concomitant medications;
- Adverse events evaluation to be started after signing of informed consent until 30 days after last study treatment. Grading by the common terminology criteria for adverse events (CTCAE) v 4.03;
- ECOG PS;
- ECG;
- Complete blood/urine examination: blood count and differential, bilirubin (total and direct), AST, ALT, alkaline phosphatase, albumin, LDH, serum creatinine, glucose, electrolytes (sodium, potassium, calcium, magnesium), TSH, FT3, FT4, International normalized ratio (INR)/Activated partial Thromboplastin Time (APTT), HIV serology, HPV serology, HBV/HCV serology, CEA, pregnancy test;
- Contrast-Enhanced chest and abdominal CT scan, or Abdomen MRI and Chest CT if contrast-enhanced CT scan is contraindicated. To be performed no more than 28 days before randomization;
- Collection of a copy of baseline CT scan (and/or abdomen MRI), digitally stored on CD-ROM if baseline radiologic assessment performed outside participating centres;
- Collection of a paraffin-embedded block of the primary tumour and/or metastases;
- Collection of blood and urine samples.

Before every cycle of treatment

- Physical examination (including height, weight, vital sign, blood pressure and heart rate);
- Concomitant medications;
- Collection of adverse events and treatment toxicity (grading by CTCAE version 4.0);
- ECOG PS;
- Complete blood examination: blood count and differential, bilirubin (total and direct), AST, ALT, alkaline phosphatase, albumin, LDH, serum creatinine, glucose, electrolytes (sodium, potassium, calcium, magnesium). International normalized ratio (INR)/Activated partial Thromboplastin Time (APTT) only if anticoaugulants ongoing.

Every 8 weeks

- Complete blood examination: blood count and differential, bilirubin (total and direct), AST, ALT, alkaline phosphatase, albumin, LDH, serum creatinine, glucose, electrolytes (sodium, potassium, calcium, magnesium), International normalized ratio (INR)/Activated partial Thromboplastin Time (APTT), TSH, fT3, fT4, CEA;
- Contrast-Enhanced chest and abdominal CT scan, or Abdomen MRI and Chest CT if contrast-enhanced CT scan is contraindicated;
- Collection of blood and urine samples.

At the end of the treatment and after evidence of PD (30 days after discontinuation ± 7days)

- Physical examination (including height, weight, vital sign, blood pressure and heart rate);
- Complete blood examination: blood count and differential, bilirubin (total and direct), AST, ALT, alkaline phosphatase, albumin, LDH, serum creatinine, glucose, electrolytes (sodium, potassium, calcium, magnesium), International normalized ratio (INR)/Activated partial Thromboplastin Time (APTT), CEA;
- Collection of adverse events and treatment toxicity (grading by CTCAE version 4.0);
- ECOG PS;
- Survival follow up.

9.3. Tabulated overview

Procedures	Screening (within 28 days before enrollment)	Cycle 1 day 1[1]	Cycle n day 1[1,2]	Every 8wks	EOT[a]
Study Drug Administration[b]		X	X		
Cycle of therapy		1	n		
Informed consent	X				
History/Demographics	X				
Physical examination [c]	X	X	X		X
Inclusion/Exclusion Criteria checked	X				
HIV and HPV serology	X				
Concomitant Medications	X	X	X		
Adverse Event evaluation	X	X	X[d]	X	X[e]
ECOG PS	X	X	X		X
12-lead ECG	X				
Complete blood examination [f]	X	X[g]	X	X	X
Radiologic evaluation[h]	X			X	
Urine Pregnancy test	X	X	X [i]		
Collection of a paraffin-embedded tissue sample	X				X [j]
Collection of blood and urine samples[k]	X			X	
Survival follow up					X

1. Blood examination can be performed up to 72 hours before the administration of study treatment.
2. Treatment cycles to be administered every 2 weeks (14 days), starting cycle 1 day 1 until disease progression or treatment discontinuation; treatment delays may be indicated as per protocol.

a) End-of-Treatment (EOT) visit should be done 30 days (± 7 days) after the last dose of study drugs administration

b) Avelumab or avelumab plus cetuximab.

c) Including: height, weight,vital signs, blood pressure and heart rate.

d) AE assessment to be started after signing of IC (Informed Consent) until 30 days after last study treatment. Grading by CTCAE v 4.03.

e) Given the potential risk for delayed immune-related toxicities, safety follow-up must be performed up to 90 days after the last dose of avelumab administration. The extended safety follow-up beyond 30 days after last avelumab administration may be performed either via a site visit or via a telephone call with subsequent site visit requested in case any concerns noted during the telephone call.

f) Blood count and differential, bilirubin (total and direct), AST, ALT, alkaline phosphatase, albumin, LDH, serum creatinine, glucose, electrolytes (sodium, potassium, calcium, magnesium), International normalized ratio (INR)/Activated partial Thromboplastin Time (APTT) (only for patients on anticoagulation therapy, otherwise can be done at screening, every 8 wks, at EOT), TSH, fT3, fT4 (thyroid hormones can be done at screening, every 8 wks, at EOT), CEA (it can be done at screening, every 8 wks, at EOT).

g) If screening complete blood examination has been performed within 72 hours from starting of study treatment cycle 1 day 1, partial blood examination on cycle 1 day 1 can be avoided.

h) Collection of a copy of baseline CT scan (and/or abdomen MRI), digitally stored on CD-ROM, if baseline radiologic assessment performed outside participating centers.

i) Urine pregnancy test for women of childbearing potential must be performed at baseline and least every month during treatment

j) Encouraged, not mandatory biopsy at PD

k) Refer to procedures detailed in Appendix 16.7

10. Safety Issues

10.1. Dose reductions and delays

Toxicities should be evaluated according to CTCAE v4.3.

Subjects should be instructed to report any delayed reactions to the Investigator as soon as possible.

10.1.1. Dose modifications for toxicities attributable to avelumab

As with all monoclonal antibody therapies, there is a risk of allergic reaction. Avelumab should be administered in a setting that allows for immediate access and administration of therapy for severe allergic/hypersensitivity reactions, such as the ability to implement immediate resuscitation measures. Steroids (dexamethasone 10 mg), epinephrine (1:1000 dilution), allergy medications (antihistamines), or equivalents should be available for immediate access.

Avelumab should be administered in a setting that allows for immediate access to an intensive care unit or equivalent environment and administration of therapy for anaphylaxis, such as the ability to implement immediate resuscitation measures. Steroids (dexamethasone 10 mg), epinephrine (1:1,000 dilution), allergy medications (IV antihistamines), bronchodilators, or equivalents, and oxygen should be available for immediate access.Infusion of avelumab will be stopped in case of Grade ≥ 2 infusion-related, allergic, or anaphylactoid reactions. Following avelumab infusions, subjects must be observed for 2 hours post infusion for potential infusion-related reactions.

If hypersensitivity reaction occurs, the subject must be treated according to the best available medical practice. Management of infusion-related reactions according to the NCI is described in the following Tables.

Table 1. Treatment Modification for Symptoms of Infusion-Related Reactions

NCI-CTCAE Grade	Treatment Modification for Avelumab
Grade 1 – mild Mild transient reaction; infusion interruption not indicated; intervention not indicated.	Decrease the avelumab infusion rate by 50% and monitor closely for any worsening.
Grade 2 – moderate Therapy or infusion interruption indicated but responds promptly to symptomatic treatment (for example, antihistamines, NSAIDs, narcotics, IV fluids); prophylactic medications indicated for ≤ 24 h.	Temporarily discontinue avelumab infusion. Resume infusion at 50% of previous rate once infusion-related reaction has resolved or decreased to at least Grade 1 in severity, and monitor closely for any worsening.
Grade 3 or Grade 4 – severe or life-threatening Grade 3: Prolonged (for example, not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for clinical sequelae. Grade 4: Life-threatening consequences; urgent intervention indicated.	Stop avelumab infusion immediately and disconnect infusion tubing from the subject. Subjects have to be withdrawn immediately from avelumab treatment and must not receive any further avelumab treatment.
- If avelumab infusion rate has been decreased by 50% or interrupted due to an infusion reaction, it must remain decreased for the next scheduled infusion. If no infusion reaction is observed in the next scheduled infusion, the infusion rate may be returned to baseline at the subsequent infusions based on investigator's medical judgment.- If hypersensitivity reaction occurs, the subject must be treated according to the best available medical practice.	

IV = intravenous; NCI-CTCAE = National Cancer Institute-Common Terminology Criteria for Adverse Event; NSAIDs = nonsteroidal anti-inflammatory drugs.

Because inhibition of PD-L1 stimulates the immune system, immuno-related AEs may occur. Treatment of immuno-related AEs is mainly dependent upon severity (NCI-CTCAE grade):

- Grades 1 to 2: treat symptomatically or with moderate dose steroids, more frequent monitoring
- Grades 1 to 2 (persistent): manage similarly to high grade AE (Grades 3 to 4)
- Grades 3 to 4: treat with high dose corticosteroids.

Treatment of immuno-related AEs should follow the guidelines in the following Tables.

Table 2. Management of Immune-mediated Adverse Reactions

Gastrointestinal irAEs		
Severity of Diarrhea/Colitis (NCI-CTCAE v4)	**Initial Management**	**Follow-up Management**
Grade 1 Diarrhea: < 4 stools/day over Baseline Colitis: asymptomatic	Continue avelumab therapy Symptomatic treatment (e.g. loperamide)	Close monitoring for worsening symptoms Educate subject to report worsening immediately If worsens: Treat as Grade 2, 3 or 4.
Grade 2 Diarrhea: 4 to 6 stools per day over Baseline; IV fluids indicated < 24 hours; not interfering with ADL Colitis: abdominal pain; blood in stool	Withhold avelumab therapy Symptomatic treatment	If improves to Grade ≤ 1: Resume avelumab therapy If persists > 5-7 days or recurs: Treat as Grade 3 or 4.
Grade 3 to 4 Diarrhea (Grade 3): ≥ 7 stools per day over Baseline; incontinence; IV fluids ≥ 24 h; interfering with ADL Colitis (Grade 3): severe abdominal pain, medical intervention indicated, peritoneal signs Grade 4: life-threatening, perforation	Withhold avelumab for Grade 3. Permanently discontinue avelumab for Grade 4 or recurrent Grade 3. 1.0 to 2.0 mg/kg/day prednisone IV or equivalent Add prophylactic antibiotics for opportunistic infections Consider lower endoscopy	If improves: Continue steroids until Grade ≤ 1, then taper over at least 1 month; resume avelumab therapy following steroids taper (for initial Grade 3). If worsens, persists > 3 to 5 days, or recurs after improvement: Add infliximab 5mg/kg (if no contraindication). Note: infliximab should not be used in cases of perforation or sepsis.
Dermatological irAEs		
Grade of Rash (NCI-CTCAE v4)	**Initial Management**	**Follow-up Management**
Grade 1 to 2 Covering ≤ 30% body surface area	Continue avelumab therapy Symptomatic therapy (for example, antihistamines, topical steroids)	If persists > 1 to 2 weeks or recurs: Withhold avelumab therapy Consider skin biopsy Consider 0.5-1.0 mg/kg/day prednisone or equivalent. Once improving, taper steroids over at least 1 month, consider prophylactic antibiotics for opportunistic infections, and resume avelumab therapy following steroids taper. If worsens: Treat as Grade 3 to 4.

Grade 3 to 4 Grade 3: Covering > 30% body surface area; Grade 4: Life threatening consequences	Withhold avelumab for Grade 3. Permanently discontinue for Grade 4 or recurrent Grade 3. Consider skin biopsy Dermatology consult 1.0 to 2.0 mg/kg/day prednisone or equivalent Add prophylactic antibiotics for opportunistic infections	If improves to Grade ≤ 1: Taper steroids over at least 1 month; resume avelumab therapy following steroids taper (for initial Grade 3).
Pulmonary irAEs		
Grade of Pneumonitis (NCI-CTCAE v4)	**Initial Management**	**Follow-up Management**
Grade 1 Radiographic changes only	Consider withholding avelumab therapy Monitor for symptoms every 2 to 3 days Consider Pulmonary and Infectious Disease consults	Re-assess at least every 3 weeks If worsens: Treat as Grade 2 or Grade 3 to 4.
Grade 2 Mild to moderate new symptoms	Withhold avelumab therapy Pulmonary and Infectious Disease consults Monitor symptoms daily; consider hospitalization 1.0 to 2.0 mg/kg/day prednisone or equivalent Add prophylactic antibiotics for opportunistic infections Consider bronchoscopy, lung biopsy	Re-assess every 1 to 3 days If improves: When symptoms return to Grade ≤ 1, taper steroids over at least 1 month, and then resume avelumab therapy following steroids taper If not improving after 2 weeks or worsening: Treat as Grade 3 to 4.
Grade 3 to 4 Grade 3: Severe new symptoms; New/worsening hypoxia; Grade 4: Life-threatening	Permanently discontinue avelumab therapy. Hospitalize. Pulmonary and Infectious Disease consults. 1.0 to 2.0 mg/kg/day prednisone or equivalent Add prophylactic antibiotics for opportunistic infections Consider bronchoscopy, lung biopsy	If improves to Grade ≤ 1: Taper steroids over at least 1 month If not improving after 48 hours or worsening: Add additional immunosuppression (for example, infliximab, cyclophosphamide, IV immunoglobulin, or mycophenolate mofetil)

Hepatic irAEs		
Grade of Liver Test Elevation (NCI-CTCAE v4)	**Initial Management**	**Follow-up Management**
Grade 1 Grade 1 AST or ALT > ULN to 3.0 x ULN and/or Total bilirubin > ULN to 1.5 x ULN	Continue avelumab therapy	Continue liver function monitoring If worsens: Treat as Grade 2 or 3 to 4.
Grade 2 AST or ALT > 3.0 to ≤ 5 x ULN and/or total bilirubin > 1.5 to ≤ 3 x ULN	Withhold avelumab therapy Increase frequency of monitoring to every 3 days.	If returns to Grade ≤ 1: Resume routine monitoring; resume avelumab therapy. If elevation persists > 5 to 7 days or worsens: Treat as Grade 3 to 4.
Grade 3 to 4 AST or ALT > 5 x ULN and/or total bilirubin > 3 x ULN	Permanently discontinue avelumab therapy Increase frequency of monitoring to every 1 to 2 days 1.0 to 2.0 mg/kg/day prednisone or equivalent Add prophylactic antibiotics for opportunistic infections Consult gastroenterologist/ hepatologist Consider obtaining MRI/CT scan of liver and liver biopsy if clinically warranted	If returns to Grade ≤ 1: Taper steroids over at least 1 month If does not improve in > 3 to 5 days, worsens or rebounds: Add mycophenolate mofetil 1 gram (g) twice daily If no response within an additional 3 to 5 days, consider other immunosuppressants per local guidelines.
Renal irAEs		
Grade of Creatinine Increased (NCI-CTCAE v4)	**Initial Management**	**Follow-up Management**
Grade 1 Creatinine increased > ULN to 1.5 x ULN	Continue avelumab therapy	Continue renal function monitoring If worsens: Treat as Grade 2 to 3 or 4.
Grade 2 to 3 Creatinine increased > 1.5 and ≤ 6 x ULN	Withhold avelumab therapy Increase frequency of monitoring to every 3 days 1.0 to 2.0 mg/kg/day prednisone or equivalent. Add prophylactic antibiotics for opportunistic infections Consider renal biopsy	If returns to Grade ≤1: Taper steroids over at least 1 month, and resume avelumab therapy following steroids taper. If worsens: Treat as Grade 4.
Grade 4 Creatinine increased > 6 x ULN	Permanently discontinue avelumab therapy Monitor creatinine daily 1.0 to 2.0 mg/kg/day prednisone or	If returns to Grade ≤1: Taper steroids over at least 1 month.

	equivalent. Add prophylactic antibiotics for opportunistic infections Consider renal biopsy Nephrology consult	
Cardiac irAEs		
Myocarditis	**Initial Management**	**Follow-up Management**
New onset of cardiac signs or symptoms and / or new laboratory cardiac biomarker elevations (e.g. troponin, CK-MB, BNP) or cardiac imaging abnormalities suggestive of myocarditis.	Withhold avelumab therapy. Hospitalize. In the presence of life threatening cardiac decompensation, consider transfer to a facility experienced in advanced heart failure and arrhythmia management. Cardiology consult to establish etiology and rule-out immune-mediated myocarditis. Guideline based supportive treatment as per cardiology consult.* Consider myocardial biopsy if recommended per cardiology consult.	If symptoms improve and immune-mediated etiology is ruled out, re-start avelumab therapy. If symptoms do not improve/worsen, viral myocarditis is excluded, and immune-mediated etiology is suspected or confirmed following cardiology consult, manage as immune-mediated myocarditis.
Immune-mediated myocarditis	Permanently discontinue avelumab. Guideline based supportive treatment as appropriate as per cardiology consult.* 1.0 to 2.0 mg/kg/day prednisone or equivalent Add prophylactic antibiotics for opportunistic infections.	Once improving, taper steroids over at least 1 month. If no improvement or worsening, consider additional immunosuppressants (e.g. azathioprine, cyclosporine A).
*Local guidelines, or eg. ESC or AHA guidelines ESC guidelines website: https://www.escardio.org/Guidelines/Clinical-Practice-Guidelines AHA guidelines website: http://professional.heart.org/professional/GuidelinesStatements/searchresults.jsp?q=&y=&t=1001		
Endocrine irAEs		
Endocrine Disorder	**Initial Management**	**Follow-up Management**
Grade 1 or Grade 2 endocrinopathies (hypothyroidism, hyperthyroidism, adrenal insufficiency, type I diabetes mellitus)	Continue avelumab therapy Endocrinology consult if needed Start thyroid hormone replacement therapy (for hypothyroidism), anti-thyroid treatment (for hyperthyroidism), corticosteroids (for adrenal insufficiency) or insulin (for Type I diabetes mellitus) as appropriate. Rule-out secondary endocrinopathies	Continue hormone replacement/suppression and monitoring of endocrine function as appropriate.

	(i.e. hypopituitarism / hypophysitis)	
Grade 3 or Grade 4 endocrinopathies (hypothyroidism, hyperthyroidism, adrenal insufficiency, type I diabetes mellitus)	Withhold avelumab therapy Consider hospitalization Endocrinology consult Start thyroid hormone replacement therapy (for hypothyroidism), anti-thyroid treatment (for hyperthyroidism), corticosteroids (for adrenal insufficiency) or insulin (for type I diabetes mellitus) as appropriate. Rule-out secondary endocrinopathies (i.e. hypopituitarism / hypophysitis)	Resume avelumab once symptoms and/or laboratory tests improve to Grade ≤ 1 (with or without hormone replacement/suppression). Continue hormone replacement/suppression and monitoring of endocrine function as appropriate.
Hypopituitarism/Hypophysitis (secondary endocrinopathies)	If secondary thyroid and/or adrenal insufficiency is confirmed (i.e. subnormal serum FT4 with inappropriately low TSH and/or low serum cortisol with inappropriately low ACTH) : • Refer to endocrinologist for dynamic testing as indicated and measurement of other hormones (FSH, LH, GH/IGF-1, PRL, testosterone in men, estrogens in women) • Hormone replacement/suppressive therapy as appropriate • Perform pituitary MRI and visual field examination as indicated **If hypophysitis confirmed:** • Continue avelumab if mild symptoms with normal MRI. Repeat the MRI in 1 month • Withhold avelumab if moderate, severe or life-threatening symptoms of hypophysitis and/or abnormal MRI. Consider hospitalization. Initiate corticosteroids (1 to 2 mg/kg/day prednisone or equivalent) followed by corticosteroids taper during at least 1 month. • Add prophylactic antibiotics for opportunistic infections.	Resume avelumab once symptoms and hormone tests improve to Grade ≤ 1 (with or without hormone replacement). In addition, for hypophysitis with abnormal MRI, resume avelumab only once shrinkage of the pituitary gland on MRI/CT scan is documented. Continue hormone replacement/suppression therapy as appropriate.
Other irAEs (not described above)		
Grade of other irAEs (NCI-CTCAE v4)	**Initial Management**	**Follow-up Management**

Grade 2 or Grade 3 clinical signs or symptoms suggestive of a potential irAE	Withhold avelumab therapy pending clinical investigation	If irAE is ruled out, manage as appropriate according to the diagnosis and consider re-starting avelumab therapy If irAE is confirmed, treat as Grade 2 or 3 irAE.
Grade 2 irAE or first occurrence of Grade 3 irAE	Withhold avelumab therapy 1.0 to 2.0 mg/kg/day prednisone or equivalent Add prophylactic antibiotics for opportunistic infections Specialty consult as appropriate	If improves to Grade ≤ 1: Taper steroids over at least 1 month and resume avelumab therapy following steroids taper.
Recurrence of same Grade 3 irAEs	Permanently discontinue avelumab therapy 1.0 to 2.0 mg/kg/day prednisone or equivalent Add prophylactic antibiotics for opportunistic infections Specialty consult as appropriate	If improves to Grade ≤ 1: Taper steroids over at least 1 month.
Grade 4	Permanently discontinue avelumab therapy 1.0 to 2.0 mg/kg/day prednisone or equivalent and/or other immunosuppressant as needed Add prophylactic antibiotics for opportunistic infections Specialty consult.	If improves to Grade ≤ 1: Taper steroids over at least 1 month
Requirement for 10 mg per day or greater prednisone or equivalent for more than 12 weeks for reasons other than hormonal replacement for adrenal insufficiency **Persistent Grade 2 or 3 irAE lasting 12 weeks or longer**	Permanently discontinue avelumab therapy Specialty consult	

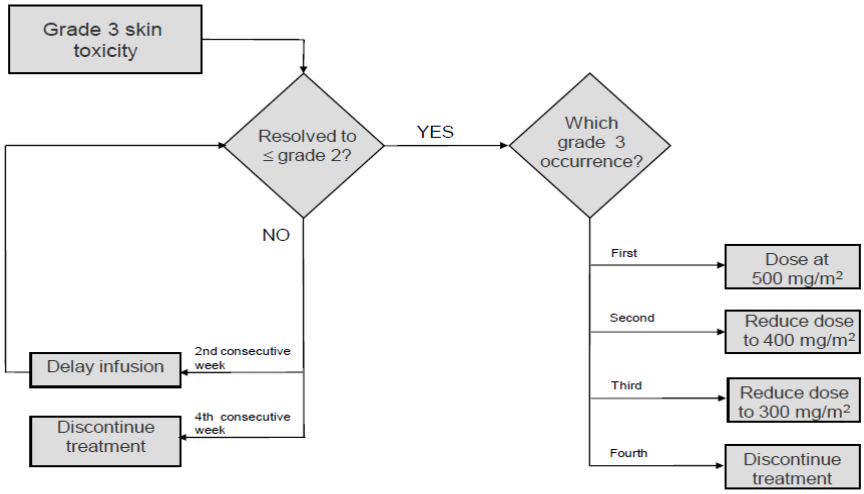
10.1.2. Dose modifications for toxicities attributable to cetuximab

For subjects who experience toxicities while on study, one or more doses of cetuximab may need to be withheld, reduced or delayed (administered at >14 day intervals). On resolution of toxicity, cetuximab doses may be re-escalated (see below). Cetuximab dose reduction are listed in the table below.

Table 3. Cetuximab dose reduction

Event	Grade	Adjustment
Skin or nail toxicity – *First Occurrence*	3 or 4	Hold cetuximab until grade ≤ 2 and restart at 100 % dose level
Skin or nail toxicity in patients treated at 100% or 80% dose level – *Recurring*	3 or 4	Restart cetuximab at 80 % dose level or 60 % dose level respectively
Symptomatic hypomagnesemia – *First Occurence*		Hold cetuximab until resolution or restart at 100 % dose level Mg^{++} supplementation
Symptomatic hypomagnesemia in patients treated at 100% or 80% dose level – *Recurring*		Restart cetuximab at 80 % dose level or 60 % dose level respectively
Diarrhea – *First Occurence*	3 or 4	Hold cetuximab until resolution and restart at 100 % dose level
Diarrhea in patients treated at 100% or 80% dose level – *Recurring*	3 or 4	Restart cetuximab at 80 % dose level or 60 % dose level respectively
Any hematologic or non-hematologic toxicity	4	Hold cetuximab until resolution

Cetuximab dose reduction for skin reactions scheme:



Criteria for withholding a dose of cetuximab

For subjects who experience a toxicity that meets the criteria for withholding a dose of cetuximab:

- Subjects are allowed to have one subsequent dose withheld for toxicity, as per scheme shown above. Even if the toxicity has resolved by the intervening week before the subsequent cycle of chemotherapy is due, cetuximab will be restarted along with Chemotherapy.

  The cetuximab dose (100% or reduced) will be defined according to the scheme shown above and described below:
- Subjects treated at 100% dose level whose toxicity resolves after 1 dose of cetuximab is withheld should be restarted at 100% dose level (recommended but not required, reduction to 80% dose is allowed as an alternative to re challenge with 100% dose).
- If toxicity recurs, subjects treated at 100% dose or 80% (400 mg/m2) dose should be restarted at 80% dose or 60% (300 mg/m2) dose, respectively, when the toxicity resolved after withholding 1 dose of cetuximab.
- Subjects who experience toxicity at the 60% dose level (300 mg/m2) will not be retreated with cetuximab and can continue with avelumab monotherapy

Cetuximab should be given on the first day of each cycle. If a cycle of avelumab is delayed, cetuximab administration should be also delayed. If avelumab is delayed greater than 4 weeks from the previous dose, and the patient has not disease progression, cetuximab monotherapy should be administered as soon as possible.

Delay of cetuximab administration greater 6 weeks from the previous dose of cetuximab are not allowed. Patients that permanently discontinue cetuximab can continue to receive avelumab monotherapy, if clinically indicated.

10.1.3 Special warning on skin toxicity

Special attention should be given to the management of skin toxicity: it is one of the Investigator's responsibilities to properly evaluate the observed reaction and to discriminate the most probable cause (from which compound it is most likely to come). If it looks more like an immune-related rash from Avelumab, then the Avelumab skin irAE treatment guidelines should be followed according to Table 2.

If the rash is more like a typical cetuximab rash, it should be treated as reported in Table 7.

10.2 Concomitant medications and management of specific adverse events

All concomitant medications taken by the subject during the trial, from the date of signature of informed consent are to be recorded in the appropriate section of the eCRF, noting the name, dose, duration and indication of each drug. Nondrug interventions and any changes to a concomitant medication or other intervention should also be recorded in the eCRF.

Permitted medicines

Any medications (other than those excluded by the clinical trial protocol) that are considered necessary to protect subject welfare and will not interfere with the trial treatment may be given at the Investigator's discretion.

The Investigator will record all concomitant medications taken by the subject during the trial, from the date of signature of informed consent, in the appropriate section of the eCRF.

Any additional concomitant therapy that becomes necessary during the trial and any change to concomitant drugs must be recorded in the corresponding section of the eCRF, noting the name, dose, duration, and indication of each drug.

Prohibited medicines

As stated for the exclusion criteria in Section 8.2, subjects must not have had prior therapy with any antibody or drug targeting T-cell coregulatory proteins (immune checkpoints) such as anti-PD-1, anti-PD-L1, or anti-cytotoxic T-lymphocyte antigen-4 antibody or concurrent anticancer treatment (for example, cytoreductive therapy, radiotherapy [with the exception of palliative bone-directed radiotherapy, or radiotherapy administered on superficial lesions], immune therapy, or cytokine therapy except for erythropoietin), major surgery (excluding prior diagnostic biopsy), or concurrent systemic therapy with steroids or other immunosuppressive agents.
In addition, the following treatments must not be administered during the trial:

- Immunotherapy, immunosuppressive drugs EXCEPT for the following:
  a. intranasal, inhaled, topical steroids, or local steroid injection (e.g., intra-articular injection);
  b. Systemic corticosteroids at physiologic doses ≤ 10 mg/day of prednisone or equivalent;
  c. Steroids as premedication for hypersensitivity reactions (e.g., CT scan premedication
  d. Systemic corticosteroids for short-term of immune-related AEs),
- Growth factors for subjects randomized to receive avelumab (granulocyte colony stimulating factor or granulocyte macrophage colony stimulating factor). Exception: erythropoietin and darbepoietin alpha may be prescribed at the Investigator's discretion
- Bisphosphonate or denosumab treatment is not allowed unless it has been initiated more than 14 days prior to receiving the first administration of avelumab or maintenance chemotherapy therapy.

If the administration of a non-permitted concomitant drug becomes necessary during the trial, the subject will be withdrawn from trial treatment (the Sponsor may be contacted to discuss whether the trial treatment must be discontinued). The subject should complete the End of Treatment visit (Section 9.2) and be followed for survival according to Section 9.2.
Medications other than those specifically excluded in this trial (see above) may be administered for the management of symptoms associated with the administration of avelumab or maintenance

chemotherapy as required. These might include analgesics, anti-nausea medications, antihistamines, diuretics, antianxiety medications, and medication for pain management, including narcotic agents.

Other interventions

The following non-drug therapies must not be administered during the trial (or within 28 days before randomization):

- Major surgery (excluding prior diagnostic biopsy)
- Herbal remedies with immunostimulating properties (for example, mistletoe extract) or known to potentially interfere with major organ function (for example, hypericin)
- Subjects should not abuse alcohol or other drugs during the trial.

Lonardi S, *et al. J Immunother Cancer* 2021; 9:e002996. doi: 10.1136/jitc-2021-002996

11. Translational Analysis

11.1. Collection and Analysis

Biological material will be collected for translation purposes. Urine, blood and tumor tissue will be collected according to Study procedures and as detailed in the dedicated Appendix 16.7.
Tissue specimens (formalin-fixed paraffin-embedded tumor blocks, together with the accompanying histological report, from primary tumor and/or metastasis) should be sent the Coordinating Center. RAS, BRAF and MSI mutational analysis will be performed on tissue specimens and centralized at the Istituto Oncologico Veneto (IOV) IRCCS.

11.2. Storage

Tumor tissue samples, blood and plasma samples will be stored at the UOC Oncologia Medica 1, Dipartimento di Oncologia Clinica e Sperimentale, Istituto Oncologico Veneto (IOV) IRCCS under the responsibility of Dr. Lonardi. Blood and plasma samples will be stored at Unit of Medical Oncology 1, Department of Medical Oncology, Oncology Institute of Veneto—IRCCS under the responsibility of Dr. Lonardi.

11.3. Other

Additional translation analysis on the above mentioned material might be described in an ancillary Protocol.

12. Ethical issues

The procedures described in this protocol, pertaining to the conduct, evaluation, and documentation of this study, are designed to ensure that the Sponsor and Investigator abide by GCP guidelines and under the guiding principles detailed in the Declaration of Helsinki. The study will also be conducted in keeping with applicable local law(s) and regulation(s).

Documented approval from appropriate IEC(s)/IRB(s) will be obtained for all participating centers before the beginning of the study, according to GCP, local laws, regulations and organizations. When necessary, an extension, amendment or renewal of the IEC/IRB approval must be obtained and also forwarded to the Sponsor. The responsible unit (eg, IEC/IRB, head of the study center/medical institution) must supply to the Sponsor, upon request, a list of the IEC/IRB members involved in the vote and a statement to confirm that the IEC/IRB is organized and operates according to GCP and applicable laws and regulations.

Strict adherence to all specifications laid down in this protocol is required for all of the aspects of the study conduction; the investigator may not modify or alter the procedures described in this protocol. Modifications to the study protocol will not be implemented by either the Sponsor or the investigator without agreement by both parties. However, the investigator or the Sponsor may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to the study subjects without prior IEC/IRB/Sponsor approval/favorable opinion. As soon as possible, the implemented deviation or change, the reasons for it and, if appropriate, the proposed protocol amendment should be submitted to the IEC/IRB/head of medical institution/Sponsor. Any deviations from the protocol must be explained and documented by the investigator.

12.1. Informed Consent

The investigator must explain to each patient (or legally authorised representative) the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits involved and any discomfort it may entail. Each patient must be informed that participation in the study is voluntary and that he/she may withdraw from the study at any time

and that withdrawal of consent will not affect her subsequent medical treatment or relationship with physician.

The informed consent will be given by means of standard written statement, written in non-technical language. The patient should read and consider the statement before signing and dating it, and should be given a copy of the signed document. If the subject cannot read or sign the document, oral presentation may be made or signature given by the subject's legally appointed representative, if witnessed by a person not involved in the study, mentioning that the patient could not read or sign documents. No patient can enter the study before her informed consent has been obtained. The informed consent is part of the protocol and must be submitted by the investigator to the local ethical committee.

A copy of the patient's signed written consent will be kept by the center in the proper section of the Investigator Site File.

12.2. Patient protection

The names of patients will not be recorded; a sequential identification number will be attributed to each patient registered in the trial. This number will identify the patient and must be included on all eCRF. In order to avoid identification errors, patients initials (maximum of 2 letters) and date of birth will also be reported on the eCRF.

Investigators will guarantee that all persons involved in this study will respect the confidentiality of any information concerning the trial subject. All parties involved in this clinical trial will maintain the strict confidentiality to assure that neither the person nor the family privacy of the patient participating in the trial is violated; appropriate measures shall be taken to avoid the access of non authorized persons to the trial data. The processing of the personal data of patients taking part in the trial, and in particular regarding data concerning consent, shall comply with local law on the privacy (Legge delega 127/2001) and with the European Directive on the Privacy of data (95/46/EC).

The patient can withdraw consent whenever he wants and further data will not be collected, even if the already collected data will be used for the study's analyses.

12.3. Confidential subject information for samples storage

For the storage of biological samples, specific means will be taken to ensure the subject's right to privacy and the pertinent guidance documents and regulations will be considered.

Subjects may withdraw their consent to store the biological samples. If the patient withdraws his consent from the study within 5 years, the biological samples will be destroyed. After 5 years, biological samples will be anonymized completely. At that time the samples cannot be identified in any way. The samples will be maintained for potential analysis for 15 years from the acquisition. Samples will be destroyed according to Fondazione GONO policies and procedures. Samples will be collected and sent to the laboratory designated for the trial where they will be processed.

Tumor tissue samples and blood and plasma samples will be stored at the Unit of Oncologia Medica 1, Istituto Oncologico Veneto IRCCS of Padova under the responsibility of Dr. Lonardi.

To maintain privacy of information collected from samples obtained for storage and future analysis, Fondazione GONO has developed secure policies and procedures to maintain subject privacy. At the clinical site, a unique Code will be placed on the blood sample for transfer to the storage facility. The Code is a random number used only to identify the biosample of each subject. No other personal identifiers will appear on the sample tube. The first Code will be replaced with a Sample Code at the Central Laboratory or at the Fondazione GONO designated facility. This sample is now a single coded sample. The Sample Code is stored separately from all previous sample identifiers. A secure code, hereinafter referred to as a "first coding key", will be utilized to match the Sample Code to the original blood code and subject number to allow clinical information collected during the course of the trial to be associated with the biosample. This "first coding key" will be transferred by the central laboratory or Fondazione GONO designated facility under secure procedures to the Fondazione GONO designated as the entrusted key-holder to maintain confidentiality of the biosamples. The Sample Code will be logged into the primary biorepository database, and in this database this identifier will not have identifying demographic data or identifying clinical information (i.e., race, sex, age, diagnosis, laboratory values) associated with it. The sample will be stored in a designated repository site with secure policies and procedures for sample storage and usage.

12.4. Ethics Committee

The Investigator must submit this protocol to the local EC and is required to forward a copy of the written approval to the CRP.

The EC approval must report, the identification of the trial (title, protocol number and version), the documents evaluated (protocol, informed consent material, advertisement when applicable) and the date of their version.

12.5. Administrative responsibilities

The coordinator center and data center (UOC Oncologia Medica 1 – Dipartimento di Oncologia Clinica e Sperimentale Istituto Oncologico Veneto IRCCS – Padova) will be responsible for:

- reviewing the protocol
- centralizing databases
- centralizing data validation according to Data Validation Plan
- controlling the quality of the reported data
- emitting Data Query Forms
- generating study program reports
- generating the Statistical Analysis Plan
- perform statistical analysis

12.6. Trial sponsorship and financing

The present study is an investigator-initiated trial, carried out by participating clinicians, who have the intellectual ownership of the results.

The study is sponsored by Fondazione GONO Cooperative Group Via G. Mameli, 3 – Genova (ITALY), who will provide the economical support for costs related to data management, statistical analysis and the other activities of central and group coordinating centers.

The Funder (Merck Serono) will contribute with 50 % of the total budget and will provide the IMPs (avelumab and cetuximab).

No funds can be provided to EC and single participating centres.

The study will be conducted according to the current regulations.

13. Study Monitoring

13.1. Quality assurance

Each participating Investigator will be responsible for ensuring data quality as planned in the Data Validation Plan document. Each reported information will be systematically checked for consistency, completeness and accuracy by the Coordinating Data Center that will issue Data Query Forms in case of inconsistent data. Local quality control will be provided by coordinating centers of each participating group, which will be responsible of monitoring the centers belonging to their group.

13.2. Responsibilities of the investigators

The Investigators undertake to perform the study in accordance with ICH Good Clinical Practice and Good Clinical Practice for Trials on Medicinal Products in the European Community (ISBN 92 - 825-9563-3).

The Investigator is required to ensure his compliance to the procedures required by the protocol with respect to the investigational drug schedule and visit schedule. The Investigator agrees to provide all information requested in the Case Report Form in an accurate and legible manner according to the instructions provided. The Investigator has responsibilities to the Health Authorities to take all reasonable steps to ensure the proper conduct of the study as regards ethics, protocol adherence, integrity and validity of the data recorded on the case report forms. The main duty of the Trial Monitor is to help the Investigator and the Coordinators to maintain a high level of ethical, scientific, technical and regulatory quality in all aspects of the study. At regular intervals during the study, the center will be contacted, through site visits, letters or telephone calls, to review the study progress, the investigators and subjects adherence to protocol requirements. During each monitoring visits, the following points will be scrutinized:

- subject informed consent;
- subject recruitment and follow-up;
- study drug allocation;

- subject compliance to the study treatment;
- study treatment accountability;
- Adverse Event documentation and reporting.

13.3. Source documents requirements

According to the guidelines on ICH Good Clinical Practice, the monitor of the study will check the case report form entries against the source documents. These personnel, bound by professional secrecy, will not disclose any personal identity or personal medical information.
Considering the primary end point of the study, independent review of objective response will be performed by an external panel. For this reason, a copy (either on CD or radiological film) of each CT or RMN scan performed during the study will be required.

13.4. Use and completion of electronic case report forms

It is the responsibility of the Investigator to prepare and maintain adequate and accurate e-CRFs for each patient enrolled in the study. All e-CRFs should be completed to ensure accurate interpretation of data.

14. Adverse events

14.1. Definition of adverse event

An adverse event is defined in the International Conference on Harmonization (ICH) Guideline for Good Clinical Practice as "any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment." (ICH E6:1.2). See below (specific table), for guidelines to drug-event relationship assessment.

The investigator is responsible for reviewing laboratory test results and determining whether an abnormal value in an individual study subject represents a change from values before the study. In general, abnormal laboratory findings without clinical significance (based on the investigator's judgment) should not be recorded as adverse events; however, laboratory value changes requiring therapy or adjustment in prior therapy are considered adverse events.

Patients will be instructed by the Investigator to report the occurrence of any adverse event.

14.2. Definition of Adverse Drug Reactions (ADR)

All untoward and unintended responses to a medicinal product related to any dose administered.

The phrase "responses to a medicinal product" means that a causal relationship between the medicinal product and the adverse event is at least a reasonable possibility, i.e. the relationship cannot be ruled out. A serious ADR (SADR) is an ADR that meets the definition of serious (provided below).

14.3. Definition of Serious Adverse Event

A serious adverse event (SAE) is defined as an adverse event that:

- is fatal;
- is life threatening (places the subject at immediate risk of death);
- requires in-patient hospitalization or prolongation of existing hospitalization;

- results in persistent or significant disability/incapacity;
- is a congenital anomaly/birth defect;
- other significant medical hazard.

A hospitalization meeting the regulatory definition for "serious" is any inpatient hospital admission that includes a minimum of an overnight stay in a health care facility. Any adverse event that does not meet one of the definitions of serious (i.e., emergency room visit, outpatient surgery, or requires urgent investigation) may be considered by the investigator to meet the "other significant medical hazard" criterion for classification as a serious adverse event. Examples include allergic bronchospasm, convulsions, and blood dyscrasias. Hospitalization for the performing of protocol-required procedures or administration of study treatment is not classified as an SAE.

All adverse events which do not meet any of the criteria for serious should be regarded as non-serious adverse events.

All serious adverse events occurring during the study treatment period must be reported within 24 hours according to the procedure described below. Any late SAE (occurring after this 30 days period) possibly or probably related to the study treatment should follow the same reporting procedure.

Progression of colorectal cancer leading to one of the above should not be reported as a serious adverse event.

14.4. Deaths reporting procedure

Any death due to SAE occurring between the randomization and 30 days following the treatment must be reported to the Sponsor within 24 hours, regardless of the relation to study drug(s). Deaths occurring later than 30 days after the treatment should be reported on the death report form section of the e-CRF regardless of cause.

14.5. Pregnancies reporting procedure

The investigator must report to the sponsor any pregnancy and lactation occurring in a study subject's partner, during the participation of subject in this study. The report should be submitted

within the same timelines as an SAE, although a pregnancy per se is not considered an SAE. For the pregnancy of a study subject's partner, all efforts should be made to obtain information on course and outcome, subject to the partner's consent.

14.6. Reporting procedure

Reporting Procedures for All Adverse Events

The investigator is responsible for ensuring that all adverse events observed by the investigator or reported by subjects are properly captured in the subjects' medical records. The following adverse event attributes must be assigned by the investigator: adverse event diagnosis or syndrome(s) (if known, signs or symptoms if not known); event description (with detail appropriate to the event); dates of onset and resolution; severity; outcome, assessment of relatedness to study treatment; and action taken.

Medically significant adverse events considered related to the treatment by the investigator or the sponsor will be followed until resolved or considered stable. It will be left to the investigator's clinical judgment to determine whether an adverse event is related and of sufficient severity to require the subject's removal from treatment or from the study. A subject may also voluntarily withdraw from treatment due to what he or she perceives as an intolerable adverse event. If either of these situations arises, the subject should be strongly encouraged to undergo an end-of study assessment and be under medical supervision until symptoms cease or the condition becomes stable.

Serious Adverse Events Reporting Procedures

Serious adverse events will be collected and recorded throughout the study period, defined as through to 6 months after the last dose of the treatment or the end of the study (including the follow-up period), whichever is longer.

The investigator should notify the Sponsor (Fondazione GONO) of all serious adverse events occurring at the site(s) in accordance with local procedures, statutes and the European Clinical Trial Directive (where applicable). The Sponsor will medically review all SAEs.

The Sponsor will ensure the notification of the appropriate Ethics Committees, Competent Authorities and participating Investigators of all serious adverse events occurring at the site(s) in accordance with local legal requirements, statutes and the European Clinical Trial Directive.

The following reportable events must be submitted to the Coordinating Center (IOV-IRCCS, PI dr. Sara Lonardi) within 24 hours (or immediately for death or life-threatening events) using the applicable safety report form provided. The Sponsor will assume responsibility for submitting the reportable event(s) to Merck as well as ensuring that any local reporting requirements are completed in parallel.

- Serious Adverse Events
- Exposure during Pregnancy or Breastfeeding (even if not associated with an adverse event)
- Occupational exposure (even if not associated with an adverse event)
- Potential drug-induced liver injury (Hy's Law cases): these events are considered important medical events and should be reported as SAEs.

Contact information for submission of reportable events to Merck:

Fax: +49 6151 72 6914

OR

E-mail: GlobalDrugSafety@merckgroup.com

Specifying:

- PROTOCOL Number and/or Title
- Merck assigned Study Number
- SUBJECT Number
- SITE Number/PI Name
- SAE ONSET DATE

The Sponsor will also provide Merck Serono with an Annual Safety Report and with a copy of any other communication or aggregate report, containing safety data generated during the course of the study, sent to the Regulatory Authorities by the Sponsor.

Furthermore, the Sponsor will provide Merck Serono with a line listing/report of all SAEs occurred in subjects exposed to Merck Serono Product on a periodic basis (but no less frequently than every

6 months) in order to fulfill the reconciliation process. The final study report should be sent to Merck no later than 1 calendar year of study completion.

14.7. Follow-up

Patients withdrawn from the study treatment due to any adverse event will be followed at least until the outcome is determined, even if it implies that the follow-up continues after the patients has left the trial, and where appropriate until the end of the planned period of follow-up.
In case of serious adverse event, the patient must be followed until clinical recovery is complete and laboratory results have returned to normal, or until symptoms have stabilized. This may imply that the follow-up will continue after the patient has left the trial.
Further information will be noted on the SAE form, by ticking the box marked “follow up” and will be sent to the Coordinating Center as information becomes available.

14.8. Post-study follow up

After study drug treatment ends, anti-cancer medications taken by the patient should be documented in the e-CRF.
Patients will be evaluated approximately every month to determine their survival status. Telephone follow-up is acceptable. Site staff must use caution when contacting the patient’s family for this information, especially if they are no longer under the care of the investigator, so as to not inadvertently cause any distress to the family of a patient who is no longer alive.
During this period, if the investigator becomes aware of a serious adverse event with any causal relationship to the investigational medicinal product that occurs after the end of the clinical trial, the investigator shall, without undue delay, report the serious adverse event to the Sponsor.
The investigator should report these events directly to the Sponsor, by completing the Serious Adverse Event Reporting Form that will be sent to the Coordinating Center. Subjects who withdraw consent from study drug treatment should enter the post-study follow-up period (unless consent to follow-up is specifically withdrawn). Details should be documented on the specified Serious Adverse Event Form.

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16. Appendix

16.1 Study synopsis

Phase of study

Phase II randomized prospective study

Indication

Unresectable locally advanced or metastatic squamous anal cancer (SCCAC) progressed after at least one systemic treatment

Rationale of the study

- The standard first line treatment in SCCAC is the association of 5-FU with cisplatin reaching a percentage of survival at 5 years of about 32% (Faivre 1999); in a recent case series of patients affected by SCCAC, the combination of 5-FU and cisplatin as first line treatment produced 34.4% objective response rate (ORR) and a 5 years survival rate of 15% (Sclafani 2017);
- No standard second line treatment exists for SCCAC;
- Cetuximab in association with irinotecan has demonstrated promising results in pretreated patients affected by SCCAC (Lukan 2009). In addition, it was recently tested in stage I-III SCCAC in association with cisplatin plus 5-FU and radiotherapy. Despite not reaching their pre-specified endpoints both studies reported an interesting activity in local control of disease, leading to hypothesize that cetuximab warrant further investigation in new strategies (Garg 2017, Sparano 2017);
- Anti-PD1 treatments such as nivolumab and pembrolizumab showed promising activity in metastatic refractory SCCAC in terms of response rate and disease control with acceptable toxicity profiles (Morris 2017, Ott 2017);
- The induction of immunogenic cell death was recently shown for cetuximab-based regimens (Pozzi 2016) and PD-L1 blockade should lead to NK cells activation enhancing cetuximab ADCC (Choncha-Benavente 2015, Choncha-Benavente 2016).

On the basis of these considerations, we designed the present randomized phase II trial of avelumab alone or avelumab plus cetuximab for previously treated unresectable locally advanced or metastatic SCCAC.

Primary Objective

To evaluate the activity of avelumab alone or in combination with cetuximab in patients with advanced SCCAC.

Secondary Objectives

- To estimate Progression-Free Survival (PFS),
- To estimate Overall survival (OS);
- To describe the safety profile of the monotherapy and of the combination;
- To collect archival tumor specimens, blood samples, newly obtained tumor specimens (optional) for translational analyses

Definition of primary endpoint

The primary endpoint is Objective Response Rate (ORR). ORR is defined as the percentage of patients, relative to the total of enrolled subjects with measurable disease, achieving a complete (CR) or partial (PR) response, according to Response Evaluation Criteria In Solid Tumors (RECIST) v1.1 criteria. The determination of the radiological response will be based on the investigator's reported measurements. Radiological responses will be evaluated every 8 weeks starting from cycle 1 day 1 of treatment until disease progression, withdrawal of consent or death for any reason, whichever occurs first.

Definition of secondary endpoints

PFS is defined as the time from study enrollment to the first documentation of objective disease progression or death due to any cause, whichever occurs first. Documentation of progressive disease is defined as per RECIST 1.1 criteria, based on investigator's assessment. PFS will be censored at the time of the last evaluable tumour assessment documenting absence of progressive disease for patients who are alive and progression free at the time of the analysis. Alive patients having no tumour assessments after baseline will have time to event censored on the date of study enrollment.

- OS is defined as the time from study enrollment to the date of death due to any cause. For patients still alive at the time of analysis, the OS time will be censored on the last date the patients were known to be alive.

- Overall Toxicity Rate is defined as the percentage of patients, out of those receiving at least one dose of treatment, experiencing any grade 3-4 adverse event, according to NCI CTCAE v4.03. Toxicity Rate is defined as the percentage of patients experiencing a specific adverse event of grade 3-4, according to NCI CTCAE v4.03.

Statistical Considerations and sample size

A Simon's two-stage Mini-Max design (Simon, 1989) will be used for both arms in parallel.

The null hypothesis that the true response rate is 5 % [p0 = 0.05] will be tested against the one-sided alternative of a true response rate of 20% [p1 = 0.20]. Type I error rate is set at 0.05 and power at 80%.

For both arms, in the first stage, 13 patients will be accrued. If there will be no responses in these 13 patients, the study will be stopped. Otherwise, 14 additional patients will be accrued for a total of 27. The null hypothesis will be rejected if 4 or more responses will be observed in 27 patients. No formal comparison between the results of the two treatment arms will be allowed.

Inclusion criteria

- Histologically proven diagnosis of SCCAC;
- Progression on or after first line systemic therapy for surgically unresectable or metastatic disease. Systemic radiosensitizing chemotherapy with curative intent in limited-stage disease should be considered equal to a first line for a patient experiencing progression during or within 6 months of completion;
- Evaluable disease lesion according to RECIST v1.1 criteria;
- Availability of tumor sample (primary and/or metastatic sites);
- Age ≥ 18 years;
- Eastern Cooperative Oncology Group – Performance Status (ECOG PS) ≤ 2;
- Life expectancy of at least 12 weeks;
- Laboratory Requirements:
    - Neutrophils ≥ 1.5 x 10^9/L;
    - Platelets ≥ 100 x 10^9/L;
    - Hemoglobin ≥ 9 g/dL;
    - Total bilirubin ≤ 1.5 time the upper-normal limits (UNL) of the normal values and ASAT (SGOT) and/or ALAT (SGPT) ≤ 2.5 x UNL (or <5 x UNL in case of liver metastases);
    - Alkaline phosphatase ≤ 2.5 x UNL (or <5 x UNL in case of liver metastases);
    - Creatinine clearance ≥ 30 mL/min according to the Cockcroft-Gault formula (or local institutional standard method) or serum creatinine ≤1.5 x UNL;
- HIV-positive patients are eligible if their $CD4^+$ cell count amounts to 300 cells per µL or more; HIV viral load has to be undetectable, and they have to be compliant with antiretroviral treatment;
- Negative serum or urine pregnancy test at screening for women of childbearing potential. Female subjects, or male subjects with female partners of child-bearing potential must be willing to use

highly effective contraception as approved by the investigator (i.e. barrier contraceptive measure or oral contraception, total abstinence) during the study and until 30 days after last study treatment;

- Written informed consent to the study procedures and to molecular analyses before patients registration;
- Will and ability to comply with the protocol.

Exclusion criteria

- Previous therapy with any drug targeting T-cell co-regulatory proteins (i.e., immune checkpoint inhibitors);
- Concurrent anticancer treatment or use of any investigational drug within 28 days before the start of the trial treatment;
- Major surgical procedure, open biopsy, or significant traumatic injury occurred within 28 days prior to study treatment start, or anticipation of the need for major surgical procedure during the course of the study;
- History or evidence upon physical examination of CNS disease unless adequately treated. Patients with treated brain metastases are eligible if their lesions were stable and asymptomatic for at least 3 months;
- Neutrophils < 1.5 x 10^9/L; platelets < 100 x 10^9/L; hemoglobin < 9 g/dL;
- Active uncontrolled infections requiring systemic therapy or other clinically relevant concomitant illness contraindicating therapy administration or putting the patient at high risk for treatment-related toxicities;
- Hepatitis B virus (HBV) or hepatitis C virus (HCV) infection at screening (positive HBV surface antigen or HCV RNA if anti-HCV antibody screening test positive)
- Patients with active autoimmune disease or history of autoimmune disease that might deteriorate when receiving an immuno-stimulatory agent or might potentially affect vital organ function, or require use of immunosuppressive treatment including chronic prolonged systemic corticosteroids (defined as corticosteroid use for ≥ 1 month). Patients with diabetes type I, vitiligo, psoriasis, or hypo- or hyperthyroid diseases not requiring immunosuppressive treatment are eligible;
- Current use of immunosuppressive medication, EXCEPT for the following: a. intranasal, inhaled, topical steroids, or local steroid injection (e.g., intra-articular injection); b. Systemic

corticosteroids at physiologic doses ≤ 10 mg/day of prednisone or equivalent; c. Steroids as premedication for hypersensitivity reactions (e.g., CT scan premedication)

- Prior organ transplantation including allogenic stem-cell transplantation;
- Vaccination within 4 weeks of the first dose of avelumab and while on trials is prohibited except for administration of inactivated vaccines;
- Known severe hypersensitivity reactions to monoclonal antibodies, any history of anaphylaxis, or uncontrolled asthma;
- Clinically significant (i.e., active) cardiovascular disease: cerebral vascular accident/stroke (< 6 months prior to enrollment), myocardial infarction (< 6 months prior to enrollment), unstable angina, congestive heart failure (≥ New York Heart Association Classification Class II), or serious cardiac arrhythmia requiring medication;
- Other severe acute or chronic medical conditions including immune colitis, inflammatory bowel disease, immune pneumonia, pulmonary fibrosis or psychiatric conditions including recent (within the past year) or active suicidal ideation or behavior; or laboratory abnormalities that may increase the risk associated with study participation or study treatment administration or may interfere with the interpretation of study results and, in the judgment of the investigator, would make the patient inappropriate for entry into this study;
- Persisting toxicity related to prior therapy (NCI CTCAE v. 4.03 Grade > 1); however, alopecia, sensory neuropathy Grade ≤ 2, or other Grade ≤ 2 not constituting a safety risk based on investigator's judgment are acceptable;
- Other co-existing malignancies or malignancies diagnosed within the last 5 years with the exception of localized basal and squamous cell carcinoma of the skin or cervical cancer in situ;
- Pregnant or lactating women;
- Any psychological, familial, sociological or geographical condition potentially hampering compliance with the study protocol and follow-up schedule.

Study treatment

Eligible patients will receive:

- **ARM A**: avelumab 10 mg/kg ev day 1
- **ARM B**: cetuximab 500 mg/m^2 plus avelumab 10 mg/kg ev day 1

In each arm the treatment will be repeated every 2 weeks (14 days), until progression of disease, refuse or inacceptable toxicity

Preliminary safety evaluation

A Data Monitoring Committee (DMC) will evaluate safety during the study.

Unblinded safety data will be reviewed by the DMC on a periodic basis, approximately every 3 months from the date of first-patient-in. In addition, the DMC will review safety data 28 days after the inclusion of the 6th patient in arm B. Safety data, including demographics, adverse events, serious adverse events, adverse events of special interest and relevant laboratory data, will be reviewed.

The DMC 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.

Study procedures overview

Procedures	Screening (within 28 days before enrollment)	Cycle 1 day 1[1]	Cycle n day 1[1,2]	Every 8wks	EOT[a]
Study Drug Administration[b]		X	X		
Cycle of therapy		1	n		
Informed consent	X				
History/Demographics	X				
Physical examination[c]	X	X	X		X
Inclusion/Exclusion Criteria checked	X				
HIV and HPV serology	X				
Concomitant Medications	X	X	X		
Adverse Event evaluation	X	x	X[d]	x	X[e]
ECOG PS	X	X	X		X
12-lead ECG	X				
Complete blood examination[f]	X	X[g]	x	X	X
Radiologic evaluation[h]	X			X	
Urine Pregnancy test	X	X	X[i]		
Collection of a paraffin-embedded tissue sample	X				X[j]
Collection of blood and urine samples[k]	X			X	
Survival follow up					X

1. Blood examination can be performed up to 72 hours before the administration of study treatment.
2. Treatment cycles to be administered every 2 weeks (14 days), starting cycle 1 day 1 until disease progression or treatment discontinuation; treatment delays may be indicated as per protocol.

a) End-of-Treatment (EOT) visit should be done 30 days (± 7 days) after the last dose of study drugs administration
b) Avelumab or avelumab plus cetuximab.
c) Including: height, weight,vital signs, blood pressure and heart rate.
d) AE assessment to be started after signing of IC (Informed Consent) until 30 days after last study treatment. Grading by CTCAE v 4.03.
e) Given the potential risk for delayed immune-related toxicities, safety follow-up must be performed up to 90 days after the last dose of avelumab administration. The extended safety follow-up beyond 30 days after last avelumab administration may be performed either via a site visit or via a telephone call with subsequent site visit requested in case any concerns noted during the telephone call.
f) Blood count and differential, bilirubin (total and direct), AST, ALT, alkaline phosphatase, albumin, LDH, serum creatinine, glucose, electrolytes (sodium, potassium, calcium, magnesium), International normalized ratio (INR)/Activated partial Thromboplastin Time (APTT) (only for patients on anticoagulation therapy, otherwise can be done at screening, every 8 wks, at EOT), TSH, fT3, fT4 (thyroid hormones can be done at screening, every 8 wks, at EOT), CEA (it can be done at screening, every 8 wks, at EOT).
g) If screening complete blood examination has been performed within 72 hours from starting of study treatment cycle 1 day 1, partial blood examination on cycle 1 day 1 can be avoided.
h) Collection of a copy of baseline CT scan (and/or abdomen MRI), digitally stored on CD-ROM, if baseline radiologic assessment performed outside participating centers.
i) Urine pregnancy test for women of childbearing potential must be performed at baseline and least every month during treatment
j) Encouraged, not mandatory biopsy at PD
k) Refer to procedures detailed in Appendix 16.7

Total number of centers

About 25 Italian Oncology Units.

Study length

Study length is planned to be about 30 months since the first patient in: enrollment is expected to be about 18 months, with a minimum period of follow-up of 12 months.

Enrollment and data management

Registration and data collection will be centralized at the U.O.Medical Oncology 1, Department of Clinical and Experimental Oncology, Veneto Oncology Institute (IOV-IRCCS).

16.2 Study synopsis (Italian Version)

Fase

Studio di fase II, randomizzato, prospettico

Popolazione di studio

Pazienti affetti da carcinoma squamoso del canale anale (SCCAC) non resecabile, localmente avanzato o metastatico, progredito dopo almeno una linea di trattamento sistemico

Razionale dello studio

- Il trattamento standard di prima linea per pazienti affetti da SCCAC è 5-FU in associazione a cisplatino. La sopravvivenza a 5 anni raggiunta con questo trattamento è del 32% circa (Faivre 1999). In una serie di casi di pazienti affetti da SCCAC la combinazione di 5-FU e cisplatino in prima linea ha prodotto un objective response rate (ORR) del 34.4% e un tasso di sopravvivenza a 5 anni del 15% (Sclafani 2017)
- Non sono disponibili trattamenti standard di seconda linea per pazienti affetti da SCCAC
- Cetuximab ed irinotecan in associazione hanno dimostrato risultati promettenti in pazienti pretrattati affetti da SCCAC (Lukan 2009). Inoltre, l'associazione di cisplatino e radioterapia è stata recentemente testata negli stadi I-III. Nonostante il mancato raggiungimento degli obiettivi prefissati, entrambi gli studi hanno evidenziato un'interessante attività di cetuximab nel controllo locale di malattia; questo ha portato ad ipotizzare che il farmaco potesse essere studiato in future strategie terapeutiche (Garg 2017, Sparano 2017)
- Farmaci anti-PD1 come nivolumab e pembrolizumab hanno dimostrato attività promettente in pazienti affetti da SCCAC metastatico e refrattario in termini di ORR e controllo di malattia con profili di tossicità accettabili (Morris 2017; Ott 2017)
- L'induzione di morte cellulare mediata da processi immunogenici è stata recentemente dimostrata in pazienti trattati con cetuximab (Pozzi 2016) ed il blocco di PD-L1 dovrebbe comportare un'attivazione delle cellule NK, aumentando la citotossicità mediata da anticorpo (ADCC) indotta da cetuximab (Concha-Benavente 2015, Concha-Benavente 2016)

Sulla base delle suddette considerazioni, è stato disegnato il presente studio randomizzato di fase II con avelumab in monoterapia o avelumab e cetuximab per pazienti affetti da SCCAC pretrattati, non resecabili, localmente avanzati o metastatici.

Obiettivo primario

Studiare l'attività di avelumab in monoterapia o in combinazione con cetuximab in pazienti con SCCAC avanzato

Obiettivi secondari

- Stima della progression free survival (PFS)
- Stima della overall survival (OS)
- Descrivere il profilo di sicurezza della monoterapia e della combinazione
- Raccogliere materiale tumorale d'archivio, campioni ematici e campioni tumorali di nuova acquisizione (opzionali) per analisi traslazionali

Definizione dell'obiettivo primario

L'obiettivo primario è l'ORR. ORR è definito come la percentuale di pazienti, relativi al totale dei soggetti arruolati con malattia misurabile che raggiungono una risposta completa (CR) o parziale (PR), secondo i criteri Response Evaluation Criteria In Solid Tumors (RECIST) v1.1. La determinazione della risposta radiologica sarà basata sulle informazioni riportate dallo sperimentatore. Le risposte radiologiche saranno valutate ogni 8 settimane iniziando dal ciclo 1 giorno 1 di trattamento fino a progressione di malattia, ritiro del consenso, o morte dovuta a qualsiasi causa, a seconda di quale si verifichi prima.

Definizione degli obiettivi secondari

La PFS è definita come il tempo che intercorre tra l'arruolamento nello studio e la prima documentazione di progressione obiettiva di malattia o morte dovuta a qualsiasi causa, a seconda di quale si verifichi prima. La progressione di malattia è da definirsi secondo i criteri RECIST v1.1, basandosi sulle valutazioni dello sperimentatore. Il dato di PFS sarà censorizzato al momento dell'ultima rivalutazione in cui verrà documentata assenza di progressione di malattia per i pazienti vivi e liberi da progressione al tempo dell'analisi. Per i pazienti vivi che non avranno eseguito una rivalutazione successiva al basale, il dato di PFS sarà censorizzato alla data di arruolamento nello studio.

- OS è definita come il tempo che intercorre tra l'arruolamento e la data di morte dovuta a qualsiasi causa. Per i pazienti in vita al momento dell'analisi, la sopravvivenza sarà censorizzata all'ultima data in cui si è a conoscenza del loro stato in vita

- il tasso di tossicità globale è definito come la percentuale di pazienti, tra quelli che avranno ricevuto almeno una dose di trattamento, che abbia sviluppato un qualsiasi evento avverso di grado 3-4 secondo

il NCI CTCAE v4.03. Il tasso di tossicità è definito come la percentuale di pazienti che abbiano sviluppato un qualsiasi evento avverso di grado 3-4 secondo il NCI CTCAE v4.03.

Considerazioni Statistiche

Il Simon's two-stage Mini-Max design (Simon, 1989) sarà utilizzato in parallelo per entrambi i bracci di trattamento.

L'ipotesi nulla, che il response rate sia del 5% [p0 = 0.05], sarà testata mediante one-sided test contro un'ipotesi alternativa di un response rate del 20% [p1 = 0.20]. Il tasso di errore di tipo I è stato definito al 0.05 e la potenza all'80%. Per ciascuno dei due bracci, saranno arruolati 13 pazienti. Se non si verificheranno risposte nei 13 pazienti, lo studio sarà interrotto. Altrimenti, altri 14 pazienti saranno arruolati per un totale di 27 pazienti. Non è permesso alcun confronto formale tra i risultati dei due bracci di trattamento.

Criteri di inclusione

- Diagnosi istologica di SCCAC;
- Progressione durante o dopo terapia di prima linea sistemica per malattia non resecabile chirurgicamente o metastatica. La chemioterapia sistemica radiosensibilizzante con intento curativo per malattia in stadio limitato, dovrà essere considerata come una prima linea di trattamento per i pazienti che progrediscano durante o entro i 6 mesi dalla fine del trattamento;
- Malattia misurabile secondo i criteri RECIST v1.1
- Disponibilità di materiale tumorale d'archivio (primitivo e/o metastasi);
- Età ≥ 18 anni;
- Eastern Cooperative Oncology Group – Performance Status (ECOG PS) ≤ 2;
- Aspettativa di vita di almeno 12 settimane
- Parametri di laboratorio:
    - Neutrofili ≥ 1.5 x 10^9/L;
    - Piastrine ≥ 100 x 10^9/L;
    - Emoglobina ≥ 9 g/dL;
    - Bilirubina totale ≤ 1.5 volte il limite superiore della norma (UNL) dei valori normali e ASAT (SGOT) e/o ALAT (SGPT) ≤ 2.5 x UNL (o <5 x UNL nel caso di metastasi epatiche);
    - Fosfatasi alcalina ≤ 2.5 x UNL (o <5 x UNL nel caso di metastasi epatiche);
    - Clearance della creatinina ≥ 50 mL/min o creatinina sierica ≤1.5 x UNL;

- I pazienti HIV-positivi sono eleggibili se la loro conta di cellule $CD4^+$ è pari o superiore a 300 cellule per µL; il titolo virale deve'essere non rilevabile; i pazienti devono inoltre seguire scrupolosamente il trattamento con farmaci antiretrovirali;
- Test di gravidanza su sangue o urine al baseline per donne potenzialmente fertili. I soggetti di sesso femminile, o maschile con partner di sesso femminile potenzialmente fertile, devono essere disposti a utilizzare adeguate misure contraccettive tra quelle proposte dallo sperimentatore (ad es. contraccettivo di barriera o a somministrazione orale o astinenza) durante lo studio e fino a 6 mesi in seguito all'ultima somministrazione;
- Firma del consenso informato alle procedure e alle analisi molecolari prima della registrazione dei pazienti nello studio
- Volontà e capacita di seguire le procedure del protocollo

Criteri di esclusione

- Precedente terapia con un farmaco diretto a proteine co-regolatrici delle cellule T (ad. Es. inibitori di checkpoint immunitari);
- Trattamento anti neoplastico o uso di altre sostanze in sperimentazione fino a 28 giorni prima dell'inizio del trattamento del trial;
- Intervento chirurgico, open biopsy, o trauma maggiore fino a 28 giorni prima dell'inizio del trattamento dello studio, o intervento chirurgico programmato durante lo svolgimento dello studio;
- Storia o riscontro all'esame obiettivo di metastasi encefaliche non adeguatamente trattate. I pazienti con metastasi encefaliche trattate sono eleggibili se le loro lesioni sono stabili e asintomatiche da almeno 3 mesi;
- Neutrofili < 1.5 x 10^9/L; piastrine < 100 x 10^9/L; emoglobina < 9 g/dL;
- Infezione attiva non controllata o altre malattie concomitanti clinicamente rilevanti che pongano controindicazione alla somministrazione della terapia o che espongano il paziente ad alto rischio di sviluppare tossicità trattamento-correlate;
- Infezione da virus dell'epatite B (HBV) o C (HCV) allo screening (positività dell'antigene di superficie dell'HBV o riscontro di HCV RNA se positività al test di screening degli anticorpi anti-HCV)
- Pazienti con malattie autoimmuni attive o storia di malattie autoimmuni le cui condizioni possano peggiorare in corso di terapia con agenti immunostimolanti o che possano ripresentarsi con potenziale coinvolgimento di organi vitali o che richiedano l'uso di trattamenti immunosoppressivi inclusi corticosteroidi sistemici in cronico o per un periodo prolungato

(definito nel caso di assunzione di corticosteroidi ≥ 1 mese). Pazienti con diabete mellito di tipo 1, vitiligine, psoriasi, o ipo/ipertiroidismo che non richiedano trattamenti immunosoppressivi sono eleggibili;

- Utilizzo di farmaci immunosoppressori, AD ECCEZIONE dei seguenti: a. Farmaci steroidei topici, intranasali o a inalazione, o iniezione localizzata di steroidei (ad es. iniezione intra-articolare); b. Corticosteroidi a dose fisiologica ≤ 10 mg/die di prednisone o equivalenti; c. Farmaci steroidei come premedicazione per reazioni di ipersensibilità (ad es. premedicazione per CT);
- Pregresso trapianto di organi incluso trapianto di cellule staminali allogeniche;
- La vaccinazione entro le 4 settimane precedenti la prima somministrazione di avelumab è proibita eccetto per somministrazione di vaccini inattivati;
- Pregresse reazioni di severa ipersensibilità a anticorpi monoclonali, storia di anafilassi o asma non controllata;
- Pazienti con insufficienza cardiaca clinicamente significativa: eventi cerebrovascolari/stroke (< 6 mesi prima dell'arruolamento), infarto miocardico (< 6 mesi prima dell'arruolamento), angina instabile, scompenso cardiaco congestizio (≥ classe II sec. New York Heart Association Classification), o aritmia cardiaca grave in terapia farmacologica;
- Altre condizioni grave acute o croniche incluse colite autoimmune, malattie infiammatorie croniche intestinali, polmoniti autoimmuni, fibrosi polmonare o condizioni psichiatriche incluse ideazioni suicidarie o comportamento suicidario recenti (entro l'ultimo anno); o anomalie laboratoristiche che possano aumentare il rischio associato alla partecipazione allo studio o alla somministrazione dei farmaci in studio o che possano aumentare il rischio associato alla partecipazione allo studio e, a giudizio dello sperimentatore, possano rendere il paziente inappropriato ad entrare in questo studio;
- Tossicità persistenti correlate alle precedenti terapie (NCI CTCAE v. 4.03 Grade > 1); comunque, sono accettabili alopecia, neuropatie sensitive di grado ≤ 2, o altre tossicità di grado ≤ 2 che non costituiscano un rischio per il paziente a giudizio dello sperimentatore;
- Altre neoplasie maligne coesistenti o diagnosticate entro gli ultimi 5 anni ad eccezione del carcinoma a cellule squamose della cute localizzato o del cancro della cervice *in situ*
- Donne incinte o in allattamento;
- Qualsiasi condizione di tipo psicologico, familiare, sociale o geografica che possa potenzialmente compromettere la compliance del soggetto col protocollo e con la schedula del follow-up.

Trattamento dello studio

I pazienti eleggibili saranno trattati con:

- **BRACCIO A**: avelumab 10 mg/kg ev giorno 1
- **BRACCIO B**: cetuximab 500 mg/m^2 più avelumab 10 mg/kg ev giorno 1

In ogni braccio il trattamento sarà ripetuto ogni 2 settimane (14 giorni) fino a progressione di malattia, rifiuto del paziente o tossicità non accettabili

Valutazione del profilo di sicurezza preliminare

Un Comitato di Data Monitoring (DMC) valuterà la sicurezza durante lo studio.

I dati di sicurezza in aperto saranno revisionati dal DMC periodicamente, approssimativamente ogni 3 mesi dalla data dell'arruolamento del primo paziente. Inoltre, il DMC revisionerà i dati relativi alla sicurezza 28 giorni dopo l'inclusione del sesto paziente nel Braccio B. Inoltre saranno revisionati dati di sicurezza, inclusi dati anagrafici, eventi avversi, eventi avversi seri, eventi avversi di particolare interesse e dati rilevanti di laboratorio.

Il DMC stabilirà l'indicazione a proseguire lo studio, ad implementare il protocollo con emendamenti o ad interrompere lo studio. La decisione finale sarà di competenza dello Sponsor.

Procedure dello studio

Procedure	Screening (entro i 28 giorni precenti l'arruolamento)	Ciclo 1 giorno 1[1]	Ciclo n giorno 1[1,2]	Ogni 8 settimane	EOT[a]
Somministrazione dei farmaci in studio[b]		X	X		
Ciclo di terapia		1	n		
Consenso Informato	X				
Anamnesi/ Anagrafica	X				
Physical examination [c]	X	X	X		X
Controllo Criteri Inclusione/Esclusione	X				
Sierologia HIV and HPV	X				
Farmaci Concomitanti	X	X	X		
Valutazione degli Eventi Avversi	X	X	X[d]		X[e]
ECOG PS	X	X	X		X
ECG a 12-derivazioni	X				
Esami del sangue completi[f]	X	X[g]	X	X	X
Valutazione Radiologica[h]	X			X	
Test di gravidanza sulle urine	X	X	X[i]		
Raccolta di materiale tumorale (Blocchetto)	X				X[j]
Raccolta di campioni ematici ed urine[k]	X			X	
Survival follow up					X

1. Gli esami del sangue possono essere eseguiti fino a 72 ore prima della somministrazione del trattamento.
2. Cicli di trattamento da somministrare ogni 2 settimane (14 giorni), iniziando dal ciclo 1 giorno 1 fino a progressione di malattia o interruzione del trattamento; il trattamento può essere rinviato come descritto nel protocollo.

a) La visita di fine trattamento (End-of-treatment, EOT) dovrà essere eseguita 30 giorni (± 7 giorni) dopo la somministrazione dell'ultima dose del farmaco in studio
b) Avelumab o avelumab e cetuximab.
c) Includendo: altezza, peso, segni vitali, pressione sanguigna e frequenza cardiaca.
d) La valutazione degli AE deve essere iniziata dopo la firma dell'IC (consenso informato) fino a 30 giorni dopo l'ultimo trattamento. Grading secondo CTCAE v 4.03.
e) Dato il potenziale rischio di tossicità immuno-correlate retardate, il safety follow up deve essere effettuato fino a 90 giorni dopo la somministrazione dell'ultima dose di avelumab. Il safety follow-up esteso oltre 30 giorni dopo la somministrazione dell'ultima dose di avelumab può esere effettuato o tramite una visita presso il centro o tramite un contatto telefonico con successiva visita presso il centro richiesta in caso di problematiche emerse durante il contatto telefonico.
f) Emocromo con formula, bilirubina (totale e diretta), AST, ALT, fosfatasi alcalina, albumina, LDH, creatinina sierica, glucosio, elettroliti (sodio, potassio, calcio, magnesio), International normalized ratio (INR)/Activated partial Thromboplastin Time (APTT) (solo per pazienti in terapia con anticoagulanti, altrimenti può essere eseguita allo screening, ogni 8 settimane e all'EOT) TSH, fT3, fT4 (ormoni tiroidei possono essere testati allo screening, ogni 8 settimane e all'EOT), CEA (può essere testato allo screening, ogni 8 settimane e all'EOT)
g) Se lo screening completo delle analisi del sangue è stato eseguito entro 72 ore dall'inizio del trattamento (ciclo 1 giorno 1), le analisi del sangue parziali al ciclo 1 giorno 1 possono essere omesse.
h) Acquisizione di una copia della TC basale (e/o RM addome), su CD-ROM, se la valutazione radiologica al basale è stata fatta al di fuori dei centri partecipanti.
i) Un test di gravidanza sulle urine per donne potenzialmente fertili deve essere effettuato al baseline e almeno ogni mese durante il trattamento
j) La biopsia a PD è incoraggiata, non obbligatoria
k) Le procedure sono dettagliate nella Appendix 16.7

Numero di centri coinvolti

Circa 25 Unità di Oncologia italiane

Durata dello studio

La durata dello studio è stata programmata per 30 mesi a partire dal primo paziente arruolato: è previsto un arruolamento della durata di 18 mesi, con un periodo minimo di follow-up di 12 mesi

Arruolamento e data management

La registrazione e la raccolta dei dati sarà centralizzata presso la UO di Oncologia Medica 1 dell'Istituto Oncologico Veneto (IOV) IRCCS

16.3 RECIST 1.1

Response and progression will be evaluated in this study using the RECIST criteria version 1.1. Changes in only the largest diameter (unidimensional measurement) of the tumor lesions are used.

Measurable Disease

Tumor lesions: Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) with a minimum size of:

- 10 mm by CT scan (CT scan slice thickness no greater than 5 mm) or MRI. If scans with slice thicknesses greater than 5mm are used, the minimum size should be twice the slice thickness;
- 20 mm by chest x-ray;
- 10 mm caliper measurement by clinical examination (lesions which cannot be accurately measured with calipers should be recorded as non-measurable).

Malignant lymph nodes: To be considered pathologically enlarged and measurable, a lymph node must be ≥ 15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

Lytic bone lesions or mixed lytic-blastic lesions: with identifiable soft tissue components that can be evaluated by CT or MRI, can be considered as measurable lesions if the soft tissue component meets the definition of measurability. All tumor measurements must be recorded in millimetres (or decimal fractions of centimetres). Tumor lesions situated in a previously irradiated area are not considered measurable unless there has been demonstrated progression in the lesion.

Non-Measurable Disease

All other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥10 to < 15mm short axis) are considered non-measurable disease. Leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitic involvement of skin or

lung, inflammatory breast disease, abdominal masses/ abdominal organomegaly identified by physical examination that is not measurable by reproducible imaging techniques and blastic bone lesions are all non-measurable.

Target Lesions: All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs should be identified as *target lesions* and be recorded and measured at baseline. These 5 lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs and should be suitable for reproducible repeated measurements. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for *all target lesions* will be calculated and reported as the baseline sum diameters. The baseline sum diameters will be used as reference to further characterize any objective tumor regression of the measurable dimension of the disease. If there are >5 measurable lesions, those not selected as *target lesions* will be considered together with non-measurable disease as *nontarget lesions*.

Non-target Lesions: All non-measurable lesions (or sites of disease) plus any measurable lesions over and above the 5 listed as *target lesions*. Measurements are not required but these lesions should be noted at baseline and should be followed as "present", "absent" or in rare cases "unequivocal progression".

Best Response: All subjects will have their BEST RESPONSE on study classified as outlined below:

- *Complete Response* (CR): Disappearance of all clinical and radiological evidence of tumor (both target and non-target). Any pathological lymph nodes (whether target or non target) must have a reduction in short axis to < 10mm.
- *Partial Response* (PR): At least a 30% decrease in the sum of diameters of target lesions taking as reference the baseline sum, no unequivocal progression of existing non target lesions and no appearance of new lesions.
- *Stable Disease* (SD): Steady state of disease. Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, no unequivocal progression of existing non target lesions and no appearance of new lesions.
- *Progressive Disease* (PD): At least a 20% increase in the sum of diameters of target lesions taking as reference the smallest sum on study (this includes the baseline sum if that is the

smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5mm. Unequivocal progression of existing non target lesions or the appearance of one or more new lesions will also constitute progressive disease.

Table 4

Response for patients with Target and Non-Target Lesions

Target Lesions	Non-Target Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Non-CR/Non-PD	No	PR
CR	Notevaluated	No	PR
PR	Non-PD or notallevaluated	No	PR
SD	Non-PD or notallevaluated	No	SD
PD	Any	Yes or No	PD
Any	Any	Yes	PD
Any	PD	Yes orNo	PD

Table 5		
Response for patients with Non-Target Lesions only		
Non-Target Lesions	**New Lesions**	**Overall Response**
CR	No	CR
Non-CR/Non-PD	No	Non-CR/non-PD*
Not evaluated	No	NE
UnequivocalPD	Yes orNo	PD
Any	Yes	PD

* Non-CR/non-PD is preferred over "stable disease" for non-target disease.

Methods of Measurement - The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up.

Clinical Lesions - Clinical lesions will only be considered measurable when they are superficial (e.g. skin nodules, palpable lymph nodes) and ≥ 10mm diameter as assessed using calipers. For the case of skin lesions, documentation by colour photography including a ruler to estimate the size of the lesion is recommended.

Chest X-ray - Lesions on chest X-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, chest CT is preferable.

CT / MRI - CT is the best currently available and reproducible methods to measure target lesions selected for response assessment. CT scans should be performed with cuts of 5 mm or less in slice thickness. When CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable. This applies to the chest, abdomen and pelvis. Head & neck and extremities usually require specific protocols.

Ultrasound - Ultrasound is not useful in assessment of lesion size and should not be used as method of measurement. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised.

Endoscopy / Laparoscopy - The utilization of these techniques for objective tumor evaluation is not advised.

Cytology / Histology - These techniques can be used to differentiate between PR and CR in rare cases (for example, residual lesions in tumor types such as germ cell tumors, where known residual benign tumors can remain).

The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

16.4 NCI Common Terminology Criteria for Adverse Events

This study will utilize the NCI Common Terminology Criteria for Adverse Events Version 4.0 for toxicity and serious adverse event reporting. A copy of the CTC Version 4.0 can be downloaded from the CTEP home page:

http/ctep.cancer.gov:protocolDevelopment:electronic_applications:ctc.htm - ctc_40)

All appropriate treatment areas should have access to a copy of the CTC Version 4.0.

16.5 ECOG

Table 6	
ECOG PERFORMANCE STATUS	**KARNOFSKY PERFORMANCE STATUS**
0 - Fully active, able to carry on all pre-disease performance without restriction	100 - Normal, no complaints; no evidence of disease 90 - Able to carry on normal activity; minor signs or symptoms of disease
1- Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work	80 - Normal activity with effort, some signs or symptoms of disease 70 - Cares for self but unable to carry on normal activity or to do active work
2 - Ambulatory and capable of all selfcare but unable to carry out any work activities; up and about more than 50% of waking hours	60 - Requires occasional assistance but is able to care for most of personal needs 50 - Requires considerable assistance and frequent medical care
3 - Capable of only limited selfcare; confined to bed or chair more than 50% of waking hours	40 - Disabled; requires special care and assistance 30 - Severely disabled; hospitalization is indicated although death not imminent
4 - Completely disabled; cannot carry on any selfcare; totally confined to bed or chair	20 - Very ill; hospitalization and active supportive care necessary 10 - Moribund
5 - Dead	0 - Dead

16.6 Risk Assessment (Italian Version)

Il presente studio ha l'obiettivo di valutare l'attività di avelumab in monoterapia o in combinazione con cetuximab in pazienti con carcinoma squamoso del canale anale (SCCAC) avanzato.

La prognosi dei pazienti in progressione dopo la prima linea di terapia rimane infausta, attestandosi sotto i 9 mesi se non trattati. Inoltre, purtroppo non esistono terapie standard per lo SCCAC a fallimento della prima linea di trattamento.

L'anticorpo monoclonale anti-EGFR cetuximab ha dimostrato attività promettente in alcuni case report e case series di pazienti affetti da SCCAC avanzato progrediti dopo almeno una linea di terapia, mostrando un profilo di tossicità favorevole (nessun evento avverso di grado 3 o 4 osservato).

Gli anticorpi monoclonali diretti contro PD-1 pembrolizumab e nivolumab, recentemente testati nei pazienti affetti da SCCAC in linee di trattamento successive alla prima, hanno raggiunto tassi di risposte obiettive notevoli. Le tossicità riportate nella maggior parte dei casi sono state di grado lieve-moderato. In particolare non sono state osservate significative differenze tra pazienti HIV negativi e pazienti affetti da HIV trattati con nivolumab.

Avelumab è un anticorpo monoclonale anti PD-L1 approvato per il trattamento del carcinoma a cellule di Merkel e recentemente testato nel carcinoma del polmone non a piccole cellule. Gli eventi avversi descritti in questi pazienti sono stati prevalentemente di grado lieve-moderato, immunocorrelati e reversibili tramite l'utilizzo di farmaci immunosoppressori e/o con l'interruzione di avelumab.

Lo scopo dello studio CARACAS è di testare l'anti PD-L1 avelumab in monoterapia e l'associazione di avelumab e dell'anti-EGFR cetuximab nel trattamento dei pazienti affetti da SCCAC avanzato in progressione dopo almeno una linea di trattamento.

Considerando il profilo di tossicità di avelumab e di cetuximab, la prognosi dei pazienti con carcinoma squamoso del canale anale dopo la I linea di terapia, l'assenza di trattamenti standard e i risultati in termini di risposta e sopravvivenza raggiunti con l'utilizzo di cetuximab o dell'immunoterapia in questi pazienti, il rapporto costo – beneficio appare propendere a favore dell'immunoterapia o di cetuximab. Non sono disponibili dati circa l'associazione di cetuximab e avelumab per questa patologia. I pazienti verranno costantemente monitorizzati al fine di

individuare e trattare precocemente l'insorgenza di eventi avversi, e di effettuare una corretta analisi del rapporto costo – beneficio della terapia con avelumab con e senza cetuximab.

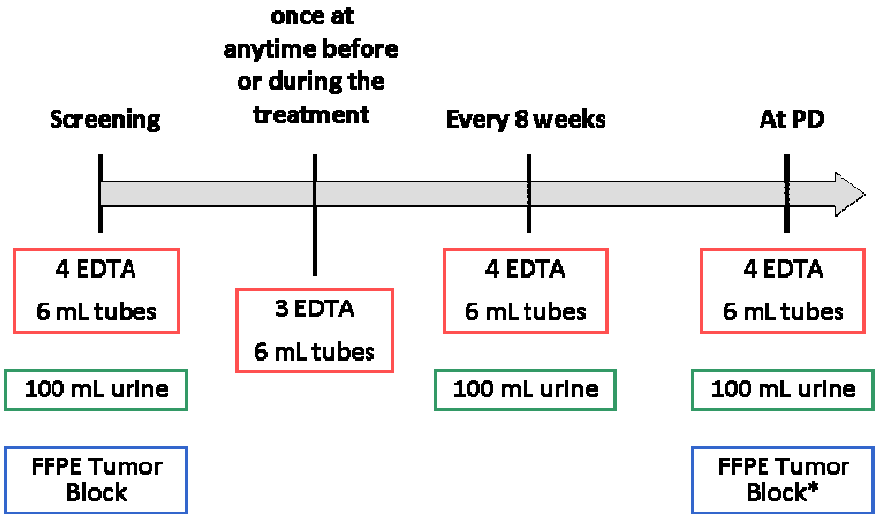
Di seguito i riferimenti scientifici principali:

- Lukan N et al. Cetuximab-based treatment of metastatic anal cancer: correlation of response with KRAS mutational status. Oncology. 2009;77(5):293-9
- Casadei Gardini A et al. (2014) KRAS, BRAF and PIK3CA Status in Squamous Cell Anal Carcinoma (SCAC). PLoS ONE 9(3): e92071
- Ott PA et al. Safety and antitumor activity of the anti-PD-1 antibody pembrolizumab in patients with recurrent carcinoma of the anal canal. Ann Oncol. 2017;28(5):1036-1041.
- Morris VK et al. Nivolumab for previously treated unresectable metastatic anal cancer (NCI9673): a multicentre, single-arm, phase 2 study. Lancet Oncol 2017;18(4):446-453
- Gulley L et al. Avelumab for patients with previously treated metastatic or recurrent non-small-cell lung cancer (JAVELIN Solid Tumor): dose-expansion cohort of a multicentre, open-label, phase 1b trial. Lancet Oncol 2017;18(5):599-610

16.7 Samples Collection

16.7.1. Biological samples overview

A scheme of the biological samples collected during the study is described below.



* if an additional biopsy is performed at PD (encouraged, not mandatory)

16.7.2. Tissue specimens collection

The collection of tissue specimens is mandatory for study entry.

A formalin-fixed paraffin-embedded tumor block, together with the accompanying histological report from primary tumor and/or metastasis is required and must be sent to the Coordinating Centre (UOC Oncologia Medica 1, Dipartimento di Oncologia Clinica e Sperimentale, Istituto Oncologico Veneto (IOV) IRCCS) during the Screening phase.

In the case in which an additional biopsy is performed at PD (encouraged, not mandatory) a formalin-fixed paraffin-embedded tumor block, together with the accompanying histological report from must be sent to the Coordinating Centre.

The shipment will be arranged by the Sponsor.

16.7.3. Blood sampling collection

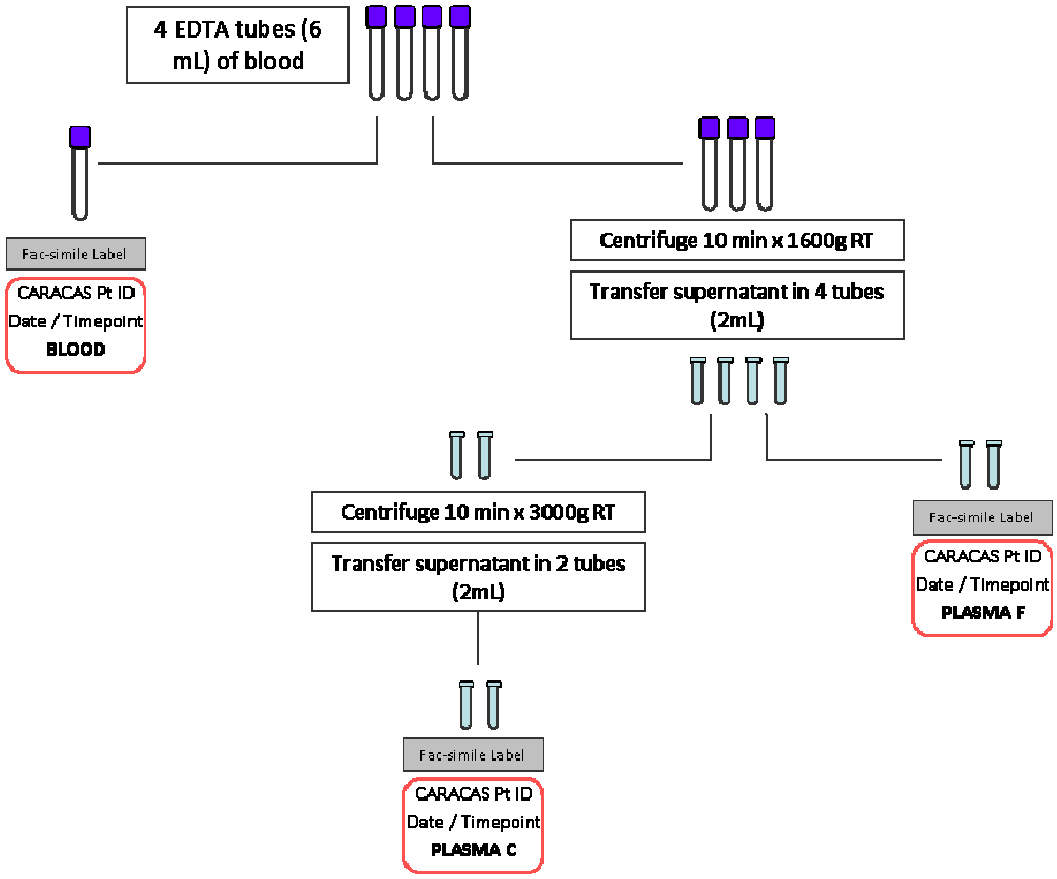
Four 6 mL EDTA tubes will be collected at the following timepoints:

- at Screening;
- during treatment, every 8-weeks;
- at the first evidence of PD.

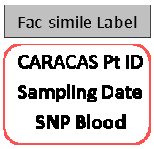
Blood samples will be processed as follows:

- Label one tube EDTA tube as "CARACAS - Patient Code/ Date/Timepoint /**Blood**" (see fac-simile) and store at -80°C until shipment to the Coordinating Center;
- Centrifuge the three additional tubes as soon as possible (1600g x 10 minutes, room temperature);
- Collect the plasma supernatant and divide it onto four aliquots;
- Label two plasma aliquots as "CARACAS - Patient Code/Date/Timepoint/**Plasma F**" (see fac-simile) and store at -80°C until shipment to the Coordinating Center;
- Centrifuge the other 2 plasma aliquots (3000 g x 10 minutes, room temperature);
- Collect the supernatant in tubes and label as "CARACAS - Patient Code/Date/Timepoint/ **Plasma C**" (see fac-simile) and stored at -80°C until shipment to the Coordinating Center.

Lonardi S, *et al. J Immunother Cancer* 2021; 9:e002996. doi: 10.1136/jitc-2021-002996



In addition, three 6 mL EDTA tubes will be collected once at anytime before or during the treatment. They will be labeled as "CARACAS-Patient Code/ Date/**SNP Blood**"(see fac-simile) and will be stored at -20°C until shipment to the Coordinating Center.



The shipment will be arranged by the Sponsor who will provide the dry ice.

16.7.4. Urine samples collection

Urine samples will be collected at the following study timepoints:

- at Screening;
- during treatment, every 8-weeks;
- at the first evidence of PD.

Tubes labeled as "CARACAS - Patient Code/ Date/Timepoint /**Urine"** containing 100 mL of urine will be collected, 10 mL of EDTA 0.5 M solution will be added to each sample and the tubes will be stored at -80°C until shipment to the Coordinating Center.
The shipment will be arranged by the Sponsor who will provide the dry ice.
The EDTA 0.5 M solution will be provided by the Sponsor.

16.7.5. Collection of CT scan images

Tumor response will be assessed by means of contrast-enhanced chest and abdomen CT scans with a contiguous slice thickness of ≤ 7mm, that will be performed in the radiology department of the study site. Abdomen MRI and chest CT scan are allowed in the case of contraindications to the use of iodine contrast agents.
In the case of clinical suspicion of disease progression, the radiographic evaluation should be performed within a maximum of 7 days to confirm objective disease progression.
CD-ROM copies of the CT scans at screening, at the time of the best response during the treatment, at the time of the evidence of PD will be collected at the Coordinating Center (UOC Oncologia Medica 1, Dipartimento di Oncologia Clinica e Sperimentale, Istituto Oncologico Veneto (IOV) IRCCS) for central review.
Site should follow their local privacy practices to de-identify all subject identifying
information (name, medical record number, ect.) prior to submitting images to Coordinating Center.