GRUPO ESPAÑOL MULTIDISCIPLINAR
EN CÁNCER DIGESTIVO

Clinical Study Report

A single arm Phase I-II multicenter trial with avelumab plus autologous dendritic cell vaccine to determine safety and preliminary efficacy of the combination in pre-treated mismatch repair-proficient (MSS) metastatic colorectal cancer patients.

Protocol Number: GEMCAD-1602 (AVEVAC)

EudraCT Number: 2016-003838-24

Clinicaltrials.gov number: NCT03152565

19/MAR/2021

CONFIDENTIAL

Signature pages for clinical study report

I have read this report and confirm that to the best of my knowledge it accurately describes the conduct and results of the study.

Signed: Date: ____/____/_____

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1. TITLE PAGE

Study title: A single arm Phase I-II multicenter trial with avelumab plus autologous dendritic cell vaccine to determine safety and preliminary efficacy of the combination in pre-treated mismatch repair-proficient (MSS) metastatic colorectal cancer patients.

Name of test drug: ADC vaccine and Avelumab

Indication studied: Pre-treated mismatch repair-proficient (MSS) metastatic colorectal cancer patients.

Study description: Single arm Phase I/II multicentric open labeled, with translational sub-study, of avelumab plus autologous dendritic cell vaccine in pre-treated mismatch repair-proficient (MSS) metastatic colorectal cancer patients. 5 Spanish Centers included patients in the study.

All subjects recruited will be administered with the following scheme:

- ADC vaccine: A dose of ADC at days 1, 14, 28, 42 and 56 (total of 5 doses), and thereafter every 6 months until disease progression (maximum of 6 additional doses) or unacceptable toxicity.
- Avelumab will be administered intravenously at a dose of 10 mg per kilogram of body weight, every 14 days until disease progression or unacceptable toxicity.

During the trial, subjects underwent follow up visits weekly during the first month of treatment and every 2 weeks thereafter.

Tumor response evaluation [through the revised response evaluation criteria in solid tumors (RECIST 1.1)], every 8 weeks (2 months) until disease progression.

Toxicity was recorded in every visit using the last version of NCI-CTCAE criteria.

A translational substudy was performed in parallel, biological samples were collected at screening period (<28d) and 2 months 60 ml of blood (50 ml for PBMC and 10 ml for serum at -80 ºC).

MSS, RAS and BRAF status from archival biopsies were determined. Biopsies before study entry and at 2 months of therapy were obtained, to evaluate pharmacodynamics changes.

Sponsors: Grupo Español Multidisciplinar en Cáncer Digestivo (GEMCAD)

Protocol Code: GEMCAD-1602 (AVEVAC)

Clinical Phase: I/II

Study dates:

Study initiation date: March 12th, 2018

First patient enrolled on: March 22nd, 2018

Last patient completed on: March 28th, 2019

Study completion date: September 15th, 2020

Coordinating investigators:

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GCP statement: This study was performed in compliance with ICH Good Clinical Practice (GCP), including the archiving of essential documents

Date of report: 19/MAR/2021

2. SYNOPSIS

NAME OF SPONSOR: Grupo Español Multidisciplinar de Investigación en Cáncer Digestivo (GEMCAD) **NAME OF FINISHED PRODUCT:** N/A **NAME OF ACTIVE INGREDIENT(S):** Avelumab + autologous dendritic cell vaccine	
Title of study	A single arm Phase I-II multicenter trial with avelumab plus autologous dendritic cell vaccine to determine safety and preliminary efficacy of the combination in pre-treated mismatch repair-proficient (MSS) metastatic colorectal cancer patients
Investigator(s)	1. Dr. Joan Maurel Santasusana 2. Dra. Elena Élez Fernández 3. Dra. Ana Isabel Ruiz Casado 4. Dr. Carlos Fernández Martos/ Dr. Ricardo Yaya Tur 5. Dr. Vicente Alonso Orduña 6. Dra. Pilar Escudero Emperador 7. Dr. Jorge Aparicio Urtasun 8. Dra. Nuria Rodríguez Salas
Study centre(s)	1. Hospital Clínic 2. Hospital Universitari Vall d'Hebron 3. Hospital Universitario Puerta de Hierro 4. Instituto Valenciano de Oncología 5. Hospital Universitario Miguel Servet 6. Hospital Clínico Lozano Blesa 7. Hospital Universitari i Politécnic La Fe 8. Hospital Universitario La Paz
Publication	***Publication in congress:*** 611TiP - Poster Display Session ESMO 2018: M. Español Rego, V. Alonso, J. Aparicio, E. Elez Fernandez, P. Escudero, C. Fernández-Martos, N. Rodríguez, A. Ruiz Casado, J. Cid, R. Cabezón, M. Lozano, A. Ginés, L. Bianchi, J. Garcia-Corbacho, X. García de Albéniz, J. Maurel, D. Benitez Ribas. AVEVAC. A PHASE I-II TRIAL

	WITH AVELUMAB PLUS AUTOLOGOUS DENDRITIC CELL (ADC) VACCINE IN PRE-TREATED MISMATCH REPAIR-PROFICIENT (MSS) METASTATIC COLORECTAL CANCER (MCRC) PATIENTS. (GEMCAD 16-02). Annals of Oncology (2018) 29 (suppl_8): viii150-viii 204. 10.1093/annonc/mdy281 *Final publication of the trial is currently ongoing.*	
Study period	Study initiation date: March 12th, 2018 First patient enrolled on: March 22nd, 2018 Last patient completed on: March 28th, 2019 Study completion date: September 15th, 2020	**Phase of development** Phase I/II
Objectives	**<u>Phase I</u>** **Primary:** To determine the recommended phase II dose (RP2D) of avelumab in combination with ADC vaccine in previously treated MSS CRC patients who have progressed at least to 2 chemotherapy lines. **Secondary:** To evaluate the safety and tolerability of avelumab in combination with ADC vaccine. **<u>Phase II</u>** **Primary:** To increase the percentage (from 20% to 40%) of pre-treated MSS mCRC patients free of progression at 6 months. **Secondary:** 1. To evaluate the safety and tolerability of avelumab in combination with ADC vaccine. 2. To identify a favourable phenotype for efficacy. 3. To evaluate pharmacodynamic changes between pre-treatment and on-treatment tumour biopsies. ◦ Modified CMS classification by NanoString. ◦ Immunophenotype signature by NanoString.	

Methodology	Single arm Phase I/II multicentric open labeled, with translational sub-study, of avelumab plus autologous dendritic cell vaccine in pre-treated mismatch repair-proficient (MSS) metastatic colorectal cancer patients in 8 Spanish Centers. Archival formalin-fixed paraffin-embedded (FFPE) tumour samples were used for central determination of MSS status, RAS and BRAF. Local determinations were also recorded when available. All the enrolled patients will receive the following treatment schedule: ● ADC vaccine: A dose of ADC at days 1, 14, 28, 42 and 56 (total of 5 doses), and thereafter every 6 months until disease progression (maximum of 6 additional doses) or unacceptable toxicity. ● Avelumab will be administered intravenously at a dose of 10 mg per kilogram of body weight, every 14 days until disease progression or unacceptable toxicity. During the trial, subjects underwent follow up visits weekly during the first month of treatment and every 2 weeks thereafter. Patients will remain on trial for a minimum of 18 months of follow-up. Tumor response evaluation was evaluated every 8 weeks (2 months) until disease progression through the revised response evaluation criteria in solid tumors (RECIST 1.1). Toxicity was recorded in every visit using version 4.03 of NCI-CTCAE criteria. The translational study was aimed to answer the need to identify biomarkers of response and to better understand pharmacodynamics changes before and after therapy, biological samples (both tumor and blood) were collected before and after 8 weeks of treatment initiation. A section of the formalin-fixed paraffin-embedded (FFPE) tumour tissue was first examined with a haematoxylin and eosin staining to determine the tumour surface area and cellularity. For RNA purification (Roche® High Pure FFPET RNA isolation kit), 1 to 8 10µm FFPE slides were cutted for each core biopsy, and macro dissection will be performed, when needed, to avoid contamination. A novel technique nCounter (NanoString Technologies) was used, that needs very few tumours and results are robust and rapidly obtained (48h). Additionally, peripheral blood samples were obtained as follows for Cytokines and Chemokines evaluation as well as lymphocytic response: 1. At screening period (<28 days from treatment initiation) and at 8 weeks 10 ml of blood (serum) will be extracted and stored at -80 ºC). A panel of 25 cytokines and chemokines (including IFN-γ,

	IL-18, CCL2, CXCL-12, IL-6, TNF and TGF-β) will be analyzed by Luminex Multiplex Cytokine Kits (Affymetrix). 2. At screening period (<28 days from treatment initiation) and at 8 weeks 50 ml of blood will be extracted for analysis of lymphocytic response. 3. MSS, RAS and BRAF status will be evaluated from archival biopsies (locally and centrally evaluation). 4. Biopsies before study entry and at 2 months (at the time of CT for response evaluation) of therapy will be done, to evaluate pharmacodynamics changes.
Number of patients	● ***Planned:*** 33 (including phase I accrual of 3-12 pts) ● ***Enrolled:*** 19 pts. ● ***Analysed for safety:*** 19 patients ● ***Analysed for efficacy:*** 19 patients
Diagnosis and main criteria for inclusion	**MAIN INCLUSION CRITERIA** 1. Written informed consent of approved by the investigator's Institutional Review Board (IRB)/Independent Ethics Committee (IEC), prior to the performance of any trial activities. 2. Histological diagnosis of MSS colorectal adenocarcinoma. 3. Metastatic disease treated with at least two chemotherapy lines, with or without targeted therapies. 4. Male or female subjects aged ≥ 18 years. 5. ECOG performance status 0 or 1. 6. Measurable disease by RECIST.1.1 criteria. 7. LDH levels <1.5 ULN (ULN=450 U/L). Maximum allowed 675 U/L. 8. Adequate hepatic function defined by a total bilirubin level ≤ 1.5 × the upper limit of normality (ULN) and AST and ALT levels ≤ 2.5 × ULN or AST and ALT levels ≤ 5 x ULN (for subjects with documented metastatic disease to the liver). 9. Negative serum pregnancy test at screening for women of childbearing potential. 10. Highly effective contraception for both male and female subjects throughout the study and for at least 30 days after last

	avelumab treatment administration if the risk of conception exists. 11. Adequate hematological function: a) Haemoglobin ≥ 9 g/dL (may have been transfused). b) Platelet count ≥ 100 × 10^9/L. c) Absolute neutrophil count (ANC) ≥ 1.5 × 10^9/L. 12. Renal: Estimated creatinine clearance ≥ 30 mL/min according to the Cockcroft-Gault formula (or local institutional standard method). 13. Female subjects must either be of non-reproductive potential (ie, post-menopausal by history: ≥60 years old and no menses for ≥1 year without an alternative medical cause; OR history of hysterectomy, OR history of bilateral tubal ligation, OR history of bilateral oophorectomy) or must have a negative serum pregnancy test upon study entry. **MAIN EXCLUSION CRITERIA** 1. Subjects with brain metastases. 2. Prior organ transplantation, including allogeneic stem-cell transplantation. 3. Presence of clinical ascites. 4. Modified Charlson score >2 (excluded cancer). 5. Significant acute or chronic infections including, among others: a) Known history of testing positive test for human immunodeficiency virus (HIV) or known acquired immunodeficiency syndrome (AIDS). b) Positive test for HBV surface antigen and / or confirmatory HCV RNA (if anti-HCV antibody tested positive). 6. Active autoimmune disease that might deteriorate when receiving an immunostimulatory agent: a) Subjects with diabetes type I, vitiligo, psoriasis, hypo- or hyperthyroid disease not requiring immunosuppressive treatment are eligible. b) Subjects requiring hormone replacement with corticosteroids are eligible if the steroids are administered

	only for the purpose of hormonal replacement and at doses ≤ 10 mg/24 h of prednisone or equivalent. c) Administration of steroids through a route known to result in a minimal systemic exposure (topical, intranasal, intraocular, or inhalation) are acceptable. 7. Local positive serologic determination to: HBsAg, Anti-HBc, HBV, HCV, HCV RNA, HIV-I RNA, Agp24 IIIV + AC IIIV ½ (MLIA) serum, IgG antigen core v. hepatitis B, RPR (Ac reagínicos Lues-RPR, serum), IgG cytomegalovirus (EIA), Ac anti HTLV I/II (if patient came from endemic zone), Ac anti Trypanosoma Cruzi, Chagas, (if patient came from endemic zone), when RPR positive or doubtful for confirmation: IgG T. pallidum (ELISA) IgM T pallidum (ELISA) , when IgG T. Pallidum doubtful: Pt confirmatory IgG/IGM, T pallidum (LIA). 8. Known severe hypersensitivity reactions to monoclonal antibodies (Grade ≥ 3 NCI-CTCAE v 4.03), any history of anaphylaxis, or uncontrolled asthma. 9. Persisting toxicity related to prior therapy of Grade >1 NCI-CTCAE v 4.03; however, alopecia and sensory neuropathy Grade ≤ 2 is acceptable. 10. Pregnancy or lactation. 11. Known alcohol or drug abuse. 12. All other significant diseases (for example, inflammatory bowel disease, uncontrolled asthma), which, in the opinion of the Investigator, might impair the subject's tolerance of trial treatment. 13. Any psychiatric condition that would impede the understanding of informed consent. 14. Vaccination other than study treatment is prohibited, within 4 weeks of the first dose of avelumab and while on trial. 15. History of other tumors in the past 5 years. 16. Active infections. 17. Current immunosuppressive treatment, 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).

	18. Known hypersensitivity to avelumab, ADC vaccines or their components. 19. Legal incapacity or limited legal capacity (patients with legal representation can be enrolled in the trial). 20. Patients with pneumonitis and pulmonary fibrosis 21. Patients with cardiac medical history: CARDIOVASCULAR DISEASE: "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." 22. Female patients who are pregnant or breastfeeding or male or female patients of reproductive potential who are not willing to employ highly effective birth control from screening to 180 days after the last dose of ADC + avelumab combination therapy.
Test product, dose, and mode of administration	● ADC vaccine: Cryopreserved autologous dendritic cells loaded with autologous tumour antigens in suspension for intradermal administration. Each vial contained $10x10^6$ of ADC plus matured DCs. ● Avelumab presented at a concentration of 20 mg/mL in a single-use glass vial contained 200 mg of avelumab administered intravenously at a dose of 10 mg per kilogram of body weight.
Duration of treatment	The study includes an initial dose escalation phase to find the RP2D. All patients started with Dose level 1: ● ADC vaccine: A dose of ADC at days 1, 14, 28, 42 and 56 (total of 5 doses), and thereafter every 6 months until disease progression (maximum of 6 additional doses) or unacceptable toxicity. ● Avelumab was administered intravenously at a dose of 10 mg per kilogram of body weight every 14 days until disease progression or unacceptable toxicity. If none of the first 3 patients experience a dose limiting toxicity (DLT) this dose will be recommended for phase 2. If 1/3 patients experience a DLT 3 more patients will be recruited. If <2/6 limiting toxicities are observed, these doses will be recommended for phase 2. If 2/3 or 2/6 patients experience a DLT, cohort -1 will be opened. Dose level -1: ● ADC vaccine: A dose of ADC at days 1, 14, 28, 42 and 56 (total of 5 doses), and thereafter every 6 months until

	disease progression (maximum of 6 additional doses) or unacceptable toxicity. ● Avelumab 3 mg/kg biweekly for until disease progression or unacceptable toxicity
Criteria for evaluation	**Phase I** To determine the recommended phase II dose (RP2D): Dose level in which <2/6 limiting toxicities were observed. The safety profile of the IMPs were assessed through the recording, reporting and analysis of baseline medical conditions, adverse events (AEs), physical examination findings including vital signs and laboratory tests. **Phase II** **Primary:** To increase the percentage (from 20% to 40%) of pre-treated MSS mCRC patients free of progression at 6 months. **Secondary:** 1. To evaluate the safety and tolerability of avelumab in combination with ADC vaccine. 2. To identify a favourable phenotype for efficacy. 3. To evaluate pharmacodynamic changes between pre-treatment and on-treatment tumour biopsies. ◦ Modified CMS classification by NanoString. ◦ Immunophenotype signature by NanoString.
Statistical methods	**Sample size considerations** **Phase 1** Phase 1 dose escalation evaluated the toxicity profile of avelumab and ADC vaccines to recommend a dose for phase 2 of the study. As the mechanisms of action and the toxicity profiles in monotherapy are different, it was expected that RP2D in combination would be the same as in monotherapy. For that reason, in cohort 1 combined Avelumab 10 mg/kg (recommended dose for trials according to Avelumab investigator brochure) and $10x10^6$ ADC vaccines (according ADC vaccines investigator brochure and NCT01413295 study). This phase followed a 3+3 design in which 2 potential cohorts (1 and -1) may recruit patients.

Phase 2

Simon's two stage design is the most widely implemented among multi-stage designs in phase II clinical trials, to assess the activity of a new treatment. It allows early stopping for futility or efficacy. In this case, two-stage Simon's minimax design has been selected because of the advantageous factor of this design, in terms of expected sample size. The only difference between the optimal and minimax designs compared with optimal models is the number of patients to enrol in the first stage; under the minimax model the number of patients to recruit is higher, without increasing total population for the trial, which makes the design more efficient.

Sample size assumptions:

- Phase II
- Simon´s two stage minimax design.
- Alpha: 0.05, Beta: 0.2.

		First Stage	Second Stage	
6-months PFS (expected)	6-months PFS			Total number of patients
20%	**40%**	**5/18**	**11/33**	**33**

Statistical considerations

Following the baseline assessment, subsequent tumour assessments according to RECIST were performed systematically every 8 weeks (±1 week) until progression disease relative to date of inclusion, according to the planned study schedule (Table 7).

Tumor assessment was also done at planned interval visit and included systematically only physical examination and PCR, albumin, CEA and LDH blood analysis. RECIST 1.1 criteria was used to assess patient response to treatment by determining progression free survival (PFS) times.

PFS and OS were analysed with the Kaplan-Meier curves and compared by a stratified log-rank test. Cox regression modeling was used for OS and PFS. Analysis was performed using SAS version 9.4 software (SAS Institute Inc., Cary, NC, USA) and the level of significance was established at the 0.05 level (two-sided).

	The safety profile of avelumab in combination with ADC vaccine was assessed through the recording, reporting and analysis of baseline medical conditions, adverse events (AEs), physical examination findings including vital signs and laboratory tests. Comprehensive assessment of any apparent toxicity experienced by each subject was performed from the time of giving informed consent and throughout the trial. The investigator reported any AEs, whether observed by the Investigator or reported by the subject.

NAME OF SPONSOR: GEMCAD **NAME OF FINISHED PRODUCT**: N/A **NAME OF ACTIVE INGREDIENT(S):** ADC Vaccines Avelumab	**INDIVIDUAL STUDY TABLE REFERRING TO MODULE 5 OF THE CTD** Volume: Page:	**(FOR NATIONAL AUTHORITY USE ONLY)**

SUMMARY CONCLUSIONS

EFFICACY RESULTS

The 6 months PFS rate was 0%. The trial reached a median PFS of 3.1 months (range 2.1-5.3). Therefore, the study treatment did not reach the pre-established threshold for significance and the study was stopped in the planned interim analysis enclosing the first 19 patients enrolled. The median OS was 12.1 months (range 3.2-22.9) and the 6 months OS rate was 73.7%. At database closure 18 (94.7%) patients were dead.

SAFETY RESULTS

The first 6 enrolled patients received the Dose level 1 (Avelumab 10mg/kg + ADC vaccine $10x10^6$). No DLTs were reported throughout the study. Thus, the initial dose schedule of Avelumab 10mg/kg biweekly until disease progression or unacceptable toxicity + $10x10^6$ ADC vaccines biweekly for 5 infusions followed by up to 6 infusions every 6 months was declared as the RP2D. All enrolled patients were administered with Dose level 1. The toxicity profile of the ADC vaccines and avelumab combination was favourable, with only 23 toxicities reported throughout the study period. Most toxicities were low grade 1-2. The most frequent toxicities (all grades) were fatigue (21.05%), fever (15.79%), and arthralgia (10.53%). Only one (5.26%) grade 3 fatigue was reported. A total of 13 SAEs in 8 (44.4%) patients were reported throughout the study period. There were no treatment-related deaths or discontinuations as of the data cutoff date. The toxicity profile was consistent with those previously reported for the study treatments avelumab and ADC vaccines in monotherapy

MOLECULAR SUBSTUDY RESULTS

After 8 weeks of avelumab and ADC vaccine treatment there was a substantial and significant decrease in VEGFc, VEGFa, MCP1 and SDF1a expression levels. VEGFc was found to be significantly reduced independently of stratification by age, gender, LDH levels, and ECOG.

CONCLUSION

Despite good tolerability and manageable safety profile, the combination of ADC vaccines and avelumab has no increased efficacy that justified further research of this combination in MSS metastatic colorectal cancer setting. The molecular substudy may suggest a potential

synergistic effect of the ADC vaccine and avelumab combination in angiogenic dependent settings or in combination with anti-angiogenic agents.

DATE OF THE REPORT: March 19th, 2021

3. TABLE OF CONTENTS

4. LIST OF ABBREVIATIONS & DEFINITION OF TERMS

Abbreviation /Acronym	Definition
ADL	Activities of Daily Living
AE	Adverse Event
AEMPS	Agencia Española del Medicamento y Productos Sanitarios
ALT	Alanine Transaminase
anti-HBc	Anti-core Antibodies
antiHBC	Antibody to Hepatitis B Core Antigen
ASCO	American Society of Clinical Oncology
ASH	American Society of Hematology
AST	Aspartate Aminotransferase
AUC	Area Under the Curve
BBDD	Database
Ca	Calcium
CA	Competent Authority
CAPA	Corrective Action Preventive Action
CD	Cluster of Differentiation
CEIm	Comité de Ética de la Investigación con Medicamentos (Independent Ethics Committee of Research with Medicines)
CI	Confidence Interval
CMR	Complete Metabolic Response

CMRr	Complete Metabolic Response with residual mass
CNS	Central Nervous System
COV	Close-out Visit
CR	Complete Response
CRAs	Clinicals Research Associates
CRO	Contract Research Organization
CSR	Clinical Study Report
CTC	Common Toxicity Criteria
CTCAE	Common Terminology Criteria for Adverse Events
CTPM	Clinical Trial Project Manager
DFS	Disease Free Survival
DNA	Deoxyribonucleic Acid
EC	Ethical Committee
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
ECOG PS	ECOG Performance Status
eCRF	Electronic Case Report Form
ECs	Ethics Committee
EDTA	EthylenediamineTetraacetic Acid
EFS	Event Free Survival
EORTC	European Organization for Research and Treatment of Cancer
EoT	End of Therapy

ESMO	European Society for Medical Oncology
FDG	Fluorodeoxyglucose
FISH	Fluorescent In Situ Hybridisation
G	Grade
G-CSF	Granulocyte-Colony Stimulating Factor
GCP	Good Clinical Practice
GGT	Gamma-Glutamyl Transferase
HBcAb	Hepatitis B Core Antibody
HBsAG	Hepatitis B Surface Antigen
HBV	Hepatitis B Virus
HCA	Hospital Central de Asturias
HCB	Hospital Clínic de Barcelona
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
ICH-GCP	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use - Good Clinical Practice
IEC	Independent Ethics Committee
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M

IHC	Immunohistochemistry
IMP	Investigational Medicinal Product
ISF	Investigator Site File
ITT	Intention To Treat
iv	Intravenous
K	Potassium
LDH	Lactate Dehydrogenase
Na	Sodium
NA	Not Available
NCI	National Cancer Institute
NCI-CTCAE	Common Terminology Criteria for Adverse Events of The National Cancer Institute
ND	Not Determined
OPS-OMS	Organización Panamericana De La Salud - Organización Mundial De La Salud Pan American Health Organization - World Health Organization
OR	Odds Ratio
OS	Overall Survival
PCR	Polymerase Chain Reaction
PET	Positron Emission Tomography
PET/CT	Positron Emission Tomography - Computed Tomography
PIS	Patient Information Sheet
PIs	Principal Investigators

PT	Prothrombin Time
PTT	Partial Thromboplastin Time
PVG	Pharmacovigilance
QA	Quality Assurance
RDT	Radiotherapy
RMS	Residual Metabolic Disease
RMV	Routine Monitoring Visit
RNA	Ribonucleic Acid
ROC	Receiver Operating Characteristic
SAE	Serious Adverse Event
sc	Subcutaneous
SD	Standard Deviation
SDV	Source Data Verification
SIV	Site Initiation Visits
Std	Standard
SUSARs	Suspected Unexpected Serious Adverse Reactions
TMF	Trial Master File
UK	Unknown
UNL	Upper Normal Limit
VEGF	Vascular Endothelial Growth Factor
WBC	White Blood Cell
WT	Wild Type

5. ETHICS AND REGULATORY APPROVAL

5.1. INDEPENDENT ETHICS COMMITTEE APPROVAL

The study protocol and all its amendments and the patient information sheet(s) were reviewed and approved by the appropriate independent ethics committees (ECs) as detailed in table 1 below.

The reference committee for this trial in Spain was the Comité Ético de Investigación con Medicamentos del Hospital Clínic de Barcelona; as reference EC, this committee collated the feedback of implied ECs and oversaw communication with the sponsor. The official positive votes for the initial submission and subsequent amendments are listed in Table 1.

Table 1. Ethics committees

Centre name and number	01 - Hospital Clínic 02 - Hospital Universitari Vall d'Hebron 04 - Hospital Universitario Puerta de Hierro 05 - Instituto Valenciano de Oncología 06 - Hospital Universitario Miguel Servet 07 - Hospital Clínico Lozano Blesa 08 - Hospital Universitari i Politécnic La Fe 09 - Hospital Universitario La Paz
Principal investigator	01 - Dr. Joan Maurel Santasusana 02 - Dr. Elena Élez Fernández 04 - Dr. Ana Isabel Ruiz Casado 05 - Dr. Carlos Fernández Martos / Dr. Ricardo Yaya Tur 06 - Dr. Vicente Alonso Orduña 07 - Dra. Pilar Escudero Emperador 08 - Dr. Jorge Aparicio Urtasun 09 - Dra. Nuria Rodríguez Salas
Ethics committee (Central)	Comité Ético de Investigación con Medicamentos del Hospital Clínic de Barcelona
Ethics committee chairman	Begoña Gómez Pérez
Date of approval of the final protocol	Version 2.1 from Jan 23rd, 2018 was approved on **March 19th, 2018** Version 2.0 from Oct 4th, 2017 was approved on **Jan 18th, 2018**
Date of approval of amendment 1	Approved on **July 31st, 2019**

5.2. ETHICAL CONDUCT OF THE STUDY

The study was performed in accordance with the current version of the declaration of Helsinki and:

- The International Conference on Harmonisation (ICH) guidelines on Good Clinical Practice (GCP).
- The Convention on Human Rights and Biomedicine. Oviedo, 4 April 1997. Entry into force: 22 October 1999 (BOE 282, 25.11.99).
- The regulation on adequate protection of personal data according to the Organic Law 15/1999 on personal data protection.
- Act 41/2002 of 14 November 2002, a basic regulating Act on the autonomy of the patient and the rights and obligations in matters of clinical information and documentation.
- The 48th General Assembly Somerset West, South Africa, October 1996, and the 52nd General Assembly, Edinburgh, Scotland, October 2000.
- Law 14/2007, 3 July on Biomedical Research.
- Royal Decree 1090/2015, of December 4, which regulates clinical trials with drugs, the Ethics Committees for Drug Research and the Spanish Registry of Clinical Studies.
- Instruction document of the Spanish Agency of Medicines and Medical Devices for conducting clinical trials in Spain.
- Regulation (EU) No. 536/2014 of the European Parliament and of the Council, of April 16, 2014, on clinical trials of medicinal products for human use.

5.3. PATIENT INFORMATION AND CONSENT

All patients provided written informed consent to participate in the study prior to being screened. The transferral of biological samples required previous written informed consent by the patient.

The patient information sheet was submitted to and approved by ECs and the AEMPS; this document detailed the procedures involved in the study (aims, methodology, potential risks, and anticipated benefits), and the investigator explained these to each patient. The patient signed the approved consent form to indicate that the information had been explained and understood. The patient was then allowed time to consider the information presented before signing and dating the informed consent form (ICF) to indicate that they fully understood the information and willingly volunteered to participate in the study. The patient was given a copy of the ICF for their information. The original copy of the ICF was kept in a confidential

file in the Investigators' site records. A sample of the patient information sheet and consent form can be found in appendix 15.1.5.

5.4. REGULATORY APPROVAL

The study was performed in compliance with the requirements of the AEMPS and the current local regulations. The study was granted full regulatory approval on March 12th, 2018. GEMCAD-1602 was issued with the following EudraCT number: 2016-003838-24. A copy of regulatory approval is provided in appendix 15.1.5.

The resolution for approval from the CA asked for some requirements to be implemented in subsequent amendments:

It was necessary to better standardize the following parameters:

- The weight of the biopsy used to obtain the tumor lysate.
- The inclusion of pre-inoculation controls as specifications or not of the finished product
- The concentration of autologous tumor lysate during cell maturation.
- The inclusion of the count and the viability at days 1, 3 and 8 within the controls in the manufacturing process.

The following information should be included in further amendments:

- Certificates of all reagents used in the preparation of dendritic cells
- Annex E. Validations
- The raw release date of the batches of product under investigation obtained in the previous clinical trial with this product.
- The relevant information that justifies the expiration date (5 years from the date of manufacture).

Finally, for future relevant modifications in the quality IMPD or new clinical trials with this product, a version with changes must be provided with respect to the latest version of PEI 09-133 authorized by the AEMPS.

6. INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

Table 2 shows the principal study personnel involved.

Table 2. Principal study personnel

Title	Name and affiliation
Coordinating Investigators	**Dr. Joan Maurel Santasusana** Oncology Department, Hospital Clinic - Barcelona Villarroel 170, 08036 – Barcelona (Spain) Email: jmaurel@clinic.cat **Dr. Daniel Benítez Ribas** Immunology Department, Hospital Clínic - Barcelona Email: dbenitezr@clinic.cat

	Dr. Javier García-Corbacho Head of Clinical trials unit. Hematology and Oncology Departments, Hospital Clinic - Barcelona Early Phase Clinical Trials Villarroel 170, 08036 – Barcelona (Spain) Email: garcia33@clinic.ub.es
Sponsor	Grupo Español Multidisciplinar de Investigación en Cáncer Digestivo (GEMCAD)
Project managers	**MFAR Clinical Research** Ana Llabrés (22.JAN.2018 - 15.NOV.2019) María Jesús González (15.NOV.2019 - current)
Clinical research associate(s)	**MFAR Clinical Research** Daniel Sanchez Jose Ignacio Ruso Bárbara Flix Elena García Francisco Roldán
Medical adviser	**Dr. Joan Maurel Santasusana** Oncology Department, Hospital Clinic - Barcelona
Data management	Daniel Sanchez - MFAR Clinical Research Verónica Roca - MFAR Clinical Research
Trial statistician	Plataforma de Estadística Médica. IDIBAPS C/ Mallorca 1º. Oficina #3. 08036, Barcelona, Spain

Abbreviation: MFAR, Marketing Farmaceutico S.L.

7. INTRODUCTION

7.1. COLORECTAL CANCER (CRC)

Colorectal cancer (CRC) is among the most frequent cancers worldwide and the second leading cause of cancer-related deaths in Western countries. Four targeted therapies (bevacizumab, cetuximab, panitumumab and aflibercept) have been approved in second line therapy in combination with chemotherapy in metastatic CRC (mCRC). Despite it less than 20% of patients are free of progression at 12 months (Peeters 2010, Sobrero 2008, Bennouna 2013, Van Cutsem 2012). In third-line therapy in two large prospective randomized trials with regorafenib or TAS102, less than 20% of patients were free of progression at 6 months (Grothey 2013, Mayer 2015).

MSI tumors have a high mutational load (and specially frame-shift mutations) that creates many neoantigens that are presented on MHC, and recognized as foreign by T cells. As a consequence, untreated MSI tumors have much higher PD-L1 expression in tumour associated macrophages (TAMs) and high presence of tumour-infiltrating lymphocytes (TIL) than mismatch repair-proficient (MSS) tumors (Llosa 2015). We know that PD1 blockade will only partially succeed (40% response rate) in the setting of a pre-existing antitumor immune responsive phenotype (MSI patients) but not in MSS colorectal cancer patients (<5% RR, median PFS of 2.5 months and <20% progression free survival at 6 months) (Le DT 2015).

There is a need to identify biomarkers of response to (PD-1 and PD-L1 blockade). PD-L1 can be induced by adapted immune resistance in high-immunogenic tumors such as melanoma or as innate immune resistance by oncogenic signaling due to STAT-3 activation (Pardoll 2013, Marzec 2008, Jiang 2013). In addition, the use of PD-L1 (B7-H1) immunohistochemistry (IHC) as a predictive biomarker has been confounded by multiple unresolved issues (e.g. variable detection antibodies, differing IHC cutoffs, tissue preparation and processing variability) that questioned PD-L1 as an exclusionary predictive biomarker.

More recently, genomic immune signatures (Tumeh 2014, Rizvi 2015) and genomic and non-genomic (transcriptomic) signatures (Hugo 2015) suggest that capturing the complexity of the immune system might be a better strategy. Our group has recently presented an immuno-signature that predicts checkpoint inhibitor efficacy, across tumour-types (Seguí 2016).

Cancer vaccines are a complementary therapeutic approach to checkpoint inhibitors. These vaccines aim to stimulate tumour antigen-specific cytotoxic T lymphocytes that recognize and eliminate cancer cells in an antigen-specific way. The active immunization can elicit adaptive antitumor immunity even in metastatic patients, who are thought to be less immune responsive. We have recently completed a phase II randomized clinical trial that compared (ADC) plus best supportive care (BSC) vs BSC in pre-treated mCRC patients (NCT01413295). Although we were unable to observe differences in survival between both arms, ADC treated patients generated a tumour-specific immune response (Caballero-Baños 2016).

7.2 AVELUMAB BACKGROUND INFORMATION:

Avelumab binds PD-L1 and blocks the interaction between PD-L1 and PD-1. This removes the suppressive effects of PD-L1 on anti-tumour CD8+ T cells, resulting in the restoration of cytotoxic T cell response.

Avelumab was administered intravenously at a dose of 10 mg per kilogram of body weight every 14 days until disease progression or unacceptable toxicity. Based on the PK results and the receptor occupancy data, sufficient trough concentrations appear to be achieved for full TO in the blood in the majority of subjects receiving the 10 mg/kg dose. Within the dose range of 1 mg/kg to 20 mg/kg, avelumab was well tolerated and is deemed to have an acceptable safety profile.

Based on Avelumab IB, a dose of 10 mg/kg Iv once every 2 weeks is considered to have a favourable risk benefit profile and thus represents an appropriate dose for further investigation in studies of avelumab.

7.3 ADC VACCINES BACKGROUND INFORMATION:

ADC vaccines aim to stimulate tumour antigen-specific cytotoxic T lymphocytes that recognize and eliminate cancer cells in an antigen-specific way. The active immunization can elicit adaptive antitumor immunity even in metastatic patients, who are thought to be less immune responsive.

Vaccines were available for administration in 7-10 calendar days from receiving source patient samples, and were stored in liquid nitrogen. Each vial containing $10x10^6$ of ADC plus matured DCs were thawed at 37ºC and immediately injected intradermally. Intradermal route of administration and dosification was selected according to the information of previous published study (NCT01413295) and IB.

Please, refer to Avelumab or ADC vaccines Investigator's Brochure (IB) for further information about the nonclinical and clinical programs and Guidance for the Investigator.

Based on the available nonclinical and clinical data to date, the conduct of the trial specified in this protocol is considered justifiable.

8. STUDY OBJECTIVES

Phase I Objectives:

Primary: To determine the recommended phase II dose (RP2D) of avelumab in combination with ADC vaccine in previously treated MSS CRC patients who have progressed at least to 2 chemotherapy lines.

Secondary: To evaluate the safety and tolerability of avelumab in combination with ADC vaccine.

Phase II Objectives:

Primary:

To increase the percentage of pre-treated MSS mCRC patients free of progression at 6 months.

Secondary:

1. To evaluate the safety and tolerability of avelumab in combination with ADC vaccine.

2. To identify a favourable phenotype for efficacy.

3. To evaluate pharmacodynamic changes between pre-treatment and on-treatment tumour biopsies.

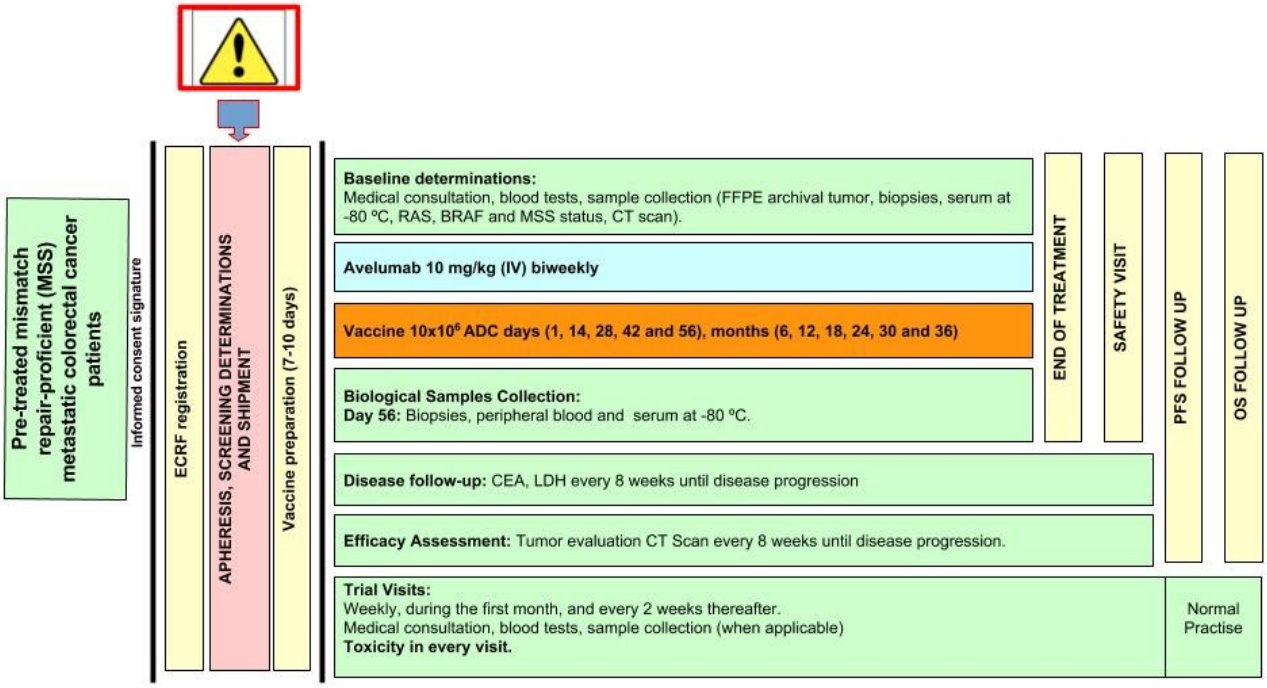
- Modified CMS classification by NanoString.
- Immunophenotype signature by NanoString.

9. INVESTIGATIONAL PLAN

9.1. OVERALL STUDY DESIGN AND PLAN

9.1.1. STUDY FLOWCHART

Figure 1. Schematic representation of study protocol



9.1.2. STUDY LOCATION

This study was conducted at the following locations:

01 - Hospital Clínic	01 - Dr. Joan Maurel Santasusana
02 - Hospital Universitari Vall d'Hebron	02 - Dr. Elena Élez Fernández
04 - Hospital Universitario Puerta de Hierro	04 - Dr. Ana Isabel Ruiz Casado
05 - Instituto Valenciano de Oncología	05 - Dr. Carlos Fernández Martos / Dr. Ricardo Yaya Tur
06 - Hospital Universitario Miguel Servet	06 - Dr. Vicente Alonso Orduña
07 - Hospital Clínico Lozano Blesa	07 - Dra. Pilar Escudero Emperador
08 - Hospital Universitari i Politécnic La Fe	08 - Dr. Jorge Aparicio Urtasun
09 - Hospital Universitario La Paz	09 - Dra. Nuria Rodríguez Salas

9.2. DISCUSSION OF STUDY DESIGN

AVEVAC is a multicentre phase I-II study that studied the safety, tolerability, pharmacodynamics and anti-tumour effects of the combination of avelumab plus autologous dendritic cells vaccines in pre-treated MSS mCRC patients.

Archival formalin-fixed paraffin-embedded (FFPE) tumour blocks were used for central determination of MSS status, RAS and BRAF. Local determinations were also recorded, when available.

Phase I was designed to evaluate the safety, tolerability, pharmacodynamics and anti-tumour effects of the combination in pre-treated MSS mCRC patients. In the phase I, patients were assigned using a standard 3x3 de-escalation criteria (level -1 if dose limiting toxicity (DLT) with avelumab 3 mg/kg every 2 weeks) to received avelumab at a dose of 10 mg/kg every 2 weeks combined to ADC vaccine at days 1, 14, 28, 42 and 56, and thereafter every 6 months until disease progression (maximum of 6 additional doses) or unacceptable toxicity. Biopsies were obtained from primary tumour or metastatic disease to prepare tumour lysate. The primary objective was to determine the maximum tolerated dose (MTD) and the efficacy of the combination.

The design of the phase I trial (i) ensured patient safety; (ii) avoided to treat patients at presumably infra-therapeutic doses; and (iii) identified the optimal drug combination for further investigation.

Phase II stage of the trial was a superiority study with the hypothesis that the treatment with the combination of avelumab plus autologous dendritic cells vaccines will exceed historic treatment in terms of 6-months PFS. To detect at least a 20% difference in PFS at 6 months (from 20% to 40%), 33 patients were needed (80% power, alpha 5%, two sided). An interim analysis (Simon's two stages) when the first 18 patients were accrued was planned.

Secondary objective included pharmacodynamics (a) NanoString 360 gene immune-signature from archival biopsy, at study entry and at 2 months therapy (b) cytokine and chemokine determination (at study entry and at 2 months therapy) and (c) Autologous tumour mixed leukocyte reaction to test the polarisation of the immune response against the combination (at study entry and at 2 months therapy).

The translational study was aimed to answer the need to identify biomarkers of response and to better understand pharmacodynamics changes before and after therapy, for that reason, samples were collected before and after 8 weeks of treatment initiation.

Taking into account the aforementioned information, the design of this Phase I/II trial was based on a strong scientific rationale to determine the potential of the combination investigated to increase 6 months PFS in MSS mCRC patients, and related secondary objectives as: potential for creating toxicities, identification of favourable phenotype for efficacy and to evaluate changes in the phenotype after therapy.

9.3. SELECTION OF THE STUDY POPULATION

9.3.1. INCLUSION CRITERIA

1. Written informed consent of approved by the investigator's Institutional Review Board (IRB)/Independent Ethics Committee (IEC), prior to the performance of any trial activities.
2. Histological diagnosis of MSS colorectal adenocarcinoma.
3. Metastatic disease treated with at least two chemotherapy lines, with or without targeted therapies.
4. Male or female subjects aged ≥ 18 years.
5. ECOG performance status 0 or 1.
6. Measurable disease by RECIST.1.1 criteria.
7. LDH levels <1.5 ULN (ULN=450 U/L). Maximum allowed 675 U/L.
8. Adequate hepatic function defined by a total bilirubin level ≤ 1.5 × the upper limit of normality (ULN) and AST and ALT levels ≤ 2.5 × ULN or AST and ALT levels ≤ 5 x ULN (for subjects with documented metastatic disease to the liver).
9. Negative serum pregnancy test at screening for women of childbearing potential.
10. Highly effective contraception for both male and female subjects throughout the study and for at least 30 days after last avelumab treatment administration if the risk of conception exists.
11. Adequate hematological function:

    a) Haemoglobin ≥ 9 g/dL (may have been transfused).

    b) Platelet count ≥ 100 × 10^9/L.

    c) Absolute neutrophil count (ANC) ≥ 1.5 × 10^9/L.
12. Renal: Estimated creatinine clearance ≥ 30 mL/min according to the Cockcroft-Gault formula (or local institutional standard method).
13. Female subjects must either be of non-reproductive potential (ie, post-menopausal by history: ≥60 years old and no menses for ≥1 year without an alternative medical cause; OR history of hysterectomy, OR history of bilateral tubal ligation, OR history of bilateral oophorectomy) or must have a negative serum pregnancy test upon study entry.

9.3.2. EXCLUSION CRITERIA

1. Subjects with brain metastases.

2. Prior organ transplantation, including allogeneic stem-cell transplantation.

3. Presence of clinical ascites.

4. Modified Charlson score >2 (excluded cancer).

5. Significant acute or chronic infections including, among others:

    a) Known history of testing positive for human immunodeficiency virus (HIV) or known acquired immunodeficiency syndrome (AIDS).

    b) Positive test for HBV surface antigen and / or confirmatory HCV RNA (if anti-HCV antibody tested positive).

6. Active autoimmune disease that might deteriorate when receiving an immunostimulatory agent:

    a. Subjects with diabetes type I, vitiligo, psoriasis, hypo- or hyperthyroid disease not requiring immunosuppressive treatment are eligible.

    b. Subjects requiring hormone replacement with corticosteroids are eligible if the steroids are administered only for the purpose of hormonal replacement and at doses ≤ 10 mg/24 h of prednisone or equivalent.

    c. Administration of steroids through a route known to result in a minimal systemic exposure (topical, intranasal, intraocular, or inhalation) are acceptable.

7. Local positive serologic determination to: HBsAg, Anti-HBc, HBV, HCV, HCV RNA, HIV-I RNA, Agp24 IIIV + AC IIIV ½ (MLIA) serum, IgG antigen core v. hepatitis B, RPR (Ac reagínicos Lues-RPR, serum), IgG cytomegalovirus (EIA), Ac anti HTLV I/II (if patient came from endemic zone), Ac anti Trypanosoma Cruzi, Chagas, (if patient came from endemic zone), when RPR positive or doubtful for confirmation: IgG T. pallidum (ELISA) IgM T pallidum (ELISA) , when IgG T. Pallidum doubtful: Pt confirmatory IgG/IGM, T pallidum (LIA).

8. Known severe hypersensitivity reactions to monoclonal antibodies (Grade ≥ 3 NCI-CTCAE v 4.03), any history of anaphylaxis, or uncontrolled asthma.

9. Persisting toxicity related to prior therapy of Grade >1 NCI-CTCAE v 4.03; however, alopecia and sensory neuropathy Grade ≤ 2 is acceptable.

10. Pregnancy or lactation.

11. Known alcohol or drug abuse.

12. All other significant diseases (for example, inflammatory bowel disease, uncontrolled asthma), which, in the opinion of the Investigator, might impair the subject's tolerance of trial treatment.

13. Any psychiatric condition that would impede the understanding of informed consent.

14. Vaccination other than study treatment is prohibited, within 4 weeks of the first dose of avelumab and while on trial.

15. History of other tumors in the past 5 years.

16. Active infections.

17. Current immunosuppressive treatment, 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).

18. Known hypersensitivity to avelumab, ADC vaccines or their components.

19. Legal incapacity or limited legal capacity (patients with legal representation can be enrolled in the trial).

20. Patients with pneumonitis and pulmonary fibrosis

21. Patients with cardiac medical history: CARDIOVASCULAR DISEASE:

“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.”

22. Female patients who were pregnant or breastfeeding or male or female patients of reproductive potential who were not willing to employ highly effective birth control from screening to 180 days after the last dose of ADC + avelumab combination therapy.

9.3.3. WITHDRAWAL OF PATIENTS FROM THERAPY OR ASSESSMENT

Subjects had the right to withdraw from the trial partially or completely, at any time, and for any cause, without any prejudice to their future medical care from the physician or the centre.

Total consent withdrawal from the trial means that the subject does not wish to receive further investigational treatment, either wishes or is unable to maintain participation in the study. Any subject has the right to total consent withdrawal, at any time throughout the trial. The investigator managed, along with the subject, the best way to withdraw in order to guarantee the subject’s health. Any subject who completely withdrew consent to participate in the trial, did not receive any further investigational treatment or underwent further study procedures, immediately after the date of application for withdrawal.

Partial consent withdrawal meant that the subject did not wish to receive further investigational treatment, but still wished to collaborate, staying in the study to offer additional data (e.g. participating in all subsequent study visits or procedures). Subjects may refuse to continue receiving the investigational product at any time during the clinical trial. However, these subjects, along with those who had discontinued the investigational product for other reasons (e.g. investigator or sponsor decision), continued with the study procedures program.

If a subject (or his/her legally authorized representative) requested or decided to withdraw from the clinical trial, all necessary efforts to complete and record observations were made, as extensively as possible, up to the withdrawal date. All the information was recorded on the case report forms and patient medical record.

IMPORTANT: It is important to discriminate between discontinuation of investigational products only (the patient remains on study) and withdrawal from study, when patients do not continue either investigational product nor clinical trial follow up.

9.3.3.1 Withdrawal from Trial Therapy

IMPORTANT: Any patient who has not yet shown objective radiological disease progression at withdrawal from IMP continued followed as per RECIST until disease progression.

A subject was withdrawn from trial therapy if any of the following occur:

- Subject withdrew consent.
- Participation in another clinical trial.
- Progressive disease.
- Unacceptable toxicity.
- Subject lost to follow up.
- Any events that unacceptably endanger the safety of the subject.
- Investigator decision based on what is considered the best for the patient.

Patients who decided to discontinue investigational products were always asked about the presence of any adverse events. If possible, they would be seen and assessed by an investigator(s). Adverse events were followed up and all study material (if any) was returned by the patient.

By discontinuation from treatment, the patient did not withdraw from the study. Patient was followed for progression (if discontinuation in the absence of progression) as per the protocol schedule.

Any patient discontinuing investigational products have been visited at 30 days post discontinuation for the evaluations outlined in the study schedule. The patients' tumour status have been assessed clinically and, if appropriate, disease progression was confirmed by radiological assessment. After discontinuation of study medication, the Investigator performed the best possible observation(s), test(s) and evaluation(s) as well as gave appropriate medication and all possible measures for the safety of the patient. In addition, they recorded on the eCRF the date of discontinuation, the reasons, manifestation

and treatment at the time of discontinuation. Patients were required to attend the treatment discontinuation visit.

After discontinuation of the study medication at any point in the study, all ongoing AEs or SAEs were followed until resolution unless, in the Investigator's opinion the condition is unlikely to resolve due to the patient's underlying disease, or the patient was lost to follow up. All new AEs and SAEs occurring during the 30 calendar days after the last dose of study medication were reported (if SAEs, they must be reported to GEMCAD appointed Contract Research Organization (CRO) for Safety within 24 hours) and followed to resolution as above.

Patients should have been seen at least 30 days after discontinuing study medication to collect and/or complete AE information. Any untoward event occurring subsequent to the 30-day follow-up AE reporting period that the investigator had assessed as possibly related to the study medication was also reported as an AE.

9.3.3.2 Withdrawal from the Trial

Patients were free to withdraw from study at any time (investigational product AND study assessments), without prejudice to further treatment (withdrawal of consent). Such patients were always asked about the presence of any adverse events. If possible, he/she was seen and assessed by an investigator. Adverse events were followed up and all study material (if any) should have been returned by the patient. The investigator may also, at his/her discretion, withdraw the subject from participating in this study at any time, or the sponsor may discontinue the study. Withdrawn patients were not replaced.

If patient withdrew consent, he/she was specifically asked if she was withdrawing consent to:

- To further participation in the study including any further follow up (e.g., survival calls).
- Withdrawal of consent to the use of her study generated data.
- Withdrawal to the use of any samples.
- Reasons for early withdrawal from the study should be documented in the CRF and patient's records as:
    - Study closed/terminated.
    - Subject lost to follow-up.
    - Investigator's decision.
    - Subject withdrew consent.
- Participation in another clinical trial.
- Major protocol violations that make patient data not valid.
- Death.

Date of withdrawal from the study, with reason for withdrawal, will be recorded on the CRF and patient's records. In the case of death, a death certificate should be obtained if possible, with the cause of death evaluated and documented.

9.3.3.3 Premature Termination of the Trial

The clinical trial may be terminated prematurely or suspended at the request of Health Authorities or if new safety or efficacy information leads to an unfavorable risk benefit judgment for any IMP. The Sponsor may discontinue the trial if it becomes unjustifiable for medical or ethical reasons, for poor enrollment, or because of discontinuation of clinical development of an IMP or withdrawal of an IMP from the market for safety reasons.

Health Authorities and Independent Ethics Committees (IECs)/Institutional Review Boards (IRBs) will be informed about the discontinuation of the trial in accordance with applicable regulations.

9.3.3.4 Definition of End of Trial

Last patient last visit as defined in this protocol, 18 months after the enrollment of last patient. Subjects may continue to receive IMPs after the cut-off because treatment cannot be discontinued for ethical reasons, in such cases, Sponsor will agree with sites further drug supply to cover patient treatment until its finalization.

A total of 28 patients were screened during the phase I of the study, 9 of them were not eligible and were excluded. Despite initial sample size calculation of 18 patients enrolled in the phase I, there were 19 patients who were considered eligible and were included in the study. All of them received the study treatment, avelumab plus ADC vaccine, and were included in the ITT cohort. The 19 patients were analyzed for safety and efficacy.

The study did not reach the efficacy cutoff established in the protocol in the intermediate efficacy cut-off point (2-stages Simon design), which prevented progress towards Phase II of the study. However the results evaluation and benefit risk evaluation were not altered by this early pre-planned termination

The main causes for the discontinuation of the investigational product or follow-up were:

Table 4. Main causes of treatment discontinuation in the Avevac trial

Reason for treatment discontinuation	N (%)
Progression disease	19

9.4. TREATMENTS

9.4.1. TREATMENTS ADMINISTERED

9.4.1.1 Avelumab

Treatment schema: Avelumab was administered intravenously at a dose of 10 mg per kilogram of body weight every 14 days until disease progression or unacceptable toxicity.

Pre-medication: 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 was mandatory (for example, 25-50 mg diphenhydramine and 500-650 mg paracetamol IV or oral). Premedication should have been administered for subsequent avelumab infusions based upon clinical judgement and presence/severity of prior infusion reactions. This may have been modified based on local treatment standards and guidelines, as appropriate.

Setting: Avelumab should have been 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.

Observation period: Following avelumab infusions, patients must have been observed for 30 minutes post-infusion for potential infusion-related reactions.

Preparation: Avelumab drug product was diluted with 0.9% saline solution; alternatively a 0.45% saline solution could be used if needed. It is recommended that the diluted avelumab solution is used immediately. If not used immediately, in-use storage times and conditions prior to administration are the responsibility of the user.

To prepare the dilutions, subsequent preparation steps were accomplished by adequate trained personnel under a laminar flow box using aseptic techniques:

Prior to the preparation of the dilution for final infusion, allow each vial to equilibrate to room temperature. Use a disposable syringe equipped with a needle of suitable size to remove a volume of sodium chloride solution to be replaced by avelumab from the infusion bag and discard the removed solution. Use a new disposable syringe equipped with a needle of suitable size to inject a volume of avelumab drug product identical to the discarded volume of sodium chloride solution into the infusion bag. Gently invert the mixture 10 times. Infusion bags must not be shaken, in order to avoid foaming or excessive shearing of the protein solution. The preparation must be carefully inspected as it should result in a homogeneous looking clear solution, free of visible particles.

Administration: Avelumab was administered as a 1-hour IV infusion, diluted with 0.9% saline solution; alternatively a 0.45% saline solution could be used if needed.

Dose modifications:

1. Infusion-Related Reactions

1. Symptoms

- Fever
- Chills
- Rigors
- Diaphoresis
- Headache

2. Management

Table 5. 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 the 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 the 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 return to baseline at the subsequent infusions based on the investigator's medical judgement. 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.	

Once the avelumab infusion rate has been decreased by 50% or interrupted due to an infusion-related reaction, it must remain decreased for all subsequent infusions. If the subject has a second infusion-related reaction Grade ≥ 2 on the slower infusion rate, the infusion should be stopped and the subject should be removed from study treatment. If a subject experiences a Grade 3 or 4 infusion-related reaction at any time, the subject must discontinue study drug.

2. Severe Hypersensitivity Reactions and Flu-Like Symptoms

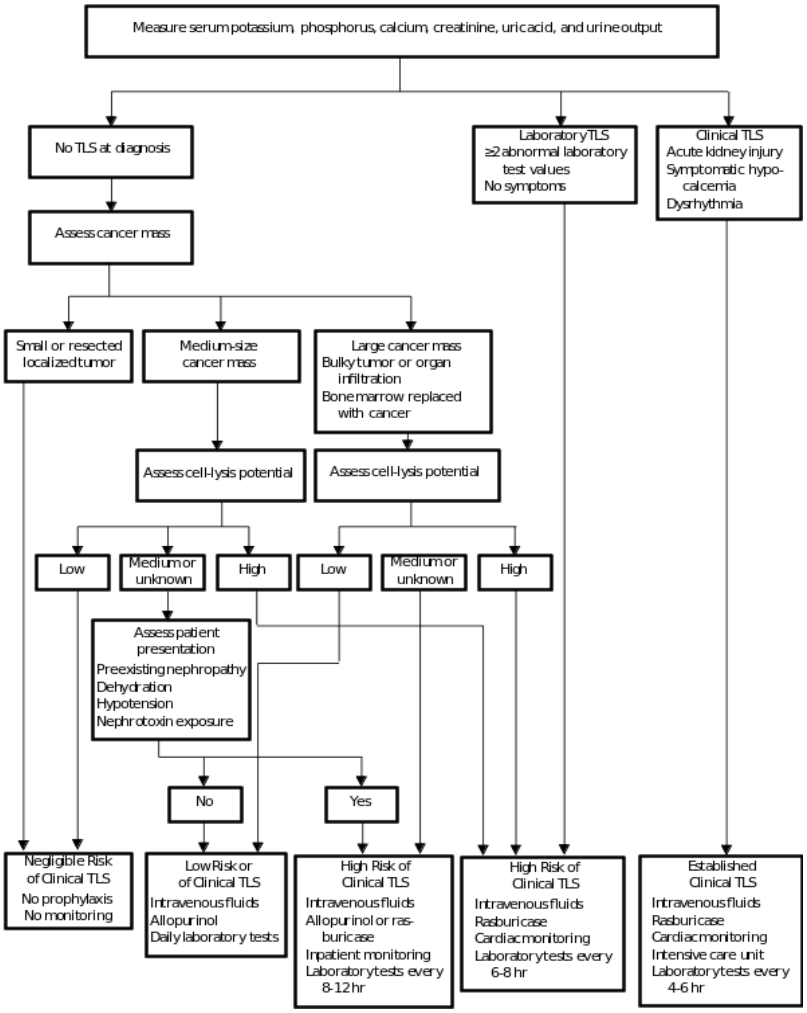
If hypersensitivity reaction occurs, the subject must be treated according to the best available medical practice. Subjects should be instructed to report any delayed reactions to the Investigator immediately.

For prophylaxis of flu-like symptoms, 25 mg of indomethacin or comparable nonsteroidal anti-inflammatory drug (NSAID) dose (for example, ibuprofen 600 mg, naproxen sodium 500 mg) may be administered 2 hours before and 8 hours after the start of each dose of avelumab IV infusion. Alternative treatments for fever (for example, paracetamol) may be given to subjects at the discretion of the Investigator.

3. Tumor Lysis Syndrome

In addition, since avelumab can induce antibody-dependent cell-mediated cytotoxicity, there is a potential risk of tumour lysis syndrome. Should this occur, subjects should be treated per the local guidelines and the management algorithm below (Howard 2011).

Assessment and Initial Management of Tumor Lysis Syndrome



4. Immune-Related Adverse Events

Table 6. 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	Reassess 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 (NCICTCAE 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 equivalent. Add prophylactic antibiotics for opportunistic infections Consider renal biopsy Nephrology consult	If returns to Grade ≤1: Taper steroids over at least 1 month.
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, restart avelumab therapy. If symptoms do not improve/worsen, viral myocarditis is excluded, and immune-mediated etiology is suspected or confirmed following cardiology consultation, 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), antithyroid treatment (for hyperthyroidism), corticosteroids (for adrenal insufficiency) or insulin (for Type I diabetes mellitus) as appropriate. Rule-out secondary endocrinopathies (i.e. hypopituitarism / hypophysitis)	Continue hormone replacement/suppression and monitoring of endocrine function as appropriate.
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), antithyroid 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	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.

	• 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.	
Other irAEs (not described above)		
Grade of other irAEs (NCICTCAE 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	

Abbreviations: ACTH=adrenocorticotropic hormone; ADL=activities of daily living; ALT=alanine aminotransferase; AST=aspartate aminotransferase; BNP=B-type natriuretic peptide; CK-MB=creatine kinase MB; CT= computed tomography; FSH=follicle-stimulating hormone; GH=growth hormone; IGF-1=insulin-like growth factor 1; irAE=immune related adverse event; IV=intravenous; LH=luteinizing hormone; MRI=magnetic resonance imaging; NCI CTCAE=National Cancer Institute Common Terminology Criteria for Adverse Events; PRL=prolactin;T4=thyroxine; TSH=thyroid stimulating hormone; ULN=upper limit of normal.

9.4.1.1 ADC Vaccines

Treatment schema: Patients received a dose of ADC at days 1, 14, 28, 42 and 56 days, and thereafter every 6 months until 6 additional doses, progressive disease or unacceptable toxicity, whichever occurs first.

Preparation: Vaccines were distributed and stored in liquid nitrogen. For administration, vaccines were thawed at 37ºC and immediately injected intradermally.

Administration: ADC vaccines were injected intradermally, once thawed at 37ºC without dilutions.

Cell injection: Thawed: keep the vaccine between gloved hands (approximately 37ºC) for 5-7 minutes.

Just right after thawing, the vaccine should be immediately administered within a maximum of 10 minutes. After thawing, the total content of the vial must be transferred to a syringe of maximum 2ml, with a needle for intradermal administration. To do this, you should tilt the vial with the needle, pull the plunger, and extract all the content. The vial should be inspected in order to confirm that no suspension remains.

Before administration, the vaccine should be visually inspected to confirm that the suspension is homogeneous. Once ready, intradermal administration of the vaccine should be performed, according to local protocols.

Dose modifications: It was not expected dose modification for ADC vaccines.

If needed, please contact coordinating investigators to any query you may have regarding IMP management or dosification (investigacion@mfar.net).

9.4.2. DESCRIPTION OF INVESTIGATIONAL PRODUCTS

1. Avelumab:

Presentation: Fully human antibody (calculated molecular weight of 143832 Dalton) of the immunoglobulin G (IgG) 1 isotype that specifically targets and blocks PD-L1, the ligand for PD-1. Avelumab drug product is a sterile, clear, and colorless concentrate for solution intended for intravenous (Iv) infusion. The drug is presented at a concentration of 20 mg/mL in single-use glass vial containing 200 mg of avelumab.

Storage conditions: Avelumab drug product must be stored at 2°C to 8°C until use, and it must not be frozen. Rough shaking of avelumab product must be avoided.

2. ADC Vaccines:

Presentation: Cryopreserved autologous dendritic cells loaded with autologous tumour antigens in suspension for intradermal administration. Each vial contains $10x10^6$ of ADC plus matured DCs.

Storage conditions: Conservation in nitrogen vapor cryotubes.

9.4.3. METHOD OF ASSIGNING PATIENTS TO TREATMENT GROUPS

During phase I 3+3 cohort, all informed consent (IC) signatures were registered immediately in the eCRF in order to reserve a patient slot, in the same way, any screening failure should immediately be registered in the eCRF to liberate the slot to other patients. During the slot reservation period, communication among sites and CRO were constant in order to inform any DLT, cohort expansion or phase II activation.

During phase II, screening and registration were open, patients were allocated according to the IC signature, however when patient enrolment was close to Simon first stage (18 patients), slot reservation period was activated.

9.4.4. SELECTION OF DOSES IN THE STUDY

Avelumab

Avelumab was administered intravenously at a dose of 10 mg per kilogram of body weight every 14 days until disease progression or unacceptable toxicity. Based on the PK results and the receptor occupancy data, sufficient trough concentrations appear to be achieved for full TO in the blood in the majority of subjects receiving the 10 mg/kg dose. Within the dose range of 1 mg/kg to 20 mg/kg, avelumab was well tolerated and is deemed to have an acceptable safety profile.

Based on Avelumab IB, a dose of 10 mg/kg Iv once every 2 weeks was considered to have a favourable risk benefit profile and thus represents an appropriate dose for further investigation in studies of avelumab.

ADC vaccines

Vaccines were available for administration in 7-10 calendar days from receiving source patient samples, and should be stored in liquid nitrogen. Each vial containing $10x10^6$ of ADC plus matured DCs was thawed at 37ºC and immediately injected intradermally. Intradermal route of administration and dosification had been selected according to the information of previous published study (NCT01413295) and IB.

Please, refer to Avelumab or ADC vaccines Investigator's Brochure (IB) for further information about the nonclinical and clinical programs and Guidance for the Investigator.

The experience from previous clinical trials showed that most of the injected cells are retained at the site of inoculation, being subsequently eliminated by macrophages from the body itself. In these studies, it has been determined that between 1-4% of the total injected cells migrate towards the lymph nodes that drain the inoculation area, in addition, the number of nodes among which the cells are distributed has been determined between 2-5 different nodes. Thus a high concentration i.e. $10x10^6$ is needed in order to achieve a significant amount of cells reaching the tumor microenvironment.

Two studies with CDs have also been developed at our institution -Hospital Clínic i Provincial de Barcelona, one for the treatment of colorectal cancer (NCT01413295; PEI 09-133), without adverse effects, and another for patients with Crohn's disease (PEI-08-049 trial), showing any side effects associated with the administration of the cells at the concentration selected for the GEMCAD-1602 trial.

There is no expectation of a sensitization against the administered DCs or a rejection of them, since it is an autologous treatment (cells from another donor are not administered). For this same reason, a graft versus host disease or an allergic reaction to the inoculation of DCs is not foreseeable.

There is no forecast of tumor induction in relation to dendritic cell transfer since dendritic cells are cells that do not proliferate, and therefore the risk of tumor generation is practically nil.

Based on the available nonclinical and clinical data to date, the conduct of the trial specified in this protocol was considered justifiable.

9.4.5. SELECTION AND TIMING OF DOSES FOR INDIVIDUAL PATIENTS

This was a single arm trial. All patients received avelumab and ADC vaccines following the same dose schedule:

Avelumab

Avelumab will be administered intravenously at a dose of 10 mg per kilogram of body weight every 14 days until disease progression or unacceptable toxicity.

Based on Avelumab IB, a dose of 10 mg/kg Iv once every 2 weeks was considered to have a favourable risk benefit profile and thus represents an appropriate dose for further investigation in studies of avelumab.

A dose of 10 mg/kg of avelumab intravenous (IV) every 2 weeks was selected for the expansion cohorts of Phase I studies (including the UC cohorts that formed the basis for approval in the indication), the Phase II pivotal study in mMCC, and most ongoing Phase III studies based on the preliminary pharmacokinetic (PK), target occupancy (TO), and safety data collected in the clinical studies.

The 10 mg/kg every 2 weeks dose used in the pivotal study of mMCC (Study EMR100070-003 Part A) is associated with substantial tumor reduction, clinically meaningful efficacy with durable responses and prolonged progression free survival (PFS)

and has an acceptable safety and tolerability profile. Based on these considerations and a favorable benefit/safety profile, a dose of 10 mg/kg every 2 weeks has been approved (cutoff date 22 March 2019).

ADC Vaccines

Vaccines will be distributed and stored in liquid nitrogen. For administration, vaccines will be thawed at 37 ºC and immediately injected intradermally. The dose of ADC vaccine will be $10x10^6$ (according to ADC vaccines investigator brochure and NCT01413295 study). Patients will receive a dose of ADC at days 1, 14, 28, 42 and 56 days (5 doses), and every 6 months, thereafter until progressive disease (maximum of 6 additional doses) or unacceptable toxicity.

The chosen schedule is consistent with the observed persistence in the body of DCs upon administration. DCs do not have proliferative capacity, they are fully differentiated and terminal cells with an approximate half-life of between 7-15 days. Therefore, their persistence in the body once inoculated is limited to that period of time and later they will be eliminated. Tumors derived from the application of these cells have not been described in humans, which indicates their low or no proliferative capacity. In addition, neither gene transfer, nor immortalizing or mutagenic agents will be used, which could modify the DCs and pose some type of risk.

Based on these considerations, the proposed schedule included 5 doses for induction of response every 14 days, to maintain DC levels and a maintenance with a dose administration every 6 months afterwards.

9.4.6. PRIOR AND CONCOMITANT THERAPY

All concomitant medications taken by the subject during the trial, from the date of signature of informed consent were recorded in the appropriate section of the CRF and in patient record, noting the name, dose, duration and indication of each drug.

The indication, dose, frequency and dates of treatment were recorded in the patient's medical records and appropriate section of the eCRF.

All medications (prescriptions or over-the-counter medications) continued at the start of the trial or started during the study or until 30 days from the end of the last protocol treatment and different from the study medication must be documented.

Permitted concomitant medication:

Any medications that were considered necessary to protect subject welfare and do not interfere with the trial medication may be given at the Investigator's discretion.

Rescue medications may be administered to address ineffective treatment, anticipated adverse reactions or anticipated emergency situations.

Pre-medication: 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 judgement and presence/severity of prior infusion

reactions. This may be modified based on local treatment standards and guidelines, as appropriate.

Prohibited concomitant medication:

Patients must not receive any other concurrent anti-cancer therapy, including investigational agents, while on study treatment.

Steroids administered at doses > 10 mg or >10 mg equivalent prednisone per day.

Vaccination within 4 weeks of the first dose of avelumab and while on trial was prohibited except for administration of study treatment.

Any other investigational treatment within the past 30 days of treatment initiation and during the trial.

9.4.7. TREATMENT COMPLIANCE

IMP compliance was assessed by reviewing the consistency of information recording at the eCRF, patient records, nurse sheets and pharmacy documentation.

Compliance of IMP schema is critical for patients and trial outcomes, any relevant deviation in IMP schema may jeopardize trial results or may affect patient safety, for that reason it may be considered as protocol deviation.

When an investigational product is dispensed in a clinical trial, the investigator or her/his designated person, will ensure high level of compliance of the investigational product administration.All stages from IMP preparation until treatment administration were documented in the corresponding forms in order to guarantee traceability during the procedure.

Patient compliance with the investigational products was recorded in the eCRFs and reviewed by clinical research associates (CRAs) at the time of the monitoring visit, when applicable.

9.5. EFFICACY AND SAFETY VARIABLES

9.5.1. EFFICACY AND SAFETY MEASUREMENTS ASSESSED

Efficacy:

At screening, the following demographic data was collected: date of birth, sex (gender), race, ethnicity, medical history: previous illness, concomitant illness at entry into the trial, allergies, prior therapies for the target indication (‘relevant previous medications’), concomitant therapies. Baseline PCR, albumin, LDH and CEA value.

MSS/MSI-H, RAS and BRAF determination were mandatory for all patients.

Tumor assessment for PFS

Following the baseline assessment, subsequent tumour assessments according to RECIST were performed systematically every 8 weeks (±1 week) until progression disease relative to date of inclusion, according to the planned study schedule.

Tumor assessment was also done at planned interval visit and included systematically only physical examination and PCR, albumin, CEA and LDH blood analysis.

Imaging modalities

At baseline, the imaging modalities used for RECIST assessment were chest and abdominopelvic CT scan with other regions as clinically indicated for the assessment of disease. During follow up, the same imaging modality was used. PET-scan could be used for disease progression assessment only if a new lesion had appeared compared to a PET-scan performed previously.

Radiological examinations performed in the conduct of this study should be retained at site as source data.

It is important to follow the assessment schedule as closely as possible. If scans were performed outside of a scheduled visit ± 1 week window interval and the patient had not progressed, every attempt should have been made to perform the subsequent scans at their scheduled time points.

Patients were evaluated until objective radiological disease progression by RECIST 1.1.

Tumor evaluation

If a patient discontinued treatment prior to progression then the patient should still continue to be followed every 8 weeks, until objective radiological disease progression as defined by RECIST 1.1.

Categorization of objective tumour response assessment was based on the RECIST criteria of response:

1. Complete response (CR).
2. Partial response (PR).
3. Stable disease (SD).
4. Progression of disease (PD).
5. Not evaluable (NE).

Target lesion (TL) progression was calculated in comparison to when the tumour burden was at a minimum (i.e. smallest sum of diameters previously recorded on study). In the absence of progression, tumour response (CR, PR, SD) was calculated in comparison to the baseline tumour measurements obtained before inclusion.

Safety:

The safety profile of the IMP was assessed through the recording, reporting and analysis of baseline medical conditions, adverse events (AEs), physical examination findings including vital signs and laboratory tests.

The AE reporting period for safety surveillance began when the subject was initially included in the trial (date of first signature of informed consent/date of first signature of first informed consent) and continued until the 30-Day Post-Treatment Safety Follow-up.

Any SAE assessed as related to Avelumab or ADC vaccines were reported whenever it occurs, irrespective of the time elapsed since the last administration of Avelumab or ADC vaccines.

At each trial visit, the subject was queried on changes in his or her condition. During the reporting period, any unfavorable changes in the subject's condition were recorded as AEs, whether reported by the subject or observed by the investigator.

Completed, accurate and consistent data on all AEs experienced for the duration of the reporting period (defined below) were reported on an ongoing basis in the appropriate section of the CRF. All SAEs and all non-serious AEs of special interest were additionally documented and reported using the appropriate Report Form.

Each AE report included a description of the event, its duration (onset and resolution dates (and times when it is important to assess the time of AE onset relative to the recorded treatment administration time), its severity, its causal relationship with the trial treatment, any other potential causal factors, any treatment given or other action taken, including dose modification or discontinuation of the IMP, and its outcome. In addition, serious cases were identified and the appropriate seriousness criteria documented. If an AE constituted a DLT this had to be documented accordingly.

Comprehensive assessment of any apparent toxicity experienced by each subject was performed from the time of giving informed consent and throughout the trial. The investigators reported any AEs, whether observed by the Investigator or reported by the subject.

Translational substudy:

A translational sub-study was carried in order to identify a more favourable phenotype for efficacy and to evaluate changes in the phenotype after therapy.

Sample collection and methodology:

1. At screening period (<28d) and at 2 months 60 ml of blood were extracted (50 ml for PBMC and 10 ml for serum at -80 ºC).

2. MSS status, RAS and BRAF were determined from archival biopsies. Biopsies before study entry and at 2 months of therapy were done, to evaluate pharmacodynamics changes before and after therapy.

All genomic analysis were performed in the Translational Genomics and Targeted Therapeutics in Solid Tumours Lab at Hospital Clínic/IDIBAPS led by Dr. Aleix Prat. Briefly, a section of the formalin-fixed paraffin-embedded (FFPE) tumour tissue was first examined with a haematoxylin and eosin staining to determine the tumour surface area and cellularity. For RNA purification (Roche® High Pure FFPET RNA isolation kit), 1 to 8 10µm FFPE slides were cut for each core biopsy, and macro dissection was performed, when needed, to avoid contamination. We used a novel technique nCounter (NanoString Technologies), that needs very few tumours, and results are robust and rapidly obtained (48h).

Storage and analyses of samples was handled according to the specifications as described in the Informed Consent Form.

Table 7. Schedule of Assessments

Study procedure	Selection		On treatment visits	End of treatment	Extended safety follow-up (up to 90 after end of treatment)	PFS evaluation (every 8 weeks until progressive disease)	OS Long-term Follow-up
	≤ 28 d before D1C1	≤ 7 d before D1C1**	Weekly for 1 month, every 2 weeks thereafter and as indicated	Safety visit* (30 ± 5 d) after end of treatment			
Medical consultation							
Informed consent signature	X						
Final Eligibility criteria assessment		X					
Medical History and CRC prior treatments	X						
Physical examination and vital signs	X		X	X		X	
ECG	X						
Comorbidities (Charlson score)	X					X	
ECOG	X	X	X	X			
Adverse events follow-up		Every visit			X		
Concomitant medication	X	Every visit					
Lab. Procedures							
Pregnancy test	X	X Monthly during treatment					
Haematology and biochemistry	X	X	X	X		X	
Free T4 and TSH, CEA, PCR, albumin, and LDH determination		X	Until PD, every 8 weeks				
MSS, RAS and BRAF status determination (local)	X						
Biological sample collection							
Apheresis for ADC vaccines (60 ml)	X						
Formalin-fixed paraffin-embedded (FFPE) central evaluation (RAS, BRAF and MSS) and phenotype (CMS and immunosignature) *Tumor archival	X		X (8 week)				
3 tubes of 4ml of peripheral blood for central virus determination (10 ml)	X						

50 ml of blood for lymphocytic response evaluation	X		X (8 week)			
Tumor biopsy (dry ice)	X					
ELISA (10 ml serum for cytokine and chemokine analysis) -80º	X		X (8 week)			
Efficacy follow-up						
CT Scan	X	Until PD according to RECIST 1.1, every 8 weeks.				
Treatment administration						
Avelumab		Every 2 weeks until PD or unacceptable toxicity				
ADC vaccine		Days 1, 14, 28, 42 and 56 and thereafter up to 6 additional doses, every 6 months, until progressive disease or unacceptable toxicity				
OS follow-up***						X

*Safety follow-up visit performed 30 days (± 5 days) after the last administration of the investigational product.

**Determination performed during “baseline assessments” can be used for the purposes of “prior treatment determination” if they are performed within 7 days before start of treatment

*** (Alive - Last visit FU / date of death) starting after progression, according to local practise.

Protocol specifications for trial determinations

Informed consent signature: It should be clearly stated that the patient consent her/his participation in the GEMCAD-1602 trial in the patient record.

ECG: Standard 12-lead ECG. According PI discretion, additionals ECG could be scheduled

Vital signs: Included the measurement of: temperature, pulse and blood pressure

Weight: It should be determined during baseline and re-measured each visit. For avelumab dose calculation modifications should be done when >10% increase or decrease in body weight.

Comorbidities: Charlson Index (cardiovascular disease, previous cancer and diabetes, pulmonary disease, dementia, etc).

ECOG performance status: To be performed as a part of physical examination, baseline ECOG performance status is critical, it is mandatory to perform and register both in patient record and eCRF.

RECIST response assessment: Response evaluation was conducted, according to the revised RECIST 1.1 criteria, every 8 weeks until disease progression. Subject follow-up should be performed regardless of discontinuation or delay of treatment.

Adverse events follow-up: Adverse events were recorded continually, until the safety follow-up visit. SAEs and significant AEs should be followed up until resolution or stabilisation.

Concomitant medication: To be recorded continually, until the safety follow-up visit, including over the counter treatments and traditionally treatments.

Pregnancy test: Women of fertile age should undergo a serum pregnancy test <7 days prior to investigational treatment initiation.

Haematology and biochemistry: Haematology tests: complete hemogram including WBC differential count and platelet count. Biochemistry tests: sodium, potassium, magnesium, creatinine, albumin, glucose, calcium, phosphorus, AST, ALT, alkaline phosphatase, LDH, total protein, total bilirubin.

CEA, LDH, PCR, albumin determination: Basally (<7 days before treatment initiation) and every 8 weeks till progressive disease.

Apheresis for ADC vaccines: Final volume of 60 ml containing mononuclear cells (>5x10^9 mononuclear cells) should be collected and processed.

50 ml of blood were obtained initially (<28 days screening period) and at 2 months to evaluate lymphocytic response

3 tubes of 4 ml of peripheral blood were obtained initially for central virus determination.

Archival Formalin-fixed paraffin-embedded (FFPE) tumour: MSS status and RAS and BRAF determination were centrally determined from archival biopsies. Local determinations if available were also recorded.

Tumour biopsy (dry ice): It was obtained by colonoscopy or from metastases, before study entry. This biopsy was used to generate the tumour lysates.

Tumour biopsy (study specific): Formalin-fixed paraffin-embedded (FFPE) should be sent to the central lab. Biopsies before study entry and at 2 months of therapy were obtained, to evaluate pharmacodynamics changes before and after therapy.

Biomarkers blood sample collection: 10 ml of blood processed to serum. Samples were processed and stored at -80 ºC. Sponsor collected the samples by dedicated courier during the trial.

CT Scan: Tumour imaging evaluations through CT-scan were performed at selection, every 8 week until disease progression. Subject follow-up by CT scan should be performed regardless of discontinuation or delay of treatment until progressive disease. Response evaluation was conducted, according to the revised RECIST 1.1 criteria.

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

Free T4 and TSH: must be performed at baseline and at least every 8 weeks during treatment and at end of treatment or 30 days post-treatment safety follow-up (if not performed in the previous 8 weeks).

Extended safety follow-up: 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.

Table 8. Sample collection chart

Day	Type of sample	Delivery condition	TIme for delivery	Rationale
-X	Archival biopsy	Room temperature	Along with paraffin tumour blocks of D-14	Centralized evaluation
-14	**Fresh frozen biopsy (Colonoscopy or M1)**	Dry ice	Immediate	Vaccine development
	Paraffin biopsy (3-4 blocks)	Room temperature	Within a week. This could be sent along with the archival biopsy.	Pharmacodynamic study
-7	**Leukapheresis (60ml, >5x10^9 mononuclear cells**	Refrigerated (4ºC)	Immediate	Vaccine development
	3 tubes of 4 ml of peripheral blood	Refrigerated (4ºC)	Immediate	Vaccine serology
	50 ml of peripheral blood	Refrigerated (4ºC)	Immediate	Lymphocytic response
	-80ºC serum	Dry Ice	Across the study	Substudy
56	**Paraffin biopsy (3-4 blocks)**	Room temperature	Within a week	Pharmacodynamic study
	50 ml of peripheral blood	Refrigerated (4ºC)	Immediate	Lymphocytic response
	-80ºC serum	Dry Ice	Across the study	Substudy

9.6. DATA QUALITY ASSURANCE

The Investigator or designee was responsible for ensuring that the data collected in the course of this trial was accurate and documented appropriately on all applicable forms. They were processed, evaluated, and stored in anonymous form in accordance with applicable data protection regulations. The Investigator must ensure that the eCRFs and any other associated documents forwarded to Sponsor or its designated organization contain no mention of any subject names.

The data was entered into a validated database. The Sponsor or its designee are responsible for data processing, in accordance with the Sponsor's data management procedures. Database lock occurred once quality control and quality assurance procedures had been completed.

This trial was monitored in accordance with the ICH GCP, and any other applicable regulations. The site Monitor performed visits to the trial site at regular intervals according to the agreed monitoring plan.

The clinical trial protocol, each step of the data capture procedure, and the handling of the data, including the final clinical study report, was subjected to independent Quality Assurance activities. Audits may be conducted at any time during or after the trial to ensure the validity and integrity of the trial data. Representatives of the Quality Assurance unit from the Sponsor or a designated organization, as well as Health Authorities, must be permitted to access all trial documents and other materials at the site, including the Investigator Site File, the completed CRFs, all IMP and IMP accountability records, and the original medical records or files for each subject.

Central reviewing of the anatomopathological diagnosis was performed as an additional quality assurance (QA) procedure for the most critical eligibility criteria of the clinical trial. MSS status and RAS and BRAF determination were centrally determined from archival biopsies. Local determinations if available were also recorded.

For QA of the data, all members of the trial were required to be trained in GCPs, clinical trial legislation, pathology, and protocol-related issues. Data quality control procedures included the need for the CRA to confirm that the data were handled appropriately by the investigator staff and that trial documentation (management templates, spreadsheets, eCRF, and sample handling), both at the beginning of the trial and when changes in the team were introduced, was properly handled.

For this trial, 4 procedures for data QA were set in place:

1. Training of CRAs in pathology: performed by the Clinical Trial Project Manager (CTPM) using the training materials available from the CRO and other materials provided by the sponsor. Several meetings took place between the coordinating investigator and CRO staff during the trial.

2. Training of CRAs in study procedures: performed by the CTPM through training materials available from the CRO and the study protocol.

3. Training of research teams: Training of site staff was maintained throughout the study, and training to staff was provided whenever the need was identified by the study monitor and was mandatory at the following times:

a. Site initiation visits (SIVs): SIV is the first exposure of the site team to the study. During these visits, the most important aspects of the trial were presented using a deck of slides previously validated by the trial coordinating investigator. The presentation included the justification of the project, the objectives, eligibility criteria, study procedures, reference to ICH-GCP (informed consent procedure, inclusions, pharmacovigilance, and protocol deviations management), drug management, the identification of CRAs, and all relevant issues.

b. Routine monitoring visits: Follow-up monitoring visits are vital because they allow CRAs to maintain contact with the site staff and to verify adherence to the protocol, ICH-GCP, and site regulations. Therefore, whenever necessary, CRAs provided training to the study team at the site; training was mandatory when there were changes in the members of the research team, in cases of substantial amendments to the protocol, patient information sheet, or study procedures, and in case of major protocol deviations. The source data validation of critical variables was performed according to the monitoring plan agreed to with the sponsor.

4. Protocol, study documents, consultation, and data management

a. Protocol: The protocol was developed from the protocol synopsis approved by the study partner using the template provided by the AEMPS and after several reviewing and validation steps by the sponsor, the biostatistician, coordinating investigators, and the coordinating team. Once the protocol was approved by the sponsor, it was submitted along with other documentation to the EC and AEMPS for evaluation. The protocol and/or synopsis was distributed to the sites to confirm their participation. Once the study was approved, trial documentation was distributed to the sites. Any comments or queries received by the CRA were recorded in the QA log, available on the study trial master file (TMF). Recording of this feedback is critical because it could identify points of improvement in the protocol and issues that could lead to protocol amendments.

b. Study documents: Includes study forms, the TMF, investigator site file (ISF) model report, etc. All improvements proposed by the teams were evaluated and implemented if necessary.

c. Inquiries: Any comments, questions, answers, and details of the concerned managing person were recorded in the QA log. This system is very effective to ensure uniformity of approach when addressing issues from the centre as requested.

d. Data management: The preparation of the CRF included a review by the coordinating investigator and study statistician to confirm that the variables collected and the methods of collection were suitable for the study. The reviewing of the CRF and guidelines for finalising the CRF were explained at the SIV.

e. Source data verification: Study data were collected by investigator and staff using eCRFs that were reviewed during monitoring visits. CRAs reviewed data collected in eCRFs and their consistency with the source data.

The source data were defined according to GCP as “all information in original records and certified copies of original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial”.

Source documents were defined according to GCP as “original documents, data, and records (e.g., hospital records, clinical and office charts, laboratory notes, memoranda, subjects’ diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate copies, microfiches, photographic negatives, microfilm or magnetic media, X-ray images, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial)”.

Source documentation is the medical record of the subject before, during, and after the trial. It confirms the eligibility criteria of the subject in the given trial. It documents the progress of the subject from consenting until the subject completes the study. It records the accountability of the investigational product dispensed, consumed, and returned by the subject. It serves as the complete medical record of the subject with reference to the treating physician at any point in time.

Finally, it forms a strong foundation for the data that got transcribed into a CRF, which ultimately gets translated into a clinical study report (CSR).

The monitoring plan included SIVs, 2 regular monitoring visits (RMV) during first stage (phase I) and 3 for second stage (phase II), and close-out visits (COVs) for each site. During RMVs, CRAs reviewed the source data. A total of 27 follow-up visits to the centers for the two phases were scheduled, those centers that had recruited at least one patient in phase I were visited by the monitor on one occasion and additional visits were necessary following the definition of risk-based monitoring (defined below) to ensure that study procedures were properly followed and data were collected appropriately.

Upon completion of Phase I, all centers that had included patients in this first phase received 1 monitoring visit after the TLDs observation period of the last patient included in this phase.

A monitoring visit was made to all centers with patients, 6 months (+/- 2 weeks) from the start of treatment of the first patient (coinciding with the CT of the evaluation of the main variable)

Closing Visit: Carried out throughout the year 2020, once the participation in the study of all the patients in each of the centers have ended.

Monitoring was performed following a RISK-BASED approach as detailed below:

The patients were selected following the criteria of “risk-based monitoring”. The selection of patients to be monitored were made by the center's CRA in accordance with the PM of the

study, analyzing the information reported by the centers collected in the eCRF, the "global trial management" spreadsheet and the previous reports of the monitoring visits.

The patients who met the highest number of the following conditions were selected in the first instance, ordered in descending order:

1. Appearance of DLTs (only for phase I)
2. Events for the study variables death and / or disease progression.
3. Non-compliance with selection criteria
4. SAEs reported / detected
5. Incorrect administration of study treatment
6. Lack of adherence to patient follow-up (visits not properly scheduled, imaging tests out of date, techniques used, etc.)
7. Delays in filling in the data in the CRD (large number of pending queries, missing data, etc.)
8. Greater number of visits pending monitoring

DATA VERIFICATION (SDV):

A 100% SDV was expected in certain variables, following the previously described risk-based monitoring criteria, these checks were expected to be performed in approximately 3 patients per center (this estimation varied according to the quality of the data reported by the research team). Any incident was reflected in the monitoring report and was conveniently reported to the Coordinating Investigator as the Sponsor's representative in this study. The procedure for the review of source documents is detailed below:

a) All patients included (patient consents will not be reviewed for screening failures):

- Informed Consent: Version review, completion of informed consent fields, name, date and signature of the principal investigator. Name, date and signature of the patient.

b) Up to a maximum of 3 patients per visit.

- Baseline tests: Local diagnosis, MSS, Localiz. tumor, ECOG, LDH.
- Primary endpoint:
    - Phase I Centers (in case of TLD): Treatment start date, TLD date, toxicity and degree of TLD.
    - Disease evaluation at 6 months: date CT scan and disease evaluation.
- Secondary variables:
    - Disease and progression evaluation: CT evaluation, date of progression and death (date and reason for death).

c) One patient per visit (selected through risk-based monitoring):

- Review of the ICF: Necessary to determine if the informed consent process of the patient is adequately collected.
- Eligibility criteria: Inclusion / exclusion criteria.

- Serious Adverse Events (SAEs) and SAEs report: Interview with the HC investigation and review team to identify potential unreported SAEs, resolution of reported SAEs with pending issues.
- Administered drug dose: One patient selected according to risk-adapted monitoring per visit. Review of date and dose of cycles administered to the patient.

d) In addition, during the visits, the investigation team will be interviewed to collect information regarding:

- Collection and storage of biological samples
- Communicate deviations from the protocol in accordance with good clinical practices and regulatory requirements, taking necessary actions to prevent the recurrence of detected deviations.

e) In the Pharmacy service / vaccine warehouse service:

- Accounting and correct vaccine administration of a patient selected by risk-based monitoring per visit
- Reconciliation and management of stock incidents.
- Review of temperature records since the last visit (only in case of reported temperature deviations)

Data cleaning: All inconsistent data were queried to the site, and all responses were answered by the site in writing; the corresponding fields in the eCRFs were amended, and new data were collected in the database. All changes were tracked in the corresponding audit trail of the eCRF.

9.7. STATISTICAL METHODS PLANNED IN THE PROTOCOL & DETERMINATION OF SAMPLE SIZE

9.7.1. STATISTICAL AND ANALYTICAL PLANS

Endpoints:

Primary:

To increase the percentage (from 20% to 40%) of pre-treated MSS mCRC patients free of progression at 6 months.

Secondary:

1. To evaluate the safety and tolerability of avelumab in combination with ADC vaccine.
2. To identify a favourable phenotype for efficacy.
3. To evaluate pharmacodynamic changes between pre-treatment and on-treatment tumour biopsies.
    - Modified CMS classification by NanoString.
    - Immunophenotype signature by NanoString.

Statistical considerations

The primary analysis for efficacy and safety was done by intention to treat (ITT). The population consisted of patients who met the criteria of selection and were exposed to at least 1 treatment cycle, regardless of the presence of deviations to the protocol or the patient's withdrawal from the study. The primary endpoint is 6 months PFS rate.

Efficacy endpoints: PFS and OS will be evaluated with the Kaplan-Meier curves and compared by a stratified log-rank test. We will fit Cox regression modeling for OS and PFS. Analysis was performed using SAS version 9.4 software (SAS Institute Inc., Cary, NC, USA) and the level of significance was established at the 0.05 level (two-sided).

Safety was evaluated as AEs and SAEs classified by type, frequency and intensity.

Further analysis was performed stratifying patients by the following baseline characteristics:

- IMMETCOLS
- GEP
- Age
- Gender
- LDH levels
- Primary tumour surgery
- Previous lines
- Genotype
- ECOG
- Affected organs at baseline

9.7.2. DETERMINATION OF SAMPLE SIZE

Sample size assumptions:

- Phase II
- Simon´s two stage minimax design.
- Alpha: 0.05, Beta: 0.2.

Table 9. sample size determination

		First Stage	Second Stage	
6-months PFS (expected)	6-months PFS			Total number of patients
20%	**40%**	**5/18**	**11/33**	**33**

During the first stage, 18 patients will be recruited including those receiving the RP2D in the phase 1. 6 months PFS will be evaluated in this population. If >13 patients have experienced progression disease, combination will be considered not effective and clinical trials will be closed. If ≥ 5 patients were identified without progressive disease at 6 months, recruitment will be continued for stage 2.

During stage 2, 15 more patients will be recruited.

9.8. CHANGES IN THE CONDUCT OF THE STUDY OR PLANNED ANALYSES

9.8.1. PROTOCOL AMENDMENTS

The initially approved protocol version was version 2.1 (Jan 23rd, 2018); which was approved on March 12th, 2018. The resolution for approval from the CA asked for some requirements to be implemented in subsequent amendments:

It was necessary to better standardize the following parameters:

- The weight of the biopsy used to obtain the tumor lysate.
- The inclusion of pre-inoculation controls as specifications or not of the finished product
- The concentration of autologous tumor lysate during cell maturation.
- The inclusion of the count and the viability at days 1, 3 and 8 within the controls in the manufacturing process.

The following information should be included in further amendments:

- Certificates of all reagents used in the preparation of dendritic cells
- Annex E. Validations
- The raw release data of the batches of product under investigation obtained in the previous clinical trial with this product.
- The relevant information that justifies the expiration date (5 years from the date of manufacture).

Finally, for future relevant modifications in the quality IMPD or new clinical trials with this product, a version with changes must be provided with respect to the latest version of PEI 09-133 authorized by the AEMPS.

The protocol was not substantially amended during the trial, so the requested and/or information requirements were not implemented/provided to the CA. Three notes to file to notify erratums were submitted as follows:

- NTF serology (Aug 14th, 2018):

Regarding the determination of IgG cytomegalovirus (CMV), it has been confirmed that positivity in this result is not relevant for this study:

Exclusion criteria 7: Local positive serologic determination to: HBsAg, Anti-HBc, HBV, HCV, HCV RNA, HIV-I RNA, Agp24 IIIV + AC IIIV ½ (MLIA) serum, IgG antigen core v. hepatitis B, RPR (Ac reagínicos Lues-RPR, serum), IgG cytomegalovirus (EIA), Ac anti HTLV I/II (if patient came from endemic zone), Ac anti Trypanosoma Cruzi, Chagas, (if patient came from endemic zone), when RPR positive or doubtful for confirmation: IgG T. pallidum

(ELISA) IgM T pallidum (ELISA) , when IgG T. Pallidum doubtful: Pt confirmatory IgG/IGM, T pallidum (LIA).

From the donor's point of view, the determination of CMV when it is positive only in the case of heart transplantation has repercussions if the recipient is CMV negative.

In the context of the vaccine, the recipient of the cells is the same donor, therefore, although the serology is positive, it does not have any significance in this context, so it is not necessary for it to be negative for the patient to participate in the clinical trial.

- NTF AESI (Aug 14th, 2018):

The Sponsor and coordinator of the study together with MERCK agreed on the following regarding the notification of adverse effects of special interest:

The adverse effects of special interest (AESIs) according to the description that appears in the protocol (section 7.4.1.1) are:

- ➔ Potential Immune-related Aes: described in section 6.5.5 of this protocol.
- ➔ Infusion-related Reactions: described in section 6.5.5 of this protocol.
- ➔ Anti-Drug Antibody Response: Anti-drug antibody, previously referred to as HAHA, data are summarized based on the data available on 19 December 2014. Post-treatment samples for evaluation of ADA response were available for 39 subjects from the dose escalation cohort and 338 subjects in the pooled expansion cohort.
- ➔ 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.

AESIs grade 3 or higher (according to the latest version of NCI-CTCAE) will be notified by completing and sending the AESI form within 24 hours.

AESIs of grade 2 or lower will be notified through a report that MFAR will send to MERCK on a monthly basis. The report will be sent every month with all the adverse effects reported in the previous month for all patients participating in the clinical trial. This report will be an extract from the eCRF that will include the following information for each event:

- ➔ Event name (CTCAE v4.0 term)
- ➔ Grade (1-5)
- ➔ Initial date
- ➔ Final date
- ➔ Related to any study drug (yes/no)
- ➔ Specify (avelumab / vaccine / both)

- NTF LDH (Dec 20th, 2018):

Regarding serology, this will be carried out centrally. The serology that will be carried out after signing the informed consent for screening and before signing the informed consent for the clinical trial will be carried out at the clinic hospital in Barcelona, for all patients from all centers participating in the clinical trial.

Regarding the determination of LDH, as soon as the patient signs the informed consent for screening, a blood sample will be collected to perform the centralized LDH determination (H. Clinic de Barcelona). The results of this determination will serve to confirm inclusion criterion # 7 (LDH levels <1.5 ULN).

The determination of LDH will be repeated again at the local level (7 days before the start of the patient's treatment). In this case, the results of the determination will no longer be able to exclude the patient.

In the protocol, inclusion criterion 7 details the following:

LDH levels <1.5 ULN (ULN = 450 U/L). Maximum allowed 675 U/L.

Currently the LDH values of the Barcelona Clinic have changed. Now the ULN value is 234 U/L so the >1.5 ULN value to allow inclusion is < 351 U/L.

10. STUDY POPULATION

10.1. DISPOSITION OF PATIENTS

Figure 1. Patient distribution (CONSORT diagram).

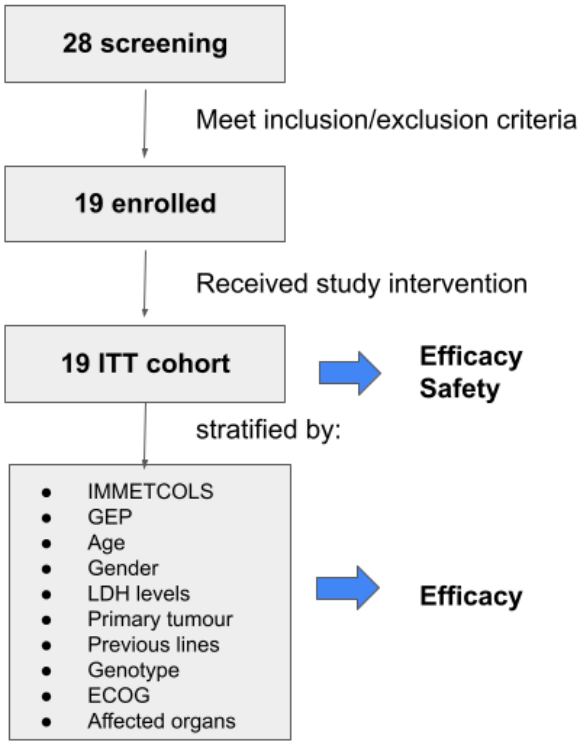


Table 10. Screening failure reasons

Screening failure reason	Screened
	n (%)
ECOG > 1	3 (10.7)
LDH > 1,5xLSN	4 (14.3)
Apheresis not viable	1 (3.6)
Brain metastasis	1 (3.6)

Table 11. Eligible patients by hospital

	Screened	Eligible
	N (%)	N (%)
Hospital Clínic de Barcelona	12 (42.9)	8 (42.1)

Hospital Universitari Vall d'Hebron	5 (17.9)	3 (15.8)
ICO Hospitalet (Hospital Duran i Reynals)	0 (0)	0 (0)
Hospital Universitario Puerta de Hierro	3 (10.7)	1 (5.3)
Instituto Valenciano de Oncología	6 (21.4)	5 (26.3)
Hospital Universitario Miguel Servet	0 (0)	0 (0)
Hospital Clínico Lozano Blesa	0 (0)	0 (0)
Hospital Universitari i Politécnic la Fe	0 (0)	0 (0)
Hospital Universitario la Paz	2 (7.1)	2 (10.5)
Total	28 (100.0)	19 (100.0)

A total of 28 patients were screened during the study, 9 of them were not eligible and were excluded. There were 19 patients who were considered eligible and were included in the study. All of them received the study treatment, avelumab plus ADC vaccine, and were included in the ITT cohort. The 19 patients were analyzed for safety and efficacy. Patients were also stratified according to their baseline characteristics to analyze efficacy according to:

- IMMETCOLS
- GEP
- Age
- Gender
- LDH levels
- Primary tumour surgery
- Previous lines
- Genotype
- ECOG
- Affected organs

Table 12. Disposition of patients

		Total
		N (%)

Patients enrolled		19 (100)
Received at least one cycle of study treatment		19 (100)
Evaluable patients		19 (100)
End of study (evaluable population)		19 (100)
Progression		19 (100)
Death		18 (94.7)
IMMETCOLS	Yes	5 (26)
	No	10 (53)
	NA/NE	4 (21)
GEP	Yes	5 (26)
	No	10 (53)
	NA/NE	4 (21)
Age ≤60 years	≤60 years	8 (42.1)
	>60 years	11 (57.9)
Age ≤62 years	≤62 years	10 (52.6)
	>62 years	9 (47.4)
Gender	Male	10 (52.6)
	Female	9 (47.4)
LDH	<234	11 (57.9)
	>234	8 (42.1)
Local Diagnosis	Rectum	6 (31.6)
	Sigma	13 (68.4)
Primary tumor surgery	Yes	13 (68.4)

	No	6 (31.6)
Previous lines ≤2	Yes	3 (15.9)
	No	16 (84.1)
Previous lines ≤3	Yes	12 (63.2)
	No	7 (36.8)
Genotype	All native	5 (26.3)
	KRAS	13 (68.4)
	BRAF	1 (5.2)
ECOG	0	13 (68.4)
	1	6 (31.6)
Number of affected organs	1	4 (21)
	>1	15 (79)
Number of affected organs	≤2	11 (57.9)
	>2	8 (42.1)

10.2. PROTOCOL DEVIATIONS

Table 13 specifies details of deviations from the study protocol by type and frequency of findings. There were 5 major protocol deviations and 36 minor deviations.

All protocol deviations were discussed with sites, codified, and escalated to Trial Coordinators with the corresponding associated information and severity assessment; when applicable, protocol deviation was reported to the competent authority (CA) according to current regulation, and corrective and preventive actions (CAPA) was applied in each instance.

Table 13. Protocol deviations

Deviation type	Minor	Major	Total
Procedures	29	0	29
Efficacy criteria	2	2	4
Informed consent form	1	3	4*
Safety	1	0	1
Eligibility criteria	3	0	3
TOTAL	36	5	41

**All findings with informed consents were managed and notified according to the current legal regulations.*

The most repeated findings were associated with study procedures and prolongation of screening period from the stipulated 28 days. Regarding informed consent, the most findings were related to incomplete completion concerning for consent of biological samples.

Major deviations consisted of:

- One patient that was enrolled in the stage 1 of the study and it was not possible to evaluate it as there were already 18 patients in that stage as stipulated per protocol. The patient was included and would have been part of the second stage if applicable.
- One patient continued the study treatment after progression due to potential clinical benefit on physician criteria
- Three patients did not sign the participation in the substudy and samples were obtained anyway.

11. EFFICACY EVALUATION

11.1. DATA SETS ANALYSED

The analysis was carried out by ITT analysis (n=19). The population analysed was composed of those patients who met the selection criteria and were exposed to at least 1 treatment cycle, regardless of the presence of deviations from the protocol or their withdrawal from the study.

All patients fulfilling the eligibility criteria (19 patients) were included in the study database. All of them received at least 1 dose of treatment and were considered for safety analysis.

11.2. DEMOGRAPHIC AND OTHER BASELINE CHARACTERISTICS

Table 14 and 15 show the demographics of the patients participating in the trial.

In the comparison between the groups in the stratified analysis by IMMETCOLS, GEP, age, gender, local diagnosis, primary tumor surgery, ECOG, genotype, LDH, previous lines, and number of affected organs, most of the baseline characteristics were balanced between groups.

When stratifying patients by IMMETCOLS, most patients in the IMMETCOLS positive group were all native (80%) whereas most patients on the IMMETCOLS negative group were BRAF or KRAS mutants (10 and 80% respectively).

When stratifying patients by GEP, most patients in the GEP positive group were all native (80%) whereas most patients on the GEP negative group were KRAS mutants (90%). ECOG was mainly 1 in GEP positive group (80%), whereas it was mainly 0 in the negative group (90%).

When stratifying patients by LDH levels, the sum of target lesions at baseline and the number of patients with hepatic lesions were distributed differentially between groups differences.

When stratified by genotype, patients with mutations in KRAS had higher proportion of positive IMMETCOLS than native genotype (61.5% vs 20% respectively) and higher proportion GEP (69.2% vs 30%).

When stratified by ECOG, patients with ECOG 1 had higher proportion of GEP (66.7% vs 7.7% respectively)(p= 0.032*) and native genotype (50% vs 15.4 % respectively) than those with lower ECOG 0.

Stratification by age, gender, tumor location, primary tumor surgery, and number of previous lines led to no relevant differences between groups in baseline characteristics.

In general, stratified analyses gave well balanced groups in terms of baseline characteristics.

Table 14. Demographics of study patients (I): Categorical variables

Baseline characteristic		n (%)
IMMETCOLS	Yes	5 (26)
	No	10 (53)
	NA/NE	4 (21)
GEP	Yes	5 (26)
	No	10 (53)
	NA/NE	4 (21)
Age ≤60 years	≤60 years	8 (42.1)
	>60 years	11 (57.9)
Age ≤62 years	≤62 years	10 (52.6)
	>62 years	9 (47.4)
Gender	Male	10 (52.6)
	Female	9 (47.4)
LDH	<234	11 (57.9)
	>234	8 (42.1)
Local Diagnosis	Rectum	6 (31.6)
	Sigma	13 (68.4)
Primary tumor	Yes	13 (68.4)
	No	6 (31.6)
Previous lines ≤2	Yes	3 (15.9)

	No	16 (84.1)
Previous lines ≤3	Yes	12 (63.2)
	No	7 (36.8)
Genotype	All native	5 (26.3)
	KRAS	13 (68.4)
	BRAF	1 (5.2)
ECOG	0	13 (68.4)
	1	6 (31.6)
Number of affected organs	1	4 (21)
	>1	15 (79)
Number of affected organs	≤2	11 (57.9)
	>2	8 (42.1)
Diagnostic stage	II	1 (5.3)
	III	2 (10.5)
	IV	16 (84.2)
Surgery for primary tumor	Yes	13 (68.4)
	No	6 (31.6)
Neo/Adjuvant CT	Yes	2 (10.5)
	No	17 (89.5)
Type of CT (n=2)	CAPOX	1 (50)
	FOLFOX	1 (50)
Fresh baseline biopsy	Yes	19 (100)
Fresh baseline biopsy location	Hepatic metastasis	10 (52.6)

	Lung metastasis	2 10.5()
	Primary tumor	5 (26.3)
	Peritoneum	1 (5.3)
	subclavicular adenopathy	1 (5.3)
Second biopsy location	Hepatic metastasis	8 (42.1)
	Primary tumor	3 (15.8)
	NA	8 (42.1)
KRAS mutation	Exon 2 - G12A	2 (10.5)
	Exon 2 - G12D	1 (5.3)
	Exon 2 - G12S	2 (10.5)
	Exon 2 - G12V	5 (26.3)
	Exon 2 - G13D	1 (5.3)
	Exon 4 - UK	1 (5.3)
	Mutant	1 (5.3)
	Native	6 (31.6)
NRAS mutation	NA	2 (10.5)
	Native	17 (89.5)
BRAF mutation	NA	2 (10.5)
	Native	16 (84.2)
	V600E	1 (5.3)
Number of affected organs	1	4 (21.1)
	2	7 (36.8)
	3	5 (26.3)

	4	3 (15.8)
Number of previous treatment lines	2	3 (15.8)
	3	9 (47.4)
	4	2 (10.5)
	5	2 (10.5)
	6	3 (15.8)
Number of new treatment lines	0	6 (31.6)
	1	7 (36.8)
	2	6 (31.6)

Abbreviations: ECOG, Eastern Cooperative Oncology Group; LDH, lactate dehydrogenase; CT Chemotherapy; GEP Gastroenteropancreatic; IMMETCOLS . NA Not Available. NE Not Evaluable

Table 15. Demographics of study patients (II): Continuous variables

	Total		
	N	**Mean (SD)**	**Median (min-Max)**
Age (years)	19	60.2 (11.3)	62 (32-77)
Number of affected organs	19	2.4 (1)	2 (1-4)
Number of previous lines	19	3.6 (1.3)	3 (2-6)
Number of new lines	19	1 (0.8)	1 (0-2)
LDH Value	19	309.3 (164.5)	266 (168-425)
CEA Value	19	91 (102.6)	43.7 (1.5-338.5)
Alkaline phosphatase Value	19	138.8 (102.6)	97 (53-472)
Sum of target lesions (mm)	19	87.4 (33.3)	88 (39-143)

Abbreviations: SD, standard deviation; LDH, lactate dehydrogenase; ECOG Eastern Cooperative Oncology Group; CEA, Carcinoembryonic antigen; FA .

11.3. CENTRAL REVIEW OF DISEASE DIAGNOSTICS

MSS, RAS and BRAF status were evaluated from archival biopsies (locally and centrally evaluation). No differences were found between the local and central mutational diagnosis.

11.4. MEASUREMENTS OF TREATMENT COMPLIANCE

Table 16 shows information regarding treatments received by patients. In general, treatment was well tolerated in evaluable patients in the trial after receiving at least 1 dose of study treatment (n=19).

Patients remained on study treatment for a median time of 55 days (range 15-356 days). A detailed information of treatment duration for each participant is provided in table 17.

The first 6 enrolled patients received the Dose level 1 (Avelumab 10mg/kg + ADC vaccine $10x10^6$). No DLT were reported throughout the study. Thus, the initial dose schedule of Avelumab 10mg/kg biweekly until disease progression or unacceptable toxicity + $10x10^6$ ADC vaccines biweekly for 5 infusions followed by up to 6 infusions every 6 months was declared as the RP2D. All enrolled patients were administered with Dose level 1.

Table 16. Measurements of treatment compliance

	Total (n=19)
Treatment disposition	
Received at least one injection (safety population)	19
Efficacy population	19
End of Treatment - reasons	
Progression Disease	19

Table 17. Treatment duration for each patient

Patient ID	Treatment start date	Treatment end date	Treatment duration (Days)
01-001	27/04/2018	20/11/2018	207
01-003	08/06/2018	06/08/2018	59
01-004	19/06/2018	01/08/2018	43
01-007	04/09/2018	14/11/2018	71

01-008	05/09/2018	05/09/2019	365
01-009	25/09/2018	29/01/2019	126
01-011	30/10/2018	20/02/2019	113
01-012	18/10/2018	07/12/2018	50
02-002	26/10/2018	15/12/2018	50
02-003	15/11/2018	05/01/2019	51
02-005	01/02/2019	16/03/2019	43
04-001	21/09/2018	10/11/2018	50
05-001	04/10/2018	17/01/2019	105
05-002	11/10/2018	5/12/2018	55
05-004	07/11/2018	14/02/2019	99
05-005	31/10/2018	22/03/2019	142
05-006	28/11/2018	27/12/2018	29
09-001	27/09/2018	16/11/2018	50
09-002	31/01/2019	15/02/19	15
		media	91
		SD	81

11.5. STUDY DURATION

The total duration of the study, considering the period between obtaining the approvals until the notification of end of study, was 38 months.

The study was approved by the AEMPS on Mar 12th, 2018 and the reference EC on Jan 18th, 2018. The first centre was activated on Mar 22nd, 2018; all the centres were activated and open to accrual except ICO Hospitalet, which was not opened. The first patient was included on Mar 22nd, 2018 and the last patient on Jan 10th, 2019.

The first patient started treatment on Apr 27th, 2018 (start of risk exposure period); the date of administration of the last patient who received treatment in the study was Sep 5th, 2019. Overall, the period of treatment exposure was 17 months.

Regarding the follow-up of patients, the last visit of the last patient was on July 31st 2020, closing the database of the study on Sep 28th, 2020.

The date of end of study was Sep 15th, 2020 and it was notified to CA/IEC on Sep 28th, 2020, after having clarified all data inconsistencies with centres and having performed the close-out visits.

11.5.1. STATISTICAL/ANALYTICAL ISSUES

11.5.1.1. HANDLING OF DROPOUTS OR MISSING DATA

A total of 19 patients were included in the study. Of these patients, none were excluded from the efficacy and safety analysis. All the eligible patients reported the administration of at least 1 infusion of study treatment and were considered for safety analysis.

The clinical database was exported from MFAR. The safety analysis was performed by MFAR. The efficacy and the molecular substudy analyses were performed by the Medical Statistics Core Facility from IDIBAPS-Hospital Clinic de Barcelona.

All inconsistencies, missing values, or clarifications were managed by CRAs at each site, and resolution issues were documented in the database, in eCRFs, and in writing with a query form (when applicable).

For evaluation of response to treatment, all patients had documented relapse at database cut-off; therefore, there was no censored data for PFS analysis.

By the time of database closure, 1 patient was alive; this patient was censored at the date of their last follow-up visit.

11.5.2. TABULATION OF INDIVIDUAL RESPONSE DATA

All demographic and relevant baseline data were collected (gender, date of birth, diagnosis date, localisation, ECOG, genotype, number of affected organs, LDH, CEA, PCR and albumin, previous treatments, ECG, Concomitant diseases and medications, disease status by CT-scan). The mutational status of MSS, RAS and BRAF was centrally reviewed.

Baseline LDH level was systematically analysed according to sites' local procedures and normal ranges and centrally in the laboratory of Hospital Clinic.

Treatment administration data were available for 19 patients analysed for efficacy; the start/end date of treatment and the number of cycles administered were recorded in the eCRF. The reasons ending treatment were also recorded and categorised.

The imaging modalities used for RECIST assessment were chest and abdominopelvic CT scan with other regions as clinically indicated for the assessment of disease. During follow up, the same imaging modality was used. PET-scan could be used for disease progression assessment only if a new lesion has appeared compared to a PET-scan performed previously. Radiological examinations performed in the conduct of this study were retained at site as source data. Response to treatment was recorded for each imaging test, taking into account the information provided by sites on the eCRF.

Progression/relapse date, progression/relapse reason (when applicable), and progression/relapse censored (and the corresponding comments field) were populated with the information provided by the sites in the eCRF.

Finally, patient status (alive, lost to follow-up, or dead) was systematically updated until database closure. Date of death or censoring, reason for death, and other comments were included in the database.

The safety database created for this study included data on all evaluable patients receiving at least 1 dose of study treatment. AEs with grades ≥3, SAEs, and suspected unexpected serious adverse reactions (SUSARs) were analysed.

Reconciliations among the safety database, SAEs, and SUSARs were performed periodically.

11.6. EFFICACY EVALUATION

11.6.1.PROGRESSION FREE SURVIVAL (PFS) RATE AT 6 MONTHS (Phase II primary endpoint)

The primary objective for efficacy of the AVEVAC trial was to increase the percentage of pre-treated MSS mCRC patients free of progression at 6 months. The trial sample size was calculated by a simon two stage model to power this variable and find a statistically significant difference, taking as reference a 20% 6 months PFS rate, described in previous trials with a population of similar characteristics, and a futility threshold of 40%. PFS was evaluated with the Kaplan-Meier curves and compared by a stratified log-rank test.

The 6 months PFS rate was 0%. The median PFS was 3.1 months (range 2.1-5.3). Therefore, the study treatment did not reach the pre-established threshold for significance and the study was stopped in the planned interim analysis enclosing the first 18 patients.

Stratifying patients by baseline characteristics such as IMMETCOLS, GEP, age, gender, local diagnosis, primary tumor surgery, ECOG, genotype, LDH, previous lines, and number of affected organs, did not give significant differences between groups in the PFS rate. There was no favourable phenotype for efficacy in terms of PFS rate (Table 18).

Table 18. PFS analysis for the study population and stratified subgroups

		N (%)	6-m PFS rate (%)	Median PFS (min-Max)
Study population		19	0%	3.1 (2.1-5.3)
IMMETCOLS	Yes	5 (26)	0%	3.1 (2.6-4.5)
	No	10 (53)	0%	3 (2.4-5)
GEP	Yes	5 (26)	0%	3.1 (2.5-4.5)

	No	10 (53)	0%	3 (2.4-5)
Age ≤60 years	≤60 years	8 (42.1)	0%	3.1 (2.4-5)
	>60 years	11 (57.9)	0%	3.1 (2.1-5.3)
Age ≤62 years	≤62 years	10 (52.6)	0%	3.1 (2.4-5.3)
	>62 years	9 (47.4)	0%	3.1 (2.1-4.9)
Gender	Male	10 (52.6)	0%	3.2 (2.1-5.3)
	Female	9 (47.4)	0%	3 (2.4-5)
LDH	<234	11 (57.9)	0%	3.1 (2.1-5.3)
	>234	8 (42.1)	0%	3.1 (2.5-4.5)
Local Diagnosis	Rectum	6 (31.6)	0%	3.7 (2.1-5.3)
	Sigma	13 (68.4)	0%	3.1 (2.4-5)
Primary tumor surgery	Yes	13 (68.4)	0%	3 (2.1-5.3)
	No	6 (31.6)	0%	3.7 (2.5-4.9)
Previous lines ≤2	Yes	3 (15.9)	0%	4.2 (3-5.3)
	No	16 (84.1)	0%	3.1 (2.1-5)
Previous lines ≤3	Yes	12 (63.2)	0%	3.1 (2.4-5.3)
	No	7 (36.8)	0%	3.1 (2.1-4.9)
Genotype	All native	5 (26.3)	0%	3.1 (2.5-4.5)
	KRAS	13 (68.4)	0%	3.1 (2.1-5.3)
ECOG	0	13 (68.4)	0%	3.1 (2.1-5)
	1	6 (31.6)	0%	3.1 (2.5-5.3)
Number of affected organs	1	4 (21)	0%	4.6 (3-5.3)
	>1	15 (79)	0%	3 (2.1-5)

Number of affected organs	≤2	11 (57.9)	0%	3 (2.4-5.3)
	>2	8 (42.1)	0%	3.1 (2.1-4.4)

11.6.1.1 OVERALL SURVIVAL (OS) (Phase II Secondary efficacy endpoint)

A total of 18 out of 19 patients died before study database closure. OS was defined as the time between inclusion in the study and death from any cause. In cases where patients withdrew from the trial or were lost to follow-up, they were censored at the date of the last contact. The patients who were still alive at the end of the study were censored at that time. OS was evaluated with the Kaplan-Meier curves and compared by a stratified log-rank test.

The 6 months OS rate was 73.7%. The trial reached a median OS of 12.1 months (range 3.2-22.9).

Stratifying patients by baseline characteristics such as IMMETCOLS, GEP, age, gender, local diagnosis, primary tumor surgery, ECOG, genotype, LDH, previous lines, and number of affected organs, did not give significant differences between groups in the OS rate (Table 19).

Table 19. OS analysis for the study population and stratified subgroups

		N (%)	6-m OS rate (%)	Median OS (min-Max)
Study population		19	73.7%	12.1 (3.2-22.9)
IMMETCOLS	Yes	5 (26)	80%	10.1 (5.1-20.2)
	No	10 (53)	70%	12 (4.1-20.8)
GEP	Yes	5 (26)	80%	13.7 (4.1-20.2)
	No	10 (53)	70%	10.3 (4.2-20.8)
Age ≤60 years	≤60 years	8 (42.1)	81.8%	13.7 (4.1-22.9)
	>60 years	11 (57.9)	62.5%	12 (3.2-20.8)
Age ≤62 years	≤62 years	10 (52.6)	77.8%	13.7 (4.1-19.2)
	>62 years	9 (47.4)	70%	12 (3.2-22.9)

Gender	Male	10 (52.6)	80%	11.1 (3.2-22.9)
	Female	9 (47.4)	66.7%	13.7 (4.2-20.8)
LDH	<234	11 (57.9)	81.8%	15.7 (4.1-22.9)
	>234	8 (42.1)	62.5%	9.4 (3.2-20.2)
Local Diagnosis	Rectum	6 (31.6)	83.3%	14.7 (4.2-22.9)
	Sigma	13 (68.4)	69.2%	11.9 (3.2-20.8)
Primary tumor surgery	Yes	13 (68.4)	69.2%	15.7 (4.1-22.9)
	No	6 (31.6)	83.3%	10.3 (3.2-20.2)
Previous lines ≤2	Yes	3 (15.9)	66.7%	20.2 (5.1-22.9)
	No	16 (84.1)	75%	12 (3.2-20.8)
Previous lines ≤3	Yes	12 (63.2)	75%	16.4 (4.1-22.9)
	No	7 (36.8)	71.4%	10.1 (3.2-15.7)
Genotype	All native	5 (26.3)	100%	13.7 (8.7-20.2)
	KRAS	13 (68.4)	69.2%	12.1 (3.2-22.9)
ECOG	0	13 (68.4)	76.9%	12.1 (3.2-20.8)
	1	6 (31.6)	66.7%	11.9 (4.1-22.9)
Number of affected organs	1	4 (21)	75%	13.7 (4.1-22.9)
	>1	15 (79)	73.3%	12.1 (3.2-20.8)
Number of affected organs	≤2	11 (57.9)	72.7%	10.1 (4.1-22.9)
	>2	8 (42.1)	75%	14.7 (3.2-20.2)

11.6.2. MOLECULAR SUBSTUDY: PHARMACODYNAMIC CHANGES BETWEEN PRE AND POST TREATMENT

11.6.2.1. MOLECULAR PHARMACODYNAMIC CHANGES ASSOCIATED TO TREATMENT EXPOSURE

At screening period (<28 days from treatment initiation) and at 8 weeks 10 ml of blood (serum) were extracted and stored at -80 ºC). A panel of 25 cytokines and chemokines (including IFN-γ, IL-18, CCL2, CXCL-12, IL-6, TNF and TGF-β) was analyzed by Luminex Multiplex Cytokine Kits (Affymetrix). Moreover, 50 ml of blood were extracted for analysis of lymphocytic response changes between screening period and 8 weeks after treatment initiation. Table 20 shows the levels for the molecular biomarkers pre, post study treatment and the differences between both.

Among the 19 patients enrolled in the study there were 16 patients with blood samples for the biomarker substudy available at baseline and 8 weeks after study treatment started, and 11 patients with blood samples for the analysis of lymphocytic response changes.

Upon treatment exposure, there was a substantial increase (more than 20% difference between baseline and 8-weeks post treatment) in the blood expression levels of TGFb3, IL-10 and MMP9 (Table 20). Conversely, expression levels of VEGFa, VEGFb, VEGFc, MCP1 and RANTEs were substantially decreased (Table 20).

There were no relevant changes in lymphocyte count and markers upon treatment exposure.

Table 20. Molecular sub-study: changes in serum biomarkers after study treatment exposure

Marker	Result	Pre-treatment	Post-treatment	Difference
TGFb3	N	16	16	16
	Mean (SD)	6.92 (19.01)	19.68 (61.08)	-12.76 (44.09)
VEGFb	N	16	16	16
	Mean (SD)	342.97 (432.97)	292.59 (589.12)	50.38 (486.39)
SDF1a	N	16	16	16
	Mean (SD)	11966.95 (22857.86)	11489.53 (23041.34)	477.43 (1359.21)
IL2	N	16	16	16
	Mean (SD)	3.55 (0.00)	3.55 (0.00)	0.00 (0.00)
IL6	N	16	16	16
	Mean (SD)	4.26 (1.84)	4.03 (0.00)	0.23 (1.84)

IL10	N	16	16	16
	Mean (SD)	0.75 (0.00)	2.92 (8.86)	-2.17 (8.86)
IL17a	N	16	16	16
	Mean (SD)	0.94 (0.00)	0.94 (0.00)	0.00 (0.00)
INFg	N	16	16	16
	Mean (SD)	6.08 (0.00)	6.08 (0.00)	0.00 (0.00)
MCP1	N	16	16	16
	Mean (SD)	44.85 (33.61)	32.92 (37.36)	11.93 (17.47)
FasL	N	16	16	16
	Mean (SD)	1.04 (0.00)	1.04 (0.00)	0.00 (0.00)
IL23	N	16	16	16
	Mean (SD)	6.59 (0.00)	6.59 (0.00)	0.00 (0.00)
VEGFa	N	16	16	16
	Mean (SD)	938.68 (726.60)	591.28 (447.87)	347.40 (859.99)
MMP9	N	16	16	16
	Mean (SD)	24141.10 (20417.71)	30363.66 (18381.74)	-6222.56 (23411.04)
RANTES	N	16	16	16
	Mean (SD)	50042.84 (43742.72)	43960.76 (37721.63)	6082.08 (65021.03)
TGFb	N	16	16	16
	Mean (SD)	727.79 (464.36)	660.65 (402.57)	67.15 (483.84)
VEGFc	N	16	16	16
	Mean (SD)	1.09 (0.53)	0.60 (0.27)	0.49 (0.41)
Naive T cell (CD62L+ CCR7+ CD45RA+ CD45RO-)	N	11	11	11
	Mean (SD)	0.14 (0.09)	0.16 (0.10)	-0.02 (0.09)
T stem cell memory (CD62L+ CCR7+ CD45RA+ CD45RO+)	N	11	11	11
	Mean (SD)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
T central memory (CD62L+ CCR7+ CD45RA- CD45RO+)	N	11	11	11
	Mean (SD)	0.09 (0.05)	0.08 (0.05)	0.01 (0.05)

T effector memory (CD62L- CCR7- CD45RA- CD45RO+)	N	11	11	11
	Mean (SD)	0.38 (0.12)	0.35 (0.09)	0.02 (0.10)
T effector (CD62L- CCR7- CD45RA+ CD45RO-)	N	11	11	11
	Mean (SD)	0.15 (0.05)	0.14 (0.05)	0.01 (0.05)
T cell	N	11	11	11
	Mean (SD)	0.55 (0.17)	0.49 (0.16)	0.06 (0.25)
CD3+ CD4+	N	11	11	11
	Mean (SD)	0.63 (0.20)	0.63 (0.19)	0.00 (0.10)
T regulatory (regarding T cell) CD4+ CD25++ CD127-	N	11	11	11
	Mean (SD)	0.02 (0.01)	0.02 (0.02)	0.00 (0.02)
CD3+ CD8+	N	11	11	11
	Mean (SD)	0.27 (0.21)	0.24 (0.16)	0.03 (0.08)
CD3+ PD1+	N	11	11	11
	Mean (SD)	0.02 (0.01)	0.02 (0.03)	-0.01 (0.03)
CD3+ CTLA4+	N	11	11	11
	Mean (SD)	0.01 (0.00)	0.01 (0.01)	-0.00 (0.01)

11.6.2.2. MOLECULAR PHARMACODYNAMIC CHANGES ASSOCIATED TO TREATMENT EXPOSURE IN STRATIFIED ANALYSIS

Patients were stratified according to their baseline characteristics and the changes after study treatment in the molecular biomarkers were analyzed and compared within each group using Wilcoxon-signed test. The baseline characteristics used for stratification were:

- IMMETCOLS
- GEP
- Age
- Gender
- LDH levels
- Primary tumour surgery
- Previous lines
- Genotype
- ECOG
- Affected organs

Most of the cytokine, chemokine and lymphocyte biomarkers did not have significant

changes upon treatment administration. Only VEGFc, VEGFa, MCP1, SDF1a and T cells were significantly reduced from baseline after 8 weeks of treatment in some stratified analysis (Table 21 and 22).

VEGFc was statistically significantly reduced after treatment in at least one of the subgroups in all the stratified analysis (Table 21 and 22). VEGFc expression levels were decreased upon treatment exposure in more favourable subgroups: i.e. patients having primary tumor surgery, 3 or less previous lines, 2 or less affected organs, ECOG 0, negative GEP/IMMETCOLS; and in the poor prognosis subgroup of KRAS mutated patients. Moreover, VEGFc was found to be significantly reduced independently of stratification by age, gender, LDH levels, and ECOG (Table 21 and 22).

Similarly expression levels of VEGFa were decreased upon treatment exposure in the subgroups of patients with ECOG 0, low LDH levels, primary surgery and KRAS mutation (Table 21 and 22).

SDF1a was reduced upon treatment in those patients with ECOG 0, lower LDH, sigma and more than 2 organs affectation. MCP1 decreased significantly after treatment exposure in patients negative for IMMETCOLS, with ECOG 0, KRAS mutation and sigma location (Table 21 and 22).

T cells were significantly decreased after 8 weeks of treatment in female and younger patient subgroups (Table 21 and 22).

Despite the significance reached in the comparison of some molecular biomarkers, the limitation of a small sample size should be taken into account.

Table 21. Stratified molecular sub-study: changes in serum biomarkers after study treatment exposure (only those with significant differences between pre and post treatment were listed)

Marker	Group	Result	Pre-treatment	Post-treatment	Difference	p value*
MCP1	IMMETCOLS yes	N	5	5	5	0.812
		Mean (SD)	46.25 (33.72)	38.70 (41.89)	7.55 (17.54)	
	IMMETCOLS no	N	8	8	8	0.039
		Mean (SD)	39.14 (27.40)	24.64 (22.77)	14.50 (15.52)	
VEGFc	IMMETCOLS yes	N	5	5	5	0.0625
		Mean (SD)	1.27 (0.64))	0.63 (0.31)	0.64 (0.53)	
	IMMETCOLS no	N	8	8	8	0.0078
		Mean (SD)	0.97 (0.55)	0.54 (0.27)	0.43 (0.36)	
VEGFc	GEP yes	N	5	5	5	0.063

		Mean (SD)	1.39 (0.62)	0.73 (0.32)	0.67 (0.51)	
	GEP no	N	8	8	8	0.008
		Mean (SD)	0.89 (0.49)	0.47 (0.21)	0.41 (0.36)	
VEGFc	≤62 years	N	7	7	7	0.016
		Mean (SD)	1.13 (0.74)	0.54 (0.25)	0.59 (0.51)	
	>62 year	N	9	9	9	0.008
		Mean (SD)	1.06 (0.32)	0.64 (0.29)	0.42 (0.32)	
T cell	≤62 years	N	7	7	7	0.016
		Mean (SD)	0.61 (0.11)	0.50 (0.11)	0.11 (0.10)	
	>62 year	N	9	9	9	0.8750
		Mean (SD)	0.44 (0.23)	0.47 (0.25)	-0.03 (0.41)	
VEGFc	Female	N	7	7	7	0.016
		Mean (SD)	1.17 (0.72)	0.58 (0.24)	0.60 (0.54)	
	Male	N	9	9	9	0.008
		Mean (SD)	1.02 (0.33)	0.61 (0.31)	0.41 (0.28)	
T cell	Female	N	7	7	7	0.031
		Mean (SD)	0.62 (0.12)	0.42 (0.19)	0.20 (0.21)	
	Male	N	9	9	9	0.438
		Mean (SD)	0.46 (0.21)	0.57 (0.08)	-0.11 (0.18)	
SDF1a	LDH <234	N	9	9	9	0.016
		Mean (SD)	14546.70 (24673.21)	13498.39 (25188.23)	1048.31 (1475.06)	
	LDH >234	N	7	7	7	1.000
		Mean (SD)	8650.14 (21718.59)	8906.70 (21621.60)	-256.56 (774.72)	
VEGFa	LDH <234	N	9	9	9	0.02
		Mean (SD)	868.28 (475.57)	407.65 (235.86)	460.64 (390.77)	
	LDH >234	N	7	7	7	0.688
		Mean (SD)	1029.18 (1000.66)	827.39 (558.28)	201.80 (1265.45)	

VEGFc	LDH <234	N	9	9	9	0.008
		Mean (SD)	0.92 (0.41)	0.55 (0.30)	0.37 (0.31)	
	LDH >234	N	7	7	7	0.016
		Mean (SD)	1.31 (0.60)	0.66 (0.22)	0.65 (0.50)	
SDF1a	Rectum	N	6	6	6	0.875
		Mean (SD)	19770.52 (29536.27)	19888.10 (29458.62)	-117.58 (984.10)	
	Sigma	N	10	10	10	0.039
		Mean (SD)	7284.81 (17921.65)	6450.38 (18096.52)	834.43 (1470.86)	
MCP1	Rectum	N	6	6	6	1.000
		Mean (SD)	40.99 (43.22)	34.01 (45.76)	6.98 (19.16)	
	Sigma	N	10	10	10	0.037
		Mean (SD)	47.16 (28.78)	32.27 (34.08)	14.89 (16.69)	
VEGFc	Rectum	N	6	6	6	0.063
		Mean (SD)	1.07 (0.46)	0.61 (0.24)	0.46 (0.40)	
	Sigma	N	10	10	10	0.002
			1.10 (0.58)	1.10 (0.58)	1.10 (0.58)	
VEGFa	primary tumor surgery Yes	N	11	11	11	0.014
		Mean (SD)	790.00 (455.77)	450.28 (310.99)	339.72 (332.43)	
	primary tumor surgery No	N	5	5	5	0.813
		Mean (SD)	1265.77 (1125.14)	901.50 (579.18)	364.28 (1580.07)	
VEGFc	primary tumor surgery Yes	N	11	11	11	0.002
		Mean (SD)	1.14 (0.40)	0.66 (0.26)	0.48 (0.37)	
	primary tumor surgery No	N	5	5	5	0.063
		Mean (SD)	0.99 (0.78)	0.47 (0.25)	0.52 (0.54)	
VEGFc	≤ 3 previous lines	N	11	11	11	0.001
		Mean (SD)	1.23 (0.56)	0.60 (0.26)	0.63 (0.42)	

	> 3 previous lines	N	5	5	5	0.125
		Mean (SD)	0.78 (0.25)	0.60 (0.31)	0.18 (0.15)	
MCP1	Native	N	5	5	5	0.625
		Mean (SD)	40.09 (39.50)	37.95 (42.58)	2.14 (15.99)	
	KRAS mut	N	10	10	10	0.027
		Mean (SD)	49.24 (33.31)	33.00 (37.83)	16.24 (17.81)	
VEGFa	Native	N	5	5	5	1.000
		Mean (SD)	1113.24 (1219.32)	946.84 (621.83)	166.40 (1551.27)	
	KRAS mut	N	10	10	10	0.002
		Mean (SD)	900.19 (417.26)	400.98 (224.16)	499.21 (278.40)	
VEGFc	Native	N	5	5	5	0.063
		Mean (SD)	1.22 (0.68)	0.57 (0.30)	0.65 (0.52)	
	KRAS mut	N	10	10	10	0.004
		Mean (SD)	0.98 (0.45)	0.56 (0.23)	0.41 (0.38)	
SDF1a	ECOG 0	N	10	10	10	0.008
		Mean (SD)	13146.17 (23680.27)	12194.94 (24103.00)	951.22 (1423.94)	
	ECOG 1	N	6	6	6	1.000
		Mean (SD)	10001.59 (23466.34)	10313.83 (23331.05)	-312.24 (833.93)	
MCP1	ECOG 0	N	10	10	10	0.014
		Mean (SD)	50.39 (32.26)	31.84 (38.45)	18.55 (18.39)	
	ECOG 1	N	6	6	6	0.438
		Mean (SD)	35.61 (36.76)	34.73 (38.98)	0.88 (8.48)	
VEGFa	ECOG 0	N	10	10	10	0.037
		Mean (SD)	1051.92 (807.95)	465.39 (402.69)	586.54 (860.82)	

	ECOG 1	N	6	6	6	0.844
		Mean (SD)	749.94 (583.47)	801.11 (474.72)	-51.17 (761.52)	
VEGFc	ECOG 0	N	10	10	10	0.004
		Mean (SD)	0.97 (0.58)	0.54 (0.26)	0.43 (0.46)	
	ECOG 1	N	6	6	6	0.031
		Mean (SD)	1.29 (0.38)	0.70 (0.28)	0.60 (0.32)	
SDF1a	≤ 2 affected organs	N	10	10	10	0.469
		Mean (SD)	18290.63 (27388.07))	18029.30 (27530.45)	261.33 (1328.22)	
	> 2 affected organs	N	6	6	6	0.031
		Mean (SD)	1427.49 (1994.65)	589.90 (587.53)	837.59 (1455.28)	
VEGFc	≤ 2 affected organs	N	10	10	10	0.002
		Mean (SD)	1.03 (0.46)	0.61 (0.27)	0.43 (0.29)	
	> 2 affected organs	N	6	6	6	0.063
		Mean (SD)	1.18 (0.65)	0.58 (0.29)	0.60 (0.57)	

Table 22. Stratified molecular sub-study: Statistically significant differences between baseline and week 8 post-treatment found in blood molecular biomarker expression.

GENE	MCP1	VEGFa	VEGFc	SDF1a	T Cell
IMMETCOLS					
GEP					
Age					
Gender					
LDH levels					
Primary tumour surgery					
Previous lines					
Genotype					
ECOG					
Affected organs					

Tumor location					

Light green (statistically significant reduction from baseline in one group from the stratification analysis); Dark green (statistically significant reduction from baseline in both groups from the stratification analysis).

11.6.2.2. STRATIFIED ANALYSIS OF MOLECULAR BIOMARKERS (BASELINE)

Patients were stratified based on their baseline characteristics and the expression levels of a set of cytokines, chemokines and lymphocyte molecular markers were compared among groups before treatment started.

Most molecular biomarkers were well balanced and no statistically significant differences were found between the groups from stratification.

12. SAFETY EVALUATION

12.1. EXTENT OF EXPOSURE

The safety profile of the study treatment was assessed through the recording, reporting and analysis of baseline medical conditions, adverse events (AEs), physical examination findings including vital signs and laboratory tests.

Comprehensive assessment of any apparent toxicity experienced by each subject was performed from the time of giving informed consent and throughout the trial. The investigator reported any AEs, whether observed by the Investigator or reported by the subject.

A total of 19 pre-treated MCC patients were enrolled and received at least one dose of avelumab plus ADC vaccines in the context of this clinical trial.

The median duration of the study treatment was 55 days (range 15-365); all patients discontinued treatment due to disease progression. At the data cut-off point for this report no patients remained on treatment.

12.2. ADVERSE EVENTS (AEs)

12.2.1. ADVERSE EVENT AND SERIOUS ADVERSE EVENT (SAE) DEFINITION

Regarding AVEVAC clinical trial, an AE was defined as any untoward medical occurrence in a subject or clinical investigation subject administered a pharmaceutical product, regardless of causal relationship with this treatment. An AE could therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

The Investigator was required to grade the severity or toxicity of each AE according to the NCI-CTCAE Toxicity (or Severity) Grades:

Investigators referenced the National Cancer Institute - Common Terminology Criteria for AEs (CTCAE), version 4.03, a descriptive terminology that can be used for AE reporting.

A general grading (severity/intensity; hereafter referred to as severity) scale is provided at the beginning of the above referenced document, and specific event grades are also provided.

If a particular AE's severity is not specifically graded by the guidance document, the Investigator was requested to use the general NCI-CTCAE definitions of Grade 1 through Grade 5 following his or her best medical judgment.

The 5 general grades are:

1. Grade 1 or Mild
2. Grade 2 or Moderate
3. Grade 3 or Severe
4. Grade 4 or Life-threatening
5. Grade 5 or Death

According to Sponsor convention, any clinical AE with severity of Grade 4 or 5 was reported as an SAE. However, a laboratory abnormality of Grade 4, such as anemia or neutropenia, was considered serious only if the condition meets one of the serious criteria described below.

If death occurs, the primary cause of death or event leading to death was recorded and reported as an SAE. "Fatal" was recorded as the outcome of this specific event and death was not recorded as a separate event. Only, if no cause of death could be reported (for example, sudden death, unexplained death), the death per se was then reported as an SAE.

Investigators also systematically assessed the causal relationship of AEs to IMP(s)/study treatment (including any other non-IMPs, radiation therapy, etc.) using the following definitions. Decisive factors for the assessment of causal relationship of an AE to the IMP/study treatment included, but were not limited to, temporal relationship between the AE and the IMP/study treatment, known side effects of IMP/study treatment, medical history, concomitant medication, course of the underlying disease, trial procedures.

Unrelated: Not reasonably related to the IMP/study treatment. AE could not medically (pharmacologically/clinically) be attributed to the IMP/study treatment under study in this clinical trial protocol. A reasonable alternative explanation must be available.

Related: Reasonably related to the IMP/study treatment. AE could medically (pharmacologically/clinically) be attributed to the IMP/study treatment under study in this clinical trial protocol.

Abnormal Laboratory Findings and Other Abnormal Investigational Findings

Abnormal laboratory findings and other abnormal investigational findings (for example, on an ECG trace) were not reported as AEs unless they were associated with clinical signs and symptoms, led to treatment discontinuation or were considered otherwise medically

important by the Investigator. If a laboratory abnormality fulfilled these criteria, the identified medical condition (for example, anemia, increased ALT) was reported as the AE rather than the abnormal value itself.

Serious Adverse Events

An SAE was any untoward medical occurrence that at any dose:

- Resulted in death.
- Was life-threatening. (Note: The term “life-threatening” refers to an event in which the subject is at risk of death at the time of the event, not an event that hypothetically might have caused death if it was more severe).
- Required inpatient hospitalization or prolonged an existing hospitalization.
- Resulted in persistent or significant disability or incapacity.
- Was a congenital anomaly or birth defect.
- Was otherwise considered to be medically important. (Note: Important medical events that may not result in death, be life-threatening, or require hospitalization were considered as SAEs when, based upon appropriate medical judgment, they may jeopardize the subject or may require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such events included allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that did not result in inpatient hospitalization, or the development of drug dependence or drug abuse).

For the purposes of reporting, any suspected transmission of an infectious agent via an IMP was also considered an SAE.

12.2.2. SUMMARY OF ADVERSE EVENT

A total of 19 patients received at least 1 dose of avelumab and ADC vaccine and were included in the safety analysis. For each AE, the number of affected patients with the highest grade recorded in the eCRF is presented, and their respective % (with respect to the total number of patients, N = 19), so each patient is only counted once in each AE, with the highest degree of it. The Table 23 includes the SAEs, which are also listed separately in Section 12.2.4. The AEs were re-categorized using version 4.03 of the CTCAE.

Table 23. Summary of all Adverse Events (AEs) reported throughout the study

AE definition (CTCAE)	Maximum grade				
	UK	1-2	3	5	Total
	N (%)	N (%)	N (%)	N (%)	N (%)
Fatigue	0 (0)	10 (52.63)	1 (5.26)	0 (0)	11 (57.89)
Fever	0 (0)	5 (26.32)	0 (0)	0 (0)	5 (26.32)
Investigations - others: LDH increased	0 (0)	4 (21.05)	0 (0)	0 (0)	4 (21.05)
Constipation	0 (0)	4 (21.05)	0 (0)	0 (0)	4 (21.05)
Back pain	1 (5.26)	3 (15.79)	0 (0)	0 (0)	4 (21.05)
Anemia	0 (0)	3 (15.79)	1 (5.26)	0 (0)	4 (21.05)
Abdominal pain	0 (0)	3 (15.79)	1 (5.26)	0 (0)	4 (21.05)
Vomiting	0 (0)	2 (10.53)	1 (5.26)	0 (0)	3 (15.79)
Nervous system disorders - Other, specify: Peripheral neuropathy NOS	1 (5.26)	2 (10.53)	0 (0)	0 (0)	3 (15.79)
Diarrhea	0 (0)	2 (10.53)	1 (5.26)	0 (0)	3 (15.79)
Anorexia	0 (0)	3 (15.79)	0 (0)	0 (0)	3 (15.79)

Urinary tract infection	0 (0)	2 (10.53)	0 (0)	0 (0)	2 (10.53)
Small intestinal obstruction	0 (0)	2 (10.53)	0 (0)	0 (0)	2 (10.53)
Pruritus	0 (0)	2 (10.53)	0 (0)	0 (0)	2 (10.53)
Pain	0 (0)	2 (10.53)	0 (0)	0 (0)	2 (10.53)
Intestinal obstruction	0 (0)	0 (0)	2 (10.53)	0 (0)	2 (10.53)
Hematuria	0 (0)	2 (10.53)	0 (0)	0 (0)	2 (10.53)
General disorders and administration site conditions - Other, clinical deterioration	1 (5.26)	0 (0)	0 (0)	1 (5.26)	2 (10.53)
Cough	0 (0)	2 (10.53)	0 (0)	0 (0)	2 (10.53)
Arthralgia	0 (0)	2 (10.53)	0 (0)	0 (0)	2 (10.53)
Urticaria	0 (0)	1 (5.26)	0 (0)	0 (0)	1 (5.26)
Upper respiratory infection	0 (0)	1 (5.26)	0 (0)	0 (0)	1 (5.26)
Toothache	0 (0)	1 (5.26)	0 (0)	0 (0)	1 (5.26)
Tooth infection	0 (0)	1 (5.26)	0 (0)	0 (0)	1 (5.26)
Skin and subcutaneous tissue disorders - Other, skin toxicity	0 (0)	1 (5.26)	0 (0)	0 (0)	1 (5.26)
Rectal hemorrhage	0 (0)	1 (5.26)	0 (0)	0 (0)	1 (5.26)
Rash	0 (0)	1 (5.26)	0 (0)	0 (0)	1 (5.26)
Psychiatric disorders - Other, specify: Mood disorder	0 (0)	1 (5.26)	0 (0)	0 (0)	1 (5.26)
Pneumonitis	0 (0)	1 (5.26)	0 (0)	0 (0)	1 (5.26)
Pleural effusion	0 (0)	0 (0)	1 (5.26)	0 (0)	1 (5.26)
Platelet count decreased	0 (0)	1 (5.26)	0 (0)	0 (0)	1 (5.26)
Peripheral sensory neuropathy	0 (0)	1 (5.26)	0 (0)	0 (0)	1 (5.26)
Perineal pain	0 (0)	1 (5.26)	0 (0)	0 (0)	1 (5.26)
Pelvic pain	0 (0)	1 (5.26)	0 (0)	0 (0)	1 (5.26)
Pain in extremity	0 (0)	1 (5.26)	0 (0)	0 (0)	1 (5.26)
Neck pain	0 (0)	1 (5.26)	0 (0)	0 (0)	1 (5.26)
Nausea	0 (0)	1 (5.26)	0 (0)	0 (0)	1 (5.26)
Myalgia	0 (0)	1 (5.26)	0 (0)	0 (0)	1 (5.26)
Musculoskeletal and connective tissue disorder - Other: tendinitis	0 (0)	1 (5.26)	0 (0)	0 (0)	1 (5.26)
Muscle weakness left-sided	0 (0)	0 (0)	1 (5.26)	0 (0)	1 (5.26)
Mucositis oral	0 (0)	1 (5.26)	0 (0)	0 (0)	1 (5.26)
Memory impairment	0 (0)	1 (5.26)	0 (0)	0 (0)	1 (5.26)
Investigations - Other, specify: Low hematocrit levels	0 (0)	1 (5.26)	0 (0)	0 (0)	1 (5.26)
Insomnia	0 (0)	1 (5.26)	0 (0)	0 (0)	1 (5.26)
Infections and infestations - Other, specify: Infected skin cyst	0 (0)	1 (5.26)	0 (0)	0 (0)	1 (5.26)
Hypothyroidism	0 (0)	1 (5.26)	0 (0)	0 (0)	1 (5.26)
Hypocalcemia	0 (0)	1 (5.26)	0 (0)	0 (0)	1 (5.26)
Hyperglycemia	0 (0)	1 (5.26)	0 (0)	0 (0)	1 (5.26)
Hepatic pain	0 (0)	1 (5.26)	0 (0)	0 (0)	1 (5.26)
Headache	0 (0)	1 (5.26)	0 (0)	0 (0)	1 (5.26)
General disorders and administration site conditions - Other: Night sweats	0 (0)	1 (5.26)	0 (0)	0 (0)	1 (5.26)
General disorders and administration site conditions - Other, port-a-cath infection	0 (0)	0 (0)	1 (5.26)	0 (0)	1 (5.26)
Flu like symptoms	0 (0)	1 (5.26)	0 (0)	0 (0)	1 (5.26)
Flank pain	0 (0)	1 (5.26)	0 (0)	0 (0)	1 (5.26)
Eye disorders - Other, specify: Decline in vision	0 (0)	1 (5.26)	0 (0)	0 (0)	1 (5.26)
Erythema	0 (0)	1 (5.26)	0 (0)	0 (0)	1 (5.26)
Ear pain	0 (0)	1 (5.26)	0 (0)	0 (0)	1 (5.26)
Dysphagia	0 (0)	1 (5.26)	0 (0)	0 (0)	1 (5.26)
Creatinine increased	0 (0)	1 (5.26)	0 (0)	0 (0)	1 (5.26)
Chills	0 (0)	1 (5.26)	0 (0)	0 (0)	1 (5.26)
Chest wall pain	0 (0)	1 (5.26)	0 (0)	0 (0)	1 (5.26)
Bullous dermatitis	0 (0)	1 (5.26)	0 (0)	0 (0)	1 (5.26)
Bone pain	0 (0)	1 (5.26)	0 (0)	0 (0)	1 (5.26)
Blood bilirubin increased	0 (0)	1 (5.26)	0 (0)	0 (0)	1 (5.26)
Aspartate aminotransferase increased	0 (0)	1 (5.26)	0 (0)	0 (0)	1 (5.26)

Anal pain	0 (0)	1 (5.26)	0 (0)	0 (0)	1 (5.26)
Alkaline phosphatase increased	0 (0)	1 (5.26)	0 (0)	0 (0)	1 (5.26)
Alanine aminotransferase increased	0 (0)	1 (5.26)	0 (0)	0 (0)	1 (5.26)
Acute kidney injury	0 (0)	0 (0)	1 (5.26)	0 (0)	1 (5.26)

No Grade 4 events were reported througout the study

The frequency and severity of AEs was consistent with those previously reported from clinical trials with similar MCC patient populations. Most AEs were low grade 1-2. The most frequent low grade AEs were fatigue (52.63%), fever (26.32%), LDH increased (21.05%), constipation (21.05%), back pain (15.79%), anemia (15.79%), abdominal pain (15.79%), and anorexia (15.79%)(Table 23).

There was only one (5.26%) grade 5 AE associated with clinical deterioration of the patient that led to death (Table 23). Grade 3 AEs included intestinal obstruction (10.53%), fatigue (5.26%), anemia (5.26%), abdominal pain (5.26%), vomiting (5.26%), diarrhea (5.26%), muscle weakness (5.26%), pleural effusion (5.26%) and port-a-cath infection (5.26%)(Table 23).

12.2.3. SUMMARY OF TREATMENT-RELATED ADVERSE EVENT (TOXICITIES)

Table 24 contains a list of all toxicities (AEs related to the treatment under study: avelumab and / or vaccines) reported throughout the study. Data is presented as the number of patients affected by each toxicity with the highest degree recorded in the eCRF, and their respective % (with respect to the total of patients, N = 19), so each patient is only counted once for each toxicity, with its highest grade. Expectedness and causal relationship with the study treatment were assessed by the investigators following the indication from the clinical trial protocol detailed in section 12.2.1 from this report and the available safety information from the IB of avelumab and ADC vaccines. The treatment related AEs were re-categorized using version 4.03 of the CTCAE.

Table 24. Summary of all Toxicities reported throughout the study

Toxicity definition (CTCAE)	Maximum grade		
	1-2	3	Total
	N (%)	N (%)	N (%)
Fatigue	3 (15.79)	1 (5.26)	4 (21.05)
Fever	3 (15.79)	0 (0)	3 (15.79)
Arthralgia	2 (10.53)	0 (0)	2 (10.53)
Pruritus	1 (5.26)	0 (0)	1 (5.26)
Pain	1 (5.26)	0 (0)	1 (5.26)
Myalgia	1 (5.26)	0 (0)	1 (5.26)
Mucositis oral	1 (5.26)	0 (0)	1 (5.26)
Hypothyroidism	1 (5.26)	0 (0)	1 (5.26)
Flu like symptoms	1 (5.26)	0 (0)	1 (5.26)
Erythema	1 (5.26)	0 (0)	1 (5.26)
Ear pain	1 (5.26)	0 (0)	1 (5.26)
Chills	1 (5.26)	0 (0)	1 (5.26)

Aspartate aminotransferase increased	1 (5.26)	0 (0)	1 (5.26)
Anorexia	1 (5.26)	0 (0)	1 (5.26)
Anemia	1 (5.26)	0 (0)	1 (5.26)
Alanine aminotransferase increased	1 (5.26)	0 (0)	1 (5.26)
Abdominal pain	1 (5.26)	0 (0)	1 (5.26)

The toxicity profile, determined by frequency and severity of treatment related adverse events, was consistent with those previously reported for the study treatments (avelumab and ADC vaccines. Overall, 23 toxicities were reported (table 24). Most toxicities were low grade 1-2. The most frequent toxicities (all grades) were fatigue (21.05%), fever (15.79%), and arthralgia (10.53%). Only one (5.26%) grade 3 fatigue was reported (Table 24).

12.2.4. SUMMARY OF SERIOUS ADVERSE EVENT (SAEs)

A total of 13 SAEs in 8 (44.4%) patients were reported throughout the study period. SAEs are listed individually on table 25. There was a grade 5 SAE identified as clinical deterioration that led to patient death. There were 9 grade 3 SAEs, one grade 1 and one unknown that led to patient hospitalization. All of them were resolved/ended in 1 months since the onset date.
Only one SAE identified as grade 1 fever was found related to avelumab.

Table 25. List of Serious Adverse Events (SAEs) throughout the study period.

	Patient ID	SAE (CTCAE)	related	drug	onset date	end date	Grade	Intensity
1	01-003	General disorders and administration site conditions - Other, clinical deterioration	No		11-SEP-2018	11-SEP-2018	5	death
2	01-004	General disorders and administration site conditions - Other, clinical deterioration	No		31-AUG-2018	12-SEP-2018	UK	Hospitalization
3	01-007	Acute kidney injury	No		16-OCT-2018	05-NOV-2018	3	Hospitalization
4	01-007	General disorders and administration site conditions - Other, port-a-cath infection	No		04-OCT-2018	10-OCT-2018	3	Hospitalization
5	01-007	Intestinal obstruction	No		09-SEP-2018	17-SEP-2018	3	Hospitalization
6	01-007	Intestinal obstruction	No		08-NOV-2018	20-NOV-2018	3	Hospitalization
7	02-003	Abdominal pain	No		12-OCT-2018	17-OCT-2018	3	Hospitalization
8	02-003	Vomiting	No		12-OCT-2018	17-OCT-2018	3	Hospitalization
9	04-001	Fever	Yes	Avelumab	26-OCT-2018	29-OCT-2018	1	Hospitalization
10	05-005	Muscle weakness left-sided	No		21-MAR-2019	23-APR-2019	3	Hospitalization
11	05-006	Pleural effusion	No		06-JAN-2019	09-JAN-2019	3	Hospitalization
12	09-001	Diarrhea	No		08-DEC-2018	09-DEC-2018	3	Hospitalization
13	09-001	Intestinal obstruction	No		15-DEC-2018	04-JAN-2019	3	Hospitalization

12.4. DEATHS

At the time of database closure, 18 deaths were reported in the trial. The 18 patients died due to reasons described below.

All deaths (100%) were associated with disease progression. Only one patient death was caused directly by a cerebrovascular event (stroke) linked to disease progression.

Table 26. Status for patients from AVEVAC trial by the database closure for the study.

Patient ID	Death	Death reason
01-001	Yes	Disease progression
01-003	Yes	Disease progression
01-004	Yes	Disease progression
01-007	Yes	Disease progression
01-008	ALIVE	-
01-009	Yes	Disease progression
01-011	Yes	Disease progression
01-012	Yes	Disease progression and stroke
02-002	Yes	Disease progression
02-003	Yes	Disease progression
02-005	Yes	Disease progression
04-001	Yes	Disease progression
05-001	Yes	Disease progression
05-002	Yes	Disease progression
05-004	Yes	Disease progression
05-005	Yes	Disease progression
05-006	Yes	Disease progression
09-001	Yes	Disease progression
09-002	Yes	Disease progression

A total of 18 out of 19 patients were dead before final data cut-off point (Table 26).

12.5. CLINICAL LABORATORY EVALUATION

At SIVs, normal laboratory ranges and laboratory certification were recorded for each site and central facilities. Baseline laboratory evaluations of patients were systematically reviewed by CRAs to monitor eligibility.

According to the protocol, blood tests included the following:

- Haematology tests: complete hemogram including WBC differential count and platelet count.

- Biochemistry tests: sodium, potassium, magnesium, creatinine, albumin, glucose, calcium, phosphorus, AST, ALT, alkaline phosphatase, LDH, CEA, PCR, total protein, total bilirubin, free T4 and TSH and coagulation.
- Only at baseline: serology.

After study initiation, all clinical laboratory evaluations were performed by PIs at each visit according to the protocol and following their local practice. All parameters of laboratory tests were characterised by PIs as “normal, ”“abnormal without clinical relevance,” and “abnormal with clinical relevance”.

CRAs reviewed the corresponding laboratory evaluations when monitoring AEs and SAEs.

Clinical laboratory toxicities were collected in the corresponding eCRF forms; they are described in the AE section of this CSR. A descriptive analysis of recorded laboratory AEs along with the percentage of incidence (%) are presented both as general AEs and categorised as SAEs. In total there were four patients with grade 1-2 increased levels of LDH (Table 23). Two patients had alterations in analytic values, increased hepatic enzyme ALT/AST values that were considered AEs related to the study treatment according to the physician criteria (Table 23).

12.5.1. VITAL SIGNS, PHYSICAL FINDINGS AND OTHER OBSERVATIONS RELATED TO SAFETY

Baseline vital signs, physical examinations, and other observations related to safety were systematically reviewed by CRAs in patients to monitor their eligibility criteria.

The safety profiles of avelumab plus ADC vaccines were assessed through the recording, reporting and analysis of baseline medical conditions, adverse events (AEs), physical examination findings including vital signs and laboratory tests.

According to the protocol, the following determinations regarding vital signs, physical examinations, and other observations related to safety were performed:

- general appearance (including height and weight)
- ECOG performance status
- respiratory system
- cardiovascular system
- abdomino-pelvic
- skin
- head and neck
- lymph nodes
- thyroid
- abdomen

- musculoskeletal (including spine and extremities) system
- neurological system
- vital signs and blood pressure

Vital signs and Blood pressure

Height was assessed at screening only. Weight was assessed at screening and was repeated according to the Study Schedule if the investigator believes that it was likely to have changed significantly.

For timings of these different assessments refer to the Study Schedule (Table 7). The date of collection and measurement was recorded on the appropriate eCRF and medical record. Any changes in vital signs were recorded as an AE, if clinically significant. Any alteration of vital signs that was considered as clinically significant were described in the AE section of this CSR. A descriptive analysis of recorded AEs along with the percentage of incidence (%) are presented both as general AEs and categorised as SAEs (if applicable) in Tables 22 and 24 respectively.

Vital signs, physical examinations and other determination of clinical consultation were performed according to local practise and were assessed and recorded in patient records.

After study initiation, evaluations were performed by principal investigators at each visit according to the protocol and following their local practice. CRAs reviewed the corresponding evaluations when monitoring AEs and SAEs.

Data on ECOG PS were available for all patients enrolled in the trial.

12.6. CONCOMITANT MEDICATION USE

Throughout the trial, investigators prescribed concomitant medication or treatment considered necessary to provide adequate supportive care, with the exception of other investigational products or prohibited medicines.

Patients must not receive:

- Any other concurrent anti-cancer therapy, including investigational agents, while on study treatment.
- Steroids administered at doses > 10 mg or >10 mg equivalent prednisone per day.
- Vaccination within 4 weeks of the first dose of avelumab and while on trial is prohibited except for administration of study treatment.
- Any other investigational treatment within the past 30 days of treatment initiation and during the trial.

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 CRF and in patient record, noting the name, dose, duration and indication of each drug.

The indication, dose, frequency and dates of treatment should be recorded in the patient's medical records and appropriate section of the eCRF.

All medications (prescriptions or over-the-counter medications, traditional treatments) continued at the start of the trial or started during the study or until 30 days from the end of the last protocol treatment (safety follow-up visit) and different from the study medication must be documented.

A follow-up of the concomitant medications used for clinically significant AEs was performed until the AEs resolved or were considered stable. CRAs reviewed concomitant medications when monitoring AEs and SAEs.

No relevant data regarding concomitant medications were observed by the sponsor. Therefore, they are not reported in this CSR.

13. DISCUSSION AND OVERALL CONCLUSIONS

AVEVAC was a single arm Phase I/II multicentric open labeled trial with an associated translational sub-study to evaluate safety and efficacy of avelumab plus autologous dendritic cell vaccine in mismatch repair-proficient (MSS) metastatic colorectal cancer patients previously treated with at least 2 chemotherapy lines.

The selected MCC trial population has poor prognosis and it is expected less than 20% of patients free of progression at 6 months (Grothey 2013, Mayer 2015). PD-L1 blockade will only partially succeed (40% response rate) in the setting of a pre-existing antitumor immune responsive phenotype (MSI patients) but not in MSS colorectal cancer patients (<5% ORR, median PFS of 2.5 months and <20% progression free survival at 6 months) (Le DT 2015).

The combination of Avelumab, and anti PD-L1, with ADC vaccine was designated to enhance immune response in those MCC with lower immunogenic tumors (MSS patients) following a Simon Two-stage design for efficacy primary endpoint (6 months PFS rate). A total of 19 patients were recruited on the first stage and all of them received at least one dose of study treatment (avelumab and ADC vaccines). The cohort reached a PFS rate at 6 months of 0%, with a median PFS of 3.1 months (range 2.1-5.3). Therefore the study did not reach the pre-specified futility threshold for efficacy (40%) and the study was stopped at the prespecified interim analysis. The median OS was 12.1 months (range 3.2-22.9) and the 6 months OS rate was 73.7%. At database closure 18 (94.7%) patients were dead. These data were consistent with previous studies in MCC patient populations treated with avelumab monotherapy or other immune checkpoint blockers (Le DT 2015)(Chen 2020)(O´Neil 2017). Thus, addition of ADC vaccines to avelumab did not seem to boost the immune system to evoke a synergistic antitumor activity.

The toxicity profile of the ADC vaccines and avelumab combination was favourable, with only 23 toxicities reported throughout the study period. Most toxicities were low grade 1-2. The most frequent toxicities (all grades) were fatigue (21.05%), fever (15.79%), and arthralgia (10.53%). Only one (5.26%) grade 3 fatigue was reported. A total of 13 SAEs in 8 (44.4%) patients were reported throughout the study period. There were no treatment-related deaths or discontinuations as of the data cutoff date. The toxicity profile was consistent with those previously reported for the study treatments avelumab and ADC vaccines in monotherapy (Powles T 2020)(Kim JH 2020)(Le DT 2015)(Caballero-Baños 2016).

At the molecular level, the study aimed to identify potential biomarkers that correlate with treatment efficacy or led to rise of treatment resistance. After 8 weeks of avelumab and ADC vaccine treatment there was a substantial and significant decrease in VEGFc and VEGFa expression levels, determined from peripheral blood samples. VEGFc was found to be significantly reduced independently of stratification by age, gender, LDH levels, and ECOG. Similarly, MCP1 and SDF1a were decreased upon treatment with ADC vaccines and avelumab. The changes reported in the molecular biomarkers indicate that the study treatment may downregulate the expression of central proteins involved in angiogenesis. The small sample size and low treatment response did not allow to correlate these changes to treatment efficacy.

These results suggest that avelumab and ADC vaccine combination may have a synergistic effect with anti-angiogenic therapies or in those tumor types that are highly dependent on angiogenesis. In fact, combined treatment of avelumab plus regorafenib, an inhibitor of multiple kinases involved in angiogenesis, achieved the mobilization of antitumor immunity in a subset of MSS colorectal cancer patients with low macrophage and high CD8 T-cell infiltration levels (Cousin S 2021). However, the overall median PFS and OS in that trial do not differ much from those reported in our study.

The main limitation of the AVEVAC trial was efficacy. The study was stopped because it did not reach the pre-specified futility threshold. Regarding safety and the biomarker substudy, the limited number of patients should be taken into consideration, as the study was not designed to power statistical differences in these endpoints. The study was designed as a single-arm, so potential bias due to indirect comparisons could not be discarded. Thus results obtained may be taken with caution and will need further research to confirm their clinical relevance.

In conclusion, despite good tolerability and manageable safety profile, the combination of ADC vaccines and avelumab has no increased efficacy that justified further research of this combination in the proposed setting. The molecular substudy may suggest a potential synergistic effect of the ADC vaccine and avelumab combination in angiogenic dependent settings or in combination with antiangiogenic agents.

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15. TABLES, FIGURES AND GRAPHS

Tables, including patient line-listings, are included in the corresponding sections of this CSR.

15. APPENDICES

15.1 STUDY INFORMATION

15.1.1. Last version of Protocol including amendments

- Protocol GEMCAD 1602 version 2.1_JAN 23rd, 2018

15.1.2. Case Report Form

15.1.3. Last version of Subject Information documents

- General patient information sheet and informed consent, version 1.0 APR 26th, 2017
- Selection patient information sheet and informed consent, version 1.0 APR 26th, 2017

15.1.4. Ethics Committees positive vote (First approval)

15.1.5. Regulatory Approval (First approval)

15.1.6. Publications based on the study

- Poster ESMO 2018

15.1.7 Protocol deviation Listing