2. SYNOPSIS

Name of Sponsor: **Institut de Recherches Internationales Servier (I.R.I.S.)**	*(For National Authority Use only)*
Name of finished product: Not applicable **Name of active ingredient:** Not applicable	
Title of study: An experimental medicine, low grade interventional, clinical study to compare peripheral immune system from subjects without cancer diagnosis and patients with advanced solid tumours Protocol No.: CL1-ONCO-001 ID-RCB No.: 2021-A01796-35 CT.gov No.: NCT05133128 The description of the study protocol given hereafter includes the modifications implemented through the 3 substantial amendments to the protocol.	
Coordinating investigator: Prof. Ghiringhelli François, Centre Georges François Leclerc (CGFL), 1 rue du Pr. Marion, 21000 Dijon - France	
Number of study centres and countries: In all, 39 participants were screened and 36 were enrolled across 2 centres [redacted] based in France.	
Studied period: Initiation date: 25 November 2021 (first participant first visit) Completion date: 04 April 2023 (last participant last visit)	
Phase of development of the study: Not applicable	
Publication (reference): Not applicable	
Background and rationale for the study: The aim of the study was to gain knowledge concerning the expression of immune markers on the immune cell subpopulation of peripheral blood mononuclear cells (PBMCs) from healthy volunteers and participants with solid tumours. Few studies have addressed the question of the difference in peripheric immune cells between these 2 populations without a specific focus on an immune cell population or an indication, and with a multiparametric approach. There are few studies available in the literature that compare PBMCs from healthy volunteers and participants with solid tumour. This multiparametric analysis allowed to assess the phenotypic and functional differences and similarities between the peripheral immune systems of healthy volunteers and participants with solid tumours of selected indications. No drug therapy was initiated in the context of this study. This study was stopped prematurely following difficulties in recruitment.	

Objectives and endpoints

Objectives	Endpoints
Primary objective: - To compare PBMCs from healthy volunteers and participants with solid tumour in terms of proportion of immune cell subtypes and expression of selected immune checkpoints.	Primary endpoints: - Standard differential blood count: Blood count was performed on whole blood samples by flow cytometry using a standardised blood count panel containing antibodies against population markers and count beads. Immune cell populations (percentages and numbers) were compared between participants with solid tumour and healthy volunteers. - General immunophenotyping of immune cell subsets (i.e., T cell subsets, myeloid cell subsets) and immune checkpoint expression on immune cell subsets by flow cytometry: For all participants, immunophenotyping was performed by flow cytometry on fresh whole blood samples. Three fluorescence-activated cell sorting (FACS) panels were developed and used to test participant whole blood if confirmed upon technical validation. The first panel aimed at measuring global immune cell subtypes (including B and T lymphocytes, natural killer, myeloid cells) and expression of selected immune checkpoints on these cell subtypes. A second panel was performed to better characterize myeloid cell subsets and the expression of immune checkpoint ligands. A third panel was performed to explore the expression of additive immune checkpoints on immune cell subtypes. Immune cell sub-populations (percentages) and expression levels of immune checkpoints in cell subtypes were compared between participants with solid tumours and healthy volunteers.
Secondary objectives: - To determine whether healthy volunteers are representative of participants with solid tumours in terms of peripheral immune system activation status (cytokine release assay [CRA]). - To assess the intra-participant variability in term of proportion of immune cell types and expression of selected immune checkpoint on PBMCs, for colorectal cancer (CRC) participants.	Secondary endpoints: - Cytokine release assay after immune cell activation: A subgroup of whole blood samples were tested for CRA following cell activation. Cell supernatant was collected after incubation of whole blood in different conditions: • Phorbol myristate acetate/Ionomycin for 3 hours • OKT3 for 20 hours • OKT3 and Nivolumab for 20 hours • Phosphate Buffer Saline (Negative control) for 20 hours Supernatants were frozen and subsequently analysed by the Sponsor using Meso Scale discovery (MSD) cytokine assay. - General immunophenotyping of immune cell subsets and immune checkpoint expression by flow cytometry on blood samples from same participants collected on different days. This was done for participants of the CRC cohort only. Immunophenotyping was performed by flow cytometry on fresh whole blood samples. The same 3 FACS panels developed to this purpose was used to test participant whole blood if confirmed upon technical validation. A standard

	differential blood count was performed at the same time of each sample.
- Biological collection/Biorepository of plasma and PBMCs for retrospective analyses related to the disease or for the development of bioanalytical methods.	- Biorepository of plasma and PBMCs: Blood samples collected during the first sampling in ethylenediaminetetraacetic acid tubes were used for PBMC isolation. Cell-free plasma was prepared from a 4 mL heparin tube. PBMCs and plasma were aliquoted in cryotubes and sent for long term storage in a central biorepository contract research organisation for a maximum of 15 years after the end of the study. The samples will be used for genomic and non-genomic analysis. For genomic analysis, only partial genome sequencing will be done (the Sponsor will not perform a complete sequencing of the genome). Plasma and PBMCs from participants will be stored in order to perform retrospective analyses on these samples. It is indeed expected that the results obtained from the tests of the study (immunophenotyping and functional cytokine release tests) will result in the need to carry out additional, more in-depth analyses on certain markers of interest. The dosage of the soluble marker in the participants' plasma (comparison of participants with solid tumours versus healthy volunteers) may thus be necessary if the expression of this marker turns out to be different on the PBMCs of the different populations studied. Carrying out additional functional tests (performed routinely on PBMCs from healthy volunteers during drug development) on PBMCs from participants with solid tumours may also be considered, particularly in the event of a strong variation of expression of a marker of interest between the 2 populations. Finally, a more detailed analysis of the expression of a marker of interest in a subpopulation of immune cells not included in the FACS panels of the study might be necessary. The material collected will therefore be valuable in order to provide essential knowledge for the development of new drugs. We will need all the available samples in order to enhance this collection and enable these research projects. Therefore, for this prospective study, we consider the collection of these samples necessary and mandatory for all participants and healthy volunteers. In case of consent withdrawal, related samples will be destroyed before any further assessment is completed.

Study design:
The study CL1-ONCO-001 was a bi-centric, low-grade interventional (category 2 according to French regulation), comparative clinical study of blood samples from participants with solid tumour(s) and healthy volunteers:

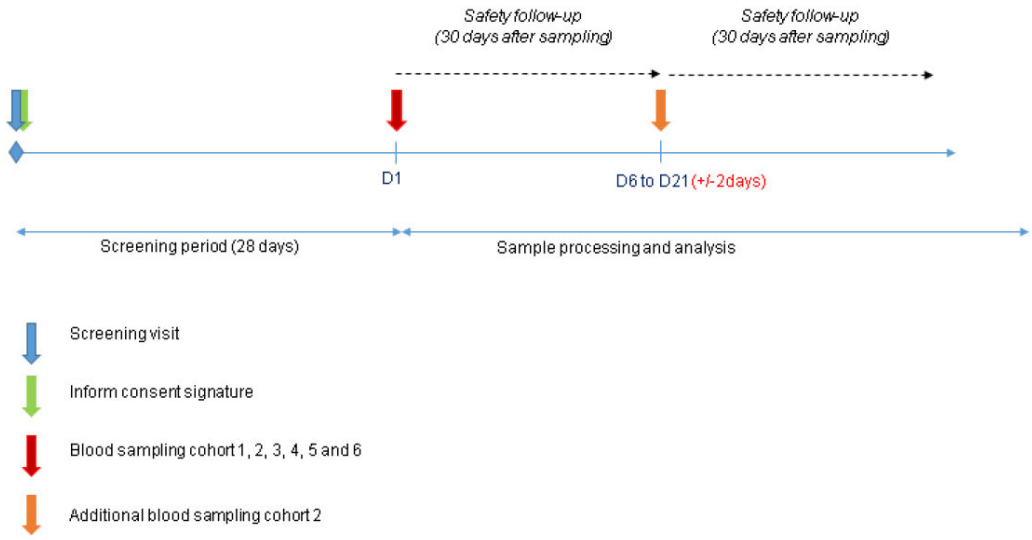
- Participants with solid tumours: 5 indications of interest were selected, i.e., non-small cell lung, colorectal, pancreatic, liver, and gastric or cholangiocarcinoma.
- Healthy volunteers: healthy participants were matched to participants with solid tumours for sex and age (at least for 66% of the participant with solid tumour population) (±5 years).

Participants had one or 2 blood sampling (depending on the cohorts). Whole blood was used to perform:

- Differential blood count.
- C-reactive protein (CRP).
- Immunophenotyping and CRA.
- Plasma and PBMCs were collected in parallel and frozen for further analyses.

No investigational medicinal product was administered during the study.

The study plan is shown below:



This study was performed in strict accordance with Good Clinical Practice. Inclusions were prematurely stopped due to difficulties in recruiting participants to the study.

Number of participants (Planned and Analysed):

Planned:
Based on preliminary internal results on expression of programmed death-ligand 1 (PDL1) and OX40 on different cell types it was estimated that we would have a power between 60% and 100% (depending on cell type and marker considered) to demonstrate the equivalence between healthy volunteers and participants with solid tumours of a specific subtype, given an equivalence margin of 5 (in percentage) and an alpha of 0.05. The number of participants to be enrolled was 25 participants for each of the following cohorts:

- Cohort 1: Participants with non-small cell lung cancer (NSCLC).
- Cohort 2: Participants with CRC.
- Cohort 3: Participants with pancreatic cancer.
- Cohort 4: Participants with liver cancer.
- Cohort 5: Participants with gastric cancer/cholangiocarcinoma.
- Cohort 6: Healthy volunteers.

Analysed:
A total of 36 participants were enrolled in the study (both Included and Biomarker Set):

- Cohort 1: 8 participants.
- Cohort 2: 7 participants.
- Cohort 3: 9 participants.
- Cohort 6: 12 participants.

Diagnosis and main criteria for inclusion/exclusion:

Main inclusion criteria for all participants (participants with solid tumours and healthy volunteers)

- For participants with unresectable, locally advanced or metastatic pancreatic cancer: men and women aged over 45 years on the day the consent was signed.
  For other cohorts: men and women should have been 45 to 70 years of age on the day the consent was signed.
  Note: Healthy volunteers should have been age-matched by ±5 years with the participant with solid tumours population.
- Adequate haematological parameters as assessed by laboratory tests within 21 days for participant and 96 hours for healthy volunteers prior to the day of blood withdrawal.
  - Absolute neutrophil count ≥1500/µL
  - Platelet count ≥100 000/µL, criteria were to be met without transfusion and thrombopoietin for at least 2 weeks prior to the day of blood withdrawal
  - Haemoglobin ≥9 g/dL, criteria were to be met without transfusion and erythropoietin for at least 2 weeks prior to the day of blood withdrawal

Main inclusion criteria for participants with solid tumours only

- Participants currently off treatment with histologically or cytologically confirmed following diagnosis:

Cohort 1	Unresectable, locally advanced or metastatic NSCLC	After at least 1 or 2 standard treatment lines (including 1 line with Immune Checkpoint Inhibitor) and being scheduled for a new anticancer treatment after progression
Cohort 2	Unresectable, locally advanced or metastatic CRC	After at least 2 treatment lines
Cohort 3	Unresectable, locally advanced or metastatic pancreatic cancer	After at least 1 or 2 treatment lines
Cohort 4	Unresectable, locally advanced or metastatic liver cancer	Before or after at least 1 treatment line
Cohort 5	Unresectable, locally advanced or metastatic gastric cancer	After at least 1 treatment line
	Unresectable, locally advanced or metastatic cholangiocarcinoma	Before or after at least 1 treatment line

Main exclusion criteria for all participants (participants with solid tumours and healthy volunteers)

- Participants with an active autoimmune disease that was currently requiring systemic anti-inflammatory treatment. Participants with autoimmune endocrinopathies that were well treated by replacement hormone therapies were allowed.
- Participants with any serious/active/uncontrolled infection, any infection requiring parenteral antibiotics, or unexplained fever >38°C within 2 weeks prior to blood withdrawal.
- Participants seropositive for and with evidence of active viral infection with Hepatitis B virus.
- Participants seropositive for and with active viral infection with Hepatitis C virus.
- Participants who had received prior systemic anticancer therapy, definitive radiotherapy, or other investigational agents or device within 14 days prior to the day of blood withdrawal.
- Participants who have received approved or investigational immunomodulators (targeting any immune cell types, such as anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), anti-PDL1, any immunomodulatory in a clinical trial) in the past 6 months (except for the NSCLC cohort).

Investigational medicinal product:
Not applicable

Comparator:
Not applicable

Trial duration:

The study was divided into the following periods:

- Participants with solid tumours:
  - A screening visit to obtain informed consent and to check the eligibility of the participants to be enrolled in the study.
  - Blood sampling visits:
    - First blood sampling at inclusion (D1).
    - Second blood sampling visit (only for the CRC cohort): 5 to 20 days after the first blood sampling visit (corresponding to D6 to D21).
  - A safety follow-up period of 30 days after blood withdrawal was performed.
- Healthy volunteers:
  - A screening visit to obtain informed consent and to check the eligibility of the participant to be enrolled in the study.
  - Blood sampling visit done at the latest 96 hours after screening visit.
  - A safety follow-up period of 30 days after blood withdrawal was performed.

Statistical methodology:

Analysis sets:

- Screened Set*:* This set corresponds to all screened participants (i.e., all participants who signed the informed consent form, whether they were enrolled or not at the end of the screening visit).
- Included Set (IS): This set corresponds to all enrolled participants (participants who had signed consent form and whose eligibility was confirmed at the end of the screening visit).
- Biomarker Set (BMKS): All participants of the IS having a first blood sampling at visit 1 and at least one available value for a biomarker among CRA data or FACS data.

Description of disposition of participants (status, protocol deviations, and analysis set), demographic data and other baseline characteristics were described in the BMKS by cohort, and in the IS for some of them to control non-inclusion criteria.

For qualitative data, number of observed values, number and percentage of participants per class were presented. For quantitative data, number of observed values, mean, standard deviation, minimum and maximum, median, first and third quartiles were presented.

Biomarker analysis:

- Blood count and immunophenotyping by FACS: blood count was performed on whole blood samples by flow cytometry using a standardised blood count panel containing antibodies against population markers and count beads.
- Between cohort analysis: for each panel, immune cell sub-populations (percentages) and expression levels of immune checkpoints in cell subtypes were compared between each cancer participants and healthy volunteers by a two one-sided test equivalence test.

Safety analysis:

A listing of all adverse event (AE) protocol-related was displayed, in the IS, with the seriousness, the requirement of added therapy and the outcome.

Summary of results and conclusions:

Disposition of participants:

A total of 39 participants were screened and 36 were enrolled in the study. Reason for non-inclusion was discontinuation due to screen failure. The overall disposition of screened participants is presented in **Table 1**.

Table 1 – Participant disposition – Screened participants (N=39)

Status		All
Screened	**n**	**39**
Included	**n (%)**	36 (92.0)
Completed	n (%)	36 (100)
Not included	**n (%)**	3 (8.0)
Discontinued	n (%)	3 (100)
Screen failure	n (%)	3 (100)

Source: Table 1-01
Percentages were based on n of considered status

Each of the 2 analysis sets (IS and BMKS) consisted in 8 participants in Cohort 1, 7 participants in Cohort 2, 9 participants in Cohort 3, and 12 participants in Cohort 6. Cohort 4 and Cohort 5 were not opened due to the premature study termination.

Baseline characteristics:

Overall, the mean ± standard deviation (SD) age of the participants was 62.0 ± 8.2 years (median = 61.5 years) and most of them (24 participants [66.7%]) were 65 years or under. The healthy volunteer's cohort trended to be younger than participants from solid tumour cohorts. Overall, the proportion of male and female participants were equal at 50%. Female participants were over-represented in the healthy volunteers' group as compared to the solid tumour cohorts. Overall, 24 participants were enrolled with metastatic disease.

The mean ± SD duration of metastatic disease since diagnosis was 1.51 ± 0.98 years (median = 1.32 years) for participants in Cohort 1, 3.19 ± 1.62 years (median = 3.21 years) for participants in Cohort 2, and 2.09 ± 1.09 years (median = 2.35 years) for participants in Cohort 3.

There was a tendency towards an increased duration of disease in participants with CRC (Cohort 2). Most participants with solid tumours (21 participants [87.5%]) had tumours originating from adenocarcinoma. Overall, only 1 participant (2.8%) with NSCLC experienced sign and symptom of the disease reported on the day of Visit 1 sampling, which included musculoskeletal chest pain and cervicobrachial syndrome.

Overall, 25 participants (69.4%) reported at least one medical history other than the studied cancer. The most commonly reported medical history (≥ 25% of the participants) by preferred term (PT) was hypertension (9 participants [25.0%]) in all solid tumour cohorts. Also, at least 1 surgical or medical procedure was reported in 13 participants (36.1%). Overall, all 24 participants of the solid tumour cohorts had received at least one previous therapy or treatment specific to the studied cancer. All the participants with NSCLC (Cohort 1) had previously received immune checkpoint (ICP) therapies (8 participants [100%]), in contrast to CRC participants (Cohort 2, no participant with previous ICP) or pancreatic cancer participants (Cohort 3, 2 participants [22.0%]).

Biomarkers results:

- PBMCs and immune markers

Overall, no equivalence (with margin 5%) between each solid tumour cohort versus healthy volunteers was observed for any of the assessed parameters. Regarding immunophenotyping parameters or immune checkpoints, non-adjusted p-values ranged from 0.433 to 0.997, and adjusted p-values ranged from 0.986 to 0.999. For the standard differential blood counts, non-adjusted p-values ranged from 0.408 to 0.999, and adjusted p-values ranged from 0.986 to 0.999.

Table 2 summarises the comparisons of immunophenotyping parameters between cohorts and healthy volunteers.

Table 2 – Immunophenotyping parameters - BMKS (N=36)
Overall - Summary of comparisons between cohorts and healthy volunteers

Parameter	Cohort 1 vs HV		Cohort 2 vs HV		Cohort 3 vs HV	
	Non-adjusted p-value	Adjusted p-value	Non-adjusted p-value	Adjusted p-value	Non-adjusted p-value	Adjusted p-value
CD45+ (Count/µL)	0.901	0.999	0.902	0.998	0.766	0.986
Monocytes (Count/µL)	0.758	0.999	0.949	0.998	0.873	0.986
Monocytes (% in CD45+)	0.652	0.999	0.766	0.998	0.723	0.986
Neutrophils (Count/µL)	0.972	0.999	0.979	0.998	0.845	0.986
Neutrophils (% in CD45+)	0.997	0.999	0.991	0.998	0.607	0.986
T CD4+ (Count/µL)	0.990	0.999	0.969	0.998	0.607	0.986
T CD4+ (% in CD45+)	0.999	0.999	0.998	0.998	0.718	0.986
T CD8+ (Count/µL)	0.963	0.999	0.834	0.998	0.595	0.986
T CD8+ (% in CD45+)	0.996	0.999	0.989	0.998	0.941	0.986
T CD4+ Treg (Count/µL)	0.408	0.999	0.761	0.998	0.877	0.986
T CD4+ Treg (% in CD45+)	0.721	0.999	0.984	0.998	0.858	0.986
T CD4+ conventional (% in CD45+)	0.997	0.999	0.988	0.998	0.544	0.986
T CD3+ (% in CD45+)	0.995	0.999	0.990	0.998	0.461	0.986
NK (% in CD45+)	0.595	0.999	0.521	0.998	0.842	0.986
OX40 (% in T CD4+ conventional)	0.979	0.999	0.659	0.998	0.986	0.986
CD73 (% in CD4+ conventional)	0.503	0.999	0.836	0.998	0.571	0.986
PD1 (% in T CD4+ conventional)	0.834	0.999	0.787	0.998	0.986	0.986
PDL1 (% in T CD4+ conventional)	0.823	0.999	0.524	0.998	0.792	0.986
41BB (% in T CD3+)	0.526	0.999	0.710	0.998	0.934	0.986
NKG2A (% in NK)	0.433	0.999	0.459	0.998	0.547	0.986

Source: Table 2-01-03
BMKS: biomarker set, CD45+: CD45+ lymphocyte, CD73: cluster of differentiation 73, FDR: false discovery rate, HV: healthy volunteers, NK: natural killer, PD1: programmed cell death 1, PDL1: programmed death-ligand 1, T CD3+: T CD3+ lymphocyte, T CD4+: T CD4+ lymphocyte, T CD8+: T CD8+ lymphocyte, TOST: two one-sided tests, Treg: regulatory T cell
Non-adjusted p-value: Two-sided p-value of the TOST equivalence test
Adjusted p-value: Two-sided adjusted p-value taking into account the Benjamini and Hocheberg FDR adjustment.

- Biochemistry

At Visit 1, abnormal values for high CRP were observed in all cohorts with no clinically relevant trends: 4 participants (66.7%) with NSCLC, 5 participants (83.3%) with CRC, 3 participants (50.0%) with pancreatic cancer, and 2 healthy volunteers (16.7%). Overall, close to half of participants (14 participants [46.7%]) had higher CRP level at Visit 1, half or more of participants in solid tumour cohorts (Cohorts 1, 2, and 3). At Visit 2, abnormal values for high CRP were observed in 4 participants (66.7%) with CRC.

Safety results:

- Adverse events

A total of 5 AEs related to the study protocol were reported across 3 participants in Cohort 6. These events were categorized under PT of 'Injection site haematoma' (3 events in 2 participants), and 'Blood pressure increased' (2 events in 1 participant). Results for AEs are described in **Table 3**.

Table 3 – Listing of all AE protocol-related - IS (N=36)
During the study

Cohort	SOC	PT
Cohort 6: Healthy volunteers	General disorders and administration site conditions	Injection site haematoma
	General disorders and administration site conditions	Injection site haematoma
	General disorders and administration site conditions	Injection site haematoma
	Investigations	Blood pressure increased
	Investigations	Blood pressure increased

Source: Listing 3-01
AE: adverse event, IS: included set, PT: preferred term, SOC: System Organ Class

None of the participants died, had serious AEs or other clinically meaningful AE related to protocol.

Conclusion:

The primary objective of this study was to compare PBMCs from healthy volunteers and participants with solid tumour in terms of proportion of immune cell subtypes and expression of selected immune checkpoints.

Taking into account the low number of participants in each cohort and the high variability, the comparative analyses between the solid tumour cohorts and the healthy volunteers suggest non-equivalence (given a 5% margin; mean in the healthy volunteers' cohort ± 5%).

Date of the report: 18 March 2024