MEDPACE

STATISTICAL ANALYSIS PLAN

Protocol Title:	OMNIA-1, A Phase 1/2 Study of ANV419 as Monotherapy or in Combination With Anti-PD-1 or Anti-CTLA-4 Antibody Following Anti-PD-1/Anti-PD-L1 Antibody Treatment in Patients With Unresectable or Metastatic Cutaneous Melanoma
Protocol Number:	ANV419-101
Protocol Version/Date:	Version 4.0/ 07 March 2023
Investigational Product:	ANV419
Sponsor:	Anaveon AG Technologiepark Basel Hochbergerstrasse 60C 4057 Basel Switzerland
SAP Version/Date:	Version v1.0/08 July 2024

SIGNATURE PAGE

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We, the undersigned, have reviewed and approved this Statistical Analysis Plan:

Signature	**Date**
[redacted]	

VERSION HISTORY

Version	Version Date	Description
1.0	08 July 2024	Original signed version

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

Abbreviation	Definition
ADA	Anti-drug antibodies
ADaM	Analysis Data Model
AE	Adverse event
AESI	Adverse event of special interest
AIDS	Acquired immunodeficiency syndrome
ATC	Anatomical therapeutic chemical
AUC	Area under the concentration-time curve
BOIN	Bayesian optimal interval
BRAF	B-type Raf proto-oncogene
CD	Cluster of differentiation
CI	Confidence interval
CL	Systemic clearance
CM	Cutaneous melanoma
C_{max}	Maximum observed serum concentration
CR	Complete response
CRF	Case Report Form
CT	Computed tomography
ctDNA	Circulating tumor deoxyribonucleic acid
CTLA-4	Cytotoxic T-lymphocyte antigen-4
DCR	Disease control rate
DLT	Dose-limiting toxicity
DNA	Deoxyribonucleic acid
DOR	Duration of response
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
eCRF	Electronic case report form
EDC	Electronic data capture
FoxP3	Forkhead box P3
HIV	Human immunodeficiency virus
ICF	Informed consent form
iCR	Immune complete response
iDCR	Immune disease control rate
iDOR	Immune duration of response
IL	Interleukin
IL-2R	Interleukin-2 receptor
iPFS	Immune progression-free survival
iPR	Immune partial response
irAE	Immune-related adverse event

Abbreviation	Definition
iRECIST	Immune Response Evaluation Criteria in Solid Tumors
IRR	Infusion-related reaction
IRT	Interactive Response Technology
iSD	Immune stable disease
iTTR	Immune Time to Response
IV	Intravenous(ly)
MedDRA	Medical Dictionary for Regulatory Activities
MTD	Maximum tolerated dose
NCI CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events
ORR	Objective response rate
OS	Overall survival
PBMC	Peripheral blood mononuclear cell
PD	Pharmacodynamic(s)
PD-1	Programmed death-1
PD-L1	Programmed death-ligand 1
PFS	Progression-free survival
PK	Pharmacokinetic(s)
PR	Partial response
[redacted]	
QTcF	Heart rate-corrected QT interval using Fridericia's formula
RECIST	Response Evaluation Criteria in Solid Tumors
RNA	Ribonucleic acid
RP2D	Recommended Phase 2 dose
RP2D(i)	Recommended Phase 2 dose for ANV419 when administered in combination with ipilimumab
RP2D(p)	Recommended Phase 2 dose for ANV419 when administered in combination with pembrolizumab
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SD	Stable disease
SDTM	Study Data Tabulation Model
SRC	Safety Review Committee
T cell	Thymus lymphocyte cell
TEAE	Treatment-emergent adverse event
TESAE	Treatment-emergent serious adverse event
TTR	Time to Response
V_{ss}	Volume of distribution at steady state
WHO	World Health Organization

1 INTRODUCTION

This Statistical Analysis Plan (SAP) describes the statistical methods implemented for the analysis of data from the OMNIA-1 study with protocol number ANV419-101 version 4.0 dated the 07 March 2023. The SAP will be finalized prior to database lock. Any deviations from the SAP after database lock will be documented in the final Clinical Study Report (CSR).

This SAP includes the analysis for all parts as described in the protocol. However, a decision to stop the trial at the end of Part 1 on 27 February 2024 was taken as the criteria to go to Part 2 was not met. As a result of not proceeding with the trial, the survival follow-up as described by protocol was stopped. The analysis that will not be performed as the trial stopped are listed in section 5.

2 STUDY OVERVIEW

2.1 Study Objectives and Endpoints

The study objectives and endpoints for Part 1: Monotherapy Dose Expansion, Part 2: Combination Dose Finding, and Part 3: Combination Dose Expansion are provided in Table 2-1, Table 2-2, and Table 2-3

Table 2-1: Study Objectives and Endpoints for Part 1: Monotherapy Dose Expansion

Part 1: Monotherapy Dose Expansion – Arms A1 and A2	
Primary Objectives	**Primary Endpoints**
Evaluate the efficacy of ANV419	ORR (CR + PR), as defined by RECIST v1.1 (hereinafter, RECIST).
Secondary Objectives	**Secondary Endpoints**
To characterize the tumor response according to modified RECIST v1.1 criteria for immune-based therapeutics (iRECIST)	Tumor response in terms of objective response rate (ORR: CR + PR) assessed by iRECIST
Expand evaluation of efficacy of ANV419	- DOR (per RECIST) and iDOR (per iRECIST[0]) measured from first response until disease progression; - DCR (DCR = CR + PR + SD), iDCR (iDCR = iCR + iPR + iSD), PFS, iPFS, and OS; - Median TTR; and - Median iTTR.
Evaluate the safety of ANV419	Incidence, frequency, and severity of AEs including the following: - SAEs; - irAEs; - AESIs; - AEs leading to discontinuation of the study; and - Changes from baseline in laboratory parameters, vital signs, ECGs, and physical examination.
Explore immunogenicity after exposure to ANV419	Incidence of immunogenicity as indicated by ADA.
Exploratory Objectives	**Exploratory Endpoints**
Explore the changes in the tumor microenvironment before and after adding ANV419 as monotherapy	Changes in immunological biomarker expression from baseline and post treatment tumor and liquid biopsies, including: - Immune cell counts, immunophenotyping (including, but not limited to, CD3, CD4, CD8, CD56, CD16, CD25, FoxP3, CD279, and CD366); - Cytokine production (including, but not limited to, IFNγ, IL-2, TNF, IL-6, IL-10); and - Immunohistochemistry (including, but not limited to, CD8, CD4, Ki67).
	Analysis of mutations in the tumor and analysis of tumor mutational burden.

	Analysis of changes in baseline ctDNA and at specified timepoints on therapy and after therapy.
	Analysis of single nucleotide polymorphisms, germline DNA for genome-wide association studies, analysis of T cell receptor repertoire, epigenetic markers, soluble CD25, and soluble PD-1/PD-L1.
Seymour L, Bogaerts J, Perrone A, et al. iRECIST: guidelines for the response criteria for use in trials testing immunotherapeutics. *Lancet Oncol*. 2017;18(3):e143-e152. AE = adverse event; AESI = adverse event of special interest; CD = cluster of differentiation; CR = complete response; ctDNA = circulating tumor deoxyribonucleic acid; DCR = disease control rate; DOR = duration of response; ECG = electrocardiogram; FoxP3 = Forkhead box P3; iCR = immune complete response; iDCR = immune disease control rate; iDOR = immune duration of response; IFNγ = interferon gamma; IL = interleukin; iPFS = immune progression-free survival; iPR = immune partial response; irAE = immune-related adverse event; iRECIST = immune Response Evaluation Criteria in Solid Tumors; iSD = immune stable disease; iTTR = immune time to response; ORR = objective response rate; OS = overall survival; PD-1 = programmed death-1; PD-L1 = programmed death-ligand 1; PFS = progression-free survival; PR = partial response; RECIST = Response Evaluation Criteria in Solid Tumors; SAE = serious adverse event; SD = stable disease; T cell = thymus lymphocyte cell; TNF = tumor necrosis factor; TTR = time to response; v1.1 = version 1.1.	

Table 2-2: Study Objectives and Endpoints for Part 2: Combination Dose Finding

Combination Dose Finding – Combination Therapy Arms (Arms B and C)	
Primary Objectives	**Primary Endpoints**
Evaluate the safety and tolerability and determine the RP2D of ANV419 in combination with pembrolizumab Evaluate the safety and tolerability and determine the RP2D of ANV419 in combination with ipilimumab	Incidence, frequency, and severity of AEs including the following: - SAEs; - TEAEs; - DLTs; - AESIs; - irAEs; - AEs leading to discontinuation of the study; and - Changes from baseline in laboratory parameters, vital signs, ECGs, and physical examinations.
Secondary Objectives	**Secondary Endpoints**
Evaluate PK and PD of ANV419 in combination with pembrolizumab or ipilimumab	PK endpoints in serum: - CL of ANV419; - V_{ss} of ANV419; - AUC of ANV419; and - C_{max} of ANV419. PD endpoints in peripheral blood: including, but not limited to, CD3, CD4, CD8, CD56, CD16, CD25, FoxP3, CD279, and CD366.
Explore immunogenicity after exposure to ANV419 in combination with pembrolizumab or ipilimumab	Incidence of immunogenicity as indicated by ADA.
Evaluate the efficacy of ANV419 in combination with pembrolizumab or ipilimumab	ORR (CR + PR), as defined by RECIST.

Expand evaluation of efficacy of ANV419 in combination with pembrolizumab or ipilimumab	DOR (per RECIST) and iDOR (per iRECIST) measured from first response until disease progression; PFS, iPFS, and OS; DCR (DCR = CR + PR + SD), iDCR (iDCR = iCR + iPR + iSD), Median TTR; and Median iTTR.
Evaluate clinical benefit in quality of life after exposure to ANV419 in combination with pembrolizumab or ipilimumab	Change in QoL at baseline and every 12 weeks while receiving ANV419 via QoL evaluations: - EQ-5D-5L; and - QLQ-C30.
Exploratory Objectives	**Exploratory Endpoints**
Explore the changes in the tumor microenvironment before and after adding ANV419 as monotherapy and in combination with pembrolizumab or ipilimumab	Changes in immunological biomarker expression from baseline and post treatment tumor and liquid biopsies, including: - Immune cell counts, immunophenotyping (including, but not limited to, CD3, CD4, CD8, CD56, CD16, CD25, FoxP3, CD279, and CD366); - Cytokine production (including, but not limited to, IFNγ, IL-2, TNF, IL-6, IL-10); and Immunohistochemistry (including, but not limited to, CD8, CD4, Ki67).
	Analysis of mutations in the tumor and analysis of tumor mutational burden.
	Analysis of changes in baseline ctDNA and at specified timepoints on therapy and after therapy.
	Analysis of single nucleotide polymorphisms, germline DNA for genome-wide association studies, analysis of T cell receptor repertoire, epigenetic markers, soluble CD25, and soluble PD-1/PD-L1.

ADA = anti-drug antibodies; AE = adverse event; AESI = adverse event of special interest; AUC = area under the concentration-time curve; CD = cluster of differentiation; CL = systemic clearance; C_{max} = maximum observed serum concentration; CR = complete response; ctDNA = circulating tumor deoxyribonucleic acid; DLT = dose-limiting toxicity; ECG = electrocardiogram; EQ-5D-5L = Euro-QoL 5 dimension 5 level; FoxP3 = Forkhead box P3; IFNγ = interferon gamma; IL = interleukin; irAE = immune-related adverse event; ORR = objective response rate; PD = pharmacodynamic(s); PD-1 = programmed death-1; PD-L1 = programmed death-ligand 1; PK = pharmacokinetic(s); PR = partial response; QLQ-C30 = quality of life core 30; QoL = quality of life; RECIST = Response Evaluation Criteria in Solid Tumors; RP2D = recommended Phase 2 dose; SAE = serious adverse event; T cell = thymus lymphocyte cell; TEAE = treatment-emergent adverse event; TNF = tumor necrosis factor; V_{ss} = volume of distribution at steady state.

Table 2-3: Study Objectives and Endpoints for Part 3: Combination Dose Expansion

Part 3: Combination Dose Expansion – Combination Therapy Arms (Arms B and C)	
Primary Objectives	**Primary Endpoints**
Evaluate the efficacy of ANV419 in combination with pembrolizumab or ipilimumab	ORR (CR + PR), as defined by RECIST.
Secondary Objectives	**Secondary Endpoints**
Expand evaluation of efficacy of ANV419 in combination with pembrolizumab or ipilimumab	- DOR (per RECIST) and iDOR (per iRECIST[0]) measured from first response until disease progression; - DCR (DCR = CR + PR + SD), iDCR (iDCR = iCR + iPR + iSD), PFS, iPFS, and OS; - Median TTR; and - Median iTTR.
Part 3: Combination Dose Expansion – Combination Therapy Arms (Arms B and C)	
Secondary Objectives	**Secondary Endpoints**
Evaluate the safety of ANV419 in combination with pembrolizumab or ipilimumab	Incidence, frequency, and severity of AEs including the following: - SAEs; - irAEs; - AESIs; - AEs leading to discontinuation of the study; and - Changes from baseline in laboratory parameters, vital signs, ECGs, and physical

	examination.
Evaluate clinical benefit in quality of life after exposure to ANV419 in combination with pembrolizumab or ipilimumab	Change in QoL at baseline and every 12 weeks while receiving ANV419 via QoL evaluations: - EQ-5D-5L; and - QLQ-C30.
Explore immunogenicity after exposure to ANV419 in combination with pembrolizumab or ipilimumab	Incidence of immunogenicity as indicated by ADA.
Exploratory Objectives	**Exploratory Endpoints**
Explore the changes in the tumor microenvironment before and after adding ANV419 in combination with pembrolizumab or ipilimumab	Changes in immunological biomarker expression from baseline and post treatment tumor and liquid biopsies, including: - Immune cell counts, immunophenotyping (including, but not limited to, CD3, CD4, CD8, CD56, CD16, CD25, FoxP3, CD279, and CD366); - Cytokine production (including, but not limited to, IFNγ, IL-2, TNF, IL-6, IL-10); and - Immunohistochemistry (including, but not limited to, CD8, CD4, Ki67).
	Analysis of mutations in the tumor and analysis of tumor mutational burden.
	Analysis of changes in baseline ctDNA and at specified timepoints on therapy and after therapy.
	Analysis of single nucleotide polymorphisms, germline DNA for genome-wide association studies, analysis of T cell receptor repertoire, epigenetic markers, soluble CD25, and soluble PD-1/PD-L1.
1. Seymour L, Bogaerts J, Perrone A, et al. iRECIST: guidelines for the response criteria for use in trials testing immunotherapeutics. *Lancet Oncol*. 2017;18(3):e143-e152. AE = adverse event; AESI = adverse event of special interest; CD = cluster of differentiation; CR = complete response; ctDNA = circulating tumor deoxyribonucleic acid; DCR = disease control rate; DOR = duration of response; ECG = electrocardiogram; EQ-5D-5L = Euro-QoL 5 dimension 5 level; FoxP3 = Forkhead box P3; iCR = immune complete response; iDCR = immune disease control rate; iDOR = immune duration of response; IFNγ = interferon gamma; IL = interleukin; iPFS = immune progression-free survival; iPR = immune partial response; irAE = immune-related adverse event; iRECIST = immune Response Evaluation Criteria in Solid Tumors; iSD = immune stable disease; iTTR = immune time to response; ORR = objective response rate; OS = overall survival; PD-1 = programmed death-1; PD-L1 = programmed death-ligand 1; PFS = progression-free survival; PR = partial response; QLQ-C30 = quality of life core 30; QoL = quality of life; RECIST = Response Evaluation Criteria in Solid Tumors; SAE = serious adverse event; SD = stable disease; T cell = thymus lymphocyte cell; TNF = tumor necrosis factor; TTR = time to response.	

2.2 Study Design

2.2.1 Overview

This is a multi-site, open-label, randomized, parallel arm, Phase 1/2 adaptive study to evaluate the efficacy and safety of ANV419 as a monotherapy and in combination with pembrolizumab or ipilimumab in patients aged 18 years or older with advanced cutaneous melanoma who have previously been treated with at least 1 line of standard of care immunotherapy, including an anti-PD-1/anti-PD-L1 antibody.

The 4 following treatment arms will be included in this study:

- Arm A1: [redacted]
- Arm A2: [redacted]
- Arm B: ANV419[redacted] in combination with pembrolizumab
- Arm C: ANV419[redacted] in combination with ipilimumab

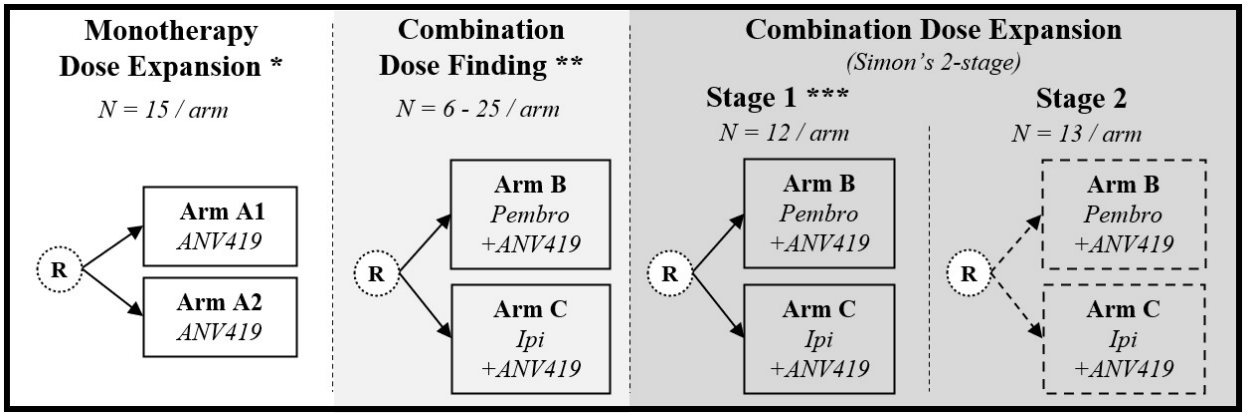
There will be up to 3 separate parts in this study:

Part 1: Monotherapy Dose Expansion with patients randomized to receive infusion of ANV419 [redacted]

Part 2: Combination Dose Finding with patients randomized to Arms B and C with infusion of ANV419 [redacted] Part 2 will open if part 1 is successful.

Part 3: Combination Dose Expansion with patients randomized to Arms B and C (see Figure 2-1). Part 3 will open if part 2 is successful.

Figure 2-1: Study Design



* = If ANV419 monotherapy is successful, the combination portion will be initiated.
** = Dose escalation increment is decided by the SRC until RP2D of ANV419 per arm is identified.
*** = Arm not meeting pre-specified ORR criteria may be stopped early after Stage 1.
In the Combination Dose Finding part, patients will be manually randomized to Arm B or Arm C. In the Monotherapy Dose Expansion part and the Combination Dose Expansion part, patients will be randomized by IRT into Arms A1 or A2 and Arms B or C, respectively.
Ipi = ipilimumab; IRT = Interactive Response Technology; ORR = objective response rate; Pembro = pembrolizumab; R = randomization; RP2D = recommended Phase 2 dose; SRC = Safety Review Committee.

2.2.1.1 Part 1: Monotherapy Dose Expansion

The Monotherapy Dose Expansion part (Arms A1 and A2) will be a parallel group part with a Bayesian sequential monitoring analysis and up to 15 patients per arm. This part will establish the efficacy and safety of ANV419 as monotherapy.

[redacted]

All responses will be assessed locally using RECIST 1.1 and used for decision making. There is 95% certainty that the true objective response rate (ORR) is greater than 10% if we observe any 1 of the following.

- 2 responses in 3 to 7 patients

- 3 responses in 8 to 13 patients
- 4 responses in 14 to 19 patients
- 5 responses in 20 to 27 patients

2.2.1.2 Part 2: Combination Dose Finding

This part will be initiated when at least 2 responses have been observed in the Monotherapy Dose Expansion part.

The Combination Dose Finding part will be comprised of 2 arms (Arms B and C) that will identify a recommended Phase 2 dose (RP2D) for ANV419 in each arm when administered in combination with pembrolizumab (Arm B) or ipilimumab (Arm C) at the prescribed dosing. The number of patients recruited to each arm will be between 6 and 25 patients depending on the number of doses increment possible. A minimum of 6 patients will be treated at RP2D. The patients from the Combination Dose Finding part will not be randomized into the Combination Dose Expansion part.

Dosing of ANV419 when administered in combination with pembrolizumab (Arm B) or ipilimumab (Arm C) will occur as shown in Table 2-4, with a dose of ANV419 currently planned to start at [redacted] Patients in the Combination Dose Finding part will be treated with a minimum of 24 hours interval between patients to allow for monitoring of acute toxicities. Dose level 1, as shown in Table 2-4 will be reviewed by the Safety Review Committee (SRC) per the safety evaluations and the SRC will make dose increment recommendations at each dose level, not exceeding 3-fold increment from on dose level to the next. The maximum dose level explored will not exceed the [redacted] studied in the ongoing ANV419-001 study. [redacted]

Table 2-4: ANV419 Dose Escalation Plan for Combination Dose Finding Part (Pembrolizumab or Ipilimumab)

Dose Level	ANV419 Dose	Pembrolizumab Dose or Ipilimumab Dose	Frequency
1	[redacted]		
2			
3			
4			
5			

Note: ANV419 will be administered by IV infusion over 15 minutes (+5 minutes).
1. Dose increments will be determined by the SRC based on a synthesis of all relevant data available from all dose levels evaluated in this study, not exceeding a 3-fold increment from one dose level to the next. The maximum dose level explored will not exceed the Q3W dose of ANV419 monotherapy studied in the ongoing ANV419-001 study.

IV = intravenous(ly); [redacted] SRC = Safety Review Committee; TBD = to be determined.

Dose escalation in the Combination Dose Finding part will follow the Bayesian optimal interval (BOIN)[1] design to find the maximum tolerated doses (MTDs)/RP2D of ANV419 when administered in combination with pembrolizumab or ipilimumab at the prescribed dose. Dose escalation decisions will be based on a synthesis of all relevant data available from all dose levels evaluated in the ongoing Combination Dose Finding part of the study.

2.2.1.3 Part 3: Combination Dose Expansion

The Combination Dose Expansion part of the study will be separate from the Combination Dose Finding part and will further evaluate the safety and efficacy of ANV419 in combination with pembrolizumab (Arm B) or ipilimumab (Arm C). This Combination Dose Expansion part will consist of 2 stages. In Stage 1, 12 patients will be randomized by interactive response technology (IRT) into each arm. At the end of this stage, an interim analysis will be performed to assess continuation of treatment arms into Stage 2 (see Table 2-5). In Stage 2, eligible patients will be randomized to the remaining treatment arms, determined at the interim analysis. Recruitment will continue until 25 patients have been randomized to a treatment arm.

Table 2-5: Continuation Decision Rules for Combination Dose Expansion Part

Treatment Arm Accrual	Discontinue if the Number of Responses is	Discontinue Criterion in Terms of ORR	Continue if the Number of Responses is at Least
Stage 1 (n=12)	≤2	Observed ORR <20%	3
ORR = objective response rate.			

Treatment with ANV419 in combination with ipilimumab can continue up to a maximum [redacted] per the approved label (United States Prescribing Information and Summary of Product Characteristics). If ipilimumab-related AEs occur, ipilimumab can be discontinued at any time. Once a patient has completed the maximum [redacted] of ipilimumab allowed, they will continue receiving ANV419 at the dose enrolled as [redacted] in the absence of disease progression or unacceptable toxicity.

2.2.2 Randomization and Blinding

Part 1: Monotherapy Dose Expansion

Patients will be randomized by IRT and stratified by locally assessed BRAF mutation status into Arms A1 or A2 on Cycle 1 Day 1.

Part 2: Combination Dose Finding part

Patients will be manually randomized into Arm B or Arm C on Cycle 1 Day 1. Steps for assigning the randomization number are as follows: The Clinical Operations team/trained individuals will use the pre-generated randomization number lists to find the next available randomization number and assign it to the patient. The team will then use the randomization number to obtain the treatment assignment from the pre-generated randomization scheme provided by the Biostatistics team. The Clinical Operations team will maintain and complete the randomization schemes throughout the course of study.

Part 3: Combination Dose Expansion part

Patients enrolled in the Combination Dose Expansion part will be randomized by IRT and stratified by BRAF mutation status into Arms B or C on Cycle 1 Day 1. For the Combination Dose Expansion part, following the interim analysis at the end of Stage 1, if 1 treatment arm is removed from Stage 2 (leaving only 1 treatment arm open for enrollment), randomization by IRT will no longer be required and eligible patients will be enrolled into the remaining treatment arm, provided there are sufficient slots remaining.

This is an open-label study. No blinding is required.

2.2.3 Study Drug

ANV419 is a stable fusion protein comprised of IL-2 fused to an anti-IL-2 mAb that sterically blocks the binding of the IL-2 to IL-2R-alpha (CD25). The ANV419 investigation product is a liquid, colorless, clear formulation with no visible particles, containing 2.5 mg/mL ANV419 protein, 20 mM histidine, 8% (w/v) sucrose, and 0.02% (w/v) PS80, pH 6.0. ANV419 is stored at -20°C (±5°C) and thawed by leaving at room temperature (15°C to 25°C) for approximately 1 hour and then further diluted into normal saline.

Patients will receive ANV419 as monotherapy (Arms A1 and A2) or in combination with pembrolizumab (Arm B) or ipilimumab (Arm C) after all Screening procedures have been completed and eligibility has been confirmed by the Investigator. Only patients enrolled in the study may receive the study drug(s). Under no circumstances will the study drug(s) be used other than as directed within this Protocol. The 3 study drugs and the administration of each are described in Table 2-6.

Table 2-6: Study Drug Administration

Treatment	Study Part	Arm	Dose/ Potency	Dose Frequency	Route of Administration/Duration	Regimen/ Treatment Period
ANV419	[redacted]					
Pembrolizumab						
Ipilimumab[Error! Reference source not found.,Error! Reference source not found.]						

2.2.4 Sample Size Determination

Part 1: Monotherapy Dose Expansion

This part of the study will use Bayesian sequential monitoring. A maximum of 15 patients will be enrolled and treated in each arm. A1 and A2 monotherapy arms will be analyzed as independent experiments, and overall, and the following assumptions are made:

- Nothing is known *a priori*: assume a uniform prior, i.e., the true ORR could be any value between 0% and 100%;
- A true ORR <6% indicates treatment failure; and
- A true ORR >10% indicates treatment success.

With a maximum of 15 patients in each arm, if no responses are observed, then the estimated true ORR would be 5.9%, with 90% confidence interval (CI) of 0.3% and 17.1% respectively. This would be sufficient evidence to suggest that the treatment is not clinically meaningful unless clinical benefit is demonstrated with other means (i.e., durable SDs > 6 months). With a maximum of 30 patients overall, if no responses were observed, then the estimated true ORR would be 3.1%, with 90% CI (0.2%, 9.2%). This would be sufficient evidence to suggest that the treatment at either dose is not clinically meaningful.

There is 95% certainty that the true ORR is greater than 10% if any of the following scenarios are observed:

- 2 responses in 3 to 7 patients
- 3 responses in 8 to 13 patients
- 4 responses in 14 to 19 patients
- 5 responses in 20 to 27 patients

Part 2: Combination Dose Finding

The Combination Dose Finding part will employ BOIN[(1)] to find the MTD/RP2D of Arm B (ANV419 in combination with pembrolizumab) and Arm C (ANV419 in combination with ipilimumab). For each arm, the following assumptions have been predefined for the dose escalation:

1. Target DLT rate of 25%;
2. Maximum of 25 patients;
3. Maximum of 9 patients treated at the same dose level; and
4. Maximum of 5 dose levels defined in **Error! Reference source not found.** to be tested.

Patients will be enrolled and treated in cohorts of 3 patients (at least). Up to 50 patients in total will be enrolled for the Combination Dose Finding part, with a minimum of 6 patients treated at RP2D.

Part 3: Combination Dose Expansion (efficacy analysis)

The sample size calculation is based on a Simon's 2-stage[(2)] design and ensures that under all possible distributions of patients to the 2 treatment arms, there is at least 80% power to correctly detect an ORR >40% and at most a Type I error rate of 10% of rejecting a null hypothesis that ORR <20%. This results in a sample size of 12 patients randomized to each treatment arm in Stage 1, followed by 13 additional patients randomized during Stage 2: a total of 25 patients per arm at the end of Stage 2.

3 STATISTICAL METHODOLOGY

3.1 General Considerations

3.1.1 Analysis Day

Analysis day will be calculated from the date of first dose of study drug. The day of the first dose of the study drug will be Day 1, and the day immediately before Day 1 will be Day -1. There will be no Day 0.

3.1.2 Definition of Baseline

Unless stated otherwise, Baseline is defined as the last non-missing measurement prior to the first dose of the study drug.

3.1.3 Unscheduled Visits

Unscheduled visits will not be included in by-visit summaries but will contribute to the derivation of best or worst-case values and will be presented in data listings. Visit windowing will not be used for handling unscheduled visits.

3.1.4 Summary Statistics

No formal hypothesis testing is planned, and summaries will in general be descriptive. Appropriate summaries will be provided for each assessment based on data type (continuous or categorical).

Categorical data will generally be summarized with number and percentages of patients. The denominator used for the percentage calculation will be clearly defined. Continuous data will generally be summarized with descriptive statistics including n (number of non-missing values), mean, median, standard deviation, minimum, and maximum.

Time-to-event endpoints (progression-free survival, duration of response, and overall survival) will be summarized using Kaplan-Meier methods and 95% confidence interval.

Unless described otherwise, analyses will in general be presented for each arm by treatment arm and BRAF Mutation status (wild-type or BRAFV600) in the Monotherapy Dose Expansion part and by dose level in the Combination Dose Finding part and Combination Dose Expansion part of, and overall using descriptive statistics.

3.1.5 Handling of Dropouts and Missing Data

Unrecorded data values will be recorded as missing. Only recorded (i.e., complete) data values will be used for statistical analyses. In general, invalid, or missing values will not be imputed unless stated otherwise.

If needed, completely or partially missing start and end dates for medical history events, including date of initial diagnosis and last date of last progression for the primary cancer history, will be imputed in a conservative fashion as follows:

Date	Type of Missing Date	Handling of Missing Date
Event Start Date (e.g., YYYY-MM-DD)	Completely missing	No imputation will be applied.
	Only YYYY is available	Use the first day of YYYY to impute the missing month and date parts of the start date
	YYYY and MM are available, but DD is missing	Use the first day of MM to impute the missing date part of the start date
Event End Date (e.g., YYYY-MM-DD)	Completely missing	No imputation will be applied. The event will be considered ongoing at the end of study.
	Only YYYY is available	Use the last day of YYYY to impute the missing month and date parts of the end date
	YYYY and MM are available, but DD is missing	Use the last day of MM to impute the missing date part of the end date

For missing or partial AEs and concomitant medications dates, conservative conventions will be applied to assign the events to corresponding periods. AEs will be considered as treatment-emergent, and the medications will be considered as concomitant if the missing/partial dates cannot definitively exclude the treatment period. Handling incomplete dates (e.g., AE, and concomitant medications dates) are described in Table 7-1 and Table 7-2 from Appendix A.

For patients lost to follow-up or with missing RECIST/iRECIST assessments, censoring rules for efficacy endpoints are defined in section 3.4.

3.2 Analysis Populations

3.2.1 Safety Population

The Safety Population is defined as all patients who receive at least 1 dose (or partial dose) of study drug(s). The Safety Population will be used for safety analyses including DLT assessments during the DLT observation period in part 2.

3.2.2 Efficacy Population

The Efficacy Population is defined as all patients who receive at least 1 dose of study drug, have at least 1 post-baseline tumor assessment, and who are part of the Monotherapy Dose Expansion part, Combination Dose Finding part, or the Combination Dose Expansion part. The Efficacy Population will be used for efficacy analyses.

3.2.3 Pharmacokinetic (PK) Population

The PK Population is defined as all patients who receive at least 1 dose of study drug and have at least 1 measured concentration for at least 1 of the analytes. The PK Population will be used for PK analyses.

3.2.4 Pharmacodynamic (PD) Population

The Pharmacodynamic Population is defined as all patients who receive at least 1 dose of study drug and have at least 1 evaluable pharmacodynamic sample. The Pharmacodynamic Population will be used for Pharmacodynamic endpoint analyses.

3.2.5 Immunogenicity Population

The Immunogenicity Population is defined as all patients who receive at least 1 dose of study drug and have at least 1 evaluable immunogenicity sample. The Immunogenicity Population will be used for immunogenicity endpoint analyses.

3.3 Patient Data and Study Conduct

3.3.1 Patient Disposition

Numbers and percentages of patients who were screened and screen failures will be summarized in total based on all screened patients. Reasons for screen failure will also be summarized.

Numbers and percentages of randomized patients (regardless of whether they receive study drug) and the patients in each analysis population will be summarized in total based on all randomized patients. Reasons for exclusion from each analysis population will also be summarized.

Numbers and percentages of patients in the following categories will be summarized based on the Safety population:

- Patients who discontinued study
- Patients who permanently discontinued ANV419
- Patients who permanently discontinued pembrolizumab
- Patients who permanently discontinued ipilimumab before completing 4 cycles

Reasons of discontinuation will also be presented.

Time on treatment (months) will be computed as (Date of the last dose administered – Date of first dose of study drug + 1) * 12/365.25.

Time on study (months) will be defined as the time the patient was followed and will be computed as (Date of end of study – Date of first dose of study drug + 1) * 12/365.25.

Time on treatment, as well as the time of study, will be summarized based on the Safety population.

Patient disposition data will be listed by patient.

3.3.2 Protocol Deviations

The protocol deviations will be reviewed by the study team and classified as “CSR Reportable” or “CSR Non-Reportable”. The number and percentage of patients with CSR Reportable protocol deviations will be summarized by category for the Safety Analysis Set.

CSR reportable protocol deviations will be listed by patient.

3.3.3 Demographic and Baseline Characteristics

The following demographic and baseline characteristics will be summarized by arm in part 1 & part 3 and by dose level in part 2.

- Age at informed consent (years)
- Sex
- Childbearing potential
- Race
- Ethnicity
- Height (cm)
- Weight (kg)

Demographic and baseline characteristics data will be listed by patient.

3.3.4 Cancer History

The following primary cancer history information will be summarized using descriptive statistics based on the Safety population:

- Histology of Primary Melanoma at initial diagnosis.
- Distant Metastasis stage at study entry based on 5 categories: M0, M1a, M1b, M1c and M1d using AJCC edition 8
- Time from initial histologic/cytologic diagnosis to study entry (months) calculated as (Date of ANV419 administration– Date of initial diagnosis+1) * 12/365.25.
- Time from last disease progression to study entry (months) calculated as (Date of ANV419 administration– Date of last progression+1) * 12/365.25.
- ECOG performance status at baseline (0, 1)
- BRAF mutation status based on two categories: Normal (Wild type), BRAFV600x mutated
- Brain metastasis status (Yes or No)
- Sum of diameters of target lesions at study entry
- Lactate dehydrogenase values at study entry (Screening)
- Lactate dehydrogenase levels compared to normal range at study entry (Screening) expressed as overall mean and categorical based on the following categories:
  - $< 1 \times ULN$
  - $\geq 1 \times ULN < 1.5 \times ULN$
  - $\geq 1.5 \times ULN \leq 2 \times ULN$
  - $> 2 \times ULN$

3.3.5 Prior Cancer Treatment

Prior cancer treatment, including (1) prior systemic cancer therapy, (2) prior cancer radiation therapy and (3) prior cancer surgery will be recorded on this eCRF.

The mean number of lines of therapies received in the metastatic setting will be reported.

The numbers and percentage of patients with any lines of prior systemic cancer therapy will be reported categorical as following

- 1, 2, 3 or ≥4 lines of therapy,
- Adjuvant line
- Metastatic lines as 1, 2, 3 or ≥4

Note that recent adjuvant treatment will be considered as a line of therapy in the metastatic setting if the patient experienced relapse/progression on therapy within 1-year prior C1D1, or within 6 months from adjuvant therapy completion and within one-year prior C1D1.

The number and percentage of patients who received a PD-1/PD-L1 or a PD-1/PD-L1 containing regimen as last treatment prior study entry.
The number lines of prior systemic cancer therapy, the best overall response of the last prior line of therapy will be summarized based on the Safety population.
The number and percentage of patients with prior cancer radiation and prior cancer surgery will be summarized.

The reported cancer therapy terms will be coded using WHO Drug Dictionary (Global B3 March 2022 or higher). Prior cancer therapies/medications will be summarized by Anatomical Therapeutic Chemical (ATC) levels and preferred term. A patient will be counted only once within an ATC classification but may contribute to two or more preferred terms in the same classification.

3.3.6 Medical History

Medical history will be collected at screening. The reported medical history term will be coded to system organ class and preferred term using the Medical Dictionary for Regulatory Activities (MedDRA) version 25.0 or higher.

Medical history data will be listed by patient.

3.3.7 Prior and Concomitant Non-cancer Medications

The used prior or concomitant non-cancer medications will be recorded in the electronic case report (eCRF) from 28 days prior to Cycle 1 Day 1 to the Safety Follow-Up Visit up to 90 days after last dose of study drug or the start date of a new cancer regimen, whichever is shorter.

Prior and Concomitant medications will be coded to anatomical therapeutic chemical (ATC) class and preferred term using the WHODrug Dictionary version Global B3, March 2022 or higher.

Numbers and percentages of patients taking prior and concomitant medications by ATC class and preferred term will be summarized for each arm by dose level in the Combination Dose Finding part of the study and by treatment arm in the Monotherapy Dose Expansion part and Combination Dose Expansion part and overall using descriptive statistics based on the Safety population.

All prior and concomitant non-cancer medication data will be listed by patient.

3.3.8 Study Drug Exposure

Exposure of ANV419, pembrolizumab and ipilimumab (only for part 2 and part 3) will be summarized.

For each component of the study treatment to following exposure parameters will be summarized for each arm by dose level in the Combination Dose Finding part of the study and by treatment arm and BRAF mutation status in the Monotherapy Dose Expansion part and Combination Dose Expansion part and overall based on the Safety population.

- The duration of exposure (days) = date of the last dose of study drug – date of the first dose of study drug + 1. The duration of exposure (months) = duration of exposure (days) * 12/365.25
  - The total number of treatment cycles initiated. [redacted]
  - The total dose administered
  - The Actual Dose Intensity (ADI) administered (mcg/day) computed as:

$$ADI = \frac{Actual\ total\ dose\ administred\ (mcg)}{Duration\ of\ exposure\ (days)}$$

  - The Planned Dose Intensity (PDI) (mcg/day) computed as:

$$PDI = \frac{Total\ Planned\ dose\ (mcg)}{Duration\ of\ exposure\ (days)}$$

  - The Relative Dose Intensity (RDI) (%) computed as:

$$RDI = \frac{ADI}{PDI} \times 100$$

  - Number and percentages of patients with dose interruption and reasons of interruption

All information collected on the electronic case report form (eCRF) related to study treatment will be listed by patient.

3.4 Efficacy Assessment

All tumor response assessment will be assessed locally by the Investigator using RECIST 1.1 criteria and iRECIST criteria (see protocol Appendix C and D).

Baseline disease assessment will include radiographic tumor measurements using CT imaging of the chest, abdomen, pelvis, or any other areas with suspected disease involvement and CT or MRI of the brain during Screening.

For Monotherapy therapy arms, on-study disease assessment should be performed [redacted]

[redacted]

Unless otherwise noted, efficacy analyses described in this section will be performed using the Efficacy population. Summarized results will be presented for each arm by dose level in the Combination Dose Finding part of the study and by treatment arm in the Monotherapy Dose Expansion part and Combination Dose Expansion part and overall.

3.4.1 Best Overall Response (BOR)

Best Overall Response (BOR) will be determined based on the overall visit responses.

BOR per RECIST v1.1 is defined as the best response in the order of: Complete Response (CR), Partial Response (PR), Stable Disease (SD), Progressive Disease (PD), and Not Evaluable (NE) for patients with measurable disease. The minimum duration of Stable Disease to be considered as the best overall response is 3 weeks from first dose of the study treatment.

iBOR per iRECIST is defined as the best response in the order of: Immune Complete Response (iCR), Immune Partial Response (iPR), Immune Stable Disease (iSD), Immune Confirmed Progression (iCPD), Immune Unconfirmed Progression (iUPD) and NE for patients with measurable disease. The minimum duration of Immune Stable Disease to be considered as the best overall response is 3 weeks from first dose of the study treatment.

3.4.2 Definition of Efficacy Endpoints

3.4.2.1 Objective Response Rate (ORR)

Objective Response Rate per RECIST v1.1 (ORR) is defined as the percentage of patients whose BOR is either CR or PR.

iORR per iRECIST is defined as the percentage of patients whose best overall response is either iCR or iPR.

3.4.2.2 Disease Control Rate (DCR)

Disease control rate (DCR) per RECIST is defined as the proportion of patients whose best overall response is CR, PR, or SD >= 4 Weeks from first dose.

DCR per iRECIST (iDCR) is defined as the proportion of subjects whose best overall response is an iCR, iPR, or iSD >= 4 Weeks from first dose.

3.4.2.3 Duration of Response (DOR)

DOR per RECIST 1.1 will be defined as time from first occurrence of CR or PR to the earliest date of first documented PD or death due to any cause, whichever occurs first. Only patients who achieved either CR or PR will be included in the analysis.

DOR per RECIST v1.1 will be calculated as:

DOR (months) = (Event/censoring date – first date of CR/PR + 1)/ 30.437

The event or censoring date will be determined based on the convention listed in Table 3-1.

Table 3-1: Date of Event or Censoring for DOR per RECIST v1.1

Situation	Date of progression or censoring	Outcome
Death or Disease progression between planned disease assessments	Date of death or first disease assessment showing disease progression, whichever occurs first	Event
Death or disease progression after missing two or more consecutively scheduled disease assessments	Date of last evaluable disease assessment visit without documentation of disease progression before the first missed visit	Censored
Initiation of subsequent treatment before disease progression or death (without disease progression beforehand)	Date of last evaluable disease assessment prior to start of alternate anti-cancer treatment	Censored
Alive and without disease progression	Date of last evaluable disease assessment	Censored

iDOR per iRECIST will be defined as time from first occurrence of iCR or iPR to the earliest date of first documented iCPD per iRECIST, initiation of alternate anti-cancer therapy or death due to any cause, whichever occurs first. If iUPD occurs, but is disregarded because of later iSD, iPR, or iCR, that iUPD date should not be used as the progression event date. In the case of an assessment of iUPD is recorded and no subsequent evaluable iRECIST assessments are recorded, the iUPD event will be treated as confirmed disease progression.

Only patients who achieved either iCR or iPR will be included in the analysis.

iDOR per iRECIST will be calculated as:

iDOR (months) = (Event/censoring date – first date of iCR/iPR + 1)/ 30.437

The event or censoring date will be determined based on the convention listed in Table 3-2.

Table 3-2: Date of Event or Censoring for iDOR per iRECIST

Situation	Date of progression or censoring	Outcome
iUPD provided that iCPD is confirmed at the next assessment on or prior to cutoff date	Date of first disease assessment showing iUPD with iCPD is confirmed at the next assessment	Event
If progression is not confirmed and there is no subsequent iSD, iPR, or iCR due to death or withdrawal	Date of first iUPD with no subsequent iSD, iPR, or iCR due to death or withdrawal	Event
Death reported without iUPD on or prior to cutoff date	Date of death	Event
Death or iUPD after more than one consecutive missed visits	Date of last disease assessment with documented non-iUPD	Censored
No post-baseline disease assessments on or prior to cutoff date	Date of the first dose	Censored

On treatment and without disease progression on or prior to the cutoff date	Date of last evaluable disease assessment with evaluable response	Censored
Initiation of alternate anticancer treatment before disease progression or death (without disease progression beforehand)	Date of last evaluable disease assessment prior to start of alternate anti-cancer treatment	Censored
Alive and withdrew on or prior to cutoff date without disease progression	Date of last evaluable disease assessment with evaluable response	Censored

3.4.2.4 Progression Free Survival (PFS)

PFS per RECIST 1.1 will be defined as the time from the first dose date to the earliest date of the first documented PD per RECIST 1.1 assess locally, initiation of alternate anti-cancer therapy, or death due to any cause, whichever occurs first.

PFS per RECIST 1.1 will be calculated as:

PFS (months) = (Event/censoring date – first dose date of ANV419 + 1)/ 30.437

For subjects who have not progressed and are still alive at time of data cutoff for study analysis or who are lost to follow-up, PFS will be right censored. The event or censoring date will be determined based on the conventions listed in Table 3-3. .

Table 3-3: Date of Event or Censoring for PFS per RECIST v1.1

Situation	**Date of progression or censoring**	**Outcome**
Death or disease progression after one missed tumor assessment	Date of death or first disease assessment showing disease progression	Event
Death or disease progression after missing two or more consecutively scheduled disease assessments	Date of last evaluable disease assessment visit without documentation of disease progression or Date of first dose of study treatment, whichever comes later	Censored
Alive and without disease progression and no initiation of alternate anticancer	Date of last evaluable disease assessment or Date of first dose of study treatment, whichever comes later	Censored
Alive with No (or Non-evaluable) post-baseline disease assessments	Date of the first dose of study treatment	Censored
Initiation of alternate anticancer treatment before disease progression or death (without disease progression beforehand)	Date of last evaluable disease assessment prior to the start of alternate anticancer treatment	Censored

iPFS per iRECIST will be defined as the time from the first dose date to the earliest date of the first documented iCPD per iRECIST, initiation of alternate anti-cancer therapy, or death due to any cause, whichever occurs first. If iUPD occurs, but is disregarded because of later iSD, iPR, or iCR, that iUPD date should not be used as the progression event date. In the case of an assessment of iUPD is recorded and no subsequent evaluable iRECIST assessments are recorded, the iUPD event will be treated as confirmed disease progression.

iPFS per iRECIST will be calculated as:

iPFS (months) = (Event/censoring date – first dose date of ANV419 + 1)/ 30.437

For subjects who have not progressed and are still alive at time of data cutoff for study analysis or who are lost to follow-up, iPFS will be right censored. The event or censoring date will be determined based on the conventions listed in Table 3-3.

The PFS and iPFS time will be derived based on the scan/assessment dates and not visit dates to determine progression. Assessments/scans contributing towards a particular visit may be performed on different dates. The following rules will be applied for this calculation:

- Date of radiological progression will be determined based on the earliest assessment/scan dates of the component that triggered the progression.
- When censoring a subject for PFS/iPFS, the subject will be censored at the latest of the Evaluable assessment/scan dates contributing to a particular overall visit assessment.

3.4.2.5 Overall Survival (OS)

OS is defined as the time from the date of first dose of ANV419 to date of death for any cause. OS will be calculated as:

OS (months) = (Death/censor date– first ANV419 dose date + 1)/30.4375

Patients who are alive or lost to follow-up as of the data cutoff date will be censored at the date the subject was last known to be alive. The last date known to be alive for each individual subject may be determined using, but not limited to, the following dates recorded on the eCRF:

- AE start and stop dates
- Study treatment start and stop dates
- Laboratory assessment dates
- Dates of vital signs, physical examination, Eastern Cooperative Oncology Group (ECOG) performance status, and electrocardiogram (ECG) assessments
- Disease assessment dates
- Start and stop dates of concomitant medications including procedures and alternative anti-cancer therapy
- Date subject last known to be alive on long-term follow-up

3.4.2.6 Time To Response (TTR)

TTR per RECIST v1.1 is defined as time from first dose to first response of CR or PR and will be calculated as:

TTR (months) = (first date of CR/PR– first treatment date+ 1)/ 30.437

Analysis of TTR will include only patients who eventually achieve CR or PR. No censoring will be performed.

iTTR per iRECIST is defined as time from first dose to first response of iCR or iPR and will be calculated as:

iTTR (months) = (first date of iCR/iPR– first treatment date+ 1)/ 30.437

Analysis of iTTR will include only patients who eventually achieve iCR or iPR. No censoring will be performed.

3.4.3 Primary Efficacy Endpoints

For Part 1 (Monotherapy Dose expansion) and Part 3 (Combination Dose Expansion), the primary efficacy endpoint is ORR using RECIST 1.1

The point estimates of ORR along with the exact Clopper-Pearson 95% CI will be presented by treatment arm in the Monotherapy Dose Expansion part and Combination Dose Expansion part and overall based on the Efficacy Population.

For Part 2 (Combination Dose Finding), the efficacy analyses are considered as secondary (see Section 3.4.4).

3.4.4 Secondary Efficacy Endpoints

3.4.4.1 Tumor response data

3.4.4.1.1 ORR for Part 2 (Combination Dose Finding)

The point estimates of ORR per RECIST 1.1 along with the exact Clopper-Pearson 95% CI will be presented for each arm by dose level in the Combination Dose Finding part based on Efficacy Population.

3.4.4.1.2 iORR

The point estimates of iORR per iRECIST along with the exact Clopper-Pearson 95% CI will be presented by treatment arm in the Monotherapy Dose Expansion part based on the Efficacy Population.

3.4.4.2 BOR, iBOR and tumor burden

The best overall response will be determined according to RECIST 1.1 criteria and according to iRECIST. The BOR/iBOR will be presented for each arm by dose level in the Combination Dose Finding part and by treatment arm in the Monotherapy Dose Expansion part and Combination Dose Expansion part and overall based on the Efficacy Population.

The tumor burden change for patients will be calculated for each patient in the Efficacy Population as the percent change from baseline in the sum of diameters (SoD) of target tumor lesions at each assessment time point based on RECIST 1.1. The best tumor burden change (decrease) will be summarized descriptively.

A waterfall plot of the best percent change (decrease) in SoD from baseline for each subject will be presented. A spider plot of the percent change in SoD from baseline at scheduled post-baseline visits will be presented. A swimmer plot will be used to show the occurrence of clinical outcomes of interest over time (response, progression, treatment duration, death, etc.).

All tumor response data will be listed.

3.4.4.3 Disease Control Rate

DCR per RECIST v1.1 and iDCR per iRECIST will be analyzed in same manner as ORR.

3.4.4.4 Duration Of Response

DOR and iDOR based on the Investigator assessment will be estimated using the Kaplan-Meier method. The Kaplan-Meier estimate of the first quartile, median, and third quartile for DOR and iDOR and the 95% CIs for the median calculated using the Brookmeyer-Crowley method will be

presented. The event-free rate with the 95% CI calculated using Greenwood's formula will be provided for selected time points.

Kaplan-Meier plots of DOR and iDOR will be presented.

3.4.4.5 Progression Free Survival

Unless specified otherwise, the analytical methods described in Section 3.4.4.3 for DOR/iDOR will be used for PFS/iPFS. PFS/iPFS summaries will be presented based on Safety Population.

In addition, the number and percentages of patients experiencing a PFS/iPFS event, types of events, and patients censored will be summarized.

3.4.4.6 Overall survival

Unless specified otherwise, the analytical methods described in Section 3.4.4.3 for DOR will be used for OS. OS summary will be presented based on Safety Population.

3.4.4.7 Time To Response

TTR/iTTR will be summarized descriptively by calculating the mean, median, standard deviation, and minimum and maximum values.

3.5 Pharmacokinetic Assessments

Pharmacokinetic assessment for this study will be performed by external vendors. The following plan will be applied.

3.5.1 Description of Pharmacokinetic Variables

Blood samples for the assessment of PK will be taken at the following visits/timepoints:

[redacted]

3.5.2 Pharmacokinetics Evaluation

The following PK parameters will be estimated by non-compartmental approach using actual elapsed time from dosing for Cycle 1 only.

Following the interim analysis of the first part of the study (Part 1) using a data cut-off of October 17th, 2023, Anaveon has decided to not pursue the development of ANV419 in unresectable or metastatic melanoma. As a result, no pharmacokinetic data were generated after October 17th, 2023.

- $AUC_{0\text{-}last}$: Area under the concentration-time curve (µg/L * hours) from time zero (pre-dose) to the last measurable concentration, calculated using the trapezoidal method (as defined in Phoenix WinNonLin manual, Certara)
- $AUC_{0\text{-}\infty}$: Area under the concentration curve (µg/L * hours) from time zero (pre-dose) to infinity, calculated trapezoidal methods with linear interpolation (as defined in Phoenix WinNonLin manual, Certara) and extrapolated to infinity by addition of the last

quantifiable concentration divided by the elimination rate constant: AUC0-last + Clast/λz. All the data measured will be reported and summarized;

- $\%AUC_{ex}$: Percent of the area extrapolated for the calculation of AUC0-∞;
- C_{max}: Maximum concentration (ng/mL), obtained directly from the observed concentration versus time data;
- T_{max}: Time to maximum concentration (hours), obtained directly from the observed concentration versus time data;
- C_{last}: Last measurable concentration (ng/mL), obtained directly from the observed concentration versus time data;
- T_{last}: Time (hours) of last concentration, obtained directly from the observed concentration versus time data;
- $t_{1/2}$: half-life (hours), determined as ln(2)/λz;
- V_{ss}: volume of distribution at steady state (L), calculated as Dose/(AUC0-∞*λz);
- λ_z: terminal elimination rate constant (1/hours), determined by linear regression of terminal points of the log-linear concentration-time curve. A minimum of three data points are required for determination of λz, with an r-square value ≥0.7. In addition, visual assessment will be used to identify the terminal linear phase of the concentration-time profile.

For calculations using the trapezoidal rule, the following formula will be used:

$$AUC = \frac{(C_1 - C_2)}{2}(t_2 - t_1)$$

For interpolation the following rule will be used if applicable

$$C^* = C_1 + \left|\frac{t^* - t_1}{t_2 - t_1}\right|(C_2 - C_1)$$

Where C* is the concentration at time t*

Pharmacokinetic data will be reported by Think Q2. Details are included below but these outputs will not be created by Medpace.

3.5.3 Serum Concentration

Raw serum concentration values will be summarized for the PK Population by study arm and dose level, visit and nominal time point using descriptive statistics, including the geometric mean and 68% range and grouped by cohort. For summaries of serum concentrations, below the limit of quantification (BLQ) values will be set to missing.

Individual and mean concentration versus time plots will be produced and displayed in linear and semi-logarithmic scales. The individual plots of all patients per cohort will be displayed in one graph. For the purposes of plotting the data, BLQ serum concentrations that are imbedded between two measurable concentrations or after the last measurable concentration will be set to

missing; however, BLQ values occurring prior to the first measurable concentration will be set to zero.

3.5.4 Pharmacokinetics Parameters

Pharmacokinetic parameters will be analyzed based on the PK Population. For each patient, the PK parameters described in Section 3.5.1 will be determined by a non-compartmental approach. For the non-compartmental PK analysis, all serum concentration BLQ values occurring prior to the first measurable concentration will be set to zero. BLQ values occurring after the first measurable serum concentration will be set to missing.

PK parameters will be listed individually and summarized by study part and dose level using descriptive statistics including geometric mean and 68% range. Additional summaries of C_{max}, AUC_s, and $t_{1/2}$ might be done for subgroup analysis that may exclude patients having biased data specifically due to either the bioanalytical aspect or other issues identified and might be done in addition to the total data (totality of the data) and reported in the dedicated section of the study report.

3.6 Pharmacodynamic Assessments and exploratory Variables

Samples for Pharmacodynamic, biomarkers, and other exploratory samples will be drawn as indicated in the Schedule of Events in the protocol. Except indicated otherwise, Pharmacodynamic analysis will be performed based on the Pharmacodynamic population. Serum cytokines analysis will be performed based on the Safety Population.

The different biomarkers tested by Flow cytometry, as well as serum cytokines are represented in Appendix C. The list presented in the Appendix is an exhaustive list and may be changed depending on the availability of the data and the consent of patients.

The absolute counts per L blood of the immune cell populations determined by flow cytometry will be computed based on the % of each population per lymphocytes. If the parent population is not lymphocytes, the corresponding parent population will be used to calculate the % of each immune cell subpopulation per lymphocytes. The absolute count (10^6/L) of each immune cell subpopulation will be calculated based on the determined percentage within lymphocytes and the lymphocytes count determined by Local labs. The different computation equations and populations are represented in Appendix D.

The change in the percentages or absolute values over time and fold change from baseline of selected biomarker will be summarized descriptively. Plots of the absolute values and the Fold change from Baseline will also be presented.

Individual pharmacodynamic and biomarkers data will be listed by patient.

3.7 Immunogenicity Assessment

Blood samples for anti-drug antibodies (ADA) will be drawn as indicated in the Schedule of Events in the protocol.

Number and percentage of prevalence and incidence of ADA, cross-reactivity with IL-2 (positive, negative), nAB will be presented as described in Section 3.1.4 based on the Immunogenicity Population at each visit. The number of patients at a visit with positive ADA and were previously negative will also be presented. The ADA titer for positive patients will be presented by a spaghetti plot in which timepoints that are nAB positive will be highlighted.

The impact of presence and titer of pre-existing, treatment-emergent, and treatment-boosted ADA and nAB on PK, safety, and efficacy read-outs will be evaluated by descriptive statistics.

A listing of all available immunogenicity data will be provided.

3.7.1 Impact of ADA on C_{max}, lymphocyte count and the pharmacodynamic response

The median of all measured ADA titers across the study will be used to categorize ADA titer levels into ADA high and ADA low levels.

The impact of ADA levels on Cmax will be analyzed based on the PK Population. The Cmax of each patient will be plotted versus high and low ADA titer groups and will be grouped for Cycle 1 Day 1, Cycle 2 Day 1, and Cycle 3 Day 1.

The impact of ADA titer levels on the lymphocyte count will be analyzed by plotting the lymphocyte count grouped by ADA high versus low for Baseline, Cycle 1 Day 1, Cycle 1 Day 2, Cycle 1 Day 4, Cycle 1 Day 8, Cycle 2 Day 1, Cycle 2 Day 4, Cycle 3 Day 1, Cycle 4 Day 1, Cycle 5 Day 1, and Cycle 5 Day 4.

The impact of ADA on the pharmacodynamic response will be analyzed by plotting the percentage of Ki67+ NK cells and Ki67+ CD8 T cells of Cycle 2 Day 4 versus ADA titer level high and low and grouped by dose levels.

3.8 Safety Assessment

Unless otherwise specified, all the data collected up to 90 days following the last dose of study drug or the start of a new cancer regimen, whichever is shorter, will be included in the safety summaries. Safety assessments include adverse events, vital signs, clinical laboratory assessments, ECGs, and physical examinations (including ECOG). Safety analyses will be summarized separately for each part as described in section 3.1.4.

3.8.1 Adverse Events (AEs)

AEs will be captured from signing of informed consent until the Safety Follow-Up Visit up to 90 days after the last dose of study drug or the start of a new cancer regimen, whichever is shorter. All AEs will be coded to system organ class and preferred term using MedDRA version 25.0 or higher. Severity of AEs will be classified using NCI-CTCAE Version 5.0 grading.

Treatment emergent adverse events (TEAEs) are defined as AEs that begin or worsen on or after the start of study.

Treatment related TEAEs are defined as having reasonable causal relationship to study treatment or with missing assessment of the causal relationship.

3.8.1.1 Overview of TEAEs

An overview of TEAEs will be provided including numbers and percentages of patients (and event counts) with the following:

- Any TEAEs (overall and by maximum severity)
- Any immune related TEAEs (overall and by maximum severity)
- Any ANV419 related TEAEs (overall and by maximum severity)
- Any Pembrolizumab related TEAEs (overall and by maximum severity)
- Any Ipilimumab related TEAEs (overall and by maximum severity)
- Any TEAEs of special interest (overall and by maximum severity)
- Any treatment-emergent serious AEs (TESEAEs)
- Any ANV419 related treatment-emergent serious AEs
- Any TEAEs leading to ANV419 discontinuation
- Any TEAEs leading to Pembrolizumab discontinuation
- Any TEAEs leading to Ipilimumab discontinuation
- Any TEAEs leading to Study discontinuation
- Any TEAEs leading to ANV419 dose reduction
- Any TEAEs leading to Pembrolizumab dose reduction
- Any TEAEs leading to Ipilimumab dose reduction
- Any TEAEs leading to ANV419 dose Interruption
- Any TEAEs leading to Pembrolizumab dose Interruption
- Any TEAEs leading to Ipilimumab dose Interruption
- Any AEs leading to death

The numbers and percentages of patients (and event number) will also be presented by system organ class (SOC) and preferred term (PT) for each of the categories in the overview. For these summaries, patients with multiple adverse events will be counted only once per SOC or PT. Note that Pembrolizumab and Ipilimumab related TEAEs will only be summarized for Combination Dose Finding and Combination Dose expansion parts.

The number of patients experiencing a DLT and the type of DLT (Hematologic or non-hematologic, Pneumonitis, Lab Abnormalities, CRS, Delay longer than 7 days for toxicity or Drug induced liver injury) will be summarized by dose level for the Combination Dose Finding part.

The following categories of Cytokine Release Syndrome adverse events will be summarized:

- Number and percent of patients with any CRS AE, and any CRS event with Grade >=2
- Number and percent of patients that experienced diagnostic parameters (Fever, Tachycardia, …) throughout the CRS event.

- Number and percent of patients with at least one treatment for an CRS event, as well as the number and percent of patients in each treatment category
- Number and percentage of CRS events treated with Corticosteroids and the duration of the Corticosteroids treatment in days
- Number and percentage of CRS events treated with Tocilizumab and the duration of the Tocilizumab treatment in days
- Duration of the CSR events onset time from the last infusion in days computed as date of start of the CSR event – date of last infusion + 1
- Duration of CRS event full recovery in days computed as the date of the full recovery – date of start of the CSR event + 1

A by-patient adverse event data listing will be provided including, but not limited to, verbatim term, preferred term, SOC, NCI CTCAE grade, and relationship to study drug. SAEs, AEs leading to death, and AEs leading to discontinuation from the treatment and from the study will also be listed.

3.8.2 Clinical Laboratory Tests

Blood and urine samples for safety analysis of urinalysis, coagulation, chemistry, serology, endocrinology, and hematology parameters (see Appendix B) will be obtained as specified in the Schedule of Events in the protocol and processed using local laboratory. For purposes of analysis and reporting, laboratory values will be standardized using International System of Units (SI).

Pregnancy tests (serum or urine) will be performed at pre-specified timepoints.

Clinical laboratory evaluations will be summarized using descriptive statistics for continuous laboratory parameters (coagulation, chemistry, hematology, and endocrinology) for the values and changes from baseline at each scheduled visit. The minimum post-baseline value, maximum post-baseline value, and last post-baseline value will also be presented. Both scheduled and unscheduled post-baseline visits will be considered for the summaries of the last, maximum, and minimum post-baseline values.

Categorical serology and urinalysis parameters will be summarized using number and percentage of patients.

Abnormal laboratory results will be graded according to NCI-CTCAE version 5 as applicable. A shift table, presenting the 2-way frequency tabulation for baseline and the worst post-treatment grade according to the NCI-CTCAE grade will be provided for selected clinical laboratory tests when applicable. Both scheduled and unscheduled post-treatment visits will be considered in tabulation of the worst post-treatment grade.

The number and percentage of patients with laboratory abnormalities will be presented for applicable hematology and serum chemistry laboratory parameters.

Additionally, the number and percentage of patients with the following potentially clinically significant abnormal liver function test will be presented:

- ALT ≥3xULN, ≥5xULN, ≥10xULN, and ≥20xULN
- AST ≥3xULN, ≥5xULN, ≥10xULN, and ≥20xULN

- Total bilirubin ≥2xULN
- Potential Hy's Law cases: ALT or AST >3xULN, total bilirubin ≥2xULN, and ALP <2xULN at the same visit

Clinical laboratory evaluations will also be listed by patient and abnormal values will be flagged.

3.8.3 Vital Signs

Vital signs will be measured as indicated in the Schedule of Events in the protocol. Vital signs will include height (Screening only), weight, systolic and diastolic blood pressure (SBP and DBP), heart rate (HR), respiratory rate, and temperature (presented in Celsius).

Descriptive statistics will be provided for the values and changes from baseline for all vital signs measurements at each scheduled visit. The last, maximum, and minimum post-baseline values will also be presented. Both scheduled and unscheduled visits will be considered for summaries of the last, minimum, and maximum. Vital signs abnormalities will be evaluated using potentially clinically significant (PCS) criteria for minimum and maximum values of SBP, DBP and HR as defined in Table 3-4. The number and proportion of patients with potentially clinically significant changes in vital signs will be presented.

Vital signs assessments will also be listed by patient.

Table 3-4: Potentially clinically significant SBP, DBP and HR

Parameter	**PCS criteria**
Systolic blood pressure (mmHg)	Minimum SBP ≤ 90 mmHg
	Minimum SBP decrease from baseline > 20 mmHg
	Maximum SBP ≥ 140 mmHg
	Maximum SBP increase from baseline > 20 mmHg
Diastolic blood pressure (mmHg)	Minimum DBP ≤ 60 mmHg
	Minimum DBP decrease from baseline > 20 mmHg
	Maximum DBP ≥ 100 mmHg
	Maximum DBP increase from baseline > 20 mmHg
Heart rate (beats per minute)	Minimum value ≤ 60 bpm
	Minimum HR decrease from baseline > 20 bpm
	Maximum value ≥ 100 bpm
	Maximum HR increase from baseline > 20 bpm

Notes:
(i) If both the baseline and values of a parameter are beyond the same PCS limit for that parameter, then the value will be considered a PCS value only if it is more extreme (farther from the limit) than was the baseline value.
(ii) If the baseline value for a parameter is missing and the absolute value of a parameter is beyond the PCS limit, then the value will be considered as PCS.
(iii) If the baseline value for a parameter is missing, the change from baseline value will be missing and PCS change criteria will not be assessed.
(iv) The missing category will count the number of patients without any values of the parameter during the period.

3.8.4 Electrocardiograms

Electrocardiograms will be performed as indicated in the Schedule of Events in the protocol. Twelve-lead ECGs will be performed locally and should be collected in triplicate, separated by approximately 5 minutes.

Continuous ECG parameters (PR, RR, QRS, QT and QTcF) will be summarized using descriptive statistics of the values and change from baseline at each scheduled visit. The last post-baseline and the maximum post-treatment values will also be presented. Both scheduled and unscheduled visits will be considered for summaries of the last and maximum post-treatment values. All triplicate ECG measurements at a particular timepoint will be averaged prior to analysis and summarization.

In addition, patients experiencing QTcF elevation or change from baseline will be tabulated by scheduled visit for the following categories:

- QTcF > 450 ms
- QTcF > 480 ms
- QTcF > 500 ms
- Increase from baseline QTcF > 30 ms
- Increase from baseline QTcF > 60 ms

ECG readings will be judged for overall clinical significance. Shift tables from baseline to the worst post-baseline result will be presented by dose cohort. Both scheduled and unscheduled visits will be used. The following categories will be used: Normal, Abnormal not clinically significant, Abnormal clinically significant.

ECG assessments will be listed by patient.

3.8.5 Physical Examinations

Physical examinations will be performed as indicated in the Schedule of Events Schedule of Events in the protocol. All physical examination data will be listed by patient.

3.9 Quality of Life Evaluation

The quality-of-life evaluation is a secondary objective in both Part 2: Combination Dose Finding and Part 3: Combination Dose Expansion. Quality of Life (QoL) evaluations (EQ-5D-5L and QLQ-C30) are to be performed for clinical benefit assessment at baseline and every 12 weeks after Cycle 1 Day 1 in combination arms (Arms B and C).

3.9.1 EQ-5D-5L

The EQ-5D-5L questionnaire consists of two parts: the EQ-5D descriptive and the EQ visual analogue scale (EQ VAS).

The EQ-5D descriptive comprises five dimensions: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression. Each dimension has 5 levels: no problems, slight problems, moderate problems, severe problems, extreme problems (i.e., unable to walk for mobility). Each patient will self-rate his current state within each dimension.

The EQ VAS records the patient's self-rated health on a vertical visual analogue scale. The VAS varies from 0 "The worst health you can imagine" to 100 "The best health you can imagine". The VAS is used as a quantitative measurement of health outcome.

The number and percentage of patients with each level in each dimension will be presented. Changes from baseline of the VAS will be summarized descriptively.

3.9.2 EORTC QLQ-C30

EORTC QLQ-C30 (version 3.0) is a well-validated instrument that includes 30 items to assess health-related quality of life (HRQOL) in cancer patients. The first 28 questions use a 4-point scale (1=not at all to 4=very much) for evaluating five functional scales (physical, role, social, cognitive, emotional), three symptom scales (fatigue, nausea/vomiting, and pain) and other single item symptom measures. The last 2 questions use a 7-point scale (1=very poor to 7=excellent) to evaluate the global quality of life. Item scales used to compute scores from 0 to 100. For functional and global QoL scales, higher scores mean a better level of functioning; for symptom-oriented scales, a higher score means more severe symptoms.

If at least half of the items from a scale are answered, it is assumed that the missing items have values equal to the average of those items that are present for that respondent. Otherwise, the scale score will be set to missing.

EORTC QLQ-C30 scores and changes from baseline will be summarized descriptively. Negative change from baseline values indicated deterioration in health status or functioning and positive changes indicated improvement.

4 INTERIM ANALYSIS

In Arm B or C, an interim analysis of the primary outcome will be performed at the end of Stage 1 of the Combination Dose Expansion part of the study (once 12 evaluable patients have completed at least 1 disease evaluation or have discontinued from the study). Any treatment arm with an ORR <20% per RECIST (i.e., treatment arms with no more than 2 complete or partial responses) will be discontinued.

Available safety, efficacy, and PD data will be reviewed on an ongoing basis by the SRC.

5 CHANGES FROM PROTOCOL-SPECIFIED STATISTICAL ANALYSES

Considering the results of the part 1, with a total of 0 response observed from the 2 monotherapy arms the following analysis will not be performed:

- All analysis related to part 2 (combination dose finding) and part 3 (combination dose expansion) as trial stopped after part 1.
- iRECIST analysis in part 1 (monotherapy dose expansion): due to lack of repeated scans once PD by RECIST is assessed, most of the PDs by iRECIST remained unconfirmed and therefore survival endpoints per RECIST will not be analysed.

6 PROGRAMMING SPECIFICATIONS

Analyses will be performed using SAS® version 9.4 or higher. All available data will be presented in patient data listings sorted by patient and visit date as applicable. Detailed Programming Specifications will be provided in a separate document.

7 APPENDIX

APPENDIX A: DATE IMPUTATION GUIDELINES

The following date imputation guidelines will apply to adverse events (Table 7-1**Error! Reference source not found.**), and for prior and concomitant medications (Table 7-2**Error! Reference source not found.**).

Table 7-1: Adverse Events date imputation guidelines

AE Start Date	AE Stop Date	Action
Known	Known/Partial/Missing	If start date < study drug start date, then not TEAE If start date ≥ study drug start date, then TEAE
Partial, but the known date components show that it cannot be on or after study drug start date	Known/Partial/Missing	Not TEAE
Partial, could be on or after study drug start date	Known	If stop date < study drug start date, then not TEAE If stop date ≥ study drug start date, then TEAE
	Partial	Impute stop date as latest date (i.e., last day of month if day is unknown or 31-Dec if day and month are unknown), then: If stop date < study drug start date, then not TEAE If stop date ≥ study drug start date, then TEAE
	Missing	Assumed TEAE
Missing	Known	If stop date < study drug start date, then not TEAE If stop date ≥ study drug start date, then TEAE
	Partial	Impute stop date as latest date (i.e., last day of month if day is unknown or 31st December if day and month are unknown), then: If stop date < study drug start date, then not TEAE If stop date ≥ study drug start date, then TEAE
	Missing	Assumed TEAE

Table 7-2: Prior and concomitant medication date imputation guidelines

CM Start Date	CM Stop Date	Action
Known	Known	If stop date < study drug start date, assign as PRIOR If stop date ≥ study drug start date, and start date ≤ end of treatment +90 days, assign as CONCOMITANT
	Partial	Impute stop date as latest date (i.e., last day of month if day unknown or 31-Dec if day and month are unknown), then: If stop date < study drug start date, assign as PRIOR If stop date ≥ study drug start date, and start date ≤ end of treatment +ç0 days, assign as CONCOMITANT
	Missing	If stop date is missing, then PRIOR will never be assumed or assigned If start date ≤ end of treatment, assign as CONCOMITANT
Partial	Known	Impute start date as earliest date (i.e., first day of month if day unknown or 01-Jan if day and month unknown), then: If stop date < study drug start date, assign as PRIOR If stop date ≥ study drug start date and start date ≤ end of treatment + 90 days, assign as CONCOMITANT
	Partial	Impute start date as earliest date (i.e., first day of month if day unknown or 01-Jan if day and month are unknown) and impute stop date as latest date (i.e., last day of month if day unknown or 31-Dec if day and month are unknown), then: If stop date < study drug start date, assign as PRIOR If stop date ≥ study drug start date and start date ≤ end of treatment + 90 days, assign as CONCOMITANT
	Missing	Impute start date as earliest date (i.e., first day of month if day unknown or 01 Jan if day and month unknown), then: If stop date is missing, then PRIOR will never be assumed or assigned If start date ≤ end of treatment + 90 days, assign as CONCOMITANT
Missing or Unknown	Known	If stop date < study drug start date, assign as PRIOR If stop date ≥ study drug start date, assign as CONCOMITANT
	Partial	Impute stop date as latest date (i.e., last day of month if day unknown or 31-Dec if day and month are unknown), then: If stop date < study drug start date, assign as PRIOR If stop date ≥ study drug start date, assign as CONCOMITANT
	Missing	Assign as CONCOMITANT

APPENDIX B: CLINICAL LABORATORY ANALYTES

Standard Safety Chemistry Panel

Alanine aminotransferase	Albumin
Alkaline phosphatase	Amylase
Aspartate aminotransferase	Blood urea nitrogen
Calcium	Chloride
Creatine kinase	Creatinine
Estimated glomerular filtration rate	Gamma-glutamyl transferase
Glucose	Inorganic phosphorus
Lactate dehydrogenase	Lipase
Potassium	Sodium
Total protein	Total and Direct bilirubin
Urea (where blood urea nitrogen is not tested)	Uric acid

Additional Chemistry Parameters

C-reactive protein	Cholesterol
Triglycerides	

Endocrinology

Thyroid-stimulating hormone	T3
T4	

T3 = triiodothyronine; T4 = thyroxine.

Hematology

Absolute basophil value	Absolute eosinophil value
Absolute monocyte count	Absolute neutrophil count
Hematocrit	Hemoglobin
Lymphocyte count	Platelets
Red blood cell count	White blood cell count and differential [2]

2. Manual microscopic review is performed only if white blood cell count and/or differential values are out of reference range.

Coagulation

D-dimer	International normalized ratio [1]
Prothrombin ratio	Prothrombin time

1. Prothrombin time/international normalized ratio should be measured daily for any patient experiencing alanine aminotransferase or aspartate aminotransferase elevations ≥3 × upper limit of normal with concomitant elevation in bilirubin ≥2 × upper limit of normal until resolution to baseline of the liver function test abnormality.

Urinalysis

Bilirubin	Blood
Glucose	Ketones
Leukocyte esterase	Microscopy [1]
Nitrite	pH
Protein	Specific gravity
Urobilinogen	

1. Urinalysis may be done by dipstick or microscopy.

Serology

Hepatitis B surface antigen	Hepatitis B virus DNA [1]
Hepatitis C virus antibody	Hepatitis C virus RNA [2]
Human immunodeficiency virus 1	Human immunodeficiency virus 2

1. Hepatitis B virus DNA will be tested by PCR in patients with positive hepatitis B surface antigen.
2. Hepatitis C virus RNA will be tested by PCR in patients with positive hepatitis C virus antibody.

DNA = deoxyribonucleic acid; PCR = polymerase chain reaction; RNA = ribonucleic acid.

APPENDIX C: LIST OF BIOMARKERS

Flow Cytometry

Total MFI CD25 (CD3+CD8+)
% CD19+ (B-Cells)
% CD19+Ki67+
% CD3+ (Total T-Cells)
% CD3+CD4+ T-Cells
% CD3+CD4+CD127dimCD25+FoxP3+ (T-Regs)
% CD3+CD4+CD127dimCD25+FoxP3+ Ki67+
% CD3+CD4+Ki67+
% CD3+CD56+ (NKT-cells)
% CD3+CD56+Ki67+
% CD3+CD8+ T-Cells
% Lymphocytes
% CD3+CD8+CD25+
% CD3+CD8+Ki67+
% CD3-CD19-CD56+ (Total NK Cells)
% CD3-CD19-CD56+Ki67+
% CD56bright CD16dim/- Ki67+
% CD56bright CD16dim/- NK Cells
% CD56dimCD16+ NK Cells
% CD56dimCD16+Ki67+
% CD56dimCD16- NK Cells
% CD56dimCD16-Ki67+
% Leukocytes

Serum Cytokines

GM-CSF
IFN-gamma
IL-1beta/IL-1F2
IL-4
IL-5
IL-6
IL-8/CXCL8
IL-10
IL-12p70
TNF-alpha

APPENDIX D: CALCULATION METHODS FOR THE FLOW CYTOMETRY ABSOLUTE VALUES

Population	Parent Population	Calculation of Absolute Value
Leukocytes	Singlets	Leukocytes absolute counts (10^6/L) by Local Laboratory
Lymphocytes	CD14- Leukocytes	Lymphocytes absolute counts (10^6/L) by Local Laboratory
CD3+ (Total T-Cells)	Lymphocytes	% CD3 T-Cells / 100 x Lymphocyte (10^6/L)
CD3+CD4+ T-Cells	Lymphocytes	% CD3+CD4+ T-Cells / 100 x Lymphocyte (10^6/L)
CD3+CD8+ T-Cells	Lymphocytes	%CD3+CD8+ T-Cells / 100 x Lymphocyte (10^6/L)
CD3+CD4+CD127dimCD25+FoxP3+ (T-Regs)	Lymphocytes	%CD3+CD4+CD127dimCD25+FoxP3+ (T-Regs) / 100 x Lymphocyte (10^6/L)
CD19+ (B-Cells)	Lymphocytes	%CD19+ (B-Cells) / 100 x Lymphocyte (10^6/L)
CD3+CD56+ (NKT-Cells)	Lymphocytes	%CD3+CD56+ (NKT-Cells) / 100 x Lymphocyte (10^6/L)
CD3-CD19-CD56+ (Total NK Cells)	Lymphocytes	%CD3-CD19-CD56+ (Total NK Cells) / 100 x Lymphocyte (10^6/L)
CD56bright CD16dim/- NK Cells	Lymphocytes	%CD56bright CD16dim/- NK Cells / 100 x Lymphocyte (10^6/L)
CD56dimCD16+ NK Cells	Lymphocytes	%CD56dimCD16+ NK Cells / 100 x Lymphocyte (10^6/L)
CD56dimCD16- NK Cells	Lymphocytes	%CD56dimCD16- NK Cells/ 100 x Lymphocyte (10^6/L)
CD3+CD4+Ki67+	CD3+CD4+ T-Cells	%CD3+CD4+Ki67+/100 x %CD3+CD4+ T-Cells/ 100 x Lymphocyte (10^6/L)
CD3+CD8+Ki67+	CD3+CD8+ T-Cells	%CD3+CD8+Ki67+/100 x %CD3+CD8+ T-Cells/ 100 x Lymphocyte (10^6/L)
CD3+CD4+CD127dimCD25+FoxP3+ Ki67+	CD3+CD4+CD127dimCD25+FoxP3+ (T-Regs)	%CD3+CD4+CD127dimCD25+FoxP3+ Ki67+/100 x %CD3+CD4+CD127dimCD25+FoxP3+ (T-Regs) / 100 x Lymphocyte (10^6/L)
CD19+Ki67+	CD19+ (B-Cells)	%CD19+Ki67+/100 x %CD19+ (B-Cells)/ 100 x Lymphocyte (10^6/L)
CD3+CD56+Ki67+	CD3+CD56+ (NKT-Cells)	%CD3+CD56+Ki67+/100 x %CD3+CD56+ (NKT-Cells)/ 100 x Lymphocyte (10^6/L)
CD3-CD19-CD56+Ki67+	CD3-CD19-CD56+ (Total NK Cells)	%CD3-CD19-CD56+Ki67+/100 x %CD3-CD19-CD56+ (Total NK Cells)/ 100 x Lymphocyte (10^6/L)
CD56brightCD16dim/- Ki67+	CD56bright CD16dim/- NK Cells	%CD56brightCD16dim/- Ki67+/100 x %CD56bright CD16dim/- NK Cells/ 100 x Lymphocyte (10^6/L)
CD56dimCD16+Ki67+	CD56dimCD16+ NK Cells	%CD56dimCD16+Ki67+/100 x %CD56dimCD16+ NK Cells/ 100 x Lymphocyte (10^6/L)
CD56dimCD16-Ki67+	CD56dimCD16- NK Cells	%CD56dimCD16-Ki67+/100 x %CD56dimCD16- NK Cells/ 100 x Lymphocyte (10^6/L)
CD3+CD8+CD25+	CD3+CD8+ T-Cells	%CD3+CD8+CD25+/100 x %CD3+CD8+ T-Cells/ 100 x Lymphocyte (10^6/L)
Total MFI CD25 (CD3+CD8+)	CD3+CD8+ T-Cells	Total Value (MFI) - No calculation needed

APPENDIX E: REFERENCES

(1) Liu S, Yuan Y. Bayesian optimal interval designs for phase I clinical trials. *J R Stat Soc Ser C Appl Stat*. 2015;64(3):507-523.

(2) Simon R. Optimal two-stage designs for phase II clinical trials. *Control Clin Trials*. 1989;10(1):1-10.