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# Phase 1 first-in-human dose-escalation study of ANV419 in patients with relapsed/refractory advanced solid tumors

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## ABSTRACT

**Background** ANV419 is a stable antibody–cytokine fusion protein consisting of interleukin-2 (IL-2) fused to an anti-IL-2 monoclonal antibody that sterically hinders binding of IL-2 to the α subunit of its receptor but has selective affinity for the receptor βγ subunits. Thus, ANV419 preferentially stimulates $CD8^+$ effector T cells and natural killer cells which are associated with tumor killing, while minimizing the activation of immunosuppressive regulatory T cells.

**Methods** ANV419-001 is an open-label, multicenter, phase 1 study to evaluate the safety, tolerability, maximum tolerated dose (MTD) and recommended phase 2 dose (RP2D) of ANV419. Secondary objectives were to characterize the pharmacokinetics, pharmacodynamics and tumor response. Adult patients with advanced solid tumors and disease progression after ≥1 previous line of systemic therapy were enrolled. ANV419 was administered by intravenous infusion once every 2 weeks, with a planned treatment duration of 12 months. The dose escalation part of the study explored doses 3, 6 and 12 µg/kg as single patient cohorts followed by 24–364 µg/kg in a 3+3 design. Interim results are reported here (data cut-off: March 22, 2023).

**Results** Forty patients were enrolled and received at least one dose of ANV419. The MTD and RP2D were determined to be 243 µg/kg. The most common ANV419-related treatment-emergent adverse events were Grade 1 and 2 fever (31 (77.5%)), chills (23 (57.5%), vomiting (14 (35.0%)), cytokine release syndrome and nausea (12 (30.0%) each). Transient and self-limiting lymphopenia due to lymphocyte redistribution was observed in all patients. In the RP2D cohort, Grade ≥3 thrombocytopenia and fever were reported by one patient (12.5%) each. All events were manageable with standard supportive care. At doses of 243 µg/kg (RP2D/MTD), the estimated $T_{1/2}$ was approximately 12 hours. At ANV419 doses ≥108 µg/kg, 64% of patients had a best response of at least SD (15 SD and 1 confirmed PR).

**Conclusions** ANV419 at doses up to 243 µg/kg (the RP2D) was well tolerated and showed signs of antitumor activity in a heavily pretreated patient population with advanced solid tumors.

**Trial registration number** NCT04855929.

### WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Recombinant interleukin-2 (IL-2, aldesleukin) is an approved cancer immunotherapy which acts by promoting the antitumor function of immune cells (effector T cells and natural killer cells) that express the βγ subunits of its receptor. However, IL-2 also potently activates immunosuppressive regulatory T cells that harbor the α subunit of its receptor. Another drawback of aldesleukin is the short half-life which necessitates frequent dosing, resulting in toxicities.

### WHAT THIS STUDY ADDS

⇒ ANV419 is a novel fusion protein which selectively signals through IL-2Rβγ, thus limiting the side effects of activating IL-2Rαβγ. This is a first-in-human study testing the safety, tolerability, pharmacokinetics and antitumor activity of ANV419 in heavily pretreated patients with advanced solid tumors.

### HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Developing a safe and effective IL-2 agonist that circumvents binding to the α subunit of the IL-2 receptor to selectively stimulate immune effector cells remains an important therapeutic goal for the treatment of patients with cancer.

## INTRODUCTION

The cytokine interleukin (IL)-2 plays an essential role in the regulation of immunity, modulating both immunostimulatory and immunosuppressive functions via multiple pathways.[1] It is indispensable for the growth and differentiation of many immune cells, including T cells, B cells and natural killer (NK) cells.[2] Its potent effects on T cells resulting not only in their expansion but also enhancement of their cytotoxic actions is the cornerstone for the application of IL-2 in cancer immunotherapy. High-dose recombinant IL-2 (aldesleukin) was the first approved cytokine-based immunotherapy for metastatic melanoma and renal cell carcinoma (RCC).[3]

IL-2 induces tumor killing by stimulating the proliferation and activity of $CD4^+$ T cells, $CD8^+$ effector T cells ($T_{effs}$) and NK cells.[2] Therapy with IL-2 (aldesleukin) has yielded long-lasting responses in patients with metastatic melanoma and RCC, with up to 20% of patients with metastatic melanoma achieving survival of 10 years or longer.[4] However, IL-2 simultaneously induces the maintenance of $CD4^+$ $Foxp3^+$ regulatory T cells ($T_{reg}$) that dampen the immune response. These pleiotropic and opposing effects of IL-2 pose a major challenge in the application and development of IL-2-directed therapies in oncology. For cancer immunotherapy, a desired outcome is to enhance the immunostimulatory effects of IL-2 (via $T_{effs}$ and NK cells that promote tumor killing) while minimizing its immunosuppressive effects (via $T_{regs}$ that are associated with a poor outcome for patients with cancer). High-dose IL-2 therapy is also limited by multiple adverse effects including capillary leak syndrome, cardiopulmonary, liver and renal toxicities, necessitating careful patient selection. Furthermore, the short half-life of IL-2 necessitates a burdensome drug administration schedule with significant toxicity.[5]

The pleiotropic effects of IL-2 are mediated through the modular structure of its receptor, components of which are expressed differentially in immune cells.[6] The IL-2 receptor (IL-2R) consists of α, β and γ subunits: IL-2Rα (CD25), IL-2Rβ (CD122), and the common IL2Rγ (CD132).[2 6] IL-2Rb and IL-2Rg are the signaling subunits and IL-2Rα increases affinity to IL-2.[7 8] The heterodimeric IL-2Rβγ has low to intermediate affinity for IL-2 and is expressed by naïve and memory $CD4^+$, $CD8^+$ T cells and NK cells.[7 8] The high-affinity heterotrimeric IL-2Rαβγ is constitutively expressed by $T_{regs}$ and vascular endothelial cells.[6 9 10] Therefore, IL-2 potently stimulates $T_{regs}$ compared with the $T_{effs}$ and NK cells that mediate antitumor actions.

For cancer immunotherapy, engineering IL-2 with the aim of enhancing its binding to $T_{effs}$ and NK cells while simultaneously bypassing its effects on $T_{regs}$ and other cell types is a key goal. Several targeted IL-2 molecules have been developed, with increased affinity for IL-2Rβγ.[6] Thus far however, these approaches have been limited by insufficient potency,[11 12] incomplete βγ selectivity,[13] or undesirable adverse effects.[14 15] ANV419 is an antibody–cytokine fusion protein consisting of IL-2 fused to an anti-IL-2 monoclonal antibody that sterically hinders binding of IL-2 to the α subunit of the receptor (IL-2Rα). Due to its selective high affinity for IL-2Rβγ, ANV419 preferentially stimulates $CD8^+$ T cells ($T_{effs}$) and NK cells over $T_{regs}$. In addition to its selectivity for $T_{effs}$, it has a good therapeutic window and an extended half-life. In vitro and in vivo, ANV419 preferentially enhanced signaling and expansion of $T_{effs}$ and NK cells over $T_{regs}$, and enhanced NK cell killing of human tumor cell lines (Murer *et al*, manuscript under submission). ANV419 is currently in development for the treatment of patients with advanced solid tumors and hematological malignancies, as a single agent and in combination with other agents.

This manuscript describes the interim results from the phase 1 dose-escalation study investigating ANV419 in patients with relapsed/refractory advanced solid tumors.

## METHODS

### Study design and objectives

ANV419-001 is an open-label, multicenter, phase 1 study with the primary objective of evaluating the safety and tolerability of ANV419, and to determine the maximum tolerated dose (MTD) and recommended phase 2 dose (RP2D). Secondary objectives are to characterize the pharmacokinetics (PK), pharmacodynamics (PD), immunogenicity, and tumor response. Exploratory analysis of circulating and intratumor biomarkers will also be performed, to assess potential correlation with safety and clinical activity.

The study consists of two parts (Part A and Part B). Part A is the single patient dose escalation portion. After the first dose in the first patient, the dose increase for each subsequent patient was based on data from the 14-day safety observation period, T-cell proliferation (Ki67-positivity), and the Day 4 lymphocyte count of the preceding patient. Dose increase was 50–100% of the prior dose. Part A dose escalation continued until the occurrence of a treatment-related adverse event (AE) of Grade 2 or higher, or a target of 50% $Ki67^+$ $CD8^+$ T cells was reached. If any new Grade ≥2 AE (or worsening of one Common Terminology Criteria for Adverse Events (CTCAE) Grade in a patient with pre-existing toxicities) occurred in the single patient cohort, that cohort and all subsequent cohorts were expanded to a standard 3+3 study design (ie, study Part B).

Part B consists of standard 3+3 dose escalation cohorts. After dosing, the first patient of each cohort was observed for at least 3 days, and subsequent patients dosed in the absence of any reported toxicities. The dose increase (ranging from 50–100% of the current dose) for the next cohort was approved by the Cohort Review Committee (which consisted of the coordinating investigator, principal investigator or representative, and the sponsor's medical monitor) on review of the 14-day safety and clinical data from all patients in the cohort. The doses were increased according to 3+3 decision rules until sufficient data for predicting the tentative RP2D dose was available or the MTD was reached.

A starting dose of 3 µg/kg ANV419 once every 2 weeks (Q2W) was selected based on data from non-clinical studies (Murer *et al*, manuscript under submission), in vitro cytokine release data from human whole blood assays and benchmarking against therapeutic doses for aldesleukin.

Results of the dose-finding parts of the ANV419-001 study (data cut-off date of March 22, 2023) are reported here. Further details on the study procedures are given in the protocol (available as online supplemental material). All participants provided written informed consent prior to enrolling in the study.

### Study population

The study population consisted of adult patients (≥18 years) with advanced solid tumors, with Eastern Cooperative Oncology Group (ECOG) performance status 0–1. The study also planned to include patients with multiple myeloma. Patients with solid tumors had to have evidence of progressive disease after at least one line of treatment (as per Response Evaluation Criteria in Solid Tumors (RECIST) V.1.1) ≤3 months prior to signing the informed consent form for the study. All patients had to have adequate pulmonary, cardiovascular, hematological, liver and renal function, and any adverse effects from prior anticancer therapies had to have resolved to CTCAE Grade 1 or below. The main exclusion criteria were symptomatic central nervous system (CNS) metastases, active second malignancy, the presence of significant uncontrolled concomitant conditions that could affect protocol compliance or interpretation of study results (ie, diabetes mellitus, relevant pulmonary disorders, hyperthyroidism) and chronic treatment with systemic immunosuppressive medications above 10 mg/day prednisolone equivalent for any reason. All male and female patients had to comply with protocol-specified contraception guidelines.

### Study treatment

ANV419 was administered by intravenous infusion over 15±5 min Q2W, with a treatment duration of 12 months, or until disease progression or intolerable toxicity if occurring earlier. The starting dose was 3 µg/kg with subsequent dose levels of 6, 12, 24, 48, 72, 108, 162, 243 and 364 µg/kg in a 3+3 cohort design. An additional cohort was enrolled to assess the 243 µg/kg dose in a Q3W dosing regimen.

### Study assessments and endpoints

The primary endpoints were the number of patients experiencing dose-limiting toxicities (DLTs) during the 14-day DLT observation period (one cycle), as well as the incidence and severity of AEs and serious AEs (SAEs), their causal relationship to ANV419, changes from baseline in laboratory, vital signs, ECG, and physical examination parameters. Key secondary endpoints were the objective response rate using RECIST V.1.1. Baseline disease assessments were performed at screening or baseline, at Cycle 3, and every 8 weeks thereafter, using CT. Imaging results were evaluated by the investigator to assess disease response (according to RECIST V.1.1[16] and iRECIST[17]). PK parameters were determined using a non-compartmental approach, and included systemic clearance, volume of distribution at steady-state ($V_{ss}$), area under the concentration-time curve (AUC), and maximum observed serum concentration ($C_{max}$). Key PD endpoints included the number of $CD8^+$ T cells, NK cells, and $T_{regs}$ as a measure of target engagement. Peripheral blood samples were also collected for assessment of biomarkers (further details are given in the online supplemental material: Biomarkers section).

Safety and tolerability assessments included the recording of AEs, SAEs, physical examinations, vital signs, 12-lead ECG assessments including heart rate and PR, QRS, and QT intervals, clinical laboratory parameters, pregnancy testing, and ECOG performance status. Concomitant medications were monitored throughout. All AEs were graded using National Cancer Institute CTCAE V.5.0 and were reported from screening/baseline to 30 days after the last dose, with an additional 90-day follow-up phone call.

### Statistical analysis

Statistical analyses were conducted using SAS V.9.4 (or later). Continuous variables were expressed descriptively; categorical variables were summarized using frequency counts and percentages. No formal sample size calculation was performed. The Safety Population consisted of all patients who received at least one dose (or partial dose) of study treatment, and this population was used for analysis of safety (including DLT determination), PK, and PD. The DLT Population consisted of all patients who completed at least one cycle of treatment or discontinued from the study treatment due to DLT. The Response Evaluable Population (for efficacy analysis) consisted of all patients who had one post-baseline assessment of tumor response or who were withdrawn due to progressive disease/death prior to the first response assessment. For the calculation of PK parameters, serum concentration-time data were analyzed by non-compartmental methods, using commercial software such as Phoenix WinNonlin.

The protocol for this study and CONSORT (Consolidated Standards of Reporting Trials) checklist are available as online supplemental material.

## RESULTS

### Study population

A total of 40 patients were enrolled at five study sites in Spain, UK and Switzerland, from June 2021.The cut-off date for this analysis was March 22, 2023. The number of patients assigned to each dose cohort is depicted in figure 1, and baseline characteristics are summarized in table 1. Median age was 59.5 years and 27 (67.5%) patients were men. Median number of prior lines of systemic therapy was 3 (range: 1–8 lines). The study population encompassed a diverse range of primary tumor types (table 1). Although the study planned to include patients with multiple myeloma, none were actually enrolled.

As of the cut-off date, 38 of 40 (95%) patients had discontinued treatment; 2 (5.0%) were still undergoing treatment in the study. The main reason for treatment discontinuation was disease progression (33 of 40 (82.5%) patients). Five (12.5%) patients withdrew consent.

### Exposure

Median duration of ANV419 treatment was 8.8 weeks (range: 1–44.3), and the median number of treatment cycles was 4.9 (range 1–17). The exposure, on-study

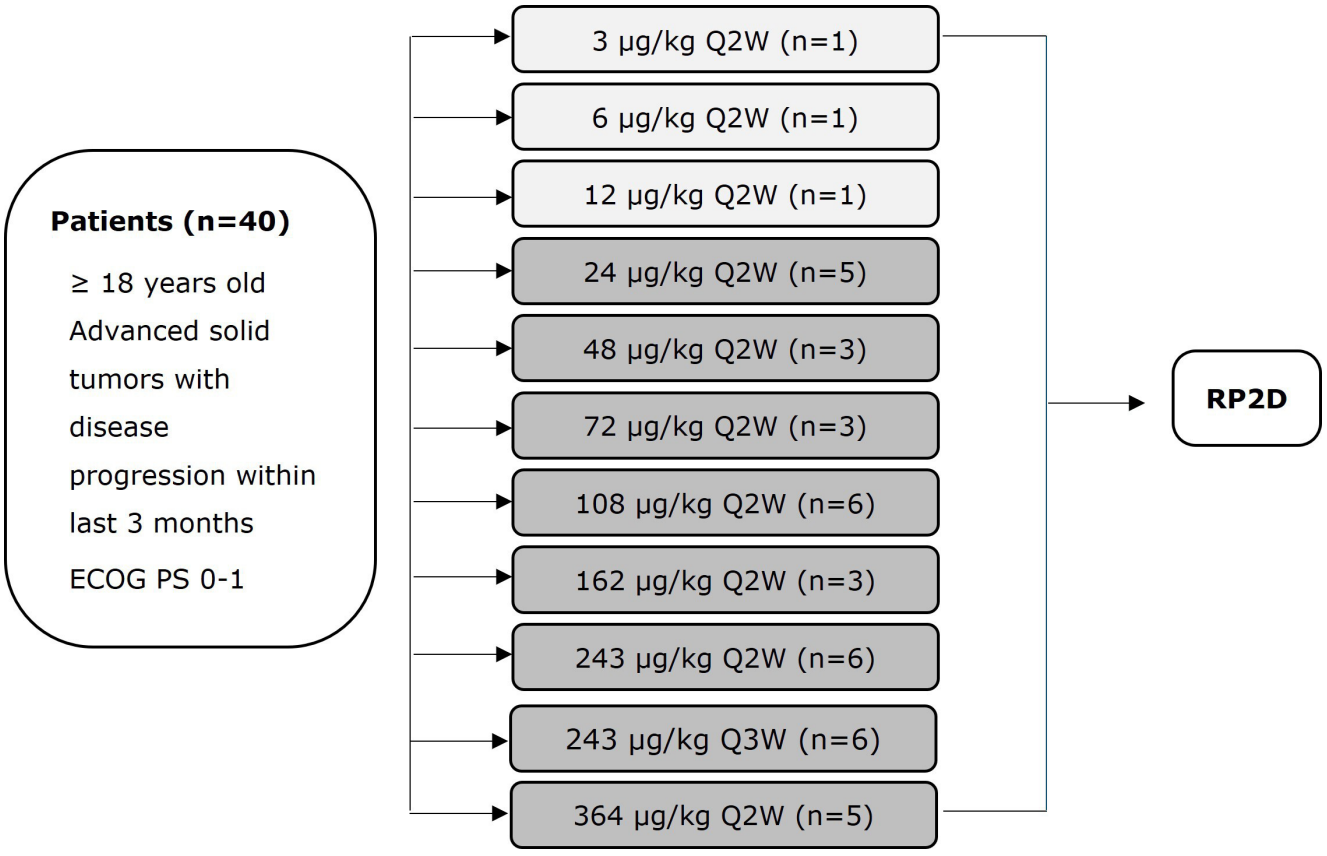


**Figure 1** Patient disposition. Q2W, once every 2 weeks; RP2D, recommended phase 2 dose.

duration and response to treatment for each individual patient are depicted in online supplemental figure 1.

### Dose-limiting toxicities

No DLTs were observed at doses up to and including 243 µg/kg. Of the five patients treated at 364 µg/kg Q2W, two experienced DLTs, and four required hospitalization within the first treatment cycle. DLTs were Grade 3 CRS in one patient and Grade 3 pemphigoid in another patient. Both patients recovered after supportive treatment. Thus, ANV419 at 243 µg/kg was considered the MTD.

### Recommended phase 2 dose

Based on the overall DLT findings from the dose-escalation portion of the study, as well as the safety, PK and PD results (discussed below), the RP2D was determined to be 243 µg/kg.

### Safety

All patients reported at least one treatment-emergent adverse event (TEAE). Regardless of attribution to the drug, 24 (60.0%) patients experienced Grade 3 events, and 3 (7.5%) patients reported Grade 4 events. Thirty-nine (97.5%) patients had TEAEs that were considered related to the study drug. All drug-related events were reversible and responded to standard supportive therapy. There were no TEAEs that led to permanent discontinuation of ANV419. TEAEs requiring dose interruption were reported in 2 (5.0%) patients, and those requiring dose reduction were reported in 6 (15.0%) patients from the overall Safety Population. A summary of the safety findings across each dose cohort is provided in table 2.

The most common drug related TEAEs were fever (31 (77.5%)), chills (23 (57.5%), vomiting (14 (35.0%)), cytokine release syndrome (CRS) and nausea (12 (30.0%) each). Transient and self-limiting lymphopenia with no clinical sequelae was observed in all patients, and was usually considered non-clinically significant by the investigator. Among the patients treated at the RP2D (243 µg/kg Q2W), the most common drug-related TEAEs were fever (7 (87.5%)), chills (5 (62.5%)), vomiting, nausea and decreased appetite (3 (37.5%) each). Among the 14 (35.0%) patients who reported at least one drug related TEAE of Grade≥3 severity, the most common events were alanine aminotransferase (ALT) increased and aspartate aminotransferase (AST) increased (4 (10.0%) each). Lymphopenia was reported in 7.5% of patients. In the 243 µg/kg (RP2D) cohort, Grade ≥3 thrombocytopenia and fever were reported by one patient (12.5%) each. Most TEAEs occurred in the first cycle. The only Grade 4 TEAE was lymphopenia. All events were self-limiting or manageable with standard supportive care. Table 3 summarizes the most frequently reported drug-related TEAEs for all dose cohorts.

Treatment-related SAEs were reported by 10 (25.0%) of patients in the Safety Population. Six patients (15.0%) reported CRS. Increased blood bilirubin and creatinine

**Table 1** Patient demographics and baseline characteristics

| | Overall patients (N=40) |
|---|---|
| Age, years | |
| Mean (SD) | 58.5 (11.75) |
| Median | 59.5 |
| Min, max | 30, 79 |
| Sex, n (%) | |
| Male | 27 (67.5) |
| Female | 13 (32.5) |
| Childbearing potential | |
| Yes | 5 (12.5%) |
| No | 8 (20.0%) |
| Ethnicity, n (%) | |
| Hispanic or Latino | 0 |
| Not Hispanic or Latino | 39 (97.5) |
| Not reported | 1 (2.5) |
| Race, n (%) | |
| American Indian or Alaska native | 0 |
| Asian | 1 (2.5) |
| Black or African American | 0 |
| Native Hawaiian or other Pacific Islander | 0 |
| White | 39 (97.5) |
| Other | 0 |
| Not reported | 0 |
| Eastern Cooperative Oncology Group performance status, n (%) | |
| 0 | 21 (52.5) |
| 1 | 19 (47.5) |
| Primary cancer diagnosis, n (%) | |
| Cancer of unknown primary | 1 (2.5) |
| Cervical cancer | 1 (2.5) |
| Colorectal cancer | 7 (17.5) |
| Head and neck cancer | 4 (10.0) |
| Hepatocellular carcinoma | 1 (2.5) |
| Melanoma | 13 (32.5) |
| Non-small cell lung cancer | 3 (7.5) |
| Esophagus carcinoma | 2 (5.0) |
| Pancreatic carcinoma | 3 (7.5) |
| Renal cell carcinoma | 5 (12.5) |
| Number of prior systemic therapy regimens, n (%) | |
| 1 | 7 (17.5) |
| 2 | 10 (25.0) |
| 3 | 10 (25.0) |
| 4 | 4 (10.0) |
| 5 | 1 (2.5) |
| 6 | 3 (7.5) |
| 7 | 3 (7.5) |
| 8 | 2 (5.0) |
| Number of prior systemic therapy regimens, n (%) | |
| n | 40 |
| Mean (SD) | 3.3 (2.07) |
| Median | 3.0 |
| Min, max | 1, 8 |

levels, and fever were reported by two patients (5.0%) each. There were no fatal TEAEs, and no TEAEs leading to withdrawal from the study.

CRS was reported by 12 (30.0%) of the Safety Population (including the 6 (15.0%) patients in whom CRS was reported as an SAE). The onset of CRS mainly consisted of Grade 1–3 fever and chills that occurred within 4–6 hours after ANV419 dosing, with nausea or hypotension. There was a trend towards increasing severity and frequency of CRS with increasing doses of ANV419. CRS symptoms resolved with anti-fever treatment, oxygen and/or steroid treatment. One patient in the 364 µg/kg cohort (above the RP2D) received tocilizumab treatment. No patient at ANV419 doses ≤243 µg/kg (RP2D/MTD) required dose reduction for CRS. Evaluation of serum cytokine concentrations (including tumor necrosis factor (TNF), IL-1β, IL-6, IL-10, and interferon (IFN)-γ) at various time points indicated low levels of cytokine production at all doses (results not shown).

Infusion-related reactions (IRRs), defined as any signs or symptoms experienced by patients during the infusion or any event occurring on the first day of drug administration, were reported by 18 (45.0%) of the Safety Population. The IRRs were distributed across various System Organ Classes, with the most frequently reported patients being chills (14 (35.0%)) and fever (11 (27.5%)) (online supplemental table 1). There was no clear association between IRRs and dose. No hypersensitivity reactions were reported.

Transient and self-limiting lymphopenia (often $\leq 0.1\times10^9$/L) was consistently observed at all doses ≥6 µg/kg, with a nadir occurring at Cycle 1 Day 2 (C1D2) and resolution of lymphocyte count to baseline, or above, between C1D4 and C1D8. No increase in infections was observed. Lymphopenia was reported as a Grade 4 TEAE in 3 (7.5%) patients. Over multiple cycles, a dose-dependent increase of lymphocyte expansion was observed (results not shown). The transient lymphopenia may be attributed to the redistribution and sequestration of lymphocytes from peripheral blood and is an expected pharmacodynamic effect of ANV419.

**Table 2** Overall summary of treatment-emergent adverse events (TEAEs)

| | | | Part B (N=37) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Overall N=40 | Part A total N=3 | 24 µg/kg Q2W N=5 | 48 µg/kg Q2W N=3 | 72 µg/kg Q2W N=5 | 108 µg/kg Q2W N=7 | 162 µg/kg Q2W N=6 | 243 µg/kg Q2W N=8 | 364 µg/kg Q2W N=5 | 162 µg/kg Q3W N=1 | 243 µg/kg Q3W N=6 |
| | n (%) | | | | | | | | | | |
| Total number of TEAEs | 702 | 59 | 65 | 33 | 17 | 81 | 112 | 150 | 88 | 4 | 93 |
| Patients with at least one TEAE | 40 (100.0) | 3 (100.0) | 5 (100.0) | 3 (100.0) | 5 (100.0) | 7 (100.0) | 6 (100.0) | 7 (87.5) | 5 (100.0) | 1 (100.0) | 6 (100.0) |
| TEAE by CTCAE Grade* | | | | | | | | | | | |
| Grade 1 (mild) | 4 (10.0) | 2 (66.7) | 0 | 1 (33.3) | 3 (60.0) | 1 (14.3) | 0 | 0 | 0 | 0 | 0 |
| Grade 2 (moderate) | 9 (22.5) | 0 | 3 (60.0) | 1 (33.3) | 0 | 3 (42.9) | 2 (33.3) | 1 (12.5) | 1 (20.0) | 1 (100.0) | 1 (16.7) |
| Grade 3 (severe) | 24 (60.0) | 1 (33.3) | 2 (40.0) | 0 | 2 (40.0) | 3 (42.9) | 2 (33.3) | 6 (75.0) | 4 (80.0) | 0 | 5 (83.3) |
| Grade 4 (life-threatening) | 3 (7.5) | 0 | 0 | 1 (33.3) | 0 | 0 | 2 (33.3) | 0 | 0 | 0 | 0 |
| Grade 5 (fatal) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| TEAE by relationship† | | | | | | | | | | | |
| Not related | 1 (2.5) | 0 | 1 (20.0) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Related | 39 (97.5) | 3 (100.0) | 4 (80.0) | 3 (100.0) | 5 (100.0) | 7 (100.0) | 6 (100.0) | 7 (87.5) | 5 (100.0) | 1 (100.0) | 6 (100.0) |
| TEAE leading to discontinuation of study drug | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| TEAE requiring dose interruption | 2 (5.0) | 0 | 1 (20.0) | 0 | 0 | 1 (14.3) | 0 | 0 | 0 | 0 | 0 |
| TEAE requiring dose reduction | 6 (15.0) | 0 | 0 | 0 | 0 | 1 (14.3) | 1 (16.7) | 1 (12.5) | 3 (60.0) | 0 | 1 (16.7) |
| TEAE leading to withdrawal from study | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Fatal TEAEs | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Patients in Part B who received more than one dose level are summarized under the last dose received prior to the onset of the event.
*Patients reporting more than one adverse event are counted only once using the highest CTCAE grade.
†Patients reporting more than one adverse event are counted only once using the closest relationship to the study drug. No related events included those reported as "unlikely" or "not related" to study drug; related events include those reported as "possibly related", "probably related", or "definitely related" to study drug or with an unknown relationship.
CTCAE, Common Terminology Criteria for Adverse Events; Q2W, once every 2 weeks.

**Table 3** Related treatment-emergent adverse events (TEAEs) with an incidence of ≥10% in the safety population

| | | | Part B (N=37) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Overall N=40 | Part A total N=3 | 24 µg/kg Q2W N=5 | 48 µg/kg Q2W N=3 | 72 µg/kg Q2W N=5 | 108 µg/kg Q2W N=7 | 162 µg/kg Q2W N=6 | 243 µg/kg Q2W N=8 | 364 µg/kg Q2W N=5 | 162 µg/kg Q3W N=1 | 243 µg/kg Q3W N=6 |
| **Preferred term** | n (%) | | | | | | | | | | |
| Patients with at least one TEAE related to study drug | 39 (97.5) | 3 (100.0) | 4 (80.0) | 3 (100.0) | 5 (100.0) | 7 (100.0) | 6 (100.0) | 7 (87.5) | 5 (100.0) | 1 (100.0) | 6 (100.0) |
| Fever | 31 (77.5) | 2 (66.7) | 4 (80.0) | 2 (66.7) | 3 (60.0) | 4 (57.1) | 3 (50.0) | 7 (87.5) | 4 (80.0) | 1 (100.0) | 6 (100.0) |
| Chills | 23 (57.5) | 3 (100.0) | 3 (60.0) | 2 (66.7) | 0 | 3 (42.9) | 2 (33.3) | 5 (62.5) | 4 (80.0) | 0 | 2 (33.3) |
| Vomiting | 14 (35.0) | 0 | 1 (20.0) | 1 (33.3) | 2 (40.0) | 2 (28.6) | 2 (33.3) | 3 (37.5) | 2 (40.0) | 0 | 1 (16.7) |
| Cytokine release syndrome | 12 (30.0) | 0 | 1 (20.0) | 0 | 1 (20.0) | 2 (28.6) | 1 (16.7) | 1 (12.5) | 2 (40.0) | 1 (100.0) | 4 (66.7) |
| Nausea | 12 (30.0) | 0 | 0 | 1 (33.3) | 1 (20.0) | 2 (28.6) | 2 (33.3) | 3 (37.5) | 3 (60.0) | 0 | 1 (16.7) |
| Alanine aminotransferase increased | 11 (27.5) | 0 | 1 (20.0) | 1 (33.3) | 0 | 1 (14.3) | 3 (50.0) | 0 | 2 (40.0) | 0 | 3 (50.0) |
| Aspartate aminotransferase increased | 11 (27.5) | 0 | 1 (20.0) | 1 (33.3) | 0 | 2 (28.6) | 2 (33.3) | 1 (12.5) | 2 (40.0) | 0 | 3 (50.0) |
| Asthenia | 8 (20.0) | 1 (33.3) | 1 (20.0) | 1 (33.3) | 0 | 0 | 1 (16.7) | 1 (12.5) | 2 (40.0) | 0 | 1 (16.7) |
| Decreased appetite | 8 (20.0) | 0 | 0 | 0 | 0 | 1 (14.3) | 2 (33.3) | 3 (37.5) | 1 (20.0) | 0 | 1 (16.7) |
| Blood bilirubin increased | 7 (17.5) | 0 | 0 | 0 | 0 | 1 (14.3) | 2 (33.3) | 1 (12.5) | 2 (40.0) | 0 | 1 (16.7) |
| Blood creatinine increased | 5 (12.5) | 0 | 0 | 0 | 0 | 0 | 0 | 1 (12.5) | 2 (40.0) | 0 | 2 (33.3) |
| Diarrhea | 5 (12.5) | 0 | 0 | 0 | 0 | 0 | 1 (16.7) | 1 (12.5) | 2 (40.0) | 0 | 1 (16.7) |
| Fatigue | 5 (12.5) | 0 | 1 (20.0) | 1 (33.3) | 0 | 0 | 1 (16.7) | 1 (12.5) | 2 (40.0) | 0 | 0 |
| Anemia | 4 (10.0) | 0 | 0 | 0 | 0 | 1 (14.3) | 0 | 1 (12.5) | 1 (20.0) | 0 | 2 (33.3) |
| Hypotension | 4 (10.0) | 0 | 0 | 0 | 0 | 2 (28.6) | 0 | 2 (25.0) | 0 | 0 | 0 |
| Myalgia | 4 (10.0) | 0 | 0 | 0 | 0 | 0 | 2 (33.3) | 0 | 2 (40.0) | 0 | 0 |
| Rash maculopapular | 4 (10.0) | 0 | 0 | 0 | 0 | 1 (14.3) | 1 (16.7) | 0 | 2 (40.0) | 0 | 0 |
| Thrombocytopenia | 4 (10.0) | 0 | 0 | 0 | 0 | 0 | 1 (16.7) | 2 (25.0) | | 0 | 1 (16.7) |
| Lymphopenia | 3 (7.5) | 0 | 0 | 1 (33.3) | 0 | 0 | 2 (33.3) | 0 | | 0 | 0 |
| Malaise | 3 (7.5) | 0 | 0 | 0 | 1 (20.0) | 1 (14.3) | 1 (16.7) | 0 | 1 (20.0) | 0 | 0 |
| Back pain | 2 (5.0) | 0 | 0 | 0 | 0 | 1 (14.3) | 1 (16.7) | 0 | | 0 | 0 |
| Blood alkaline phosphatase increased | 2 (5.0) | 0 | 0 | 1 (33.3) | 0 | 0 | 0 | 0 | | 0 | 1 (16.7) |
| Cystitis non-infective | 2 (5.0) | 0 | 0 | 0 | 1 (20.0) | 1 (14.3) | 0 | 0 | 1 (20.0) | 0 | 0 |
| Gamma-glutamyltransferase increased | 2 (5.0) | 0 | 0 | 0 | 0 | 0 | 1 (16.7) | 0 | | 0 | 1 (16.7) |
| Rash | 2 (5.0) | 0 | 0 | 0 | 0 | 0 | 0 | 1 (12.5) | 1 (20.0) | 0 | 0 |

Patients reporting more than one related adverse event are counted only once (at the level of event and preferred term). Related adverse events are those reported as "possibly", "probably", or "definitely" related to study drug, or with an unknown relationship. Patients in Part B who received more than one dose level are summarized under the dose received prior to onset of the event.
Q2W, once every 2 weeks.

Table 4 Summary of ANV419 pharmacokinetics

| Dose | | $AUC_{last}$ (h*µg/L) | $AUC_{\infty,obs}$ (h*µg/L) | $C_{max}$ (µg/L) | $T_{1/2}$ (h) |
|---|---|---|---|---|---|
| 3 µg/kg | N | 1 | 1 | 1 | 1 |
| | Geo mean | 240.2 | 306.2 | 52.5 | 3.635 |
| | 68% range | ND | ND | ND | ND |
| 6 µg/kg | N | 1 | 1 | 1 | 1 |
| | Geo mean | 808.6 | 1,572 | 152 | 7.178 |
| | 68% range | ND | ND | ND | ND |
| 12 µg/kg | N | 1 | 1 | 1 | 1 |
| | Geo mean | 2,993 | 3,134 | 250 | 5.456 |
| | 68% range | ND | ND | ND | ND |
| 24 µg/kg | N | 4 | 4 | 4 | 4 |
| | Geo mean | 6,422 | 8,611 | 542.1 | 10.22 |
| | 68% range | (4,021–10,260) | (4,851–15,290) | (374.8–784.1) | (6.015–17.38) |
| 48 µg/kg | N | 2 | 2 | 2 | 2 |
| | Geo mean | 16,860 | 39,570 | 1,155 | 24.8 |
| | 68% range | (15,790–18,000) | (20,240–77,380) | (956.5–1,394) | (13.71–44.86) |
| 72 µg/kg | N | 3 | 3 | 3 | 3 |
| | Geo mean | 29,420 | 36,130 | 1,626 | 11.64 |
| | 68% range | (17,560–49,300) | (25,480–51,250) | (1,410–1,875) | (10.27–13.18) |
| 108 µg/kg | N | 4 | 4 | 4 | 4 |
| | Geo mean | 58,620 | 66,860 | 2,517 | 11.63 |
| | 68% range | (34,350–100,100) | (48,960–91,290) | (2,074–3,054) | (9.761–13.85) |
| 162 µg/kg | N | 3 | 3 | 3 | 3 |
| | Geo mean | 86,180 | 110,500 | 3,651 | 15.71 |
| | 68% range | (48,870–152,000) | (88,240–138,400) | (3,360–3,967) | (11.3–21.83) |
| 243 µg/kg | N | 12 | 11 | 12 | 11 |
| | Geo mean | 173,100 | 179,100 | 5,729 | 12.23 |
| | 68% range | (149,900–199,800) | (153,700–208,700) | (4,902–6,696) | (8.399–17.82) |
| 364 µg/kg | N | 5 | 5 | 5 | 5 |
| | Geo mean | 200,900 | 262,600 | 7,319 | 18.44 |
| | 68% range | (151,100–267,200) | (194,300–355,000) | (6,208–8,629) | (10.42–32.62) |

AUC, area under the concentration-time curve; Cmax, maximum observed serum concentration; Geo mean, geometric mean; ND, not determined; $T_{1/2}$, half-life.

## Pharmacokinetics

Maximal concentrations of ANV419 ($C_{max}$) increased with increasing doses, ranging from 52.5 µg/L to a maximum of 7319 µg/L after dosing with 3 to 364 µg/kg, respectively. Likewise, mean exposure ($AUC_{\infty,obs}$) also increased with increasing doses with values from 306.2 h*µg/L to 262,600 h*µg/L (for the 364 µg/kg dose). At low doses, ANV419 PK was affected by target-dependent disposition, which was overcome with higher doses. The geometric mean half-life ($T_{1/2}$) varied from 3.6 hours (for the 3 µg/kg dose) to approximately 12–24 hours for higher doses. Due to the apparent non-linear elimination of ANV419, this parameter is strongly dependent on the sampling scheme used and does not reflect a terminal $T_{1/2}$. The PK characteristics of ANV419 are summarized in table 4.

At doses of 243 µg/kg (RP2D/MTD), the estimated $T_{1/2}$ was approximately 12 hours. The geometric mean volume of distribution at steady state ($V_{ss,obs}$) ranged from 1.1 L to 4.2 L across the investigated doses.

## Pharmacodynamics

Proliferation of target cells was investigated at each tested dose of ANV419 (up to 364 µg/kg), via assessment of Ki67-positivity in $CD8^+$ T cells, NK cells, and $T_{regs}$ in blood.

Three days after dosing (with ≥108 µg/kg Q2W), the majority of $CD8^+$ T cells (up to 92.1%) and NK cells

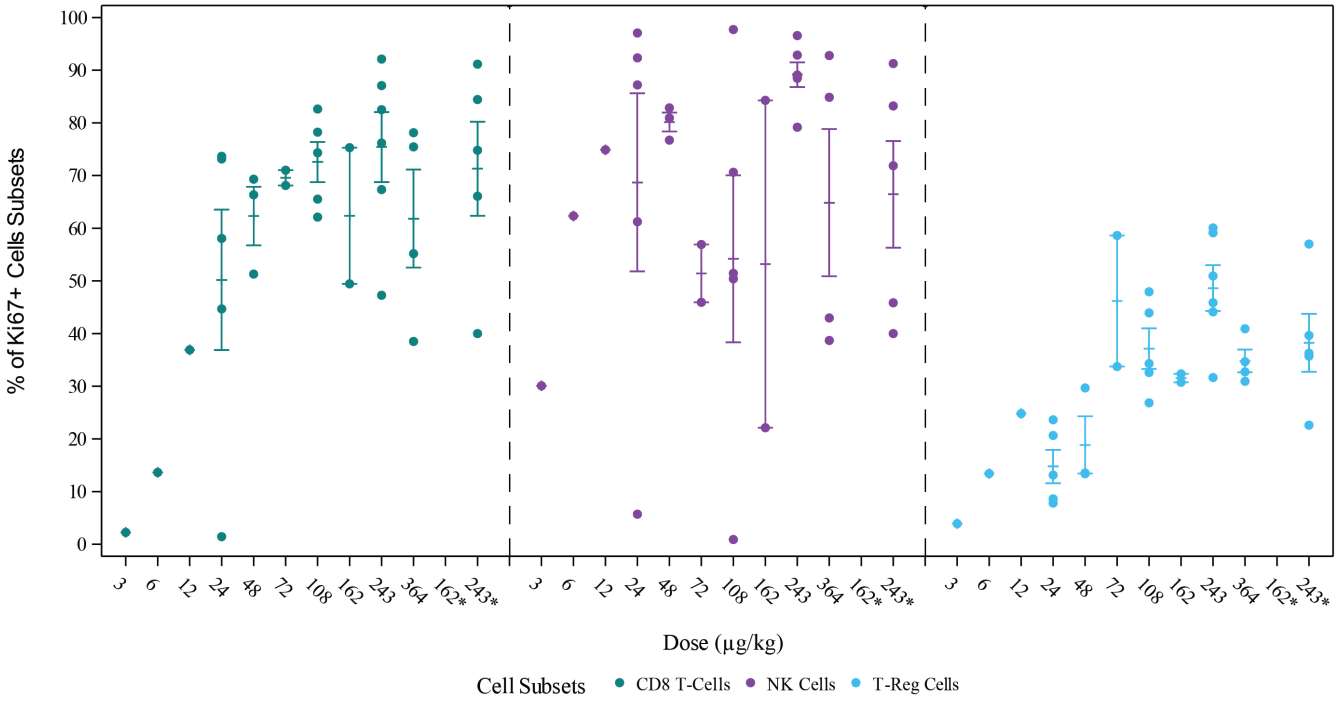


**Figure 2** Cell proliferation over time following ANV419 dosing (Safety Population). The figure depicts mean (±SE), as well as individual results for the proliferation of $CD8^+$ T cells, NK cells and $T_{reg}$ cells on Cycle 1 Day 4. Q3W, once every 3 weeks. *Q3W dosing. CD, cluster of differentiation; NK, natural killer; T-reg, regulatory T cells.

(up to 99.2%) were observed to be $Ki67^+$. At the RP2D of 243 µg/kg Q2W, the mean proliferation rate at C1D4 was 75.4% in $CD8^+$ T cells, 89.2% in NK cells and 48.6% in $T_{regs}$. The cell proliferation in blood reached a plateau starting from doses of 48 µg/kg for $CD8^+$ T cells and 24 µg/kg for NK cells. At the same time, a lower and dose-dependent proliferation of $T_{regs}$ was observed. The data show an increase of $\%Ki67^+$ $T_{regs}$ above baseline with a plateau at approximately 40% on treatment with ANV419, with a corresponding approximately twofold to fourfold increase in absolute $T_{reg}$ counts in the blood. This is much lower than what is observed for treatment with high-dose aldesleukin where the rate of $Ki67^+$ $T_{regs}$ reaches 80–100% with a corresponding increase of greater than 30-fold in the absolute $T_{reg}$ counts.[18]

A similar proliferation rate for $CD8^+$ T cells and NK cells were observed at Cycle 2 with doses >108 µg/kg (figure 2). In general, the cell proliferation levels at C2D4 were comparable to those from C1D4 at doses >108 µg/kg. Taken together, these findings indicate that dosing with ANV419 results in a selective and dose-dependent proliferation of $CD8^+$ T cells and NK cells, accompanied by a lower increase in proliferating $T_{regs}$.

Neither an obvious association between the percentage of $Ki67^+$ NK with tumor shrinkage nor between the percentage of $Ki67^+$ CD8 T cells with efficacy was observed (data not shown).

### Antitumor activity

Among the 40 patients enrolled, 39 were evaluable for tumor response using RECIST V.1.1 criteria. All patients had received between 1 and 8 prior lines of systemic therapy including immunotherapy (75%), targeted therapy (57.5%), and chemotherapy (65%) and the primary tumor type was heterogeneous across the population. Best response was SD in 20 patients and a confirmed PR in 1 patient (figure 3). At ANV419 doses ≥108 µg/kg, 15 patients had SD and 1 patient a confirmed PR. One patient with metastatic non-small cell lung cancer, post-chemoimmunotherapy, experienced a durable partial response of almost 7 months.

A total of 5 patients with RCC (2 patients with clear cell RCC, 2 patients with non-clear cell RCC; 1 patient was unclassified) and 13 patients with advanced melanoma (6 patients with cutaneous, 5 with uveal and 2 with mucosal melanoma) were enrolled in the study. Patients received doses ranging from 3 to 364 µg/kg. One-third (33%) of the RCC and melanoma patients were exposed at the RP2D dose of 243 µg/kg.

Overall, 2/18 patients with RCC or melanoma had SD lasting ≥24 weeks as best response: 1 patient with cutaneous melanoma treated with increasing doses of ANV419 (6 µg/kg, escalated to 12 and 24 µg/kg) had SD for 24 weeks, and 1 RCC patient treated with ANV419 at 162 µg/kg had SD for 28.6 weeks.

## DISCUSSION

The use of high-dose IL-2 as cancer immunotherapy was first established in the treatment of metastatic melanoma and renal carcinoma, but is currently expanding to the treatment of other malignancies.[19] Despite long-standing

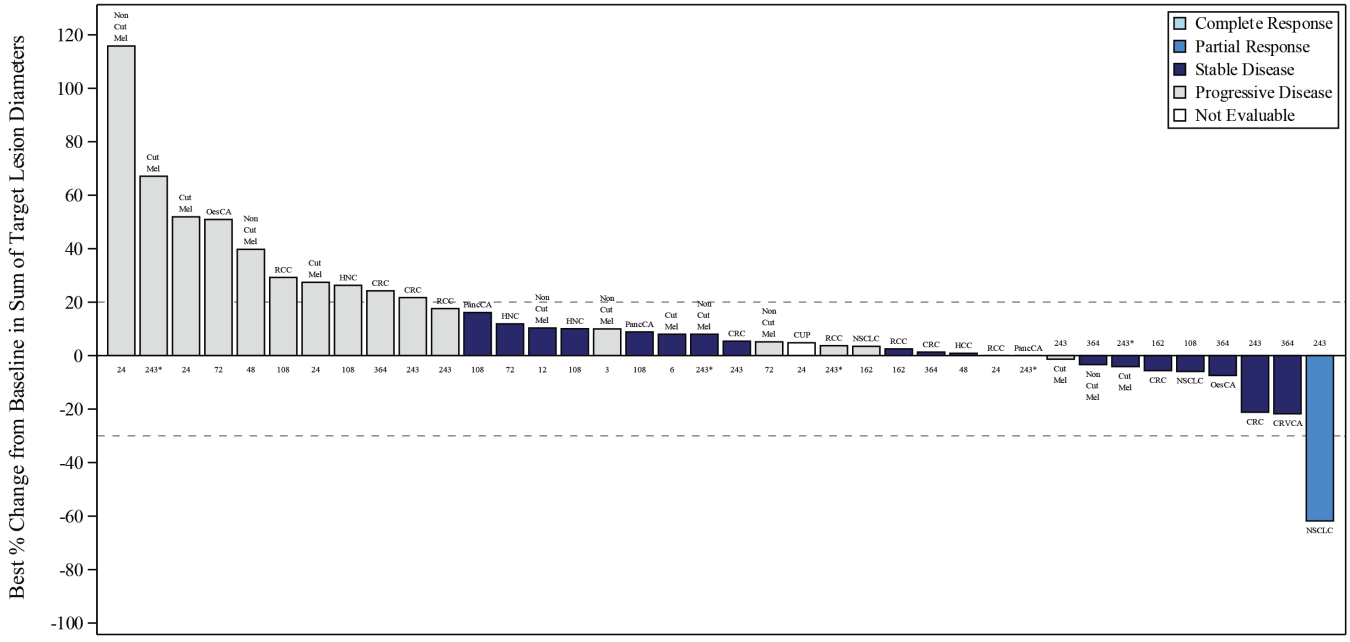


**Figure 3** Waterfall plot depicting the best tumor change from baseline (Response Evaluable population). Q2W, once every 2 weeks.*Q3W dosing. All other doses administered Q2W. A single overall response of SD obtained <2 weeks after the first dose of ANV419 is considered NE (due to the minimum criteria for SD not being met). Two response evaluable patients who did not have the sum of target diameters (due to not all lesions being assessed) were not included. CR, complete response; CRC, colorectal cancer; CRVCA, cervical cancer; CUP, cancer of unknown primary; Cut Mel, cutaneous melanoma; HCC, hepatocellular carcinoma; HNC, head and neck cancer; NE, not evaluable; Non Cut Mel, non-cutaneous melanoma; NSCLC, non-small cell lung cancer; OesCa, esophagus carcinoma; PancCA, pancreatic carcinoma; PD, progressive disease; PR, partial response; RCC, renal cell carcinoma; SD, stable disease.

clinical experience with IL-2 in this context, several key hurdles remain to be overcome, namely, the ability of IL-2 to activate immunosuppressive $T_{regs}$ and its suboptimal PK characteristics.[18] Together with the need for optimal patient selection, these lower treatment efficacy and potentiate toxicity. Currently, different approaches that leverage the potential of high-dose IL-2 immunotherapy for the treatment of metastatic cancer are in development.[3] Despite the numerous candidates undergoing clinical testing, none have yet demonstrated efficacy in Phase 3 trials.[3] ANV419, a selective and potent stable antibody-cytokine fusion protein, has been designed to overcome the limitations of recombinant IL-2. This first-in-human, phase 1 study demonstrated that ANV419, was well-tolerated and showed encouraging signals of clinical activity. In ANV419, the IL-2 component is fused to an anti-IL-2 monoclonal antibody resulting in steric hindrance of IL-2 binding to the α subunit of its receptor. Due to its high selectivity for IL-2Rβγ, ANV419 preferentially stimulates $CD8^+$ T cells ($T_{effs}$) and NK cells over $T_{regs}$. This was reflected in the high level of proliferation (Ki67 index) seen in $CD8^+$ T cells and NK cells, compared with $T_{regs}$.

Based on the overall safety and tolerability findings from the dose-escalation portion of the study, as well as PK and PD results and encouraging signals of efficacy, the RP2D for ANV419 administered Q2W was determined to be 243 µg/kg. Among the patients treated at the RP2D (243 µg/kg Q2W), the most common related TEAEs were fever (7 (87.5%)), chills (5 (62.5%)), vomiting, nausea and decreased appetite (3 (37.5%) each). In the 243 µg/kg (RP2D) cohort, Grade ≥3 thrombocytopenia and fever were reported by one patient (12.5%) each. Two patients treated with ANV419 doses ≤243 µg/kg (RP2D/MTD) required dose reduction for drug-related TEAEs.

In this study, ANV419 exhibited favorable PK properties, including a long half-life (approximately 12 hours at the RP2D), good tissue distribution ($V_{ss,obs}$ at steady-state ranging from 1.1 L to 4.2 L), and sustained plasma concentrations as reflected in the serum concentration-time profiles. Based on the DLT findings in the dose escalation portion of the study, 243 µg/kg was the MTD. Historical data using IL-2 in cancer immunotherapy highlights the importance of maximizing dose intensity to obtain the desired outcome (tumor cell destruction by $T_{effs}$ and NK cells). In patients with cancer, previous attempts at lowering the dose of IL-2 in the hopes of ameliorating its side effects resulted in lowered efficacy.[2] High-dose aldesleukin shows a rapid distribution and tissue penetration, but its fast clearance and short half-life (85 mins) entail frequent dosing.[20 21] The high doses needed in order to achieve clinical efficacy are accompanied by broad organ toxicity, which appear to be exposure-related.[22] The molar equivalents of IL-2 delivered by ANV419 are similar to those delivered at the recommended dose and administration schedule of aldesleukin, but exposure is

significantly higher for ANV419 due to its comparatively longer half-life (online supplemental table 2). Furthermore, the PK properties of ANV419 enable the use of a Q2W dosing regimen.

Conventional IL-2-based therapies are associated with capillary leak syndrome and CNS toxicities.[23] These side effects were not among the TEAEs related to ANV419. The most common TEAEs related to ANV419 were fever (77.5%), chills (57.5%), and vomiting (35.0%). Among the related TEAEs of Grade ≥3 severity, the most common events were increases in ALT and AST. All events were self-limiting and manageable with standard supportive care. Lymphopenia was observed in patients treated with ANV419. Lymphocyte counts reached a nadir between 2 and 4 days after the first dose of ANV419, and gradually increased by Day 8. Lymphopenia is well-established as a side effect of IL-2 treatment.[24 25] This is assumed to be secondary to IL-2 induced sequestration and redistribution of lymphocytes from peripheral blood,[24 25] and is an expected pharmacodynamic effect of ANV419. Importantly, all the cases of lymphopenia that occurred in this study were transient and self-limiting, with no clinical sequelae such as increased infections or sepsis. CRS is an acute systemic inflammatory response, characterized by high fever and a variety of other symptoms associated with multiple organ dysfunction (ie, nausea, headache, dyspnea, tachycardia, hypotension, and myalgia/arthralgia).[26] The advent of IL-2-based cancer immunotherapy predated our understanding of CRS as the clinical entity recognized today.[4 27] The etiology of CRS is bound to the increased levels of serum cytokines, including IL-6, IFN-γ and TNF-α, that arise as a consequence of therapy with immunostimulatory agents including IL-2.[28] There are no standard diagnostic criteria for IL-2-induced CRS. Low-grade CRS is managed by supportive care, whereas moderate-to-severe CRS is treated with the IL-6R-blocking antibody tocilizumab with or without immunosuppression with corticosteroids.[28] Low levels of cytokine production were observed in patients and all of the cases of CRS reported in this study resolved with standard supportive therapy. None of the patients who received ANV419 at or below the RP2D required treatment with tocilizumab. Additional characterization of CRS reported with ANV419 is ongoing, to help differentiate between an uncontrolled cytokine cascade and cytokine infusion syndrome.

ANV419 showed preliminary signals of clinical efficacy. At ANV419 doses ≥108 µg/kg, 64% of patients achieved at least SD (15 SD and 1 PR). Due to its suboptimal PK characteristics (necessitating frequent dosing) and significant adverse effects, aldesleukin treatment is limited to patients with good performance status and organ function.[29 30] The protocol of the present study has been amended to explore dose intensification of ANV419 and the combination with ipilimumab. Studies are ongoing to evaluate ANV419 as single agent Q2W and as combination therapy with standard of care in melanoma (OMNIA-1; NCT05578872) and multiple myeloma (OMNIA-2, NCT05641324).

## CONCLUSIONS

This first-in-human, phase 1 study demonstrated that ANV419, a selective and potent antibody–cytokine fusion protein, was well-tolerated and showed evidence of clinical activity at the RP2D of 243 µg/kg administered Q2W.

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**Competing interests** MJ received support for attending meetings and/or travel from Roche and Takeda; participation on a Data Safety Monitoring Board or Advisory Board for Novartis, Innomedica, Debiopharm, AstraZeneca and BMS. EC is employed by START and HM Hospitales Group and holds a leadership role at START, Pharma Mar, EORTC, Sanofi, BeiGene, Novartis and Merus NV; is a stockholder of START and Oncoart Associate, received grants from START, received consulting fees or honoraria from Nanobiotix, Janssen-Cilag, Roche/Genentech, TargImmune Therapeutics, Servier, Bristol-Myers Squibb, Amunix, Adcendo, Anaveon, AstraZeneca/MedImmune, Chugai Pharma, MonTa, MSD Oncology, Nouscom, Novartis, OncoDNA, T-Knife, Elevation Oncology, PharmaMar, Ellipses Pharma, Syneos Health, Genmab, Diaccurate and HM Hospitales Group. HL received consulting fees from BMS, Palleon, MSD; support for attending meetings and/or travel from Amgen. JL received consulting fees for participation in an Advisory Board for Roche Genentech, GSK, Basilea and Pierre-Faber. ECdlF, GA, VSP and DH have no disclosures. DK received consulting fees from AstraZeneca; payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events from Amgen and Sanofi; support for attending meetings and/or travel from Amgen, Roche and Sanofi; participation on a Data Safety Monitoring Board or Advisory Board from AstraZeneca, Merck, MSD. CB and SJ were employees and held stock options of Anaveon AG during the conduct of this study. EG received grants/contracts from Novartis, Roche, Thermo Fisher, AstraZeneca, Taiho, BeiGene, Janssen; consulting fees from Roche, Ellipses Pharma, Boehringer Ingelheim, Janssen Global Services, Seattle Genetics, Thermo Fisher, MabDiscovery, Anaveon, F-Star Therapeutics, Hengrui, Sanofi, Incyte; payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or education events from Merck Sharp & Dohme, Roche, Thermo Fisher, Lilly, Novartis, SeaGen; Principal Investigator or Insitutional Co-for studies sponsored by: Adaptimmune LLC, Affimed Gmbh, Amgen SA, Anaveon AG, AstraZeneca AB, Bicycletx Ltd, BioInvent International AB, Biontech SE, Biontech Small Molecules Gmbh, Boehringer Ingelhem International Gmbh, Catalym Gmbh, Cyclacel Biopharmaceuticals, Cytovation AS, Cytomx, F.Hoffmann La Roche Ltd, F-Star Beta Limited, Genentech Inc, Genmab B.V., Hifibio Therapeutics, Hutchison Medipharma Limited, Icon, Imcheck Therapeutics, Immunocore Ltd, Incyte Corporation, Incyte Europe Sàrl, Janssen-Cilag International NV, Janssen-Cilag SA, Laboratorios Servier SL, Medimmune Llc, Merck & Co, Inc, Merck Kgga, Novartis Farmacéutica, S.A, Peptomyc, Pfizer Slu, Relay Therapeutics, Replimmune, Ribon Therapeutics, Ryvu Therapeutics SA, Seattle Genetics Inc, Sotio as, Sqz Biotechnologies, Symphogen A/S, Taiho Pharma Usa Inc and T-Knife Gmbh.

**Patient consent for publication** Not applicable.

**Ethics approval** The study was conducted according to the principles of the Declaration of Helsinki and Good Clinical Practice (GCP). The study was approved by the Spanish Agency of Medicines and Medical Devices (C/Campezo, 1-Edificio 8, 28022 Madrid, Spain), HM Hospitals Drug Research Ethics Committee, Madrid, Spain) HM code 20.12.1736-GHM, Ethikkommission Nordwest- und Zentralschweiz (Hebelstrasse 53, 4056 Basel, Switzerland) (project ID 2021-00911), Swiss Agency for Therapeutic Products (Hallerstrasse 7, 3012 Bern, Switzerland), London-Surrey Borders Research Ethics Committee (The Old Chapel, Royal Standard Place, Nottingham NG1 6FS, UK) REC reference 21/LO/0213, IRAS Project ID 1003590 and the MHRA (10 South Colonnade, Canary Wharf, London E14 4PU, UK). Participants gave informed consent to participate in the study before taking part.

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**Data availability statement** All data relevant to the study are included in the article or uploaded as supplementary information.

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## REFERENCES

1 Overwijk WW, Tagliaferri MA, Zalevsky J. Engineering IL-2 to give new life to T cell Immunotherapy. *Annu Rev Med* 2021;72:281–311.
2 Choudhry H, Helmi N, Abdulaal WH, *et al*. Prospects of IL-2 in cancer Immunotherapy. *Biomed Res Int* 2018;2018:9056173.
3 Hernandez R, Pöder J, LaPorte KM, *et al*. Engineering IL-2 for Immunotherapy of Autoimmunity and cancer. *Nat Rev Immunol* 2022;22:614–28.
4 Rosenberg SA. IL-2: the first effective Immunotherapy for human cancer. *J Immunol* 2014;192:5451–8.
5 Konrad MW, Hemstreet G, Hersh EM, *et al*. Pharmacokinetics of recombinant interleukin 2 in humans. *Cancer Res* 1990;50:2009–17.
6 Wolfarth AA, Dhar S, Goon JB, *et al*. Advancements of common gamma-chain family Cytokines in cancer Immunotherapy. *Immune Netw* 2022;22:e5.
7 Mitra S, Leonard WJ. Biology of IL-2 and its therapeutic modulation: mechanisms and strategies. *J Leukoc Biol* 2018;103:643–55.
8 Waters RS, Perry JSA, Han S, *et al*. The effects of Interleukin-2 on immune response regulation. *Mathematical Medicine and Biology* 2018;35:79–119.
9 Arumugam P, Carroll KL, Berceli SA, *et al*. Expression of a functional IL-2 receptor in vascular smooth muscle cells. *J Immunol* 2019;202:694–703.
10 Krieg C, Létourneau S, Pantaleo G, *et al*. Improved IL-2 Immunotherapy by selective stimulation of IL-2 receptors on lymphocytes and endothelial cells. *Proc Natl Acad Sci U S A* 2010;107:11906–11.
11 Ptacin JL, Caffaro CE, Ma L, *et al*. An engineered IL-2 Reprogrammed for anti-tumor therapy using a semi-synthetic organism. *Nat Commun* 2021;12:4785.
12 Sharma P, Goswami S, Raychaudhuri D, *et al*. Immune Checkpoint therapy-current perspectives and future directions. *Cell* 2023;186:1652–69.
13 Charych D, Khalili S, Dixit V, *et al*. Modeling the receptor pharmacology, pharmacokinetics, and pharmacodynamics of NKTR-214, a Kinetically-controlled Interleukin-2 (Il2) receptor agonist for cancer Immunotherapy. *PLoS One* 2017;12:e0179431.
14 Klein C, Waldhauer I, Nicolini VG, *et al*. Cergutuzumab Amunaleukin (CEA-Il2V), a CEA-targeted IL-2 variant-based Immunocytokine for combination cancer Immunotherapy: overcoming limitations of Aldesleukin and conventional IL-2-based Immunocytokines. *Oncoimmunology* 2017;6:e1277306.
15 Ribba B, Boetsch C, Nayak T, *et al*. Prediction of the optimal dosing regimen using a mathematical model of tumor uptake for Immunocytokine-based cancer Immunotherapy. *Clin Cancer Res* 2018;24:3325–33.
16 Eisenhauer EA, Therasse P, Bogaerts J, *et al*. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer* 2009;45:228–47.
17 Seymour L, Bogaerts J, Perrone A, *et al*. iRECIST: guidelines for response criteria for use in trials testing Immunotherapeutics. *Lancet Oncol* 2017;18:e143–52.
18 Sim GC, Martin-Orozco N, Jin L, *et al*. IL-2 therapy promotes suppressive ICOS+ Treg expansion in Melanoma patients. *J Clin Invest* 2014;124:99–110.
19 Wrangle JM, Patterson A, Johnson CB, *et al*. IL-2 and beyond in cancer Immunotherapy. *J Interferon Cytokine Res* 2018;38:45–68.
20 Jeal W, Goa KL. Aldesleukin (recombinant Interleukin-2): a review of its pharmacological properties, clinical efficacy and tolerability in patients with renal cell carcinoma. *BioDrugs* 1997;7:285–317.
21 Kirchner GI, Franzke A, Buer J, *et al*. Pharmacokinetics of recombinant human Interleukin-2 in advanced renal cell carcinoma patients following subcutaneous application. *Br J Clin Pharmacol* 1998;46:5–10.
22 Dutcher JP, Schwartzentruber DJ, Kaufman HL, *et al*. High dose Interleukin-2 (Aldesleukin) - expert consensus on best management Practices-2014. *J Immunother Cancer* 2014;2:26.
23 Schwartz RN, Stover L, Dutcher JP. Managing toxicities of high-dose Interleukin-2. *Oncology (Williston Park)* 2002;16(11 Suppl 13):11–20.
24 Weil-Hillman G, Hank JA, Rosenthal NS, *et al*. Transient decrease in IL-2-responsive lymphocytes 24 hours after initiation of continuous IL-2 infusion in cancer patients. *J Biol Response Mod* 1988;7:424–37.
25 Ménétrier-Caux C, Ray-Coquard I, Blay J-Y, *et al*. Lymphopenia in cancer patients and its effects on response to Immunotherapy: an opportunity for combination with Cytokines *J Immunother Cancer* 2019;7:85.
26 Tvedt THA, Vo AK, Bruserud Ø, *et al*. Cytokine release syndrome in the Immunotherapy of hematological malignancies: the biology behind and possible clinical consequences. *J Clin Med* 2021;10:5190.
27 Rosenberg SA, Lotze MT, Muul LM, *et al*. Observations on the systemic administration of Autologous lymphokine-activated killer cells and recombinant Interleukin-2 to patients with metastatic cancer. *N Engl J Med* 1985;313:1485–92.
28 Morris EC, Neelapu SS, Giavridis T, *et al*. Cytokine release syndrome and associated neurotoxicity in cancer Immunotherapy. *Nat Rev Immunol* 2022;22:85–96.
29 Amaria RN, Reuben A, Cooper ZA, *et al*. Update on use of Aldesleukin for treatment of high-risk metastatic Melanoma. *Immunotargets Ther* 2015;4:79–89.
30 Sullivan RJ, Atkins MB, Kirkwood JM, *et al*. An update on the society for Immunotherapy of cancer consensus statement on tumor Immunotherapy for the treatment of cutaneous Melanoma: version 2.0. *J Immunother Cancer* 2018;6:44.