

## Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our [Editorial Policies](#) and the [Editorial Policy Checklist](#).

### Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a Confirmed

- ☐ ☒ The exact sample size ( $n$ ) for each experimental group/condition, given as a discrete number and unit of measurement
- ☐ ☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- ☐ ☒ The statistical test(s) used AND whether they are one- or two-sided  
*Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
- ☐ ☒ A description of all covariates tested
- ☐ ☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- ☐ ☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- ☐ ☒ For null hypothesis testing, the test statistic (e.g.  $F$ ,  $t$ ,  $r$ ) with confidence intervals, effect sizes, degrees of freedom and  $P$  value noted  
*Give  $P$  values as exact values whenever suitable.*
- ☒ ☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- ☒ ☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- ☐ ☒ Estimates of effect sizes (e.g. Cohen's  $d$ , Pearson's  $r$ ), indicating how they were calculated

*Our web collection on [statistics for biologists](#) contains articles on many of the points above.*

### Software and code

Policy information about [availability of computer code](#)

Data collection

Data analysis

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

### Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

For eligible studies, qualified researchers may request access to individual patient level clinical data through a data request platform. At the time of writing, this request platform is Vivli (<https://vivli.org/ourmember/roche/>). For up-to-date information on Roche's Global Policy on the Sharing of Clinical Information and how to request access to related clinical study documents, see here: [https://go.roche.com/data\\_sharing](https://go.roche.com/data_sharing). Source data for all main figures and extended data figures have been provided as source data files. All other data supporting the findings of this study, along with model specifications, files and qualification, including final

modeling parameters, are available from the corresponding author on reasonable request. RNA sequencing data cannot be made available due to regional secondary data use restrictions, data privacy and subject re-identification risks. Medical imaging data (DICOM images) are considered sensitive personal health information (PHI) and there is currently no safe environment for sharing this kind of PHI. Anonymized records for individual patients across more than one data source external to Roche cannot, and should not, be linked due to a potential increase in risk of patient re-identification.

## Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender	Demographic data included self-reported sex (Table 1). Stratification or analysis by self-reported sex was not performed.
Reporting on race, ethnicity, or other socially relevant groupings	Demographic data included self-reported race/ethnicity (data not presented). Stratification or analysis by self-reported race/ethnicity was not performed.
Population characteristics	Demographic data are presented in Table 1.
Recruitment	Eighty patients with advanced and/or metastatic solid tumors were enrolled across 10 sites within five countries. Participants were identified for potential recruitment by the local investigators using pre-screening enrollment logs and institutional databases.
Ethics oversight	The design and conduct of study WP42627 (NCT04857138) and the imaging substudy WP42627/IMG (conducted at a single site in Spain) complied with all relevant regulations regarding the use of human study participants and were conducted in accordance with the criteria set by the Declaration of Helsinki. The studies were approved by the Institutional Review Boards and/or local Ethics Committees of the participating centers (IRB/EC [CEIC de Navarra: EC_2021/2; HRA & HCRW: 21/FT/0031; De VK Region Hovedstaden: H-21017757; SNUH IRB: H-2104-078-1211; ASM IRB S2021-0747-0001; CPP Ile de France I: CPPIDF1-2022-DI21-cat.1]). The imaging substudy was approved as an amendment to the main study in Spain (CEIC de Navarra: EC_2021/2). All patients provided written informed consent before enrollment and were not compensated for taking part in the study. Human specimen collection and their subsequent evaluations were in accordance with the informed consent agreements.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

## Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://nature.com/documents/nr-reporting-summary-flat.pdf)

## Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	A total of 80 patients were enrolled into the main study (dose-escalation cohorts: n=29; backfill cohorts: n=43) and imaging substudy (n=8). No statistical methods were used to pre-determine sample sizes. Sample sizes for the dose-escalation cohorts were determined by the operating characteristics of the CRM model, as in similar Phase I dose-escalation trials. Sample sizes for the backfill cohorts were determined empirically, with the aim of collecting at least 10 evaluable paired biopsies, which would be sufficient to detect a difference of one standard deviation.
Data exclusions	Patients B, M and Q were excluded from tumor biomarker analysis, as the biopsy samples did not fulfill the QC criteria defined (more details in M&M); For patient L, all DC-LAMP+ values had to be removed due to false detection in this channel. This resulted in a reduction to n=20 or n=19 depending on the analysis, this is detailed in the respective sections/ figure legends.
Replication	Not applicable in view of the first-in-human nature of the study.
Randomization	Not applicable. There was no simultaneous enrollment into several cohorts.
Blinding	Not applicable. The study had an open-label design.

## Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

## Materials &amp; experimental systems

n/a	Involvement in the study
<input type="checkbox"/>	<input checked="" type="checkbox"/> Antibodies
<input checked="" type="checkbox"/>	<input type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology and archaeology
<input checked="" type="checkbox"/>	<input type="checkbox"/> Animals and other organisms
<input type="checkbox"/>	<input checked="" type="checkbox"/> Clinical data
<input checked="" type="checkbox"/>	<input type="checkbox"/> Dual use research of concern
<input checked="" type="checkbox"/>	<input type="checkbox"/> Plants

## Methods

n/a	Involvement in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> ChIP-seq
<input type="checkbox"/>	<input checked="" type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging

## Antibodies

Antibodies used	All antibody reagents were obtained from commercial suppliers. Manufacturers, clones and catalogue numbers for all antibody reagents have been included in Table S4, S5, S7 and S8. All biomarker data were generated applying appropriately qualified/validated standard operating procedures and/or methods, including the use of specific and appropriately diluted antibody reagents. Qualification or validation reports can be made available upon request.
Validation	Methods for the assessment of RO and TBNK+Monocytes were developed, validated and executed at Q Squared Solutions, a qualified and certified CRO according to Good Clinical Practice Guidelines. Validation reports can be made available upon request.

## Clinical data

Policy information about [clinical studies](#)

All manuscripts should comply with the ICMJE [guidelines for publication of clinical research](#) and a completed [CONSORT checklist](#) must be included with all submissions.

Clinical trial registration	NCT04857138 (EudraCT: 2020-004489-21)
Study protocol	A redacted version of the protocol has been provided with the submission
Data collection	Eighty patients with advanced and/or metastatic solid tumors were enrolled between May 2021 and January 2023 at 10 centers in five countries.
Outcomes	The primary objective of the study was evaluation of RO7300490 safety and tolerability. Secondary objectives were assessment of pharmacokinetics, immunogenicity, and anti-tumor activity. Pharmacodynamics were assessed as an exploratory objective. Outcome measures for all objectives are described in detail in the Methods section.

## Plants

Seed stocks	<i>Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.</i>
Novel plant genotypes	<i>Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.</i>
Authentication	<i>Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosaicism, off-target gene editing) were examined.</i>

## Flow Cytometry

## Plots

Confirm that:

- ☐ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- ☐ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- ☐ All plots are contour plots with outliers or pseudocolor plots.
- ☐ A numerical value for number of cells or percentage (with statistics) is provided.

## Methodology

### Sample preparation

Blood samples were collected in Sodium Heparin Blood Collection Tubes (BD Biosciences) at the sites and sent at ambient temperature for sample preparation and flow cytometry analysis according to validated assay protocols. All incubation steps were conducted at room temperature up to and including the cell lysis stage, and at 4°C thereafter. Anti-coagulated whole blood (100 µL) was transferred to each assay tube. The saturation tube was incubated for one hour with the drug. Cells were lysed with BD Pharm Lyse (BD Biosciences) to remove red blood cells. Cells were washed in phosphate-buffered saline (PBS), blocked with FCR blocking (Miltenyi Biotec) and stained with the appropriate antibody cocktail mixture. Cells were washed and resuspended in PBS before acquisition.

### Instrument

Data acquisition for the CD40 RO assay was conducted using FACS Canto II (10 color, 3 laser) instruments (BD Biosciences). Data acquisition for the phenotyping assay was conducted using FACS Canto II (8 color, 3 laser) instruments (BD Biosciences).

### Software

Analysis was carried out using FACSDiva (BD Biosciences) acquisition templates specific to each assay and FACSDiva software.

### Cell population abundance

The percentage of RO on B cells and monocytes are calculated using the following equation: RO calculation of MFI values %  
$$RO = ((MFI \text{ bound} - MFI \text{ Ctrl}) / (MFI \text{ total} - MFI \text{ Ctrl})) * 100$$

### Gating strategy

The gating strategies of the two assays used are summarised in the Methods.

☐ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.