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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	Absorbance data from the human TLR agonist evaluation were collected on a BMG LABTECH POLARstar Omega microplate reader using OMEGA Software (v5.70 R2). PBMC and monocyte stimulation assays were acquired with Magellan software for ELISA readouts, and cytokine multiplex measurements were acquired with xPONENT software (v4.3, update 1; Luminex). Plasma IgE levels in the murine efficacy study were quantified on a BioTek PowerWave XS microplate spectrophotometer using KCjunior software. Wheal diameters were measured from photographic images using Fiji (ImageJ, v2.16.0). Serum total and specific IgE and specific IgG4 concentrations were quantified by fluoroimmunoassay with Phadia 250 running on Phadia Prime v2 while total IgG4 was quantified by nephelometry on a BN ProSpec® System running BN ProSpec® System software. Regulatory T cells were analyzed on Kaluza C software v1.2.
Data analysis	Statistical and graphical analyses were performed using GraphPad Prism v8.0.1. Quantitative analysis of the biodistribution images was conducted with Syngo MI Applications TrueD software (Siemens). Flow cytometry standard files were analyzed using Kaluza C software (v1.2). Microsoft excel was used for data management and basic descriptive calculations.

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

The data supporting the findings off the present study are available within the paper and its supplementary information. The datasets generated and analyzed during this study, including preclinical data and clinical individual participant-level data, are not publicly available due to privacy and confidentiality constraints. De-identified participant-level data supporting the findings of this study may be made available upon reasonable request to the corresponding authors (sgomez@innoupfarma.com and mferrerp@unav.es), subject to signing of a data use agreement and approval by Innoup Farma S.L. Requests will be considered on a case-by-case basis, and data will remain available for 5 years following publication. The trial protocol and statistical analysis plan are included as Supplementary Information.

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	Sex was recorded as a biological variable for all participants based on self-report and is reported in the baseline demographic table, disaggregated by cohort and treatment arm (active vs. placebo). Gender identity was not specifically collected or analyzed. Enrollment was open to participants of any sex meeting the eligibility criteria, and the sex distribution of the enrolled population is presented in the demographic table (Table 1).
Reporting on race, ethnicity, or other socially relevant groupings	Race and ethnicity were not collected, as this exploratory phase I study was not designed or powered to assess outcome differences across these groupings and no stratified analyses were prespecified.
Population characteristics	The study population comprised participants aged ≥12 years with a documented history of peanut allergy, a positive skin prick test (>3 mm), peanut-specific IgE >0.35 kUA/L, and a positive double-blind placebo-controlled food challenge at screening. Key demographic and clinical characteristics are reported in Table 1.
Recruitment	Participants were recruited at two clinical sites in Spain (Clínica Universidad de Navarra and Hospital Universitario de Navarra) in accordance with the study protocol. Recruitment occurred sequentially by dose-escalation cohort, based on predefined inclusion and exclusion criteria, and was not influenced by sex, race, or ethnicity.
Ethics oversight	The trial was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines, and with applicable regulatory requirements. The study was approved by the independent Research Ethics Committee for Medicinal Products of Navarra (CEIm Navarra) and authorized by the Spanish Agency of Medicines and Medical Devices (AEMPS). All participants, or their legally authorized representatives, provided written informed consent prior to enrolment and before the initiation of any study-related procedures. Details of the approvals are provided below: Spain: Research Ethics Committee for Medicinal Products of Navarra (CEIm Navarra); Spanish Agency of Medicines and Medical Devices (AEMPS). EudraCT No. 2018-003665-34 (submitted 26 April 2019); ClinicalTrials.gov identifier NCT04163562.

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

Life sciences study design

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

Sample size	The sample size was 52 participants to assess safety and tolerability of escalating oral doses of INP20. Forty participants were assigned to the INP20 group and twelve were assigned to the placebo group. To assess pharmacodynamic effects of INP20, thirty-one participants were assigned to the INP20 group and nine to the placebo group.
Data exclusions	All participants who received at least one dose of INP20/placebo were included in the safety analysis. Pharmacodynamic analyses were only performed in participants who completed the 14-day treatment period. Participants from cohort F were not included in the pharmacodynamic analyses owing to the limited number of evaluable participants (n=5; 3 active and 2 placebo).

Replication	Since this is a first-in-human, multicenter, double-blind, randomized, placebo-controlled Phase I/II trial, study replication is not applicable.
Randomization	This is a first-in-human, multicenter, double-blind, randomized, placebo-controlled Phase I/II trial. Participants were randomized in a 6:2 ratio to receive INP20 or placebo in each cohort.
Blinding	This is a first-in-human, multicenter, double-blind, randomized, placebo-controlled Phase I/II trial. INP20 and placebo were identically packaged and labelled; participants, investigators, and site staff remained blinded to treatment allocation until database lock.

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 & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☐	☒ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	Neutralizing monoclonal antibodies against human Toll-like receptors were used for TLR-blocking experiments on primary human CD14⁺ monocytes isolated from PBMCs: anti-hTLR1-IgG (InvivoGen, Cat. No. mabg-htlr1), anti-hTLR2-IgA2 (InvivoGen, Cat. No. htlr2-mab7), anti-hTLR4-IgG (InvivoGen, Cat. No. mabg-htlr4), and anti-hTLR6-IgG (InvivoGen, Cat. No. mabg-htlr6). Cytokines were measured in cell culture supernatants using commercial sandwich ELISA kits containing matched capture and detection antibody pairs supplied by the manufacturer: human IL-10 (BD OptEIA™, BD Biosciences, Cat. No. 555157, Lot. 5252822). For multiplex cytokine quantification, analytes included IFN-γ, IL-4, and IL-10, measured with the MILLIPLEX Human Cytokine/Chemokine/Growth Factor Panel A (Millipore Sigma, Cat. No. HCYTA-60K. Kit Lot. 4089881), which contains pre-coupled bead-based capture and biotinylated detection antibody pairs supplied by the manufacturer (clones proprietary). Mouse IgE levels in serum from the preclinical efficacy experiment were quantified using the BD OptEIA™ Mouse IgE ELISA Set (BD Biosciences, Cat. No. 555248, Lot. 5307935). The kit was used according to the manufacturer's instructions. For regulatory T-cell immunophenotyping, a pre-formulated, dry-format DuraClone tube (Beckman Coulter, Cat. No. B53346, Lots: 080224_01, 260423_27, 220622_03, 100622_15, 021121_65, 110320, and 61219) was used. The panel consisted of the following antibody–fluorochrome conjugates, all supplied within the same lyophilized tube: CD45RA-FITC (clone 2H4LDH11LDB9(2H4)), CD25-PE (clone B1.49.9), CD39-PC5.5 (clone BA54), CD4-PC7 (clone SFCI12T4D11(T4)), FoxP3-Alexa Fluor 647 (clone 259D), CD3-APC-AF750 (clone UCHT-1), Helios-Pacific Blue (clone 22F6), and CD45-Krome Orange (clone J33). Individual catalog numbers and lots are not available, as antibodies are provided pre-mixed and pre-titrated by the manufacturer within the DuraClone format. Specific immunoglobulin levels in patient serum were quantified using β-galactosidase-conjugated mouse monoclonal antibodies against human IgE (ThermoFisher, Cat. No 10-9316-01, Lots: U59K, TZPW, TR4A, TF55, T8B4, SXWW, STEG, SGZW, S0HS, RP9S, R7ZH, PBWH, P66H, NTMF, NNWK, NE71, ND9E, BGR13, BGR15, BGR1B, BGR1G, BGR1H, BGR1K, BGR1T, BGR2A, BGR2D, BGR2S, BGROL, BGROP, BGRYN, BGRYS, BGRZM, and BGRZV) and IgG4 (ThermoFisher, Cat. No 10-9549-01, Lots: UCGG, TBJC, SYZR, SF7Z, RZ8M, RAR8, NSZ4, N336, BS, ECEC9, C8UC3, C8UC4, ECECC, ECECG, and ECECE). Bound antibodies were detected through the enzymatic activity of β-galactosidase on a fluorogenic substrate. Antibody specificity and assay performance were validated by the manufacturer.
Validation	All antibodies used in this study were commercially available and validated by the manufacturers for their intended applications.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	HEK-Blue™ hTLR2 (Cat. hkb-htlr2), HEK-Blue™ hTLR4 (Cat. hkb-htlr4), and HEK-Blue™ Null1 (Cat. hkb-null1) reporter cell lines were obtained from InvivoGen (Toulouse, France).
Authentication	The cell lines were obtained directly from the commercial supplier (InvivoGen). Functional authentication was performed in each experiment by confirming the expected SEAP response to receptor-specific reference agonists (Pam2CSK4 for hTLR2 and LPS-K12 for hTLR4) and the absence of response in the HEK-Blue™ Null1 control line.
Mycoplasma contamination	The cell lines were obtained from InvivoGen, which routinely tests its cell lines for mycoplasma contamination as part of its quality control. Cultures were periodically monitored for changes in morphology or growth consistent with contamination; no evidence of mycoplasma contamination was observed.

Commonly misidentified lines (See ICLAC register)	*Name any commonly misidentified cell lines used in the study and provide a rationale for their use.*

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals	For the murine efficacy study, six-week-old male and female ICR mice weighing approximately 20 g were obtained from Envigo (Barcelona, Spain). Mice were housed in groups of six per cage under conventional conditions at the animal facility of the Center for Applied Medical Research (CIMA), University of Navarra, with controlled temperature, humidity, and a standard light/dark cycle, with food and water provided ad libitum. The ICR outbred strain was selected because these mice are immunologically non-polarized and therefore more representative of the heterogeneous human population. Previous in-house studies have demonstrated successful induction of peanut allergy in this strain. All procedures were approved by the Ethics Committee for Animal Experimentation of the University of Navarra (protocol 006-15) and complied with European Directive 2010/63/EU on the protection of animals used for scientific purposes and with Spanish Royal Decree 53/2013. All animal experiments were conducted and reported in accordance with the ARRIVE guidelines. For the biodistribution study, three female Wistar rats weighing approximately 200 g were housed in the microPET facilities of the Clínica Universidad de Navarra (CUN) under social housing conditions in compliance with Spanish Royal Decree 53/2013. Animals were maintained under controlled environmental conditions with 15 air changes per hour, a 12-hour light/dark cycle, relative humidity of 50 ± 20%, and temperature maintained at 22 ± 3°C. All procedures were approved by the Ethics Committee for Animal Experimentation of the University of Navarra (protocol 036-16) and complied with European Directive 2010/63/EU and Spanish Royal Decree 53/2013.
Wild animals	No wild animals were used in this study.
Reporting on sex	Both male and female ICR mice were used in the preclinical efficacy experiment (38 total; 18 males and 20 females), with balanced sex distribution across the three treatment groups: control (6M/7F), peanut extract (7M/7F), and INP20 (5M/6F). The clinical trial included participants of both sexes. For the biodistribution study in rats, only female Wistar rats (n=3) were used. Given the qualitative nature of the study and the small sample size, a single-sex design was adopted to minimise inter-individual variability, and females were selected for this purpose. Sex-disaggregated analysis was therefore not applicable.
Field-collected samples	No field-collected samples were used in this study.
Ethics oversight	All animal procedures were approved by the Ethics Committee for Animal Experimentation of the University of Navarra (protocol 006-15 for the murine efficacy study and protocol 036-16 for the biodistribution study) and were performed in compliance with European Directive 2010/63/EU and its transposition into Spanish law (Royal Decree 53/2013).

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

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	The study was registered at ClinicalTrials.gov prior to patient enrolment (ClinicalTrials.gov identifier: NCT04163562).
Study protocol	The study protocol has been provided as Supplementary Information document.
Data collection	Clinical data were collected prospectively at the two participating sites (Clínica Universidad de Navarra and Hospital Universitario de Navarra) using a sponsor-approved electronic data capture (EDC) system in accordance with Good Clinical Practice guidelines. Data collected included baseline demographic and clinical characteristics, allergic disease history, treatment exposure, safety assessments (adverse events, vital signs, physical examination, and laboratory parameters), pharmacodynamic and immunologic measurements (peanut-specific IgE, IgG4, and skin prick test responses), and results of double-blind placebo-controlled food challenges. Data quality, monitoring, and source data verification were performed by an independent Contract Research Organization (CRO) according to predefined procedures. Preclinical data were collected directly by laboratory investigators using standardized assay protocols and stored on secure institutional servers.
Outcomes	Primary outcomes were to determine the maximum tolerated dose (MTD) and to evaluate the safety and tolerability of escalating oral doses of INP20, and to establish the recommended oral dose for further development. Secondary outcomes were pharmacodynamic effects on serum peanut-specific IgG4 concentrations. All outcomes were prespecified in the study protocol.

Plants

Seed stocks — *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.*

Novel plant genotypes — *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.*

Authentication — *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, mosiacism, off-target gene editing) were examined.*

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 — Briefly, 50 µL of whole blood was added to the dried antibody tube and incubated for 15 min at room temperature protected from light. Following surface staining, cells were washed with PBS and subsequently fixed and permeabilized for intracellular staining using the FOXP3 / Transcription Factor Staining Buffer Set (Invitrogen, Thermo Fisher Scientific). Briefly, 1 mL of fixation/permeabilization buffer was added, and cells were incubated for 30 min at room temperature protected from light. Cells were then washed using the supplied permeabilization buffer and subsequently incubated with the dried intracellular antibody tube (FoxP3 AF647 and Helios PB) for 40 min at room temperature protected from light. After final washing steps with permeabilization buffer, cells were resuspended in the same buffer prior to acquisition.

Instrument — Samples were acquired on the following flow cytometers, depending on equipment availability over the study period: Navios EX (Beckman Coulter), Omnicyt Accoustic Focusing - lasers BRVX (Cytognos), and finally DxFlex - lasers BRV (Beckman Coulter). Fluorescence intensity was corrected over time on Beckman equipment using FlowSet beads (Beckman Coulter, A69184).

Software — Acquisition software was the instrument own software: Navios v1.3, Attune™ NxT v2.5 and, for data analysis, Kaluza C v1.2 was used.

Cell population abundance — Based on literature, T regulatory cells (Tregs), defined as CD3+CD4+CD25high FoxP3+, typically represent 1–3% of total CD4+ T cells in healthy adult peripheral blood.

Gating strategy — Constant flow over time was selected, afterwards leukocyte region was selected (based on FSC/SSC and CD45+ cells) and lymphocyte region (based on SSC). T lymphocytes (CD3+CD4+) were selected and finally T regulatory cells as CD25high and FoxP3+.

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