nature portfolio

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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	Zelta EDC was used to collect the clinical data. Immunospot counter S6 for ELISpot. BD LSRFortessa to for flow cytometry. Data collection was provided by Personalis Inc per internal vendor software and has not been made available. All data was collected at each of the participating academic institutions following start of enrollment (Oct 2020) through completion of the trial and database lock (December 2021).

Data analysis	SAS v9.4 was used to analyze the clinical data. Immunospot software v7.037.0 Professional for ELISpot. BD FACSDiva software v9.0 and FlowJo v10.5.3/v10.8 for flow cytometry. TCR data analysis was conducted in R(v4.1.3) using RStudio(v2024.04.2, Build764) with the immunach(v0.9.0) package. CloneTrack methodology was adopted from Rojas et al. (Personalized RNA neoantigen vaccines stimulate T cells in pancreatic cancer. Nature 618, 144–150 (2023)) with all work conducted in R(v4.1.3) using R Studio(v2024.04.2, Build764). Custom code used in this analysis can be found on GitHub (https://github.com/Inovio-Pharmaceutical/CloneTrack.Adopted.git) GSEA software v4.3.2[build13] was used for all GSEA analysis. ssGSEA was conducted in R(v4.1.3) using RStudio (v2024.04.2, Build 762) with the GSVA package(v1.50.0) Ingenuity Pathway Analysis (IPA), Winter 2023 release for RNAseq. Morpheus, https://software.broadinstitute.org/morpheus for RNAseq. GraphPad Prism v9 through v10.2.3 for graphing.

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 sequencing data generated in this study have been deposited in the Gene Expression Omnibus database under accession code GSE275788 [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE275788]. The de-identified individual patient clinical data are available under restricted access for on-going research, regulatory, and privacy reasons, due to ethical concerns related to the sensitive nature of the participant information. Interested investigators can obtain and certify a data transfer agreement and submit requests by emailing Jeffrey Skolnik (Jeffrey.Skolnik@inovio.com). The raw individual patient data are protected and are not available due to data privacy laws. The processed de-identified immunology data are available under restricted access for privacy, ethical and on-going research concerns. Interested investigators can obtain and certify a data transfer agreement and submit requests by emailing Matthew Morrow (Matthew.Morrow@inovio.com). Responses to data requests will be provided within 30 days of receipt.

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	Both male and female participants were eligible for inclusion in the study. In this rare disease, the male-to-female ratio is estimated to be 4:1, aligning with the population enrolled. Sex was determined based on assigned biological sex. Sample size (N=32) does not permit for meaningful separate analyses based upon biological sex.
Reporting on race, ethnicity, or other socially relevant groupings	No sex or gender analyses were carried out. The sample size (N=32) does not permit for meaningful separate analyses based upon race, ethnicity, or other socially relevant groupings. There are too few individuals in any one group other than White to assess specific response by race, ethnicity or other.
Population characteristics	Please see demographic table provided in the manuscript. Subjects represent the general patient population of those with the disease under investigation (recurrent respiratory papillomatosis), which has a male predilection, and the trial enrolled only adults aged 18 years or older.
Recruitment	Subjects were recruited between October 2020 and November 2021 by the investigators at each site by pre-identifying trial participants through pre-screening. Site staff then reach out to the pre-screened patients to confirm eligibility and interest in participation. Patients were enrolled after meeting all of the inclusion criteria and none of the exclusion criteria. No specific recruitment tools were used for this trial. The small sample size and rarity of the condition under investigation implied that the investigator(s) knew most of the subjects recruited or were chronic patients of those investigator(s).
Ethics oversight	The study was conducted in accordance with the Declaration of Helsinki, Good Clinical Practice guidelines, and local and national regulatory requirements. The protocol was reviewed and approved by the relevant Institutional Review Board/Ethics Committee at each participating site (NYU Langone; University of Texas Southwestern; Washington University School of Medicine; University of California, Davis; Johns Hopkins University School of Medicine; Mayo Clinic Arizona; Emory University; University of Cincinnati Medical Center). All patients provided written informed consent.

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	As this was a Phase 1/2 trial sample size was determined by defining the likelihood of seeing an adverse event in the population under investigation. No formal power analysis was applicable to this study, as descriptive statistics were to be used to summarize the data. For 30 INO-3107 administered subjects, the trial provided 95% confidence that the true incidence of SAEs is <12% if no SAEs are observed.
Data exclusions	No data were excluded from the analysis.
Replication	With respect to Translational Analyses, limitation of the availability of samples precluded multiple runs of the same sample (i.e. sample was used up during the first runs of each assay).
Randomization	Not applicable; the study was open-label, single-arm, non-randomized.
Blinding	Blinding was not relevant to this study as it was an open-label single arm clinical trial.

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

Antibody Conjugate Dilution Supplier Catalog Number Clone
Live/Dead Aqua BV510 1:125 ThermoFisher L34957 N/A
CD14 BUV395 1:160 BD Biosciences 740286 M5E2
CD16 BUV395 1:160 BD Biosciences 563785 3G8
CD19 BUV395 1:80 BD Biosciences 563549 SJ25C1
CD3 BUV737 1:60 BD Biosciences 612752 SK7
CD4 APC-Cy7 1:160 BioLegend 344616 SK3
CD8a BV650 1:60 BD Biosciences 563821 RPA-T8
CD69 BV711 1:40 BioLegend 310944 FN50
CD137 APC 1:60 BioLegend 309810 4B4-1
CD38 BV786 1:40 BD Biosciences 563964 HIT2
Ki67 BV605 1:160 BioLegend 350522 Ki-67
PD1 PE-Dazzle594 1:40 BioLegend 329940 EH12.2H7
Tim3 PE 1:40 Novus Biological FAB2365P 344823
Lag3 PerCP-eFluor710 1:40 ThermoFisher 46-2239-42 3DS223H
Granulysin AF488 1:15 BD Biosciences 558254 RB1
Perforin BV421 1:16 BioLegend 353307 B-D48
Granzyme A PE-Cy7 1:12 ThermoFisher 25-9177-42 CB9
Granzyme B AF700 1:30 BD Biosciences 560213 GB11

Validation

Antibodies were purchased in 2021 and 2022 and validated per the supplier's reporting. Briefly, the specificity of antibodies was tested against isotype control in human PBMCs or cell lines.

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	NCT04398433
Study protocol	Full protocol has been posted to: https://www.clinicaltrials.gov/study/NCT04398433 and is appended to the end of the Supplementary Information.
Data collection	Data was collected according to GCP/ICH standards at each of the enrolling investigational sites by qualified personnel representative of the Sponsor.
Outcomes	The primary endpoint was safety and tolerability, assessed by reported treatment-emergent adverse events (TEAEs) and serious adverse events (SAE). Secondary endpoints included: (1) efficacy as assessed by number of RRP surgical interventions in the year following Day 0 compared with the prior year (surgeries on Day 0 were considered to have been performed during the prior year); (2) efficacy as assessed by changes in staging assessments measured by the RRP Severity Score (modified) over time; and (3) antigen-specific cellular immune responses assessed by T-cell phenotype and lytic potential in peripheral blood mononuclear cells (PBMCs) by flow cytometry.

Plants

Seed stocks	Plants were not used in this study
Novel plant genotypes	Plants were not used in this study
Authentication	Plants were not used in this study

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	Whole blood is obtained in ACD-A tubes. PBMCs are separated from blood taken from these tubes using a Ficoll gradient and are subsequently washed with PBS before counting and plating for stimulation.
Instrument	LSR-II
Software	BD FACSDiva v6.1.3
Cell population abundance	Cell population frequencies are reported within all Flow Cytometry figures.
Gating strategy	Gating strategies for all cell populations starting with singlets down to terminal gating is shown for stimulated and unstimulated PBMCs in Supplemental Material. Gates identifying positive populations are shown for all stains.

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