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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	Single-cell sequencing: 10X Genomics Chromium 5'Kit V2, sequenced on Illumina NovaSeq 6000 CapTCRseq: sequenced on an Illumina NovaSeq 6000
Data analysis	Single-cell sequencing: Alignment using 10X cellranger v6.1.2, v7.0.1 and v8.0.1 (as indicated in Methods), normalization, integration, and clustering Seurat v4.3.0 and SCTransform v0.3.5, harmony v0.1.1. Downstream data analyses and visualizations umap v0.2.10.0, vegan (v2.6-4), tidyverse v2.0.0, pheatmap v1.0.12, ggplot2 v3.4.2, ggalluvial v0.12.5, packcircles v0.3.7. All performed using R v4.2.1. All figures were processed using Adobe Illustrator 2025. Survival analysis: performed in R v4.2.1 using survival v3.4.0, survminer v0.5.0, and PHInfiniteEstimates v2.9.5. CapTCRseq: data was aligned using MiXCR v4.6.0, bcl2fastq v2.20, downstream analysis performed in R v4.2.1 and packages listed above. PreGame training and predictions: performed in python v3.9.6 using imblearn v0.11.0, numpy v1.26.3, pandas v2.2.0, sklearn v1.1.3. Other statistical analyses not listed above were performed using GraphPad Prism (v10.6.1). Custom code generated in this study can be found on Zenodo (https://doi.org/10.5281/zenodo.20028241).

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

Single-cell CITE + VDJ sequencing and CapTCRseq data from MM patients enrolled in the ALGONQUIN clinical trial have been deposited in the European Genome-Phenome Archive (EGA) database under accession code EGAS50000001889. Single-cell CITE + VDJ sequencing and CapTCRseq data from MM patients in the PreGame cohort generated in this study have been deposited in EGA under accession code EGAS50000001888. Single-cell RNA and TCR sequencing data from MCC patients referenced in this study can be found in the European Genome-Phenome Archive under accession code EGAD50000000137. Previously published γδ TCR CDR3 sequences used in this study were obtained from https://gdt.moffitt.org (pan-cancer) and from Gene Expression Omnibus (GEO) under accession code GSE235090 (Merkel cell carcinoma). CapTCR-seq data from the plasma cfDNA of healthy donor samples used in this study can be found in the European Genome-Phenome Archive under accession code EGAS00001003280. Bulk RNA sequencing data from 66 MM cell lines was previously generated and obtained from the Keat's lab (https://www.keatslab.org/data-repository).

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	The sex of each patient is included in Supplemental Table 1
Reporting on race, ethnicity, or other socially relevant groupings	Not reporting.
Population characteristics	Bone Marrow and Peripheral Blood samples were obtained from MM patients as indicated in Supplemental Table 1. There is a mixture of patients of different age, sex, and previous treatment status.
Recruitment	Patients were recruited by participating investigators. Investigators obtained written informed consent for each patient before any study-specific activity was performed. Potentially eligible patients who are receiving care for a diagnosis of multiple myeloma or related plasma cell dyscrasias at the Princess Margaret Cancer Centre may be approached for participation in this study. The inclusion criteria are: 1. Patients must have a diagnosis of MM or related malignancy. 2. Patient must be ≥ 18 years old. 3. Patients are undergoing standard of care bone marrow aspirate and blood draw. 4. All patients must have signed and dated an informed consent form: either a paper version or an identical electronic version using REDCap. Endpoints assessments are uniformed according to the International Myeloma Working Group (IMWG) response criteria, this eliminated observer bias, ensured comparability between baseline and subsequent assessments; a consistent laboratory method of assessment and the same technique was used when assessing response.
Ethics oversight	The study was conducted in accordance with the Declaration of Helsinki, International Council Harmonisation Good Clinical Practices Guidelines. The study protocol, amendments and informed consent were approved by the University Health Network Institutional Review Board (REB Numbers 16-5260, 18-5911 and 17-5357). The study complied with local regulation governing the conduct of clinical studies, and institutional guidelines. 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	No predetermined sample size calculation was performed. Sample size for developing the algorithm was determined based on availability and previous publications involving alpha beta T cell algorithms. For analysis of patient samples in the ALGONQUIN trial, all available blood samples were used. All available bone marrow samples were used for patients with availability of both baseline and cycle 2 day 1 biopsies,

	with the exception of patient ALQ-19-019, where poor viability of the baseline bone marrow biopsy prohibited single-cell CITE+VDJ sequencing. For in vitro experiments , sample sizes of at least n=3 were selected to allow for statistical analysis.
Data exclusions	Low quality single-cells were excluded from downstream analysis as described in the methods. When cloning TCRs into the Jurkat cells, a small number of TCRs demonstrated extremely high background or were unable to be expressed in the Jurkat cells. These TCRs were likely recognizing an antigen expressed on the Jurkat cells and so we were unable to accurately evaluate their reactivity to myeloma cells and excluded them from the manuscript.
Replication	All experiments were replicated in at least 2 independent repeats as indicated in each figure legend.
Randomization	No randomization occurred as the study was exploratory and observational.
Blinding	Investigators performing in vitro tumor-reactivity assays and running the FACs were blinded to expected reactivity of TCRs tested. The computational data analysis was exploratory and observational results are presented, therefor blinding is not relevant.

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	Antibodies used in this study for flow cytometry were as indicated: CD3 (OKT3; BioLegend cat# 317308 or 317322, 1:400), B2M (A17082A; BioLegend cat# 395716, 1:800), CD69 (FN50; BioLegend cat# 310910, 1:400), CD8 (RPA-T8; BioLegend cat# 301049, 1:400), CD4 (OKT4; BioLegend cat# 317420, 1:200), NGFR (ME20.4; BioLegend cat# 345132, 1:200), γδTCR (B1; BioLegend cat# 331210, 1:100), αβTCR (IP26; BioLegend cat# 306720, 1:100), CD14 (61D3; Thermofisher cat# 53-0149-42, 1:50), CD2 (RPA-2.10; BioLegend cat# 300230, 1:75), CD235 A/B (HIR2; BioLegend cat# 306614, 1:200), CD138 (MI15; BioLegend cat# 356504, 1:100), CD319 (235614; BD cat# 750838, 1:20), HLA-ABC (W6/32; BioLegend cat# 311405, 1:50), HLA-A (1082C5; BD cat# 568024, 1:100), HLA-C(DT-9; BD cat# 566372, 1:100), BTN3 (232-5; BD cat# 566006, 1:100), Bw4 (REA274; Miltenyi cat# 130-123-760, 1:100), KIR3DL1 (DX9; BioLegend cat# 312707, 1:100), CD94 (HP-3D9; Invitrogen cat# 17-5094-4210, 1:100) GPR56 (CG4; BioLegend cat# 358205, 1:100), 4-1BB (4B4; BioLegend cat# 309809, 1:200), CD1D (51.1; BioLegend cat# 350305, 1:100) or CD1C (L161; BioLegend cat# 331505, 1:100). Trustain Fc Block (BioLegend cat# 422302) was used to block Fc receptors, and fixable viability dye (Invitrogen cat# 65-0865-14), with or without Annexin V staining (BioLegend cat# 640920), were used to identify live and apoptotic cells. Unless used for cell sorting or Annexin V staining, cells were fixed with 4% paraformaldehyde (PFA) prior to analysis. Flow cytometry data were acquired on a BD LSR Fortessa X20 or Beckman Coulter CytoFLEX instrument and analyzed using FlowJo v10 software (BD Biosciences)
Validation	Antibodies were obtained from commercial sources. The antibodies were validated and QCed by the companies to bind the human protein and indicated by the companies to be used for flow cytometry of human samples.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	The MM cell-lines RPMI8226 and MM1R were purchased from ATCC, ALMC-1 was purchased from Sigma, and INA-6, JJN3, EJM and AMO-1 were purchased from the DSMZ. All MM cell lines were cultured in complete IMDM which is IMDM (Gibco) supplemented with 10% fetal calf serum (FCS), 2 mM L-glutamine, 1% non-essential amino acids, 1% Pen/Strep, 10 ug/mL gentamicin, and 55 uM β-ME (all purchased from Gibco). Human IL-6 (Biolegend) was added to the culture media for ALMC1 (1 ng/mL) and INA6 (10 ng/mL) cells. To generate CD1D-over expressing myeloma cell-lines, the human CD1D full-length construct was transduced into MM cell lines using lentivirus, and the CD1D+ cells were purified with FACS. Jurkat 76 cells were obtained from Dr. Naoto Hirano (Princess Margaret Cancer Center, Toronto) and cultured in the same complete IMDM as the myeloma cell lines. The melanoma cell lines A-375 (ATCC), A2058 (ATCC), 624Mel, 526Mel, 888Mel (provided by Dr. S. Rosenberg, National Cancer Institute), WM3670 (Rockland), the lung cancer cell lines A549, H1568, H1573, H1437, H1792, H1944 and H2030 (all from ATCC), the Glioblastoma multiforme (GBM) cell lines LN-229 (ATCC) and A-172 (ATCC) were cultured in complete DMEM (Gibco) + 1% Glutamax (Gibco) and supplemented in the same manner as the complete IMDM. The lung cancer cell line H1568 (ATCC), Small-cell lung carcinoma (SCLC) cell line H69PR (ATCC), Mesothelioma cell lines H2452 (ATCC) and MSTO-211H (ATCC), breast cancer cell lines HCC1143 (ATCC) and HCC1937 (ATCC), pancreatic cancer cell

line ASPC1 (ATCC) and colon cancer cell lines LoVo (ATCC) and Colo205 (ATCC) were cultured in complete RPMI-1640 (Gibco) supplemented in the same manner as the complete IMDM. The pancreatic cancer cell line CFPAC-1(ATCC) was cultured in complete IMDM medium. The Merkel cell carcinoma cell lines MCC14-2, MCC26, MS-1 and MCC13 were purchased from Sigma and cultured in RPMI-1640 (Gibco) supplemented with 20% FCS, 2mM L glutamine, 1% Pen/Strip, 25 mM HEPES and 10 ug/mL gentamicin. Lenti-X 293T were purchased from Takara and cultured in complete DMEM as above. Healthy primary cells used for screening were obtained from healthy donors and obtained from ATCC and Sigma and cultured according to the manufacturers' recommended media and protocol. Tumor cell lines used were confirmed to be mycoplasma free.

Authentication	None of the cell lines were authenticated
Mycoplasma contamination	All cell lines were confirmed to be mycoplasma free
Commonly misidentified lines (See ICLAC register)	No commonly misidentified lines were used

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	NCT03715478
Study protocol	The study protocol can be found in Trudel et al. 2024 PMID: 38177852
Data collection	The Algonquin study was conducted at 9 Canadian sites in accordance with the Declaration of Helsinki, International Council Harmonisation Good Clinical Practices Guidelines, local regulation governing the conduct of clinical studies, and institutional guidelines. All patients provided written informed consent. The data were collected by the sponsor (Canadian Myeloma Research Group). A total of 99 patients were enrolled and treated on the Algonquin study. This included 61 patients in the Part 1 dose-exploration phase (not included in this study), and 26 in the Part 2A and 12 in the Part 2B dose-expansion phase which comprise the 38 patients included in the correlative component of the study. One patient enrolled in Part 2A withdrew consent and was not included in any component of this study. Patient enrollment ended on September 14, 2022. The clinical data cut-off date for the presented analysis was October 28, 2025
Outcomes	Clinical-primary:identification of recommended part 2 dose (RP2D) and ORR; secondary: safety, PFS and OS Exploratory: To explore the relationship between dynamic changes in immune cells and response to GSK2857916 in combination with pomalidomide and dexamethasone. AEs were assessed for severity using CTCAE v.5.0, with the exception of visual acuity by the Snellen method and corneal epithelium changes observed on ophthalmic examination, which were graded by the prespecified KVA scale. Clinical activity of the combination was assessed by the treating physician in accordance with the International Myeloma Working Group Uniform Response Criteria for Multiple Myeloma.

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	Samples were stained in FACS Buffer (PBS + 2% FCS + 0.09% Sodium Azide) for 30 minutes at 4C according to the

Sample preparation	manufacturers recommended dilution of each antibody. Prior to staining, samples were FC blocked for 10 minutes at 4C in FACs Buffer. After surface staining, samples were stained with viability dye in PBS for 30 minutes at 4C. After staining, samples were washed with FACs Buffer and used for cell sorting, or cells were fixed in 4% PFA at room temp for 15 minutes followed by washing with FACS buffer and storage in FACS buffer until acquisition.
Instrument	Flow cytometry data was acquired on a BD LSR Fortessa X20 or Beckman Coulter CytoFLEX. BD Fusion was used for cell sorting.
Software	FlowJo v10 (BD Biosciences)
Cell population abundance	Purity was not assessed in post sort fractions due to limited post-sort cell number. The entire population of sorted cells were sent for scRNA sequencing.
Gating strategy	Gating strategy is outlined in Extended Data Figure 4A and Extended Data Figure 5E

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