

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 (<i>n</i>) 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
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| ☒ | ☐ 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted
<i>Give P values as exact values whenever suitable.</i> |
| ☒ | ☐ 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 <i>d</i> , Pearson's <i>r</i>), 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	MACRO database software v 4.9.1 was used for data collection.
Data analysis	Statistical analyses were conducted using STATA version 17.0. Additional genomic and laboratory data were plotted and analyzed using Prism 8 (GraphPad), and ggplot2 in R version 4. DNA sequencing: Genome Analysis ToolKit (GATK) was used for realigning around known indels to improve indel calling and base quality score recalibration for adjusting systematic errors made by the sequencer when estimating quality scores of each base call. HaplotypeCaller is used for variant calling in germline sample (limit of detection ~10%) and Mutect2 is used for tumor-normal paired somatic analysis. RNA sequencing used STAR alignment software (v2.7.6a) was used to align reads to the reference genome, HTSeq-count (HTSeq v0.12.4) was used to count the number of reads mapping unambiguously to genomic features in each sample. DESeq2 (v1.34.0). Dispersion estimations were corrected using the fdrtool (v1.2.17). Gene Set Enrichment and Pathway Analysis was carried out in R using DOSE (v3.20.1), pathview (v1.34.0) and clusterProfiler (v4.2.2) packages, and using GSEA (v4.3.2). Volcano plots were generated using EnhancedVolcano v1.16.0. Heatmaps were constructed using the pheatmap package (v1.0.12). Cell-type deconvolution was performed using CIBERSORTx. Immunohistochemistry analysis was performed using HALO image analysis (Indica Labs). Quantification of tumor-infiltrating lymphocytes was performed using QuPath. Flow cytometry analyses were performed using FlowJo v10.

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](#)

Individual participant data that underlie the figures in this article is supplied in the article supplementary data file. DNA/RNA sequencing is restricted by privacy regulations and will be provided to researchers who provide a methodologically sound proposal. Requests should be submitted to magnus.dillon@icr.ac.uk, a signed data access agreement will be required.

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	Gender was self-reported. Sex and/or gender-based analyses have not been performed. Sample size is insufficient to reveal sex and/or gender differences in response or safety.
Reporting on race, ethnicity, or other socially relevant groupings	Racial identity was provided by participants. Classification terms were provided by the researchers. Terms were: caucasian/white, mixed race, african-caribbean, asian, other-specify.
Population characteristics	This is provided in table 1 of the manuscript
Recruitment	Participants were approached by site investigators and given information about the study. Participants were given adequate time to make a decision as per GCP guidance.
Ethics oversight	NRES Committee London - City and East, reference 14/LO/0465

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://www.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	Sample size for this phase I trial is not based on a formal sample size calculation, as no formal statistical hypothesis is being tested. The dose escalation component sample size used a 3+3 design and was contingent on the number of patients experiencing dose-limiting toxicity. The expansion cohorts allowed a maximum of 50 patients, aiming to recruit patients with putative markers of sensitivity to the drug being tested.
Data exclusions	All participants receiving at least one dose were included in the safety analysis. Dose-limiting toxicities and response assessment were limited to participants receiving at least 80 % of planned treatment and/or at least 1 response assesment, This was pre-specified in the protocol.
Replication	Replication studies have not taken place.
Randomization	Not randomised
Blinding	Not blinded

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

IHC
 phospho-(S635)Rad50 (Cell Signaling 14223)
 γH2AX (S139, Cell Signaling 9718)
 ATM (Abcam ab32420)
 Cyclin E1 (Invitrogen HE12, MA514336)
 Ki67 (Mib-1, Dako M7240)
 ARID1A (EPR13501, abcam ab182560)

Immunofluorescence
 anti-phospho-(S345)Chk1 [133D3] (Cell Signaling 2348)
 anti-gamma-H2A.X(S139) (Abcam ab11174)

Validation

phospho-(S635)Rad50 (Cell Signaling 14223) Jones, G.N., et al., pRAD50: a novel and clinically applicable pharmacodynamic biomarker of both ATM and ATR inhibition identified using mass spectrometry and immunohistochemistry. Br J Cancer, 2018.
 γH2AX (S139, Cell Signaling 9718), multiple citations for IHC on <https://www.cellsignal.com/products/primary-antibodies/phospho-histone-h2a-x-ser139-20e3-rabbit-mab/9718>

ATM (Abcam 32420) knockout validated and multiple citations at <https://www.abcam.com/products/primary-antibodies/atm-antibody-y170-ab32420.html>

Cyclin E1 (Invitrogen HE12) Oncotarget 2016 Jan 5;7(1):550-64. doi: 10.18632/oncotarget.6375

Ki67 (Mib-1, Dako M7240) J Natl Cancer Inst. 2011 Nov 16; 103(22): 1656–1664.

ARID1A (EPR13501, abcam ab182560) - knockout validated and validated for ARID1A mutations: Khaliq, S., et al., Optimised ARID1A immunohistochemistry is an accurate predictor of ARID1A mutational status in gynaecological cancers. J Pathol Clin Res, 2018. 4(3): p. 154-166

anti-phospho-(S345)Chk1 [133D3] (Cell Signaling 2348) Nat Commun 2017 Dec 14;8(1):2118. doi: 10.1038/s41467-017-01805-9; Nat Commun 2017 Nov 9;8(1):1392. doi: 10.1038/s41467-017-01401-x

anti-gamma-H2A.X(S139) (Abcam ab11174) multiple citations at <https://www.abcam.com/products/primary-antibodies/gamma-h2ax-phospho-s139-antibody-ab11174.html>

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

Clinicaltrials.gov: NCT02223923
 EudraCT: 2013-003994-84

Study protocol

supplied with manuscript

Data collection

All data was collected in UK NHS sites. Recruitment took place between January 2016 and December 2020.

Outcomes

Primary outcome measure was identifying the maximum tolerated dose of ceralasertib in combination with palliative radiation schedules. This was assessed by assessing participants for dose-limiting toxicity during the dose-escalation. Secondary endpoints were (i) determining causality of each adverse event in relation to the study medication and grading severity according to CTCAE v4.03; (ii) single and multiple dose pharmacokinetics assessed by measurement of plasma levels of ceralasertib at pre-defined intervals in the dose-escalation part of the study in order to establish pharmacokinetic parameters; (iii) Any response (stable disease,

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	Peripheral blood was drawn into 8 mL EDTA tubes (Vacutainer, BD) and analyzed within 24 hours. ACK-lysed whole blood (for myeloid panel) or PBMC from density gradient centrifugation (for lymphocyte panel) were used. Samples were surface-stained with antibodies for 30 mins at 4 degrees C
Instrument	LSR Fortessa (BD Biosciences)
Software	FlowJo v10
Cell population abundance	Cells are expressed as a proportion of parent population as described in the figure legends.
Gating strategy	For lymphocyte panel: FSC/SSC gates were used to gate the lymphocyte population, single cells were identified, then gated for CD45-positive. CD3-CD56 gating was used to gate T-cells and NK cells and then CD4-CD8 gating was used for subpopulations. Markers expressed on these populations are shown in the figures. For myeloid panel: FSC/SSC gating was used to exclude debris, FSC-A/FSC-H to isolate single cells, CD45+ Lin (CD3, CD56, CD19, CD20)- cells to exclude T, B, NK cells. FSC/SSC was used to gate monocytes and CD14/CD16 was used to subclassify into classical, intermediate or nonclassical. CD14/CD16 negative cells were gated by HLA-DR and CD11c to identify dendritic cells. CD14/CD33 was used to identify MDSC: CD14+CD33+ HLA-DR Low: mMDSC. CD14-CD33-CD15+CD11b+ gMDSC.

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