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	Clinical data were acquired, stored and analysed with hospital-wide General Electric (GE) Picture Archiving and Communication System (PACS). Cerenkov Images were collected using Oxford Instruments Andor Solis Software V4.23.30003.0.
Data analysis	Clinical data were acquired, stored and analysed with hospital-wide General Electric (GE) Picture Archiving and Communication System (PACS). Cerenkov Images were analysed with a macro in ImageJ 1.53c. Analysed data were collected and plotted in Prism 9.2.0. System-testing analysis was also performed using custom code in MATLAB (2019b, MathWorks, CA, USA).

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 generated and analysed during the study were funded by NIH R01CA18395305, and de-identified data can be made available by the corresponding author on reasonable request. The code used to process and overlay Cerenkov images is available from Zenodo at https://doi.org/10.5281/zenodo.6261583.

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 preclinical study design was based on an n = 3 for most technical replicates, with animal size limited to 5 mice in the chamber at one time, and the length of imaging time necessary. The initial clinical sample size was based on a power calculation to determine that the signal intensity was greater than two-fold over noise. The study was arbitrarily expanded to 128 to accommodate the protocol amendment to include three new radiotracers 223RaCl2, [177Lu]-Lu-DOTATATE, and [68Ga]Ga-DOTATATE).
Data exclusions	Patients were excluded from the intensity-analysis portion of the study only if insufficient information was provided about the patient's scan, such as activity administered and activity measured, or if no scan was conducted. Patients that withdrew their consent were also excluded from any study analysis.
Replication	Preclinical Cerenkov well-plate experiments were replicated more than 3 times, with the last experiment used for the Supplementary figures. Animal-imaging studies were done twice, once with increasing amounts of 18FDG, whereas the second experiment was with a fixed 30 µCi amount for filter analysis. Patients were imaged in one session per consent, with between one and seven Cerenkov images acquired during that session. Session length was dependent on patient participation, and patients could withdraw at any time. Patients that were imaged for more than one session were asked to re-consent.
Randomization	Patients were separated into groups, based on the radiotracer that they received.
Blinding	The investigators were not blinded to the patients prior to performing the PET/CT or SPECT/CT scans. Imaging was conducted by focusing on the area of disease. Image analysis was conducted with a macro script written for ImageJ, to streamline image analysis and to reduce variabilities in processing.

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
☐	☒ Human research participants
☐	☒ Clinical data
☒	☐ Dual use research of concern

Methods

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

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	HT1080 cells were procured from American Tissue Type Culture Company (ATCC).
Authentication	The cell line HT1080 was authenticated by STR profiling.
Mycoplasma contamination	The cell line was tested and found to be free of mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	Athymic female 4–6 week-old nude mice from the Jackson Laboratories were used for the intratumoural [18F]F-DG CLI assessment in the preclinical enclosure.
Wild animals	The study did not involve wild animals.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	The procedures with mice were approved by the MSKCC Institutional Animal Care and Use Committee (IACUC), under protocol 08-07-014.

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

Human research participants

Policy information about studies involving human research participants

Population characteristics	Between May 2018 and March 2020, 96 patients consented and were imaged with the Cerenkov fiberscope. Of the 96 patients consented, 106 imaging sessions were conducted with the five radiopharmaceuticals detailed in Table 1. Patients were 42% female and 58% male, with a median age of 58.2 (26–87) years old. Concerning race, 78% were white, 9% Black or African American, 6% Asian, and 7% unknown; in terms of ethnicity, 11% identified as Hispanic. Thirteen patients were available for subsequent imaging sessions, allowing for longitudinal surveillance of therapy (eight [177Lu]Lu-DOTATATE, four [68Ga]Ga/[177Lu]Lu-DOTATATE, and one [18F]F-DG).
Recruitment	Potential research subjects were identified by a member of the patient's treatment team, the protocol investigator, or the research team. If the investigator was a member of the treatment team (for example, a primary/treating physician), s/he screened for suitable research study participants (that is, those that met the eligibility criteria) and discussed the study and their potential for enrolling in the research study during their routine clinic visit. The study CRC was available in these clinics to approach patients after he/she had spoken with the primary/treating physician who had already mentioned the study to the patient. The principal investigator may also have screened the medical records of patients with whom they do not have a treatment relationship for the limited purpose of identifying patients who would be eligible to enroll in the study and to record appropriate contact information in order to approach these patients regarding the possibility of enrolling into the study. The investigator/research staff may also have reviewed portions of their medical records at MSKCC in order to further assess eligibility. They may have used the information provided by the patient and/or medical record to confirm that the patient was eligible and to contact the patient regarding study enrollment. If the patient was ineligible for the research study, the research staff destroyed all information collected on the patient during the initial conversation and medical records review, except for any information that must be maintained for screening-log purposes. Patients were given ample time to review and consider participation, and it was emphasized to the patient that their participation in the study would not influence in any way their future care management at MSKCC. Patients scheduled for 131I therapy, 223Ra (Xofigo) or [177Lu]Lu-DOTATATE were approached by their treating Nuclear Medicine Physician and given information with regards to the study. A consenting professional presented the study and discussed the potential risks/benefits, which were the same as the risks for a regular PET/CT exam. Upon their return for their scheduled 131I scan, patients were asked for their participation. Participants had to meet the eligibility criteria in order to be considered for the protocol objectives. If a patient was found to be ineligible, the patient was taken off the study and replaced. In most cases, the initial contact with the prospective subject was conducted by the treating physician/investigator or the research staff (CRC) working in consultation with the treating physician during the patient's routine clinic visit. The recruitment process outlined presents no more than minimal risk to the privacy of the patients who are screened, and minimal PHI was maintained as part of a screening log. For these reasons, we sought a (partial) limited waiver of authorization for the purposes of (1) reviewing medical records to identify potential research subjects and obtain information relevant to the enrollment process; (2) conversing with patients regarding possible enrollment; (3) handling of PHI contained within those records and provided by the potential subjects; and (4) maintaining information in a screening log of patients approached (if applicable).
Ethics oversight	This imaging study was approved by the MSKCC Institutional Review Board (IRB) under protocol 17-538.

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	https://clinicaltrials.gov/ct2/show/record/NCT03484884

Study protocol	The full protocol is available on request. Minimal eligibility and study information are available at https://clinicaltrials.gov/ct2/show/record/NCT03484884.
Data collection	Data were collected at the Memorial Hospital (single site) between May 2018 through March 2020, with PET and SPECT imaging equipment connected to the hospital-wide General Electric (GE) Picture Archiving and Communication System (PACS). Cerenkov images were acquired and stored on the clinical server of the radiology department.
Outcomes	This study is a follow-up to an initial study, which evaluated if the acquisition of Cerenkov signal from patients having received radiotracers (for routine clinical studies) is at all possible. We showed that clinical Cerenkov imaging is indeed feasible. In this study we explored several questions that arose from the initial study: (i) can we image Cerenkov with the fibrescope concept; (ii) what are the spectral characteristics of the Cerenkov signal and can we use it to obtain information on the depth of the signal; (iii) can we also obtain signal from deeper lying regions in the body (below the subcutaneous and axillary fat). The data was analysed to determine if the clinical dose of [18F]F-DG for PET/CT, 131-I, 223Ra, [68Ga]Ga-DOTATATE, or [177Lu]Lu-DOTATATE are sufficient for Cerenkov luminescence imaging with a fiberscopic device, which is the basis for future applications. Furthermore, we obtained spectral information on the Cerenkov signal (that is, in what part of the spectrum is the maximum) and evaluated if the spectral information (particularly the shift from the predominantly blue Cerenkov light to a more transmitted red part of the spectrum through scatter and absorption from overlying tissue) can provide information on the depth of the region. The Cerenkov tumour/background signal was obtained by comparing the ROI of the tumour area to that of the background anatomy. The initial outcomes were to determine feasibility, and to compare and correlate the Cerenkov imaging data with that of quantitative PET. The signal was assumed to be sufficient if the tumor/background signal was 2.0 or higher. We recruited 128 evaluable patients.