Dynamic changes in gene alterations during chemotherapy in metastatic castrate resistant prostate cancer

Winston Tan1,4, Tiantian Zheng2,4, Amy Wang2, Joanna Roacho2, Seng Thao2, Pan Du2, Shidong Jia2, Jianjun Yu2, Bonnie L. King2* & Manish Kohli3*

Docetaxel chemotherapy is a standard treatment option for metastatic castrate resistant prostate cancer (mCRPC) patients. To date, the genomic perturbations underlying the emergence of resistance in mCRPC patients during chemotherapy treatment have not been fully characterized. Previous studies have established that AR, TP53, RB1 and PTEN gene alterations are frequent at this stage of progression and that TP53, RB1 and PTEN, but not AR alterations are associated with poor outcome. However, the clonal dynamics of these key driver cancer genes during chemotherapy in mCRPC patients have not been described. Toward this goal, we performed a retrospective analysis of serially profiled cell-free DNA (cfDNA) alterations in blood samples collected from mCRPC patients before and after starting chemotherapy who were followed for response and clinical outcomes. While AR alterations and measures of mutational load were significantly reduced in patients with stable or decreased PSA levels after 3 cycles of chemotherapy, reductions in RB1, TP53 and PTEN alterations were relatively modest, which may represent the persistence of a clonal signature associated with the emergence of treatment-induced lineage plasticity (TILP) underlying resistance. The ability to monitor these driver gene clonal dynamics during chemotherapy may have utility in the clinical setting.

Prostate cancer (PCa) accounted for greater than 34,000 deaths in US males1 and over 325,000 deaths worldwide2 in 2021, with nearly all cancer-related mortality occurring in the metastatic state. The initial clinical management of metastatic prostate cancer is based on disruption of the androgen receptor (AR) signaling axis, and prior to 2015 this was achieved through the use of single agent androgen deprivation therapy (ADT). However, the clinical landscape has evolved rapidly and now includes treatment regimens based on a combinatorial approach of ADT with multiple drugs3. While ADT based combination treatments slow progression of disease, they inevitably fail in most patients with the emergence of castrate resistance4. Current standard-of-care treatment options for this stage of prostate cancer progression include different chemotherapeutic agents such as docetaxel and cabazitaxel, the androgen receptor signal inhibitors (ARSIs) abiraterone and enzalutamide, and radionuclides (Radium-223)3–7. However, there are currently no approved guidelines for choosing between these multiple approved drug treatment options8. Biomarkers are urgently needed to define which patients will benefit from the different treatment options in a first line setting, define the optimal sequence and combination of therapies, and track the response and emergence of treatment resistance. Toward that end, a variety of biomarkers including PSA8, alkaline phosphatase (ALP)8, βIII-tubulin9, CTC numbers10,11 and molecular alterations including AR-V7 splice variants12–17, plasma ctDNA alterations18–26 and plasma cell-free DNA (cfDNA)27–29 have been evaluated to predict and monitor response. However, none of these biomarkers have been clinically validated for choosing between therapies and their remains a critical need to understand the molecular profile and driver gene clonal dynamics during chemotherapy in order to maximize treatment accuracy and benefit.

In the present study, we used a targeted NGS-based liquid biopsy approach to profile serially obtained plasma samples collected prospectively and analyzed retrospectively in a cohort of mCRPC patients before and after undergoing docetaxel chemotherapy. Here we describe the dynamics of key driver gene alterations during chemotherapy.

1Department of Medicine, Mayo Clinic, Jacksonville, USA. 2Predicine, Inc., 3555 Arden Road, Hayward, CA 94545, USA. 3Division of Oncology, Department of Medicine, Jack R. and Hazel M. Robertson Presidential Endowed Chair, Huntsman Cancer Institute, University of Utah, 2000 Circle of Hope Dr. Rm. 4263, Salt Lake City, UT 84112, USA. 4These authors contributed equally: Winston Tan and Tiantian Zheng. *email: bking@predicine.com; Manish.kohli@hci.utah.edu
chemotherapy in association with treatment response and outcome, which may underlie the emergence of resistance and impact survival.

**Materials and methods**

**Patient enrollment and sample collection.** The current study was approved by the IRB of the Mayo Clinic. Metastatic prostate cancer patients were prospectively enrolled at a single tertiary-level cancer center (Mayo Clinic, Rochester, MN) after obtaining informed consent in an Institutional Review Board-approved study (MC IRB # 09-001889; “Study of Molecular Circulatory Biomarkers in Hormone Sensitive and Castration Recurrent Prostate Cancer”). All methods were carried out in accordance with relevant guidelines and regulations, and all experimental protocols were approved by the above-cited IRB-approved study at the Mayo Clinic. Serial blood samples were collected between September 2009 and January 2013 as previously described. Enrolled patients were followed for outcomes until June 2021. Methods for extracting cell-free (cfDNA) and germline DNA (gDNA) have been previously published and are also detailed in Supplementary Methods.

**Next generation sequencing (NGS) methods.** The details of library preparation, amplification, capture and sequencing, as well as the analysis of sequencing data have been previously described and are also provided in Supplementary Methods. Briefly, libraries were constructed using extracted cfDNA and fragmented gDNA, amplified by PCR and then subjected to hybrid capture using the PredicineCARE panel (Supplementary Table 1). Sequencing data were analyzed using Predicine’s in-house analysis pipeline encompassing the initial analysis of raw sequencing data base call files through variant calling. Details on variant calling, copy number variation (CNV) estimation methods, and the calculation of circulating tumor DNA (ctDNA) fraction and plasma Tumor Mutational Burden (pTMB) have been previously reported. Total alteration counts represent the total number of single nucleotide variants (SNVs) and CNVs detected per sample across all genes in the PredicineCARE panel. Top alteration counts represent the total number of SNVs and CNVs detected per sample across a set of top 32 most frequently altered genes. Variant allele frequencies (VAFs), representing the fraction of altered/total alleles, were normalized to ctDNA fractions.

**Statistical analysis methods.** Survival analysis was performed to evaluate associations of ctDNA-based alterations measured prior to the commencement of chemotherapy in the mCRPC state with overall survival (OS). This extends our pre-defined and published analysis reported after a median follow up for 94.67 months at a data-freeze date of June 2018, to June 2021 for a median follow-up of 130.6 months. OS was calculated from the date of first sample collection to the date of death or to the last follow-up for alive patients at the time of the cutoff date of the analysis. We also performed a retrospective exploratory analysis to determine clonal dynamic changes with treatment, in serially captured and previously sequenced ctDNA samples from mCRPC patients. Kaplan–Meier plots were used to show survival and the log-rank test was used for comparing survival differences between groups. Associations of variables with OS were also evaluated by univariate and multivariate analyses using Cox proportional hazards regression and the log-rank test. Scaled Schoenfeld residuals and variance residuals with time were examined to ensure the validity of the Cox regression assumptions. To account for multiple hypothesis testing, adjusted P-values using the Benjamini and Hochberg procedure are reported. Age, ctDNA fraction, Gleason score and alkaline phosphatase levels were included in the multivariate analysis of OS in the mCRPC state. To dichotomize the patient cohorts for certain variables, the upper quartile cutoff was used for ctDNA fraction, and the median cutoff was used for alkaline phosphatase levels. A Gleason Score of 8 or above was defined as high, and a score of less than or equal to 7 was defined as low. Paired Wilcoxon tests were performed to examine differences of ctDNA, pTMB, total number of alterations, total SNV counts, total CNV counts and VAFs between paired patient samples collected before and after three months of chemotherapy. McNemar’s test was used to evaluate differences in the frequency of alterations in specific genes across paired patients before and during chemotherapy. The Wilcoxon test was used to examine differences of ctDNA fraction, pTMB and total alteration counts in unpaired patients before vs. during chemotherapy. The Fisher’s exact test was used to evaluate differences in the frequency of mutations across specific genes in unpaired patients before and during chemotherapy. All statistical analyses were performed using R version 3.5.3 and all tests of statistical significance were two-tailed with a significance set at p ≤ 0.05.

**Results**

**Patient enrollment, study design and treatment groups.** Three hundred and three metastatic prostate cancer patients were prospectively enrolled at the Mayo Clinic from September 2009 to January 2013, and followed until death, with a cutoff date of June 13, 2021, for analyses. This study sub-cohort is composed of 101 of the 303 patients who experienced clinical progression (defined by biochemical failure and/or the appearance of new radiographic metastases) during androgen deprivation therapy (ADT), and subsequently underwent chemotherapy for the mCRPC state. All patients with sufficient plasma sample volumes for cfDNA extraction were included. The workflow for plasma samples collected and processed for analyses from these patients is shown in Supplementary Fig. S1. Samples were collected prior to the commencement of chemotherapy, and after 3–4 chemotherapy treatments to allow adequate drug exposure (Fig. 1a). Of the 52 samples collected prior to chemotherapy, 30 patients were sampled serially a second time during chemotherapy, creating a subset of paired samples (before chemotherapy, n = 30; after at least 3 cycles of chemotherapy, n = 30). An additional 49 samples were collected from a separate cohort of mCRPC patients when they had received at least three to four cycles of chemotherapy, but did not have matched pre-chemotherapy samples (unpaired sample set, n = 49). The demographic data for all patients (n = 101) are described in Table 1.
Figure 1. Landscape of somatic alterations detected in mCRPC patients before and during chemotherapy. (a) Plasma samples were collected from mCRPC patients following clinical progression on androgen deprivation therapy (ADT), prior to the initiation of chemotherapy (n = 52). Thirty of these patients were serially sampled during chemotherapy to create a subset of paired samples (before chemotherapy, n = 30; after 3–4 chemotherapy cycles, n = 30). Forty-nine samples were collected from an independent group of patients following 3–4 chemotherapy cycles (n = 49). (b) Venn diagram illustrating the overlap of the 4 most frequently altered genes (AR, TP53, RB1 and PTEN) detected at baseline in individual patients. (c) Heatmap of somatic alterations including SNVs and CNVs detected across all samples collected before and after 3–4 cycles of chemotherapy. The percentage of alterations detected in each gene across all patient samples is shown to the right of the heatmap. The total number of alterations detected in each patient sample is shown in the bar graph above the heatmap. This figure was created by manuscript authors using the Adobe Illustrator 2020 (https://www.adobe.com/products/illustrator.html) (a), VennDiagram (version 1.7.1) (https://cran.r-project.org/web/packages/VennDiagram/index.html) (b), and Bioconductor (https://www.bioconductor.org/packages/release/bioc/html/mapttools.html) (version 3.14) (c) software packages.
Genomic landscape of ctDNA-based alterations across all mCRPC (paired and unpaired) patient sub-cohorts. To examine the spectrum of genomic alterations in mCRPC patients before and during chemotherapy, we extracted cell-free DNAs from patient plasma. SNVs and CNVs were profiled with a targeted NGS panel (PredicineCARE). We determined measures of ctDNA fraction, pTMB and total alteration counts across all patient samples collected before and during chemotherapy. Figure 1b shows overlap of the top 4 most frequently altered genes (AR, TP53, RB1 and PTEN) in some patients. Figure 1c shows the top 20 most frequently altered genes in ≥ 5% of all study patient samples before and during chemotherapy. The most frequently altered genes across all patient samples were AR (44%), TP53 (38%), RB1 (23%), PTEN (18%), ATM (15%), PIK3CA (15%), BRCA2 (12%), CDH1 (12%) and BRAF (10%). These most frequently altered genes were selected for analysis of association with the overall survival for the sub-cohort of 52 patients whose initial samples were collected prior to chemotherapy.

Table 1. Clinical characteristics of metastatic castrate resistant prostate cancer (mCRPC) patients undergoing chemotherapy following clinical progression on androgen deprivation therapy (ADT).
Patient outcomes based on somatic alterations detected before chemotherapy. To investigate the potential prognostic and predictive value of ctDNA-based alterations, we evaluated overall survival (OS) in association with cfDNA yield, ctDNA fraction, pTMB, SNVs and CNVs assessed in the sub-cohort of 52 patient samples collected prior to initiating chemotherapy. High (upper quartile) ctDNA fraction was associated with shorter OS (p = 0.0008) (Supplementary Fig. S2), whereas no significant reduction was observed in association with cfDNA yield or pTMB (Supplementary Fig. S3). At the univariate level, the presence of a CNV or SNV in RB1 (p < 0.0001), TP53 (p = 0.003), AR (p < 0.0001) or CDH1 (p = 0.0005) (Supplementary Fig. S2), was significantly associated with shorter OS. In multivariate analyses (Supplementary Table 2), after adjustment for age, ctDNA fraction, Gleason Score and alkaline phosphatase levels, the presence of a SNV or CNV in the TP53 (p = 0.009), CDH1 (p = 0.007), RB1 (p = 0.01), or PTEN (p = 0.01) genes independently remained significantly associated with shorter OS. Genes significantly associated with OS before and after adjustment for multiple clinical and genomic covariates are listed in Supplementary Table 2.

Comparison of ctDNA-based profiles across the unpaired patient sub-cohorts. To compare the landscape of ctDNA alterations in mCRPC chemotherapy-naïve patients vs. patients after the initiation of chemotherapy treatment, we analyzed genomic profiles across the unpaired patient sub-cohorts. No differences were detected in ctDNA-based profiles between the independent groups of patients who had not started chemotherapy vs. patients who had received 3–4 cycles of chemotherapy (Supplementary Fig. 4).

Pharmacodynamic changes in ctDNA-based alterations during chemotherapy in the paired patient sub-cohort. In the paired cohort, to identify changes associated with PSA-based response to chemotherapy, we compared ctDNA profiles across the set of patients with serial samples collected before and after 3–4 cycles of treatment. For associating changes with clinical outcomes in this sub-cohort of paired samples from the same patient, we classified patients into two groups based on PSA response after 3–4 treatments. Patients exhibiting a 25% or greater increase in PSA level at the time of the serial sample collection after three to four chemotherapy treatments were classified as 3-month PSA non-responders, and the remaining patients were classified as the “PSA stable to decrease” group who continued on the same chemotherapy in line with standard of care decided by the treating physician.

The landscape of ctDNA-based alterations across the paired patient sample sets collected from both groups is presented in heatmaps in Fig. 2a. Figure 2b shows that the frequency of alterations in most of the top-mutated genes trended lower after 3–4 cycles of chemotherapy, but most of these trends were not significant. The one exception to this trend was a non-significant increase in the frequency of RB1 alterations in the patients with stable or decreased PSA levels on chemotherapy. Notably, the only gene alteration to decrease significantly after chemotherapy in the paired samples was AR. After 3–4 cycles of treatment the frequency of AR alterations decreased substantially in patients with stable or decreased PSA at 3-months (53–12%; p = 0.03) but not in patients in the 3-month PSA non-responder group (50–42%; p = 1). The high frequency of AR alterations across patients enabled additional comparison of individual AR VAFs, which were also significantly reduced during chemotherapy across all patients (p = 0.002) (Fig. 3a). Reductions in VAF levels were also observed in patients with stable or decreased PSA levels (p = 0.08) and patients with increased PSA levels (p = 0.02), although due to the small number of AR SNVs in each group, distinct differences could not be detected between the two groups (Fig. 3b,c). Both ctDNA fraction (p = 0.001) and pTMB (p = 0.004) were also significantly reduced across all serial samples (Fig. 4a). While ctDNA fraction reductions were similar in both 3-month PSA groups (Fig. 4b), the reduction in pTMB was significantly greater in patients with stable or decreased PSA levels (p = 0.001) than in non-responders (p = 0.91) (Fig. 4c). Total alteration counts (including SNVs and CNVs) were also reduced in serial samples after chemotherapy in patients with stable or decreased PSA levels (p = 0.03) and in 3-month PSA non-responders (p = 0.06), (Fig. 4d). When total alterations were assessed for the top 32-mutated genes only, top alteration counts were significantly reduced in patients with stable or decreased PSA levels (p = 0.007), but to a lesser degree in the 3-month PSA non-responders group (p = 0.08) (Fig. 4e). Similar comparisons focusing exclusively on SNV or CNV counts revealed significant decreases in SNV counts after chemotherapy in the patients with stable or decreased PSA levels (p = 0.01) but not in the PSA non-responders (p = 0.57). No significant reductions were observed for CNV counts in either group (Supplementary Fig. 5).

Discussion
In this study we confirmed the association of RB1, TP53 and PTEN, but not AR gene alterations detected in mCRPC patients prior to initiating chemotherapy with poor OS. We also observed that dynamic changes in these and other gene alterations can be tracked during chemotherapy treatment, which has not been previously reported. Our results illustrate the potential utility of NGS-based sequencing to monitor the clonal evolutionary changes during treatments, which could potentially be useful for developing adaptive strategies that target the emergence of resistance. Recent therapeutic advances in the treatment of mCRPC have prolonged survival and increased quality of life, but therapeutic benefits are temporary, and further research is needed to develop genome-based biomarkers that may be used to optimize benefit and maximize treatment accuracy. Given the high frequency of acquired therapeutic resistance during metastatic progression, with the highest frequency of alterations most consistently observed in AR, TP53, RB1, PTEN, APC and DNA repair genes, in keeping with findings reported by others24–26. Although genomic features of this cohort have been reported previously33,37,38,
the analyses undertaken in this study focuses on the effect of serial sequencing using a targeted and sensitive NGS panel, which has not been explored. In this study we have characterized genomic profiles of mCRPC patients before and after initiating chemotherapy. Following treatment, we observed significant reductions in several genomic indices, including ctDNA fraction (presumably reflecting reductions in tumor cell numbers), total variant counts and pTMB. We were also able to track VAFs for individual AR mutations, which were also significantly reduced following chemotherapy. To further evaluate these indices and alterations as potential biomarkers for monitoring chemotherapy response, we also compared post-chemotherapy profiles in patients classified as on the basis of post-treatment PSA levels. This comparison revealed significantly greater reductions in AR alterations, pTMB and total alteration counts (for the top 32 mutated genes) in patients with stable or decreased PSA after three months of chemotherapy vs. those patients experiencing a PSA increase, suggesting that AR alterations and comprehensive measures of mutational load are promising candidate biomarkers of response to chemotherapeutic drugs.

We also demonstrated that the presence of TP53, RB1 and PTEN but not AR alterations at baseline in mCRPC patients undergoing chemotherapy significantly defined survival outcomes. Interestingly, we observed that patients responding to systemic chemotherapy at 3-months based on PSA response alone had a notable decrease in AR, but not RB1, TP53 or PTEN alterations. The frequency of AR alterations was significantly reduced in

Figure 2. Pharmacodynamic changes in genomic alterations in response to chemotherapy. (a) Heatmaps of somatic alterations detected in paired mCRPC samples collected before (top) and after (bottom) 3–4 cycles of chemotherapy from patients who were classified for response to chemotherapy on the basis of PSA levels at the time of second sample collection. Patients exhibiting stable or decreased PSA levels were classified as “PSA Stable/Decrease” and patients exhibiting increased PSA levels as “PSA Non-Responders”. The frequency of alterations observed in a given gene across all patients is listed to the right of the heatmaps. (b) Graphical representation of heatmap data for the top 10 most frequently altered genes across all paired patients combined (n = 29), patients with stable or decreasing PSA (n = 17) and patients with increasing PSA levels (n = 12) before and after 3–4 cycles of chemotherapy. The frequency of alterations detected in the AR gene was significantly reduced after chemotherapy in the group of patients with stable/decreasing PSA (p = 0.03). Comparisons were made using the McNemar’s test with significance set at p ≤ 0.05. The heatmaps in this figure were created by manuscript authors using the Bioconductor ComplexHeatmap software package (version 3.14) (https://www.bioconductor.org/packages/release/bioc/html/ComplexHeatmap.html).
patients with stable or declining PSA values, with AR alterations found in 53% of patients before vs. 12% after chemotherapy. Based on these observations, while it is unclear if chemotherapy selectively targeted cancer cells bearing AR alterations, it significantly reduced them, while less robust reductions were observed for other key clonal alterations such as TP53, RB1 and PTEN. As these gene alterations may be drivers of resistance in the mCRPC state, pharmacodynamic profiling, if performed in real time in future patients, might present a clearer picture for identifying adaptive selection of therapeutic drugs. Of note, the presence of TP53, RB1 and PTEN alterations have previously been associated with poor patient outcomes related to TILP and the generation of an aggressive neuroendocrine or adeno-neuroendocrine tumor phenotype  and39–41. Although in our cohort we did not perform metastatic tissue biopsies to identify amphicrine prostate cancer histology, reduction in AR alterations in blood on serial monitoring in parallel with less dramatically altered levels of TP53, RB1 and PTEN alterations suggest clonal dynamics consistent with the association of poor patient outcomes with these gene alterations in our study.

A primary limitation of our study includes the small size of our exploratory, retrospective analysis of patients with serial samples, which limited the comparison of clonal dynamics between patients with stable versus increasing PSA after 3 months of chemotherapy. In addition, we were not able to correlate plasma profiles with metastatic tissue sampling and limited our sequencing to a targeted panel of genes. Nevertheless, the serial NGS-based sequencing was able to detect candidate molecular biomarkers during a PSA-based response to chemotherapy and identify dynamic clonal patterns that may have relevance to monitoring for the development of TILP. These will need to be systematically determined in larger prospective cohorts, and if successful may enable the future development of adaptive therapeutic strategies.

**Data availability**
The annotated sequencing data that support the findings of this study are available in an Excel file labeled “Raw Sequencing Data” in the supplementary materials.
Figure 4. Pharmacodynamic changes in ctDNA fraction, pTMB and total alteration counts in response to chemotherapy. Comparisons were made in patients who were classified for response to chemotherapy on the basis of PSA levels at the time of second sample collection following 3–4 cycles of treatment. Patients exhibiting stable or decreased PSA levels were classified as “PSA Stable/Decrease” and those exhibiting increased PSA levels as “PSA Non-Responders”. (a) Median ctDNA fraction ($p = 0.001$) and pTMB ($p = 0.004$) were significantly reduced in the group of combined patients ($n = 29$) after 3–4 cycles of docetaxel. (b) Similar significant reductions in ctDNA fraction were observed for the “PSA Stable/Decrease” patients ($n = 17$, $p = 0.03$) and the “PSA Non-Responder” patients ($n = 12$, $p = 0.03$). (c) Median pTMB was significantly reduced in the “PSA Stable/Decrease” patients ($p = 0.001$) but not the “PSA Non-Responder” patients ($p = 0.91$). (d) Similar reductions were observed in median total alteration counts for the “PSA Stable/Decrease” patients ($p = 0.03$) and the “PSA Non-Responder” patients ($p = 0.06$). When this comparison was restricted to the top 32 most altered genes, values were significantly reduced in the “PSA Stable/Decrease” patients ($p = 0.007$), and modestly in the “PSA Non-Responder” patients ($p = 0.08$). Comparisons were made using the paired Wilcoxon test with significance set at $p \leq 0.05$.

Received: 14 September 2021; Accepted: 22 February 2022
Published online: 18 March 2022

References
1. Siegel, R. L., Miller, K. D., Fuchs, H. E. & Jemal, A. Cancer statistics, 2021. CA Cancer J. Clin. 71, 7–33. https://doi.org/10.3322/caac.21654 (2021).
2. Sung, H. et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J. Clin. https://doi.org/10.3322/caac.21660 (2021).
3. Mottet, N. et al. EAU-EANM-ESTRO-ESUR-SIOG guidelines on prostate cancer-2020 update. Part 1: Screening, diagnosis, and local treatment with curative intent. Eur Urol 79, 243–262. https://doi.org/10.1016/j.eururo.2020.09.042 (2021).
4. Tan, J.-L. et al. Androgen receptor targeted therapies in metastatic castration-resistant prostate cancer—the urologists’ perspective. Urol. Sci. 28, 190–196 (2017).
41. Beltran, H. et al. Circulating tumor DNA profile recognizes transformation to castration-resistant neuroendocrine prostate cancer. *J. Clin. Investig.* **130**, 1653–1668. https://doi.org/10.1172/JCI131041 (2020).

**Author contributions**
Conception and design: M.K., B.K.; Acquisition of data: W.T., M.K.; Sample analysis: A.W., J.R., S.T.; Data generation: PD; Analysis and interpretation of data: W.T., T.Z., B.K., M.K., P.D., J.Y.; Drafting of the manuscript: W.T., M.K., B.K.; Critical revision of the manuscript for important intellectual data: W.T., J.Y., S.J., M.K., B.K.; Obtaining funding: M.K.

**Funding**
This study was supported by the National Institute of Health- RO1-CA212097 (to Manish Kohli).

**Competing interests**
WT and MK have no conflicting interests to report. TZ, AW, JR, ST, BK, JY, PD and SJ are employees and stockholders of Predicine, Inc.

**Additional information**

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1038/s41598-022-08520-6.

**Correspondence** and requests for materials should be addressed to B.L.K. or M.K.

**Reprints and permissions information** is available at www.nature.com/reprints.

**Publisher’s note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

**Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2022, corrected publication 2022