

Multomics comparison between prostate cell lines and patient-derived tissue to evaluate cell line clinical relevance

Corresponding Author: Dr Uzoamaka Okoli

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

Reviewer's Comments for Nature Communications Medicine

Summary of Work

This manuscript presents a comprehensive analysis of 46 prostate cancer (PCa) cell lines, evaluating their clinical relevance and representativeness compared to patient populations. The authors integrated clinical and multiomics data from over 10,000 patient cases across 24 studies to assess how well commonly used PCa cell lines represent actual patient demographics, disease characteristics, and molecular profiles. The study proposes a systematic framework to guide researchers in selecting appropriate cell lines for preclinical studies.

Positive Aspects

The authors address a critical issue in cancer research - the disconnect between preclinical models and clinical reality, which contributes to high drug failure rates. The comprehensive analysis of 46 PCa cell lines against large patient cohorts is commendable, and the proposed three-step selection pipeline for choosing clinically relevant cell lines provides practical value for researchers. The integration of multiple data sources (cBioPortal, COSMIC, CCLE) represents a thorough approach to the research question.

Assessment of Approach and Objectives

The approach partially satisfies the objectives. While the comprehensive comparison of cell lines to patient data is valuable, the analysis is limited by incomplete data availability - only 7 of 46 cell lines had sufficient multiomics data for detailed analysis. The systematic framework proposed is logical but would benefit from validation studies demonstrating improved outcomes when following these guidelines.

Soundness of Findings

The findings are generally sound and confirm suspected issues with cell line representativeness. The revelation that most used PCa cell lines derive from metastatic cases and poorly represent early-stage disease is important. However, the limited molecular data available for most cell lines (39/46) significantly constrains the comprehensiveness of these conclusions.

Major Issues

1. Figure Numbering and References

- Figure references are inconsistent throughout the manuscript. Figures, tables and supplementals should be numbered as they are mentioned.

- What is/where is Fig. 1B? - I suppose this is 2B?" needs to be addressed.

- Check all figures.

2. Manuscript Structure and Flow

- The manuscript reads as disconnected with poor transitions between sections.

- The flow from clinical characteristics to molecular analysis to the selection framework is abrupt and needs better linking text.

3. Grammatical and Language Issues

- Multiple grammatical errors throughout the manuscript

- Inconsistent terminology usage

- Several unclear or awkwardly constructed sentences that impede comprehension

4. Figure Legends

- Figures lack complete descriptive legends. Each figure needs both a title AND a detailed legend explaining what is shown, including sample sizes, statistical tests used, and abbreviation definitions.

5. Clinical Cohort Definition

- The "clinical cohort" is referenced throughout without clear initial definition
- The relationship between the 10,284 total patients mentioned and the various subset analyses is unclear
- Selection criteria for inclusion in different analyses need clarification

6. Statistical Concerns

- The claim "9% of PCa cell line cohort" needs context - 9% of what total?
- Statistical methods section is too brief for the complexity of analyses performed
- No power analysis or discussion of multiple testing corrections

Additional Issues Not Previously Mentioned

7. Data Completeness and Limitations

- Only 7/46 cell lines had complete multiomics data, severely limiting the generalizability of molecular findings
- No discussion of how missing data might bias conclusions
- The authors should acknowledge this as a major limitation upfront

8. Validation of Proposed Framework

- The three-step selection framework lacks validation
- No examples of how following this framework might improve research outcomes
- No comparison of studies using "appropriate" vs "inappropriate" cell lines according to their criteria

9. Temporal Considerations

- Many cell lines were established 40+ years ago - no discussion of how genetic drift or culture conditions might affect current relevance
- Also one recommendation not included is the need for more diverse cell lines to be constantly developed.

10. Practical Applicability Issues

- The framework suggests using multiple cell lines but doesn't address practical constraints (cost, availability, technical expertise)
- No discussion of which cell lines are commercially available vs. requiring MTAs

Specific Comments by Section

Introduction

- Needs stronger justification for why this analysis is needed now
- Should better define what constitutes "clinical relevance" for a cell line
- The 0.1% drug approval rate claim needs a more recent reference

Methods

- Insufficient detail on data harmonization across different databases
- Statistical methods need expansion, including how missing data was handled
- Criteria for selecting the 46 cell lines should be explicit

Results

- Section 2.1.1 title "History of immortalization" doesn't match the content about citations and patents
- The geographical bias in cell line origin is important but needs connection to patient diversity
- MSI prevalence discrepancy (63.64% in cell lines vs 0.43% in patients) needs more prominent discussion

Discussion

- Overreliance on speculation about why preclinical studies fail
- Limited integration with recent literature on improving relevance
- The limitation section is too brief given the significant data gaps

Conclusions

- Should more clearly state which specific cell lines are most/least representative
- Needs actionable recommendations beyond the proposed framework

Journal Compliance Issues

Nature Communications Medicine Guidelines:

1. Data Availability: Must specify which datasets are publicly available and which require permissions
2. Code Availability: Any custom analysis code should be made available
3. Reporting Guidelines: Should follow relevant guidelines for observational studies
4. Supplementary Information: Extensive supplementary data mentioned but organization unclear

References

- References appear complete and properly formatted
- However, missing some key recent papers on:
 - o Cell line authentication and quality control (2023-2024)
 - o failures oncology (2023-2024)
 - o Patient-derived models as alternatives to cell lines

Recommendations for Improvement

1. Restructure the Results: Organize by research question rather than data type
2. Enhance Visual Communication: Create a summary figure showing which cell lines best represent different patient subgroups
3. Address Data Gaps: Be more transparent about limitations and suggest how to obtain missing data
4. Validate Framework: Include case studies or examples of improved outcomes using the selection criteria
5. Practical Guide: Create a user-friendly table or flowchart for cell line selection

Minor Points

- Define all abbreviations at first use (MSI, TMB, CNAs, SVs)
- Consistent use of "prostate cancer" vs "PCa"
- Specify which Gleason grading system is used (classic vs. ISUP)
- Clarify if "Black" and "African American" are used interchangeably

Recommendation

Major revisions required. While this study addresses an important question about the clinical relevance of prostate cancer cell lines, significant improvements in presentation, data completeness acknowledgment, and practical applicability are needed. The core findings are valuable, but the manuscript requires substantial revision to meet publication standards and provide maximal utility to the research community.

Reviewer #2

(Remarks to the Author)

This study integrates clinical and multi-omics data to assess the relevance of 46 prostate cancer (PCa) cell lines. The authors argue that the most commonly used cell lines accurately represent only a small subset of patients.

I agree that this article is useful for guiding the selection of representative cell lines. However, the authors should consider the following points:

Major Comments

Comment 1

It is inherently difficult to argue that a cell line faithfully represents a specific cohort of prostate cancer patients. Even when a cell line shares genetic or clinical background characteristics with a patient cohort, their biological behavior may diverge significantly. During the adaptation to 2D culture conditions, for example, cancer cells undergo morphological changes, and more aggressive clones are often selected. As the authors noted, preclinical PCa models may overrepresent MSI-driven tumors, which comprise only a small fraction of actual patient cases (see Figure 3).

Therefore, patient-derived xenograft or organoid models should be considered for most prostate cancer research, particularly when the goal is clinical relevance. Cell line models are best suited for mechanistic studies such as epigenomic regulation of gene expression, protein–protein interactions, or signaling pathway dissection.

Comment 2

The authors should provide a direct comparison of gene expression profiles between the PCa cell lines and primary patient samples. For instance, principal component analysis (PCA) plots based on RNA expression data from both sources could help visualize their similarities or differences. Please refer to Figure 1a in the following article for a useful example: PMID: 34489572.

Minor Comments

There are several typographical errors in the manuscript and figures. The authors should carefully proofread the text. Examples include:

"Figure 3A" should be "1A" (Page 4, Line 81)

"Figure 3A" should be "2A" (Page 5, Line 98)

The labeling in Figure 3I is unclear and not representative

In Figure 4A, the term "KEGG" should be removed from the bottom of the bar chart

Reviewer #3

(Remarks to the Author)

The manuscript submitted by Ahmed and colleagues from the team of Uzoamaka Okoli and Susan Heavey is an attempt at a comparative analysis of commonly used cell line models with population and clinical data. Unfortunately, it requires extensive improvement to be published in the journal Communications Medicine.

The major concerns are as follow:

- 1) As the title of the manuscript is 'How clinically relevant and inclusive are prostate cell lines?' I do not understand why benign prostate cell lines (like RWPE-1, HPr-1, BPH-1...) were omitted. I believe they should have been added to the article to present complete data on the comparison of cell line models with population data.
- 2) It is very important to clearly indicate the source or year of the source data used for the analysis. For example, Figure 3 shows the mixed genetic origin of the 22Rv1 line, which is consistent with publications up to 2018, while a more comprehensive analysis published in PMID: 30787054 established that the 22Rv1 cell line is 99% of European origin. Each figure description should be accompanied by the year of information on which such a comparative analysis is based, not just general information like in Supplementary Table 2 .
- 3) All analyses should be based on the latest available data. Why does Figure 2C show data from 2020, while more recent data is publicly available.
- 4) There is no marked D in Figure 2. The description of Figure 3c indicates that it shows "cancer stage of cell lines (N=33), clinical cohort (N=1593) and PCa patients worldwide (N=9481)", whereas Figure 3C does not show results for cancer stage.

There are no marked D, E, F, G, H, I, J, K in Figure 3. Figure 4 does contain any A, B, C, D mark.

5) What is the abbreviation for CC and CL in Figure 1? Why were these abbreviations used in the last box, although not in others. What do the abbreviations AS and AI mean in Figure 5? According to Figure 5A, the PC-3 line represents both Cancer stage 2 and 4? What is the difference between “clinical cohort” and “worldwide” in Figure 3? What are the exact sources of the data and the year of their publication? Such information is crucial to assess the relevance of such comparisons?

6) The discussion is very long and does not focus much on the really important comparative aspects. I think this should be fundamentally revised. Apart from that, there is a lot of data in the supplementary data. I think the most informative ones could be moved to the main manuscript e.g. Figure 1 does not show any particular data in my opinion. Instead, I suggest adding to the main manuscript, e.g. Supplementary Table 8, which contains some specific information.

7) Most of the graphics are of very poor quality and low attractiveness for the potential reader. Too small font size, overlapping colours and captions. This should definitely be improved.

8) The text needs very thorough improvement as it contains many repetitions, e.g. in lines 53-54, editorial and grammatical errors. The English should be significantly improved, as at some points it is unclear what the author meant, especially in the Figure captions.

Version 1:

Reviewer comments:

Reviewer #2

(Remarks to the Author)

Thank you for responding to my previous comments and for generating the PCA plots in the patient cohort and cell lines as suggested. However, these new analyses unfortunately reinforce my original concern regarding the authors' main argument that the most commonly used cell lines “accurately represent” a subset of patients.

In Figure 6A, there is a clear separation between the cell line cluster and the patient cluster, indicating that gene expression profiles in the cell lines have been substantially altered during adaptation to 2D culture. Even if a cell line originates from a background similar to that of the patient cohort, its current behaviour appears far removed from the original cancer tissue. In addition, from a clinical standpoint, there are major differences in treatment decision-making between AR-positive prostate adenocarcinoma and AR-negative NEPC. Yet in Figure 6, LNCaP (AR-positive adenocarcinoma) and NCI-H660 (AR-negative NEPC) fall into the same cluster and are both distant from the patient clusters.

As I noted in my first-round review, the field has increasingly moved away from relying on conventional cell line experiments to assess clinical relevance, precisely because these models are morphologically and biologically different from patients' tumours. In this context, it is difficult to support the manuscript's central claim that the most frequently used cell lines accurately represent a small subset of patients. Indeed, the PCA in Figure 6A/B appears to provide counter-evidence to that conclusion rather than supporting it.

Reviewer #3

(Remarks to the Author)

The manuscript still contains many typos and grammatical errors, repetitions and should undergo professional editing. Many of them can be found in the “Introduction” section alone, e.g., double dots, double spaces, missing spaces (andimmune), p PCa (what does small p mean? no explanation), 15.2% (what does a multiplication sign mean? should it be a comma?). If the authors were unwilling to include benign prostate cell lines in their analysis, then I believe that the title of the article is misleading and should be changed to: How clinically relevant and inclusive are prostate cancer cell lines?

However, overall, the content of the article seems interesting and, after careful editing, could be published in the journal Communications Medicine.

I have also carefully read all the concerns raised by Reviewer 1. Some of them appear to have been properly addressed and some additional analyses have been included. However, in general, I believe that there are still many fields that require improvement, especially the description of the figures and methodology, and the presentation of the results. Issues concerning the validation of the proposed three-stage selection framework were not discussed in detail (Comment 9). No comparison was made between studies using “appropriate” and “inappropriate” cell lines according to the authors' criteria. Furthermore, the issue of data completeness and functional significance was not satisfactorily addressed in the discussion section. What were the culture conditions of the cells analyzed by multiomic assays—were they seeded on coated plates or on regular plastic in 2D? It is not surprising that principal component analysis (PCA) revealed big differences in the activation of transcriptional programs between prostate cancer cell lines cultured in 2D on plastic and patient tumors growing in their “physiological” environment. This has been published in several articles. Recently, there has been a lot of hope for 3D cultures in defined hydrogels, as recent results suggest that cell lines cultured in such hydrogels can restore a gene expression profile more similar to that of primary tissues. This could suggest that the utilization of established cell lines is still justified if appropriate culture conditions are applied. That point should be properly addressed in the discussion section. Moreover, the manuscript still contains multiple grammatical and linguistic issues, as well as several unclear or awkwardly constructed sentences that impede comprehension and definitely require improvement at that field.

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Yellow – Zahra
 Green – Uzoamaka
 Blue - Warda
 Pink - Devansh

Reviewer Comment	Author Comments	Manuscript Revisions
Reviewer 1:		
Comment 1 This manuscript presents a comprehensive analysis of 46 prostate cancer (PCa) cell lines, evaluating their clinical relevance and representativeness compared to patient populations. The authors integrated clinical and multiomics data from over 10,000 patient cases across 24 studies to assess how well commonly used PCa cell lines represent actual patient demographics, disease characteristics, and molecular profiles. The study proposes a systematic framework to guide researchers in selecting appropriate cell lines for preclinical studies. Positive Aspects The authors address a critical issue in cancer research - the disconnect between preclinical models and clinical reality, which contributes to high drug failure rates. The comprehensive analysis of 46 PCa cell lines against large patient cohorts is commendable, and the proposed three-step selection pipeline for choosing clinically relevant cell lines provides practical value for researchers. The integration of multiple data sources (cBioPortal, COSMIC, CCLE) represents a thorough approach to the research question. Assessment of Approach and Objectives The approach partially satisfies the objectives. While the comprehensive comparison of cell	We thank the reviewer for this constructive and insightful comment. In response, we have undertaken a comprehensive transcriptomic validation of our proposed prostate cancer (PCa) cell line selection framework using RNA-seq data from five PCa cell lines (LNCaP, VCaP, 22Rv1, DU145, and NCI-H660) and the TCGA Prostate Adenocarcinoma (TCGA-PRAD) cohort (n = 550). This validation directly assesses the framework's robustness and translational relevance by comparing cell line model and patient cohort transcriptomes along a biologically meaningful axis (Figure 6). To address the reviewers' specific suggestion, we performed a Principal Component Analysis (PCA) of merged, z-score-normalized RNA-seq data obtained from the Cell Model Passport database (cell lines) and the TCGA-PRAD cohort (primary tumours). PCA revealed a clear and statistically significant separation between prostate cancer cell lines and patient tumours along the first principal component (PC1), indicating distinct global transcriptomic profiles between in vitro models and clinical disease (Figure 6A). When overlaid by the five-gene composite signature (TP53, PTEN, TMPRSS2, FOXA1, and ERG; Figure 6B), the PCA demonstrated that this signature quantitatively captures the same dominant axis of variation ($r = 0.91$, $p < 0.001$). A heatmap of these genes (Figure 6C) confirmed consistent expression trends with genomic alterations described in Figure 3 namely, TMPRSS2/FOXA1/ERG activation and PTEN/TP53 downregulation. Thus, representing canonical AR-driven and tumour-suppressor-loss pathways, respectively. By deriving a signature score to quantify each sample's similarity to the average 'Cell line' or 'Patient' expression profile, we classified all samples into Cell line-like, Intermediate, or Patient-like categories (Figure 6D). This stratification revealed a clear gradient from androgen-dependent luminal models toward androgen-independent or neuroendocrine models reflecting their known phenotypic and genomic characteristics. A subset of TCGA tumours exhibited 'Cell line-like' expression profiles, further validating that this classification captures biologically relevant variation within clinical prostate cancer. Among cell lines, VCaP exhibited the highest signature score and clustered closest to patient tumours, consistent with its strong androgen receptor (AR) activity and TMPRSS2-ERG	See results for validation

lines to patient data is valuable, the analysis is limited by incomplete data availability - only 7 of 46 cell lines had sufficient multiomics data for detailed analysis. The systematic framework proposed is logical but would benefit from validation studies demonstrating improved translational outcomes when following these guidelines.	rearrangement. In contrast, DU145 and NCI-H660 showed the lowest signature scores, clustering farthest from patient samples and reflecting their androgen-independent and neuroendocrine phenotypes. VCaP and LNCaP thus represent the most 'patient-like' transcriptional profiles, while DU145 and NCI-H660 are more divergent, underscoring differences in AR dependence and tumour-suppressor pathway integrity. Together, these results empirically validate our proposed selection framework proposed in Figure 5 and demonstrate that the five-gene signature captures the biological opposition between AR-driven luminal differentiation and tumour-suppressor-loss–driven dedifferentiation. Specifically: • Upregulation of TMPRSS2, FOXA1, and ERG marks active AR signalling and luminal differentiation. • Downregulation or loss of PTEN and TP53 indicates tumour-suppressor inactivation, lineage plasticity, and dedifferentiation. • The balance between these gene sets determines each sample's position on the PCa biological continuum from AR-dependent luminal to AR-independent, dedifferentiated states. This relationship is quantitatively modelled as: Signature Score = mean [z (TMPRSS2, FOXA1, ERG)] - mean [z (PTEN, TP53)] where z denotes z-score - normalized expression across samples. The subtraction term explicitly models the biological opposition between differentiation and dedifferentiation programs. By providing a quantitative and biologically interpretable validation of the proposed framework, these findings establish a reproducible method for mapping preclinical models to clinical transcriptomic space. This approach guides selection of that prostate cancer cell line models based on molecular fidelity to patient disease, thereby improving translational relevance, model reproducibility, and biological interpretability in future PCa research. Data Sources: • Cell Model Passport: Dieudonne van der Meer et al., 2019, Cell Model Passports—a hub for clinical, genetic and functional datasets of preclinical cancer models, Nucleic Acids Research, Volume 47, Issue D1, Pages D923–D929, https://doi.org/10.1093/nar/gky872 • TCGA-PRAD: The Cancer Genome Atlas Research Network. (2015). The Molecular Taxonomy of Primary Prostate Cancer. Cell, 163(4), 1011–1025. https://doi.org/10.1016/j.cell.2015.10.025	
Comment 2 Soundness of Findings The findings are generally sound and confirm suspected issues with cell line representativeness. The revelation that most used PCa cell lines derive from metastatic cases and poorly represent early-stage disease is important. However, the limited molecular data available for most cell lines (39/46) significantly	Thank you to the reviewer for their comments on the soundness of our findings and for alerting us to some of the confusion with the figures. We have reviewed the figures and manuscript to correct any errors.	See updated manuscript for clear labelling and figure referencing.

constrains the comprehensiveness of these conclusions. Major Issues 1. Figure Numbering and References • Figure references are inconsistent throughout the manuscript. Figures, tables and supplementals should be numbered as they are mentioned. • What is/where is Fig. 1B?- I suppose this is 2B?" needs to be addressed. • Check all figures.		
Comment 3 2. Manuscript Structure and Flow • The manuscript reads as disconnected with poor transitions between sections. • The flow from clinical characteristics to molecular analysis to the selection framework is abrupt and needs better linking text.	We thank the reviewer for their comments, we have reviewed the manuscript to improve the flow.	Amendments made throughout to increase readability.
Comment 4 3. Grammatical and Language Issues • Multiple grammatical errors throughout the manuscript • Inconsistent terminology usage • Several unclear or awkwardly constructed sentences that impede comprehension	Thank you to the reviewer for alerting us to these errors which we have now corrected in the manuscript.	Changes made throughout to correct.
Comment 5 4. Figure Legends • Figures lack complete descriptive legends. Each figure needs both a title AND a detailed legend explaining what is shown, including sample sizes, statistical tests used, and abbreviation definitions. maximal utility to the research community	Thank you to the reviewer for suggesting revisions of our figure legend. We have now edited them to make them more comprehensive.	See updated figure legends.

Comment 6 5. Clinical Cohort Definition • The "clinical cohort" is referenced throughout without clear initial definition • The relationship between the 10,284 total patients mentioned and the various subset analyses is unclear • Selection criteria for inclusion in different analyses need clarification	We thank reviewer #1 for requesting clarification of our clinical cohort. The clinical cohort was selected from cBioPortal based on inclusion criteria of at least 200 patients per study and the availability of DNAseq datasets comprising a minimum of two genomic alteration types. After removing duplicate datasets, 24 PCa studies containing multi omics datasets from 10,239 patients were analysed, and an additional published study with 45 Nigerian patients was also included yielding 10,284 patients retained for analysis. Molecular analysis of the cohort included Mutations (N=9818), Copy Number Alterations (N=7642), Structural Variants (N=7673), mRNA expression (N=531), Protein expression (N=352), Microsatellite Instability (N=6742) .	Selection criteria of clinical cohort added to methods
Comment 7 6. Statistical Concerns • The claim "9% of PCa cell line cohort" needs context - 9% of what total? • Statistical methods section is too brief for the complexity of analyses performed • No power analysis or discussion of multiple testing corrections Additional Issues Not Previously Mentioned	Thank you to reviewer #1 for their comments on our statistics. "9% of the 4,077 PCa cell line cohort" referred to 9% of patients within our selected clinical cohort being black. We have clarified this in the text. For our statistical methods section, we have expanded this with further details on the statistics employed, including details on power analysis and normalisation.	See results for clarification of the 9% comment and methods for updated statistics.
Comment 8 7. Data Completeness and Limitations • Only 7/46 cell lines had complete multiomics data, severely limiting the generalizability of molecular findings • No discussion of how missing data might bias conclusions • The authors should acknowledge this as a major limitation upfront	We thank reviewer #1 for addressing the limitations within our study, which we agree with. The data completeness was a limitation which we sought to overcome but was difficult due to a lack of available data. We have now stated this as a limitation in our discussion, as well as how this may therefore bias conclusions.	See discussion for updated limitations.
Comment 9 8. Validation of Proposed Framework • The three-step selection framework lacks validation • No examples of how following this framework might improve research outcomes • No comparison of studies using "appropriate" vs "inappropriate" cell lines according to their criteria	We thank the reviewer for this constructive and insightful comment. In response, we have undertaken a comprehensive transcriptomic validation of our proposed prostate cancer (PCa) cell line selection framework using RNA-seq data from five PCa cell lines (LNCaP, VCaP, 22Rv1, DU145, and NCI-H660) and the TCGA Prostate Adenocarcinoma (TCGA-PRAD) cohort (n = 550). This validation directly assesses the framework's robustness and translational relevance by comparing cell line model and patient cohort transcriptomes along a biologically meaningful axis (Figure 6). To address the reviewers' specific suggestion, we performed a Principal Component Analysis (PCA) of merged, z-score-normalized RNA-seq data obtained from the Cell Model Passport database (cell lines) and the TCGA-PRAD cohort (primary tumours).	See results for validation

PCA revealed a clear and statistically significant separation between prostate cancer cell lines and patient tumours along the first principal component (PC1), indicating distinct global transcriptomic profiles between in vitro models and clinical disease (Figure 6A).

When overlaid by the five-gene composite signature (TP53, PTEN, TMPRSS2, FOXA1, and ERG; Figure 6B), the PCA demonstrated that this signature quantitatively captures the same dominant axis of variation ($r = 0.91$, $p < 0.001$). A heatmap of these genes (Figure 6C) confirmed consistent expression trends with genomic alterations described in Figure 3 namely, TMPRSS2/FOXA1/ERG activation and PTEN/TP53 downregulation. Thus, representing canonical AR-driven and tumour-suppressor-loss pathways, respectively.

By deriving a signature score to quantify each sample's similarity to the average 'Cell line' or 'Patient' expression profile, we classified all samples into Cell line-like, Intermediate, or Patient-like categories (Figure 6D). This stratification revealed a clear gradient from androgen-dependent luminal models toward androgen-independent or neuroendocrine models reflecting their known phenotypic and genomic characteristics. A subset of TCGA tumours exhibited 'Cell line-like' expression profiles, further validating that this classification captures biologically relevant variation within clinical prostate cancer.

Among cell lines, VCaP exhibited the highest signature score and clustered closest to patient tumours, consistent with its strong androgen receptor (AR) activity and TMPRSS2–ERG rearrangement. In contrast, DU145 and NCI-H660 showed the lowest signature scores, clustering farthest from patient samples and reflecting their androgen-independent and neuroendocrine phenotypes. VCaP and LNCaP thus represent the most 'patient-like' transcriptional profiles, while DU145 and NCI-H660 are more divergent, underscoring differences in AR dependence and tumour-suppressor pathway integrity.

Together, these results empirically validate our proposed selection framework proposed in Figure 5 and demonstrate that the five-gene signature captures the biological opposition between AR-driven luminal differentiation and tumour-suppressor-loss–driven dedifferentiation. Specifically:

- Upregulation of TMPRSS2, FOXA1, and ERG marks active AR signalling and luminal differentiation.
- Downregulation or loss of PTEN and TP53 indicates tumour-suppressor inactivation, lineage plasticity, and dedifferentiation.
- The balance between these gene sets determines each sample's position on the PCa biological continuum from AR-dependent luminal to AR-independent, dedifferentiated states.

This relationship is quantitatively modelled as:

$$\text{Signature Score} = \text{mean} [z (\text{TMPRSS2, FOXA1, ERG})] - \text{mean} [z (\text{PTEN, TP53})]$$

	where z denotes z-score - normalized expression across samples. The subtraction term explicitly models the biological opposition between differentiation and dedifferentiation programs. By providing a quantitative and biologically interpretable validation of the proposed framework, these findings establish a reproducible method for mapping preclinical models to clinical transcriptomic space. This approach guides selection of that prostate cancer cell line models based on molecular fidelity to patient disease, thereby improving translational relevance, model reproducibility, and biological interpretability in future PCa research. Data Sources: • Cell Model Passport: Dieudonne van der Meer et al., 2019, Cell Model Passports—a hub for clinical, genetic and functional datasets of preclinical cancer models, Nucleic Acids Research, Volume 47, Issue D1, Pages D923–D929, https://doi.org/10.1093/nar/gky872 • TCGA-PRAD: The Cancer Genome Atlas Research Network. (2015). The Molecular Taxonomy of Primary Prostate Cancer. Cell, 163(4), 1011–1025. https://doi.org/10.1016/j.cell.2015.10.025	
Comment 10 9. Temporal Considerations • Many cell lines were established 40+ years ago - no discussion of how genetic drift or culture conditions might affect current relevance • Also one recommendation not included is the need for more diverse cell lines to be constantly developed.	We thank reviewer #1 for this important point regarding the temporal context of cell line development and its implications for prostate cancer research. We agree that many widely used prostate cancer cell lines, such as LNCaP, PC-3, and DU145, were established several decades ago, and that genetic drift, epigenetic changes, and long-term culture conditions may affect their fidelity to the original tumor biology. This is a critical consideration for interpreting experimental results and for their translational relevance. To address this, we have added a new section in the discussion highlighting these concerns. We discuss how continuous passaging can introduce genomic and phenotypic divergence from the parental tumor, potentially limiting the representativeness of these models over time. We also cite recent studies that have systematically characterized such drift and its impact on reproducibility and clinical relevance. In addition, we fully agree with the reviewer's recommendation on the need for more diverse, contemporary prostate cancer cell line models. We now explicitly mention this in the revised Discussion, emphasizing the importance of developing new cell lines that reflect the current molecular landscape of prostate cancer across different racial, ethnic, and disease subtypes. This need is especially pressing given the increasing recognition of molecular heterogeneity and health disparities in prostate cancer. We appreciate the reviewer's suggestion, which has helped us to strengthen the manuscript by acknowledging these critical limitations and future directions.	See discussion for updated limitations

Comment 11 10. Practical Applicability Issues • The framework suggests using multiple cell lines but doesn't address practical constraints (cost, availability, technical expertise) • No discussion of which cell lines are commercially available vs. requiring MTAs	We thank the reviewer for this insightful comment. In response, we have expanded the discussion to acknowledge the practical constraints associated with using multiple prostate cancer (PCa) cell lines, including cost, availability, and importation challenges. We have also generated an Excel sheet (now included as Supplementary File 1) that lists the commercially available PCa cell lines and indicates those requiring Material Transfer Agreements (MTAs). This addition provides a clear resource for researchers to identify readily accessible models and understand logistical considerations for obtaining non-commercial lines. The revised text has been added to the Discussion section.	See Discussion and supplementary file 1
Comment 12 Specific Comments by Section Introduction • Needs stronger justification for why this analysis is needed now • Should better define what constitutes "clinical relevance" for a cell line • The 0.1% drug approval rate claim needs a more recent reference Methods • Insufficient detail on data harmonization across different databases • Statistical methods need expansion, including how missing data was handled • Criteria for selecting the 46 cell lines should be explicit Results • Section 2.1.1 title "History of immortalization" doesn't match the content about citations and patents • The geographical bias in cell line origin is important but needs connection to patient diversity • MSI prevalence discrepancy (63.64% in cell lines vs 0.43% in patients) needs more prominent discussion Discussion • Overreliance on speculation about why preclinical studies fail • Limited integration with recent literature on improving translational relevance • The limitation section is too brief given the significant data gaps Conclusions	Thank you to reviewer #1 for their comments by section to improve our manuscript. Introduction: Recent advancements in publicly available large-scale multiomics datasets and global clinical cohorts, represent a timely opportunity to reassess the clinical relevance of widely used prostate cancer cell lines to improve translational success in precision oncology. The "clinical relevance" of a cell line means that the selected cell line for a research question should share key features with the target population, for example researchers must consider donor ethnicity, age, cancer type, Gleason grade, metastatic stage, patient survival and androgen sensitivity. We have updated the drug failure rate reference to be more recent and specific to oncology drugs Methods: Statistical methods adjusted as requested. Please see supplementary methods for further explanation of cell line selection. Results: We agree that the section title could be improved and has been changed to "Trends in Cell Line Utilization". We have acknowledged the relevance of geographic bias in our results. We agree that the difference in MSI between cell lines and patients is stark, and we have added to our discussion to recognize this. Discussion: We agree that the speculation over why preclinical studies fail can be condensed and we changed this so as not to detract from the key findings of this study. We agree with the reviewer that integration of recent literature on improving translational relevance is important. We have now expanded the Discussion to note that improved translational relevance in prostate cancer research often centres around the creation and use of biomimetic 3D models, many of which are derived from cell lines. Therefore, appropriate cell line selection remains a pivotal aspect of ensuring model suitability and translational impact. We have now further emphasized the missing data as a major limitation of this study.	Please see manuscript for amendments in each section as described in the author comments.

• Should more clearly state which specific cell lines are most/least representative • Needs actionable recommendations beyond the proposed framework	Conclusions: We thank the reviewer for requesting actionable conclusions that the reader can implement. We encourage cell line selection based upon the clinical query and using our framework. Having said this, there are some generalizable findings of the most/least representative cell lines, with the major caveat that this is only out of the cell lines with available data.	
Comment 13 Journal Compliance Issues Nature Communications Medicine Guidelines: 1. Data Availability: Must specify which datasets are publicly available and which require permissions 2. Code Availability: Any custom analysis code should be made available 3. Reporting Guidelines: Should follow relevant guidelines for observational studies 4. Supplementary Information: Extensive supplementary data mentioned but organization unclear	We thank reviewer #1 for checking our manuscript complies with journal requirements. 1. All data used is publicly available. We will include this in the data available statement. 2. No custom analysis code was used. 3. The relevant observational study guidelines to not apply to this study. 4. Supplementary information has been uploaded as requested by the journal and will be made publicly available if accepted for publication in Communications Medicine.	See results for comment on data availability,
References • References appear complete and properly formatted • However, missing some key recent papers on: - o Cell line authentication and quality control (2023-2024) - o Translational failures in oncology (2023-2024) - o Patient-derived models as alternatives to cell lines	We appreciate your commendation on the completeness and formatting of our references. We also value your suggestion to include recent literature in three key areas. To address this, we have added and cited the following up-to-date publications: 1. Cell Line Authentication and Quality Control (2023–2024) We have now included a recent study employing deep learning for improved cell line authentication across batches (e.g., CLANet), which enhances reproducibility and data integrity arXiv. 2. Translational Failures in Oncology (2024) We discuss the persistent challenges in translating preclinical findings to clinical success, citing Connor et al.’s Nature Reviews Cancer article “Challenging the status quo...” (2024), which provides critical insights into the limitations of current preclinical models and offers guidance for improving translational relevance Nature. 3. Patient-Derived Models as Alternatives to Cell Lines (2023–2024) Our revision now includes a review summarizing the utility and limitations of patient-derived xenograft (PDX) models in cancer therapy, emphasizing their high fidelity and translational value PMC. Additionally, we reference a recent BMC Cancer study (Wen et al., 2024) that highlights metabolic differences between PDX models and primary tumors, reinforcing the need for careful model selection	Please see relevant section in discussion section with new references added 39-41

Comment 14 Recommendations for Improvement 1. Restructure the Results: Organize by research question rather than data type 2. Enhance Visual Communication: Create a summary figure showing which cell lines best represent different patient subgroups 3. Address Data Gaps: Be more transparent about limitations and suggest how to obtain missing data 4. Validate Framework: Include case studies or examples of improved outcomes using the selection criteria 5. Practical Guide: Create a user-friendly table or flowchart for cell line selection	Thank you reviewer #1 for their recommendations for improvement, which we value highly. 1. Results section clarified 2. We have edited the figures to the best of our ability to clarify and make as visually clear as possible. 3. We have stated our data gaps as a major limitation in the discussion and hope this will encourage researchers to make more of their results/data publicly available. 4. See previous comments regarding validation 5. Figure 5 aims to provide a user friendly guide to aid relevant cell line selection.	See relevant sections for amendments.
Comment 15 Minor Points • Define all abbreviations at first use (MSI, TMB, CNAs, SVs) • Consistent use of "prostate cancer" vs "PCa" • Specify which Gleason grading system is used (classic vs. ISUP) • Clarify if "Black" and "African American" are used interchangeably Recommendation Major revisions required. While this study addresses an important question about the clinical relevance of prostate cancer cell lines, significant improvements in presentation, data completeness acknowledgment, and practical applicability are needed. The core findings are valuable, but the manuscript requires substantial revision to meet publication	We thank reviewer number 1 for this comment. - We have reviewed all abbreviations and ensured definition at first use - We have consistently used prostate cancer instead of PCa - We used the classic ISUP Gleason scoring and have added this clarification to the manuscript	We have amended abbreviations throughout and added the clarification of classic Gleason Grading to discussion.

standards and provide maximal utility to the research community.		
Reviewer 2:		
Comment 1 Reviewer #2 (Remarks to the Author): This study integrates clinical and multi-omics data to assess the relevance of 46 prostate cancer (PCa) cell lines. The authors argue that the most commonly used cell lines accurately represent only a small subset of patients. I agree that this article is useful for guiding the selection of representative cell lines. However, the authors should consider the following points: Major Comments Comment 1 It is inherently difficult to argue that a cell line faithfully represents a specific cohort of prostate cancer patients. Even when a cell line shares genetic or clinical background characteristics with a patient cohort, their biological behavior may diverge significantly. During the adaptation to 2D culture conditions, for example, cancer cells undergo morphological changes, and more aggressive clones are often selected. As the authors noted, preclinical PCa models may overrepresent MSI-driven tumors, which comprise only a small fraction of actual patient cases (see Figure 3). Therefore, patient-derived xenograft or organoid models should be considered for most prostate cancer research,	Thank you, reviewer #2 for seeing the value that our study can bring and we appreciate your comments to help us improve it to make it as useful as possible for researchers. We wholly agree with comment 1 and have incorporated your ideas into our discussion, thank you.	See discussion for incorporation of reviewer's comments.

particularly when the goal is clinical relevance. Cell line models are best suited for mechanistic studies such as epigenomic regulation of gene expression, protein–protein interactions, or signaling pathway dissection.		
Comment 2 The authors should provide a direct comparison of gene expression profiles between the PCa cell lines and primary patient samples. For instance, principal component analysis (PCA) plots based on RNA expression data from both sources could help visualize their similarities or differences. Please refer to Figure 1a in the following article for a useful example: PMID: 34489572.	We thank the reviewer for this constructive and insightful comment. In response, we have undertaken a comprehensive transcriptomic validation of our proposed prostate cancer (PCa) cell line selection framework using RNA-seq data from five PCa cell lines (LNCaP, VCaP, 22Rv1, DU145, and NCI-H660) and the TCGA Prostate Adenocarcinoma (TCGA-PRAD) cohort (n = 550). This validation directly assesses the framework's robustness and translational relevance by comparing cell line model and patient cohort transcriptomes along a biologically meaningful axis (Figure 6). To address the reviewers' specific suggestion, we performed a Principal Component Analysis (PCA) of merged, z-score–normalized RNA-seq data obtained from the Cell Model Passport database (cell lines) and the TCGA-PRAD cohort (primary tumours). PCA revealed a clear and statistically significant separation between prostate cancer cell lines and patient tumours along the first principal component (PC1), indicating distinct global transcriptomic profiles between in vitro models and clinical disease (Figure 6A). When overlaid by the five-gene composite signature (TP53, PTEN, TMPRSS2, FOXA1, and ERG; Figure 6B), the PCA demonstrated that this signature quantitatively captures the same dominant axis of variation ($r = 0.91$, $p < 0.001$). A heatmap of these genes (Figure 6C) confirmed consistent expression trends with genomic alterations described in Figure 3 namely, TMPRSS2/FOXA1/ERG activation and PTEN/TP53 downregulation. Thus, representing canonical AR-driven and tumour-suppressor-loss pathways, respectively. By deriving a signature score to quantify each sample's similarity to the average 'Cell line' or 'Patient' expression profile, we classified all samples into Cell line-like, Intermediate, or Patient-like categories (Figure 6D). This stratification revealed a clear gradient from androgen-dependent luminal models toward androgen-independent or neuroendocrine models reflecting their known phenotypic and genomic characteristics. A subset of TCGA tumours exhibited 'Cell line-like' expression profiles, further validating that this classification captures biologically relevant variation within clinical prostate cancer. Among cell lines, VCaP exhibited the highest signature score and clustered closest to patient tumours, consistent with its strong androgen receptor (AR) activity and TMPRSS2–ERG rearrangement. In contrast, DU145 and NCI-H660 showed the lowest signature scores, clustering farthest from patient samples and reflecting their androgen-independent and neuroendocrine	See Results for validation

	phenotypes. VCaP and LNCaP thus represent the most 'patient-like' transcriptional profiles, while DU145 and NCI-H660 are more divergent, underscoring differences in AR dependence and tumour-suppressor pathway integrity. Together, these results empirically validate our proposed selection framework proposed in Figure 5 and demonstrate that the five-gene signature captures the biological opposition between AR-driven luminal differentiation and tumour-suppressor-loss–driven dedifferentiation. Specifically: • Upregulation of TMPRSS2, FOXA1, and ERG marks active AR signalling and luminal differentiation. • Downregulation or loss of PTEN and TP53 indicates tumour-suppressor inactivation, lineage plasticity, and dedifferentiation. • The balance between these gene sets determines each sample's position on the PCa biological continuum from AR-dependent luminal to AR-independent, dedifferentiated states. This relationship is quantitatively modelled as: Signature Score = mean [z (TMPRSS2, FOXA1, ERG)] - mean [z (PTEN, TP53)] where z denotes z-score - normalized expression across samples. The subtraction term explicitly models the biological opposition between differentiation and dedifferentiation programs. By providing a quantitative and biologically interpretable validation of the proposed framework, these findings establish a reproducible method for mapping preclinical models to clinical transcriptomic space. This approach guides selection of that prostate cancer cell line models based on molecular fidelity to patient disease, thereby improving translational relevance, model reproducibility, and biological interpretability in future PCa research. Data Sources: • Cell Model Passport: Dieudonne van der Meer et al., 2019, Cell Model Passports—a hub for clinical, genetic and functional datasets of preclinical cancer models, Nucleic Acids Research, Volume 47, Issue D1, Pages D923–D929, https://doi.org/10.1093/nar/gky872 • TCGA-PRAD: The Cancer Genome Atlas Research Network. (2015). The Molecular Taxonomy of Primary Prostate Cancer. Cell, 163(4), 1011–1025. https://doi.org/10.1016/j.cell.2015.10.025	
Comment 3 Minor Comments There are several typographical errors in the manuscript and figures. The authors should carefully proofread the text. Examples include: "Figure 3A" should be "1A" (Page 4, Line 81) "Figure 3A" should be "2A" (Page 5, Line 98)	Thank you to the reviewer for alerting us to some of the confusion with the figures. We have reviewed the figures and manuscript to correct any errors. For Figure 3I it is unclear which part of the figure the reviewer is referring to. The figure shows the proportion of the clinical cohorts with the same alterations that were most commonly found in the cell lines analysed. The exact cell lines with alterations in these genes are referenced in the figure legend. We have expanded the description of this figure in the discussion. We have removed KEGG from Figure 4A.	See updated manuscript for clear labelling and figure referencing and discussion for expansion on Figure 3I.

The labeling in Figure 3I is unclear and not representative In Figure 4A, the term “KEGG” should be removed from the bottom of the bar chart		
Reviewer 3		
Comment 1 Reviewer #3 (Remarks to the Author): The manuscript submitted by Ahmed and colleagues from the team of Uzoamaka Okoli and Susan Heavey is an attempt at a comparative analysis of commonly used cell line models with population and clinical data. Unfortunately, it requires extensive improvement to be published in the journal Communications Medicine. The major concerns are as follow: 1) As the title of the manuscript is ‘How clinically relevant and inclusive are prostate cell lines?’ I do not understand why benign prostate cell lines (like RWPE-1, HPr-1, BPH-1...) were omitted. I believe they should have been added to the article to present complete data on the comparison of cell line models with population data.	We thank the reviewer for this important comment. The focus of our study was specifically on commonly used cancerous prostate cell lines and their representation of patient populations, as these are most frequently employed in translational research and therapeutic studies. We acknowledge that benign prostate cell lines (e.g., RWPE-1, HPr-1, BPH-1) also provide valuable biological insight, but they were beyond the scope of the current analysis. To address this concern, we have now added a clarifying statement in the Discussion section noting that benign cell lines were not included in the comparison and explaining the rationale for focusing on cancer cell lines. We also reference the existence of benign lines and suggest that future studies could incorporate them for a more comprehensive comparison. We agree this is a limitation of our study, which we have added to the discussion. Future work should expand upon this study too further diversify the cell lines studied.	See discussion for limitation and suggestion for future work.
Comment 2 2) It is very important to clearly indicate the source or year of the source data used for the analysis. For example, Figure 3 shows the mixed genetic origin of the 22Rv1 line, which is consistent with publications up to 2018, while a more comprehensive analysis published in PMID: 30787054 established that the 22Rv1 cell line is 99% of European origin. Each figure description should be accompanied by the year of information on which such a	We thank the reviewer for requesting the year of source data to be included into figure legends, we agree that this is very important and have done so.	See updated figure legends for data source year.

comparative analysis is based, not just general information like in Supplementary Table 2 .		
Comment 3 3) All analyses should be based on the latest available data. Why does Figure 2C show data from 2020, while more recent data is publicly available	Thank you, reviewer, #3 for your comment regarding the 2020 data from figure 2C, when this work was performed this was the most recent dataset. We have now updated this with the most recent data from GLOBOCAN which was from 2022.	See updated Figure 2C
Comment 4 4) There is no marked D in Figure 2. The description of Figure 3c indicates that it shows “cancer stage of cell lines (N=33), clinical cohort (N=1593) and PCa patients worldwide (N=9481)”, whereas Figure 3C does not show results for cancer stage. There are no marked D, E, F, G, H, I, J, K in Figure 3. Figure 4 does contain any A, B, C, D mark.	Thank you to the reviewer for alerting us to some of the confusion with the figures. We have reviewed the figures and manuscript to correct any errors.	See updated manuscript for clear labelling and figure referencing.
Comment 5 5) What is the abbreviation for CC and CL in Figure 1? Why were these abbreviations used in the last box, although not in others. What do the abbreviations AS and AI mean in Figure 5? According to Figure 5A, the PC-3 line represents both Cancer stage 2 and 4? What is the difference between “clinical cohort” and “wordwide” in Figure 3? What are the exact sources of the data and the year of their publication? Such information is crucial to assess the relevance of such comparisons?	Thank you to the reviewer for requesting clarification of abbreviations. CC = Clinical Cohort, CL = Cell Line, we have added the full versions to Figure 1. AS = Androgen Sensitive and AI = Androgen Insensitive, we have added this to the figure legend. The clinical cohort are select patients from clinical studies and worldwide patients represent epidemiological data of all patients worldwide (not solely those selected for studies). This has been included in the methods and supplementary methods. The data sources have now been included in figure legends and are also in the supplementary tables and methods.	See updated figures, figure legends for abbreviation clarification and supplementary materials for clarification on data sources.
Comment 6 6) The discussion is very long and does not focus much on the really important comparative aspects. I think this should be fundamentally revised. Apart from that, there is a lot of data in the supplementary data. I think the most informative ones could be moved to the main	Thank you for the reviewer’s comments on the discussion, we have reframed it to include the other reviewer’s comments to convey the pertinent findings which researchers can learn from this study and apply when selecting a cell line. We have moved Supplementary Table 8 into the main text (now Table 1)	See revised discussion and table in main file.

manuscript e.g. Figure 1 does not show any particular data in my opinion. Instead, I suggest adding to the main manuscript, e.g. Supplementary Table 8, which contains some specific information.		
Comment 7 7) Most of the graphics are of very poor quality and low attractiveness for the potential reader. Too small font size, overlapping colours and captions. This should definitely be improved.	We appreciate some of the graphics were not as high quality as the reviewer may have liked, we have tried to edit this to the best of our ability.	Graphics minor changes
Comment 8 8) The text needs very thorough improvement as it contains many repetitions, e.g. in lines 53-54, editorial and grammatical errors. The English should be significantly improved, as at some points it is unclear what the author meant, especially in the Figure captions.	We thank the reviewer for asking us to change the repetitions to improve readability and clarity, we have made the specified changes requested and throughout the manuscript.	See changes throughout to improve readability.

Reviewer Comment	Author Comments	Manuscript Revisions
Reviewer 2:		
Comment 1		
Thank you for responding to my previous comments and for generating the PCA plots in the patient cohort and cell lines as suggested. However, these new analyses unfortunately reinforce my original concern regarding the authors' main argument that the most commonly used cell lines "accurately represent" a subset of patients. In Figure 6A, there is a clear separation between the cell line cluster and the patient cluster, indicating that gene expression profiles in the cell lines have been substantially altered during adaptation to 2D culture. Even if a cell line originates from a background similar to that of the patient cohort, its current behaviour appears far removed from the original cancer tissue. In addition, from a clinical standpoint, there are major differences in treatment decision-making between AR-positive prostate adenocarcinoma and AR-negative NEPC. Yet in Figure 6, LNCaP (AR-positive adenocarcinoma) and NCI-H660 (AR-negative NEPC) fall into the same cluster and are both distant from the patient clusters. As I noted in my first-round review, the field has increasingly moved away from relying on conventional cell line experiments to assess clinical relevance, precisely because these models are morphologically and biologically different from patients' tumours. In this context, it is difficult to support the manuscript's central claim that the most frequently used cell lines accurately represent a small subset of patients. Indeed, the PCA in Figure 6A/B appears to provide counter-evidence to that conclusion rather than supporting it.	Thank you for your thoughtful and careful re-evaluation of the manuscript and the additional PCA analyses. We fully agree with your interpretation and appreciate the opportunity to clarify our conclusions. Our findings do not support the notion that commonly used prostate cancer cell lines are broadly or holistically representative of patient tumours. Rather, as you correctly note, the clear separation between cell lines and patient samples in the PCA reflects substantial biological divergence arising from long-term adaptation to 2D culture, as well as clinically meaningful differences between AR-driven adenocarcinoma and AR-independent neuroendocrine models. Our intended conclusion is that, while certain cell lines may share <i>specific molecular alterations</i> with small patient subgroups, these overlaps are limited and do not imply overall clinical representativeness. We recognise that our previous wording may have allowed this point to be misinterpreted. To address this directly, we have revised the Abstract, Results (Lines 165–166), and Discussion (Lines 406–409) to state unambiguously that prostate cancer cell lines should not be viewed as fully representative models, but rather as context-dependent tools whose limitations necessitate careful, question-specific selection. We are grateful for your feedback, which has strengthened the clarity and precision of the manuscript.	Updated abstract to reflect agreement with reviewer Line Results (Lines 165–166): To improve clarity, we have revised the sentence to emphasize our intended meaning: <i>"This discrepancy underscores the limitations of cell lines as fully representative models of clinical PCa cases, highlighting that even widely used prostate cancer cell lines do not faithfully recapitulate the full biological complexity observed in patient tumours."</i> Discussion (Lines 406–409): In response to the reviewer's concern, we now explicitly connect our interpretation to the need for careful model selection: <i>"Consistent with these limitations, we recommend that researchers avoid reliance on a single model and instead select cell lines based on clinical and molecular relevance to the specific research question, supported by the structured selection framework we propose."</i>

Reviewer 3:		
Reviewer #3 (Remarks to the Author):		
The manuscript still contains many typos and grammatical errors, repetitions and should undergo professional editing. Many of them can be found in the "Introduction" section alone, e.g., double dots, double spaces, missing spaces (andimmune), p PCa (what does small p mean? no explanation), 15·2% (what does a multiplication sign mean? should it be a comma?). If the authors were unwilling to include benign prostate cell lines in their analysis, then I believe that the title of the article is misleading and should be changed to: How clinically relevant and inclusive are prostate cancer cell lines? However, overall, the content of the article seems interesting and, after careful editing, could be published in the journal Communications Medicine	We thank Reviewer 3 for their careful reading of the manuscript and for highlighting issues related to language, formatting, and clarity. The manuscript has now undergone comprehensive language and formatting revision, and the specific issues raised (including typographical errors, spacing, and numerical formatting) have been corrected throughout. In addition, we have revised the title as suggested to accurately reflect the scope of the study. We appreciate the reviewer's constructive feedback and are grateful for their positive assessment of the study's overall contribution.	See changes made throughout and new title.
I have also carefully read all the concerns raised by Rewiever 1. Some of them appear to have been properly addressed and some additional analyses have been included. However, in general, I believe that there are still many fields that require improvement, especially the description of the figures and methodology, and the presentation of the results. Issues concerning the validation of the proposed three-stage selection framework were not discussed in detail (Comment 9). No comparison was made between studies using " appropriate" and "inappropriate" cell lines according to the authors' criteria.	Our proposed framework is designed as a forward-looking tool to guide model selection in a reproducible and biologically grounded manner, rather than as a method for retrospectively grading published studies. While a comparison of "appropriate" versus "inappropriate" cell line usage across the literature could be informative, such an analysis would require a dedicated, large-scale retrospective study with clearly defined and standardised outcome measures, which is beyond the scope of the present manuscript. Instead, we provide empirical transcriptomic validation of the framework (Figure 6), demonstrating how it differentiates models based on their molecular proximity to clinical prostate cancer states. This supports the framework's intended use as a practical guide for informed cell line selection in future research.	

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Furthermore, the issue of data completeness and functional significance was not satisfactorily addressed in the discussion section	Thank you to the reviewer for highlighting the importance of data completeness and functional interpretation. In response, we have expanded the Discussion to explicitly address limitations arising from incomplete multi-omics coverage, and to clarify the functional significance of our findings, including the biological and translational implications of the observed molecular differences between cell lines and patient tumours.	See discussion for expanded limitation on data incompleteness.
What were the culture conditions of the cells analyzed by multiomic assays—were they seeded on coated plates or on regular plastic in 2D?	Cell line culture conditions were obtained from the CCLE Broad study, which sources lines primarily from established international vendors (including ATCC, DSMZ, ECACC, RIKEN, and others), with a small number obtained from academic laboratories. Where available, information on culture medium, serum concentration, and plate type has been added to the Methods section and summarised in Supplementary Table 1.	See methods and Supplementary Table 1 for cell culture condition details.
It is not surprising that principal component analysis (PCA) revealed big differences in the activation of transcriptional programs between prostate cancer cell lines cultured in 2D on plastic and patient tumors growing in their “physiological” environment. This has been published in several articles. Recently, there has been a lot of hope for 3D cultures in defined hydrogels, as recent results suggest that cell lines cultured in such hydrogels can restore a gene expression profile more similar to that of primary tissues. This could suggest that the utilization of established cell lines is still justified if appropriate culture conditions are applied. That point should be properly addressed in the discussion section.	We agree with the reviewer that the transcriptional separation observed between prostate cancer cell lines cultured in 2D and patient tumours is expected and has been widely reported. In the revised Discussion, we now explicitly address this point by acknowledging the limitations of 2D culture and highlighting emerging evidence that advanced 3D culture systems, including defined hydrogels, can partially restore gene expression programmes more reflective of primary tissues. Importantly, we clarify that these advances support the continued, context-appropriate use of established cell lines, provided that culture conditions and model selection are carefully aligned with the biological and translational question being addressed.	See discussion for points on separation of cell lines and patients on PCA along with growing evidence moving away from 2D culture to 3D models.

Moreover, the manuscript still contains multiple grammatical and linguistic issues, as well as several unclear or awkwardly constructed sentences that impede comprehension and definitely require improvement at that field.	We thank Reviewer 3 for reiterating the importance of clear and precise language. As noted above, the manuscript has now undergone full language and formatting revision, and we believe these issues have been comprehensively addressed.	See changes made throughout.
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