Cell Proliferation

WILEY

EDITORIAL OPEN ACCESS

Human Gallbladder Cancer Organoids

Dongxi Xiang[1,2,3] | Ting Gong[4] | Yinjie Zhu[5] | Bin Jia[6] | Chen Zhang[7] | Ning Tang[8] | Weiping Wang[9] | Yalong Wang[10] | Laiping Zhong[11] | Yanmei Zou[12] | Jian Zhang[13] | Zunqiang Zhou[14] | Ligang Xing[15] | Changchun Zhou[16] | Dongyuan Zhu[17] | Zengjun Liu[17] | Gang Chen[18] | Xiaoguang Wang[19] | Zhan Wang[20] | Aina He[21] | Yonggang Wang[21] | Zebing Liu[22] | Jiabei Wang[23] | Feng Xu[24] | Ganglong Gao[2] | Xiaonan Kang[25] | Shuai Gong[26] | Xianming Kong[27] | Chunyan Dong[28] | Aijin Ma[29] | Gengming Niu[30] | Guiying Wei[30] | Hongling Zhao[31,32] | Xiongxiong Lu[33] | Jie Cao[34] | Tongbiao Zhao[31,32,35] | Zizhen Zhang[3] | Yingbin Liu[2]

Correspondence: Dongxi Xiang (dxiang@shsmu.edu.cn) | Xiongxiong Lu (simone515night@126.com) | Jie Cao (caojie@renji.com) | Tongbiao Zhao (tbzhao@ioz.ac.cn) | Zizhen Zhang (zhangzizhen@renji.com) | Yingbin Liu (laoniulyb@shsmu.edu.cn)

Received: 2 March 2026 | **Revised:** 2 March 2026 | **Accepted:** 18 March 2026

The clinical management of patients with gallbladder cancer remains challenging [1]. Conventional chemotherapeutic regimens and targeted therapies have demonstrated limited efficacy, whereas immunotherapies have yet to yield substantial therapeutic benefit in this maliganancy [1]. Consequently, their is an urgent, unmet clinical need for precision-medicine approaches tailored to individual gallbladder cancer patients. Over the past decade, significant advances have been achieved in the developed of patient-derived organoids as in vitro models that recapitulate key features of human tumors [2]. Patient-derived organoids preserve the physiological, pathological and genomic characteristics of their parental tumour tissues, providing unprecedented opportunities for translational research, therapeutic discovery and precision oncology [2]. The 'Human Gallbladder Cancer Organoids' guideline represents an integral component of Human Cancer Organoids Guidelines series in China, which was jointly developed by experts from the Chinese Society for Cell Biology and its affiliated branches [3]. The Chinese version of this guideline was officially released on 28 October 2024. This standard document establishes comprehensive frameworks covering the terminologies, technical requirements, evaluation criteria and quality control procedures for the genaration and application of human gallbladder cancer organoids. The publication of the English version of this standard aims to facilitate global dissemination and adoption of standardized practices for human gallbladder cancer organoids. By promoting methodological consistency and quality assurance, this guideline is expected to support international collaboration, accelerate translational research, and advance the integration of GBCUnidentified organoid into biomedical research, drug development, and precision clinical applications.

1 | Scope

This document specifies the general requirements, technical requirements, testing methods, inspection rules, instructions for use, labelling, transportation and storage requirements for human gallbladder cancer organoids.

2 | Normative References

The provisions of the following documents constitute essential requirements of this document through normative references. For dated references, only the edition cited edition applies. For undated references, the latest edition of the referenced document (including all amendments) applies.

Pharmacopoeia of the People's Republic of China (2025 Edition, Part III).

Dongxi Xiang, Ting Gong, Yinjie Zhu, Bin Jia, Chen Zhang, and Ning Tang contribute equally to this work.

For affiliations refer to page 4.

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2026 The Author(s). *Cell Proliferation* published by Beijing Institute for Stem Cell and Regenerative Medicine and John Wiley & Sons Ltd.

3 | Terms and Definitions

The following terms and definitions apply to this document.

3.1 | Organoids

Three-dimensional (3D) in vitro cultures derived from stem cells or progenitor cells that process self-organisation and self-renewal capabilities, comprise organ-specific cell types and recapitulate the structural features and specific functions of the original tissue [4].

3.2 | Human Gallbladder Cancer Organoids

Organoids established from gallbladder tumour cells from patients with pathologically confirmed gallbladder cancer, which recapitulate the biological characteristics of original gallbladder cancer tissues.

3.3 | Organoid Passage

The process of dissociating established organoids into smaller fragments or single cells via physical, chemical or biological methods, followed by inoculation and in vitro expansion under the same culture conditions as the original culture.

3.4 | Organoid Cryopreservation

The process of preserving organoids under low temperature in an inactive state to maintain their cellular compositions, gene expression profiles, and functional properties.

3.5 | Organoid Thawing

The process by which cryopreserved organoids recover growth capability and metabolic activity from an inactively state.

4 | Abbreviations

The following abbreviations apply to this document.

3D: Three dimension

DAB: Diaminobenzidine

DMSO: Dimethyl sulfoxide

DNA: Deoxyribonucleic acid

PBS: Phosphate-buffered saline

PCR: Polymerase chain reaction

STR: Short tandem repeat

H&E: Haematoxylin and eosin

5 | General Requirements

5.1 | Raw Materials

5.1.1 The acquisition of raw materials shall comply with nationally recognized ethical standards and applicable local laws and regulations [5, 6].

5.1.2 According to the intended application, donor evaluation criteria shall be established for the research and production of human gallbladder cancer organoids.

5.2 | Process and Information Management

5.2.1 Critical factors that may affect product quality during raw material acquisition, preparation, testing, transportation and storage shall be documented. Unidentified. A unique identification system shall be established to ensure full-process traceability.

5.2.2 The minimum retention period for relevant records shall be clearly defined to ensure the integrity, confidentiality, and security of documentation.

6 | Technical Requirements

6.1 | Morphology

Human gallbladder cancer organoids exhibit diverse morphological features including dense-cystic, spherical or tubular shapes under optical microscopy.

6.2 | Pathological Features

6.2.1 The organoid cells shall retain the atypical characteristics of tumour cells from the original tumour tissue, including hyperchromatic nuclei, abnormal mitotic figures and altered nuclear-cytoplasmic ratio.

6.2.2 Immunohistochemical testing for relevant biomarkers shall be performed on organoids, and the results shall be generally consistent with those of the original tumour tissues. The tested markers include, but are not limited to, CK19 and CK7.

6.3 | Genetic Characteristics

Genetic variation analysis shall be performed on organoids, and the results shall be highly consistent with those of the original tumour tissue. Genes tested include, but are not limited to, *FGFR2* and *IDH1/2*.

6.4 | In Vitro Culture and Growth

6.4.1 Human gallbladder cancer organoids derived from gallbladder cancer patient tissues or cells shall be capable of being passaged for at least three generations after the initial culture in vitro.

6.4.2 Organoids after passage shall be capable of reconstructing new organoid structures in vitro, with morphology, pathological characteristics, and genetic characteristics consistent with those of the parental organoids.

6.5 | Survival Rate of Organoids

The viable number of thawed human gallbladder cancer organoids after cryopreservation shall not be less than 50% of the number before freezing. The surviving organoids shall retain the ability for in vitro passaged.

6.6 | Microorganisms

Organoids shall be negative for fungi, bacteria, mycoplasma and viral contamination testing.

6.7 | STR

The identity of organoids shall be consistent with that of the donor tissue based on STR analysis.

7 | Testing Methods

7.1 | Morphology

Organoids shall be cultured under three-dimensional in vitro conditions and observed using optical microscopy.

7.2 | Pathological Features

Representative testing methods are provided in Supplementary Annex A.

7.3 | Genetic Characteristics

Representative testing methods are provided in Supplementary Annex B.

7.4 | In Vitro Culture and Growth

Three-dimensional cultured organoids shall be photographed using optical microscopy. Scale bars shall be included for diameter measurement and quantitative analysis.

7.5 | Survival Rate of Organoids

Representative testing methods are provided in Supplementary Annex C.

7.6 | Microorganisms

7.6.1 Fungi and Bacteria

The '1101 Sterility Inspection Method' specified in *Pharmacopoeia of the People's Republic of China* (2020 Edition, Part III) shall be followed.

7.6.2 Mycoplasma

The '3301 Mycoplasma Inspection Method' specified in *Pharmacopoeia of the People's Republic of China* (2020 Edition, Part III) shall be followed.

7.6.3 Exogenous Viral Factors

The '3302 Exogenous Viral Factors Inspection Method' specified in *Pharmacopoeia of the People's Republic of China* (2020 Edition, Part III) shall be followed.

7.7 | STR

Representative testing methods are provided in Supplementary Annex D.

8 | Instructions for Use

The instructions shall include at least the following information:

(a) Organoid code;
(b) Passage number;
(c) Organoid quantity;
(d) Production date;
(e) Batch number;
(f) Manufacturing organization;
(h) Transportation conditions;
(i) Contact information;
(j) Usage instructions;
(k) Execution standard number;
(l) Production address;
(m) Postal code;

Note: Endotoxin results should be provided upon user request.

9 | Labelling

The labels shall include at least the following information:

(a) Organoid code;
(b) Passage number;
(c) Organoid quantity;
(d) Batch number;
(e) Manufacturing organization;
(f) Production date.

10 | Transportation and Storage

10.1 | Transportation

10.1.1 The transportation mode and conditions shall be selected according to the intended requirements for human gallbladder cancer organoids to ensure the preservation of their biological characteristics, safety, stability and functional properties.

10.1.2 The transportation of human gallbladder cancer organoids shall consider, but not be limited to, factors including

organoid characteristics, organoid containers, transportation routes, transportation conditions, transportation equipment, transportation modes, potential risks and necessary protective measures.

10.1.3 Control measures for transportation conditions shall include, but not be limited to, temperature range control, vibration management, contamination prevention, equipment performance assurance and appropriate packaging.

10.1.4 Relevant inspection reports and technical guidance documents shall be provided upon user request.

10.1.5 Package shall be inspected during transportation. When necessary, additional cryogenic materials (e.g., dry ice and liquid nitrogen) shall be supplemented to maintain the required transportation temperature.

10.2 | Storage [7, 8]

10.2.1 Optimised cryopreservation protocols and procedures shall be applied to minimise damage to human gallbladder cancer organoids during cryopreservation and thawing, ensuring minimal impact on their normal biological functions.

10.2.2 Cryopreservation records for human gallbladder cancer organoids shall be documented, including but not limited to:

(a) Organoid code;
(b) Batch number;
(c) Organoid quantity;
(d) Passage number;
(e) Freezing date;
(f) Cryoprotectant composition;
(g) Name of the operator.

10.2.3 10.2.3 The storage conditions for human gallbladder cancer organoids should be documented, including but not limited to:

a. Storage conditions;
b. Storage date;
c. Storage duration;
d. Name of the operator.

Author Contributions

Dongxi Xiang, Ting Gong, Yinjie Zhu, Bin Jia, Chen Zhang, Ning Tang, Weiping Wang, Yalong Wang, Laiping Zhong, Yanmei Zou, Jian Zhang, Zunqiang Zhou, Ligang Xing, Changchun Zhou, Dongyuan Zhu, Zengjun Liu, Gang Chen, Xiaoguang Wang, Zhan Wang, Aina He, Yonggang Wang, Zebing Liu, Jiabei Wang, Feng Xu, Ganglong Gao, Xiaonan Kang, Shuai Gong, Xianming Kong, Chunyan Dong, Aijin Ma, Gengming Niu, Guiying Wei, Hongling Zhao, Xiongxiong Lu, Jie Cao, Tongbiao Zhao, Zizhen Zhang, Yingbin Liu critically read and revised the manuscript.

Funding

This work was supported by the National Natural Science Foundation of China (32130036, 82573430, 82173358, 62202304, 82403651), the Shanghai Municipal Health Commission (2024ZZ2032, 2025ZZ1023), the Science and Technology Commission of Shanghai Municipality (23141901000), the School of Medicine, Shanghai Jiao Tong University (02.101005.001.29.38A) and the Laboratory of Early Prevention and Treatment for Regional High-Frequency Tumor (GKE-KF202307, GKE-KF202404).

Affiliations

[1]State Key Laboratory of Systems Medicine for Cancer, Shanghai Cancer Institute, Shanghai Jiao Tong University School of Medicine, Shanghai, China | [2]Department of Biliary-Pancreatic Surgery, Renji Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, China | [3]Department of Gastrointestinal Surgery, Renji Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, China | [4]Department of Oncology, Tianjin Medical University General Hospital, Tianjin, China | [5]Department of Urology, Renji Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, China | [6]Lung Cancer Department, Tianjin Cancer Hospital, Tianjin, China | [7]Department of Hepatobiliary Surgery, Liver Transplant Center, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China | [8]School of Health Science and Engineering, University of Shanghai for Science and Technology, Shanghai, China | [9]Department of Pharmacology and Pharmacy, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, China | [10]Guangzhou National Laboratory, Guangzhou, China | [11]Department of Stomatology, Oralmaxillofacial Head and Neck Surgery, Huashan Hospital, Fudan University, Shanghai, China | [12]Department of Oncology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China | [13]Department of Medical Oncology, Fudan University Shanghai Cancer Center, Shanghai, China | [14]Department of Surgery, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai, China | [15]Department of Radiation Oncology, Shandong Cancer Hospital and Institute, Shandong First Medical University, Shandong Academy of Medical Science, Jinan, China | [16]Biobank, Cancer Research Center, Shandong Cancer Hospital and Institute, Shandong First Medical University, Shandong Academy of Medical Sciences, Jinan, China | [17]Rare Tumors Department, Shandong Cancer Hospital and Institute, Shandong First Medical University and Shandong Academy of Medical Sciences, Jinan, China | [18]Jiaxing Key Laboratory of Basic Research and Clinical Translation on Orthopedic Biomaterials, Department of Orthopaedics, The Second Affiliated Hospital of Jiaxing University, Jiaxing, China | [19]Department of Hepatobiliary Surgery, Affiliated Hospital of Jiaxing University, Jiaxing, China | [20]Department of Medical Oncology, Changzheng Hospital, Second Military Medical University, Shanghai, China | [21]Department of Oncology, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai, China | [22]Department of Pathology, Renji Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, China | [23]Department of Hepatobiliary Surgery, the First Affiliated Hospital of USTC, Division of Life Sciences and Medicine, University of Science and Technology of China, Anhui, China | [24]Department of Medical Oncology, Shanghai Gongli Hospital, Second Military Medical University, Shanghai, China | [25]Department of Biobank, Renji Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, China | [26]Division of Gastroenterology and Hepatology, Renji Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, China | [27]Collaborative Research Center for Biomedicines, Shanghai University of Medicine and Health Sciences, Shanghai, China | [28]Department of Oncology, East Hospital Affiliated to Tongji University, Tongji University School of Medicine, Shanghai, China | [29]Beijing Technology and Business University, Beijing, China | [30]Shanghai OneTar Biomedicine, Shanghai, China | [31]Beijing Institute for Stem Cell and Regeneration Medicine, Beijing, China | [32]Standards Committee, Chinese Society for Cell Biology, Shanghai, China | [33]Department of General Surgery, Pancreatic Disease Center, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China | [34]Department of Hepatology Surgery, Renji Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, China | [35]Key Laboratory of Organ Regeneration and Reconstruction, State Key Laboratory of Stem

Cell and Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing, China

Acknowledgements

This work was supported by grants from the National Natural Science Foundation of China (32130036 to Y.L., 82573430 to D.X., 82173358 to A.H., 62202304 to X.L., 82403651 to B.Y.), the Shanghai Municipal Health Commission (2024ZZ2032 to A.H., 2025ZZ1023 to D.X.), the Science and Technology Commission of Shanghai Municipality (23141901000 to X.K.), the Shanghai Jiao Tong University School of Medicine (02.101005.001.29.38A), the Laboratory of Early Prevention and Treatment for Regional High-Frequency Tumour (Guangxi Medical University) and the Ministry of Education (GKE-KF202307, GKE-KF202404). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Ethics Statement

The authors have nothing to report.

Consent

The authors have nothing to report.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

References

1. J. C. Roa, P. García, V. K. Kapoor, S. K. Maithel, M. Javle, and J. Koshiol, "Gallbladder Cancer," *Nature Reviews Disease Primers* 8, no. 1 (2022): 69, https://doi.org/10.1038/s41572-022-00398-y.

2. D. Xiang, A. He, R. Zhou, et al., "Building Consensus on the Application of Organoid-Based Drug Sensitivity Testing in Cancer Precision Medicine and Drug Development," *Theranostics* 14, no. 8 (2024): 3300–3316, https://doi.org/10.7150/thno.96027.

3. H. Lin, Y. Wang, C. Cheng, et al., "Standard: Human Intestinal Cancer Organoids," *Cell Regeneration* 12, no. 1 (2023): 24, https://doi.org/10.1186/s13619-023-00167-6.

4. National Health and Family Planning Commission of the People's Republic of China, Measures for the Ethical Review of Biomedical Research Involving Humans (2016).

5. Y. Peng, A. Ma, Z. Xiao, et al., "Technical Specifications for Ethics Review of Human Stem Cell Research," *Cell Proliferation* 57, no. 3 (2024): e13556, https://doi.org/10.1111/cpr.13556.

6. J. Hao, A. Ma, L. Wang, et al., "General Requirements for Stem Cells," *Cell Proliferation* 53, no. 12 (2020): e12926, https://doi.org/10.1111/cpr.12926.

7. H. Gao, Q. Chen, P. Zhai, et al., GB/T 37864–2019, General Requirements of Quality and Competence for Biobank (2019).

8. T. Zhao, J. Hao, Q. Zhou, et al., GB/T 42466–2023, Biobanking Technical Requirement for Management of Pluripotent Stem Cells (2023).

Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Annex A (Informative):** Organoid histopathology testing (paraffin embedding method). **Annex B (Informative):** Organoid gene mutation testing. **Annex C (Informative):** Quantification of organoid survival rates (Calcein-AM staining method). **Annex D (Informative):** Organoid authentication by STR profiling.