FRIGID ZONE **MEDICINE**
Review • DOI: 10.1515/fzm-2024-0008

Cryoablation techniques in bladder cancer: A review

Binglei Ma[1,2], Wilhem Teixeira[1,2], Lijuan Jiang[1,2*]

Abstract

Bladder cancer (BC) ranks as the tenth most common cancer globally. Histopathologically, BC is broadly categorized into urothelial and non-urothelial BC. Urothelial carcinoma represents over 90% of BC in most regions worldwide. The standard treatment procedure for diagnosing and treating non-muscle-invasive bladder cancer (NMIBC) is transurethral resection of bladder tumors (TURBT). Currently, the standard of care for muscle-invasive bladder cancer (MIBC) is neoadjuvant chemotherapy followed by radical cystectomy. Cryoablation therapy is a medical technique that uses extremely low temperatures to destroy diseased tissue. This treatment serves as a therapeutic tool for both benign and malignant diseases in organs such as the kidney, prostate gland, lung, liver, and breast, and is particularly effective for unresectable tumors, offering less trauma, quick recovery, good tolerability, and symptom control. However, cryoablation has its limitations. Over the past few years, cryoablation therapy has emerged as a new method for treating early BC. This treatment is minimally invasive, precise, and offers quick recovery, providing patients with a new treatment option. Although randomized studies are still limited, increasing evidence suggests its potential application in bladder cancer combined with transurethral resection (TURBT) or medication. Cryoablation is not standard therapy for bladder cancer. Treatment decisions should be discussed by a multidisciplinary team of urologists, oncologists, and interventional physicians and require more randomized controlled trials to define patient selection criteria and treatment approaches.

Keywords

bladder tumor; transurethral resection of bladder tumors; muscle-invasive bladder cancer; non-muscle-invasive bladder cancer; cryoablation

Received 21 November 2023, accepted 1 April 2024

[1]*State Key Laboratory of Oncology in South China, Collaborative Innovation Center of Cancer Medicine, Sun Yat-sen University Cancer Center, Guangzhou 510060, China*
[2]*Department of Urology, Sun Yat-sen University Cancer Center, Guangzhou 510060, China*

**Corresponding author Lijuan Jiang, Email: jianglij@sysucc.org.cn*

1 Bladder cancer

Bladder cancer (BC) ranks as the tenth most common cancer globally and climbs to seventh among males. The global age-standardized incidence rate (per 100,000 person-years) is 9.5 in men and 2.4 in women[1]. Histopathologically, BC is broadly divided into urothelial and non-urothelial types, with squamous cell carcinoma (SCC) being the most common in the latter category. Urothelial carcinoma represents over > 90% of BC cases in Europe and North America, while SCC predominates in Middle Eastern regions where Schistosoma haematobium is endemic. Almost 80% of BC cases present as non-muscle invasive disease. Among these, 60% are confined to the bladder mucosa (pTa), 30% invade the submucosa (pT1), and 10% present as carcinoma in situ[2-4]. Patients with non-muscle-invasive bladder cancer (NMIBC) have a higher prevalence due to longer survival times and a lower risk of cancer-specific mortality compared to those with muscle-invasive bladder cancer (MIBC)[5-6]. The five-year survival rate for BC correlates with the disease stage at diagnosis, ranging as high as 95.8% for carcinoma in situ and as low as 4.6% for metastatic disease, underscoring the importance of accurate and timely diagnosis for the prognosis of BC patients[7].

The standard treatment for diagnosing and managing NMIBC is transurethral resection of bladder tumors (TURBT)[8-9]. While TURBT alone can completely remove NMIBC, recurrence and progression to muscle-invasive are common. Significant variation in the 3-month recurrence rate suggests incomplete TURBT or frequent recurrences in many patients[10]. MIBC is an aggressive form requiring timely management. The current standard of care is neoadjuvant chemotherapy followed by radical cystectomy, an approach associated with significant morbidities. However, refinements in the chemotherapy regimens, perioperative care, and surgical techniques have led to improved overall toxicity

profiles and faster recovery[11-13]. The 5-year overall survival rate for patients undergoing radical cystectomy with locally advanced bladder cancer ($pT_{3-4}N_{-}$, or pN_{+}) is approximately 50%[14]. Most local recurrences or distant metastases occur within 2 years after radical cystectomy[15]. BC that progresses to metastasis is generally incurable and has a poor prognosis. Patients receiving platinum-based chemotherapy for metastatic disease have a median survival of 9-26 months[16-18]. However, better outcomes (28%-33% survival at 5 years) have been observed in patients with oligometastatic disease treated with total metastatic tumor (TMT), including metastasectomy[19-20]. Notably, bladder preservation in carefully selected patients can lead to acceptable oncological outcomes and better quality of life[21-23]. Optimizing bladder preservation protocols and accurately identifying patients who tolerate and respond well to various treatment modalities will significantly enhance survival in the future[24-31].

Ablation therapy stands as one of the earliest and widely used minimally invasive treatment methods for cancer in various locations[32]. Local ablation therapy encompasses thermal ablation and cryoablation, with this study focusing primarily on cryoablation technology. Cryoablation proves most effective for small tumors. Cryotherapy serves as a therapeutic tool for both benign and malignant diseases in organs such as the kidney, prostate gland, lung, liver, and breast. It is particularly useful for unresectable tumors, offering less trauma, quick recovery, good tolerability, and effective symptom control[33-36]. This study reviews research advancements in cryoablation for bladder cancer.

2 Cryoablation

Cryoablation therapy, also known as cryotherapy, is a medical procedure that employs extremely low temperatures to destroy diseased tissue. This technique is primarily utilized for treating certain cancers, including those of the liver, kidney, prostate, as well as both benign and malignant skin tumors[37-40].

Cryoablation operates on the Joule-Thompson principle, a concept in physics first applied in 1993 to induce cell death through cold exposure[32]. Typically conducted under the guidance of imaging techniques such as ultrasound or CT scans, this procedure involves inserting one or more slender probes (cryoprobes) into the targeted cancer or tissue. Liquid nitrogen or argon gas circulates through the probes, freezing the tissue and forming ice balls that destroy the cells. The formation of these "ice balls" is monitored in real-time using CT scans during multiple freezing and thawing cycles, which is a unique advantage in treating nonaerated organs[41-42]. Multiple cycles and several probes may be employed for larger tumors to ensure full ablation[43]. Cryotherapy destroys cells by extracting heat, utilizing methods like liquid nitrogen or pressurized argon gas, both of which reach temperatures around -185°C. The key to cryotherapy is low temperatures. All cells, except those prepared for cryopreservation, are destroyed at temperatures around -40°C[33]. Cytotoxic ice temperatures have been observed around 4-5 mm within the ice ball's perimeter[44-45].

The advantages of this treatment include less trauma, faster recovery, and fewer side effects compared to traditional surgery. Typically minimally invasive, it can be done under local anesthesia, reducing the patient's hospital stay and recovery time. Vasoconstriction around the frozen area may also reduce the risk of bleeding during treatment. Post-treatment symptoms, like pain, swelling, fever, and other reactions, are generally mild and resolve with symptomatic treatment[46-50].

However, cryoablation has its limitations. It may not work for all types of cancer, especially cases that have metastasized widely. The treatment's efficacy may be affected by the size and location of the tumor, with larger tumors, tumors near significant blood vessels, and tumors in vital organs posing challenges for complete ablation[51]. Additionally, there is a risk of tumor recurrence in the treated area[52]. Therefore, cryoablation is more suitable for early malignant tumors with small size and moderate location.

In summary, the key factors in cryoablation are freezing temperature, speed, and duration[33]. Cryotherapy is mainly performed percutaneously with minimal harm and no absolute contraindications. This makes it an effective complementary treatment for patients unsuitable for surgery, aiding in tumor control or managing oligometastatic disease. The decision depends on the specific cancer characteristics, the tumor's location, and the patient's overall health.

3 Cryoablation in the treatment of bladder cancer

Bladder cancer, a common malignant tumor of the urinary system, is treated through various methods including surgery, systemic medicine therapy, radiotherapy. In recent years, advancements in medical technology have introduced cryoablation therapy as a novel method for treating early bladder cancer (Fig. 1). This minimally invasive approach offers precision and rapid recovery, presenting a new treatment option for patients.

Prior to cryoablation, a thorough cystoscopy is essential to assess tumor size, location, and quantity, guiding the selection of the type of cryoprobe and treatment plan. During the procedure, patients are typically under general anesthesia. A cryoablation probe is placed into the tumor tissue *via* cystoscopy. Post-treatment symptoms such as poor urination and bladder irritation are common, requiring dietary adjustments and pharmacological

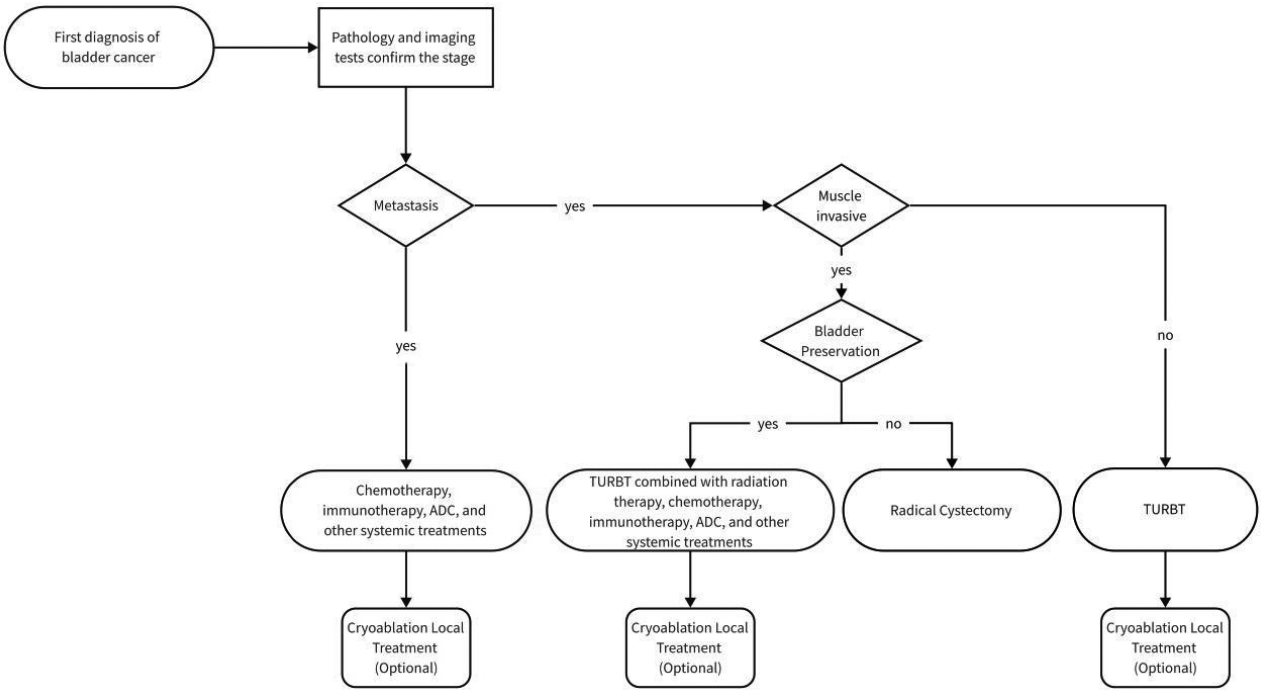

Fig. 1 Strategy for choosing cryoablation treatment for bladder cancer

intervention[53-56].

Cryoablation has been a supplementary treatment option for BC for over seventy years. McDonald *et al.* explored cryoablation's impact on bladder cancer in dogs in 1950, noting the formation of characteristic necrotic lesions[57]. Permpongkosol *et al.* demonstrated the morphological changes from percutaneous full-thickness bladder cryoablation in pigs, confirming reliable tissue destruction without bladder perforation. The initial data indicated feasibility and safety of trials in treating bladder tumors using cystoscopic, laparoscopic, or image-guided techniques in 2006[58]. At the same time, it showed controllable transmural necrosis in both single and repeated cycles without bladder perforations[58]. Nevertheless, there is limited evidence regarding the use of cryotherapy in treating intraluminal tumors, such as bladder cancer, whether metastatic, MIBC, or NMIBC[59]. Factors potentially hindering its clinical implementation include clear ice ball visibility on CT scans, precise coverage matching complete necrosis, and preventing damage to bladder, prostate, and uterine structures[58,60-61]. Existing reports are scattered and primarily consist of case reports[62-63], and a limited amount of clinical data[64-66].

One feasibility study reported the success of CT imaging–guided percutaneous argon-helium cryotherapy in treating 32 patients with MIBC[64]. All patients had clinical stage $T_{2\text{-}4a}N_0M_0$. Bladder cryoablation was technically successful in all 32 cases, and 30 cases required only one treatment session. No major complications were observed, and bladder integrity was maintained in all patients. This study provides preliminary evidence suggesting that this minimally invasive method for ablating bladder tumors using CT imaging-guided percutaneous argon-helium cryoablation is promising and safe for treating patients with muscle-invasive bladder cancer[64].

Cryoablation may also be a practical option for metastatic bladder cancer. A retrospective study involving 23 patients who underwent comprehensive cryosurgery for metastatic bladder cancer showed a progression-free survival (PFS) of 14 ± 8 months over four years of follow-up. The study suggested outcomes surpassing those for platinum-based chemotherapy, with differentiation status and tumor size influencing the efficacy of percutaneous cryoablation. However, tumor location and histopathology had no effect on PFS[65].

Cryoablation is a therapeutic approach that employs extreme cold to induce necrosis and apoptosis in living cells[67]. The necrosis caused by extreme cold has the potential to trigger a systemic anti-tumor immune response by releasing tumor antigens. This implies that cryoablation might serve as an autologous tumor vaccine[68-69]. Recently, there have been changes in preferred medications, yet there have been limited advancements in the domain of medical devices for bladder cancer. Endoscopic balloon cryoablation (EBCA) showed a promising minimally invasive technique for intraluminal cancer. EBCA is presently being assessed as an innovative option for treating bladder cancer[66].

There is a prospective randomized clinical trial (RCT) that involves combining cryotherapy with the standard transurethral resection (TUR) for patients with bladder cancer. This study is the first to demonstrate that EBCA is a safe and effective adjuvant therapy when used alongside TUR for NMIBC. The cryoballoon, which uses liquid nitrogen, was specifically designed for use under a cystoscope and resectoscope. The feasibility and safety of using cryoablation for bladder tumors were confirmed through experiments on animal models and a small, single-arm preclinical trial before[70-71]. Subsequent research has revealed that the tumor-specific immune responses induced by cryoablation could reduce tumor recurrence and metastasis[59].

4 Conclusion

Cryoablation is a minimally invasive surgical procedure offering notable advantages for localized tumor control compared to traditional surgery or systemic drug treatments. While randomized studies remain limited, increasing evidence suggests its potential application in bladder cancer management, particularly when combined with transurethral resection (TURBT) or pharmacological therapies.

Currently, cryoablation is not a standard therapy for bladder cancer. Treatment decisions need to be discussed by a multidisciplinary team of urologists, oncologists, and interventional specialists. There is a pressing need for more randomized controlled trials to define precise patient selection criteria and treatment approaches.

Author contributions

Jiang L J drafted, edited, and reviewed the manuscript. Ma B L edited the manuscript. Teixeira W summarized relevant articles and data.

Source of funding

This work was supported by the 2023 Guangzhou Basic and Applied Basic Research Project (2023A04J2132).

Conflict of interest

The authors declare no conflicts of interest.

Data availability statement

Data used to support the findings of this study are available from the corresponding author upon request.

References

[1] Burger M, Catto J W, Dalbagni G, *et al.* Epidemiology and risk factors of urothelial bladder cancer. Eur Urol, 2013; 63: 234-241.
[2] Georgantzoglou N, Pergaris A, Masaoutis C, *et al.* Extracellular vesicles as biomarkers carriers in bladder cancer: diagnosis, surveillance, and treatment. Int J Mol Sci, 2021; 22(5): 2744.
[3] Torre L A, Bray F, Siegel R L, *et al.* Global cancer statistics, 2012: Global Cancer Statistics, 2012. CA Cancer J. Clin, 2015; 65: 87-108.
[4] Dahm P, Gschwend J E. Malignant non-urothelial neoplasms of the urinary bladder: a review. Eur. Urol, 2003; 44: 672-681.
[5] Comperat E, Larre S, Roupret M, *et al.* Clinicopathological characteristics of urothelial bladder cancer in patients less than 40 years old. Virchows Arch, 2015; 466: 589-594.
[6] IARC, Cancer Today. Estimated number of new cases in 2020, worldwide, both sexes, all ages. Accessed on March 2022. https: //gco.iarc.fr/today/online-analysis-table.
[7] Bray F, Ferlay J, Soerjomataram I, *et al.* Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin, 2018; 68: 394-424.
[8] Logan C, Brown M, Hayne D. Intravesical therapies for bladder cancer - indications and limitations. BJU Int, 2012; 110 Suppl 4: 12-21.
[9] Flaig T W, Spiess P E, Agarwal N, *et al.* NCCN guidelines insights: bladder cancer, version 5.2018. J Natl Compr Canc Netw, 2018; 16: 1041-1053.
[10] Brausi M, Collette L, Kurth K, *et al.* Variability in the recurrence rate at first follow-up cystoscopy after TUR in stage Ta T1 transitional cell carcinoma of the bladder: a combined analysis of seven EORTC studies. Eur Urol, 2002; 41: 523-531.
[11] Siegel R L, Miller K D, Jemal A. Cancer statistics, 2019. CA Cancer J Clin, 2019; 69(1): 7-34.
[12] Charlton M E, Adamo M P, Sun L, *et al.* Bladder cancer collaborative stage variables and their data quality, usage, and clinical implications: a review of SEER data, 2004-2010. Cancer, 2014; 120(Suppl 23): 3815-3825.
[13] Chang S S, Bochner B H, Chou R, *et al.* Treatment of non-metastatic muscle-invasive bladder cancer: AUA/ASCO/ASTRO/SUO guideline. J Urol, 2017; 198(3): 552-559.
[14] Sternberg C N, Skoneczna I, Kerst J M, *et al.* Immediate versus deferred chemotherapy after radical cystectomy in patients with pT3-pT4 or N+ M0 urothelial carcinoma of the bladder (EORTC 30994): an intergroup, open-label, randomised phase 3 trial. Lancet Oncol, 2015; 16: 76-86.
[15] Stenzl A, Cowan N C, De Santis M, *et al.* The updated EAU guidelines on muscle-invasive and metastatic bladder cancer. Eur Urol, 2009; 55: 815-825.
[16] Vrooman O P, Witjes J A. Follow-up of patients after curative bladder cancer treatment: guidelines vs. practice. Curr Opin Urol, 2010;

20: 437-442.
[17] Cagiannos I, Morash C. Surveillance strategies after definitive therapy of invasive bladder cancer. Can Urol Assoc J, 2009; 3: S237-242
[18] M J Mathers J Z, S Wyler, S Roth, *et al.* Is there evidence for a multidisciplinary follow-up after urological cancer? An evaluation of subsequent cancers. World J Urol, 2008; 26: 251-256.
[19] Bekku K, Saika T, Kobayashi Y, *et al.* Could salvage surgery after chemotherapy have clinical impact on cancer survival of patients with metastatic urothelial carcinoma? Int J Clin Oncol, 2013; 18: 110-115.
[20] Lehmann J, Suttmann H, Albers P, *et al.* Surgery for metastatic urothelial carcinoma with curative intent: the German experience (AUO AB 30/05). Eur Urol, 2009; 55: 1293-1299.
[21] Ghandour R, Singla N, Lotan Y. Treatment options and outcomes in nonmetastatic muscle invasive bladder cancer. Trends Cancer, 2019; 5(7): 426-439.
[22] Alfred Witjes J, Lebret T, Compérat E M, *et al.* Updated 2016 EAU guidelines on muscle-invasive and metastatic bladder cancer. Eur Urol, 2017; 71(3): 462-475.
[23] Flaig T W, Spiess P E, Agarwal N, *et al.* NCCN guidelines insights: bladder cancer, version 5.2018. J Natl Compr Canc Netw, 2018; 16(9): 1041-1053.
[24] Ploussard G, Daneshmand S, Efstathiou J A, *et al.* Critical analysis of bladder sparing with trimodal therapy in muscle-invasive bladder cancer: a systematic review. Eur Urol, 2014; 66(1): 120-137.
[25] Dunst J, Sauer R, Schrott K M, *et al.* Organ-sparing treatment of advanced bladder cancer: a 10-year experience. Int J Radiat Oncol Biol Phys, 1994; 30(2): 261-266.
[26] Shipley W U, Prout G R Jr, Einstein A B, *et al.* Treatment of invasive bladder cancer by cisplatin and radiation in patients unsuited for surgery. JAMA, 1987; 258(7): 931-935.
[27] Hussain S A, Stocken D D, Peake D R, *et al.* Long-term results of a phase II study of synchronous chemoradiotherapy in advanced muscle invasive bladder cancer. Br J Cancer, 2004; 90(11): 2106-2111.
[28] Choudhury A, Swindell R, Logue J P, *et al.* Phase II study of conformal hypofractionated radiotherapy with concurrent gemcitabine in muscle-invasive bladder cancer. J Clin Oncol, 2011; 29(6): 733-738.
[29] Hoskin P J, Rojas A M, Bentzen S M, *et al.* Radiotherapy with concurrent carbogen and nicotinamide in bladder carcinoma. J Clin Oncol, 2010; 28(33): 4912-4918.
[30] Fransen van de Putte E E, Pos F, Doodeman B, *et al.* Concurrent radiotherapy and panitumumab after lymph node dissection and induction chemotherapy for invasive bladder cancer. J Urol, 2019; 201(3): 478-485.
[31] Gakis G, Efstathiou J, Lerner S P, *et al.* ICUD-EAU International consultation on bladder cancer 2012: radical cystectomy and bladder preservation for muscle-invasive urothelial carcinoma of the bladder. Eur Urol, 2013; 63(1): 45-57.
[32] Amoils S P. The Joule Thomson cryoprobe. Arch Ophthalmol, 1967; 78: 201-207.
[33] Coldwell D M, Sewell P E. The expanding role of interventional radiology in the supportive care of the oncology patient: from diagnosis to therapy. Semin Oncol, 2005; 32: 169-173.
[34] Mu F, Niu L, Li H, *et al.* Percutaneous comprehensive cryoablation for metastatic hepatocellular cancer. Cryobiology, 2013; 66: 76-80.
[35] Niu L Z, Li J L, Zeng J Y, *et al.* Combination treatment with comprehensive cryoablation and immunotherapy in metastatic hepatocellular cancer. World J Gastroenterol, 2013; 19: 3473-3480.
[36] Niu L, Mu F, Zhang C, *et al.* Cryotherapy protocols for metastatic breast cancer after failure of radical surgery. Cryobiology, 2013; 67: 17-22.
[37] Kwak K, Yu B, Lewandowski R J, *et al*. Recent progress in cryoablation cancer therapy and nanoparticles mediated cryoablation. Theranostics, 2022; 12(5): 2175-2204.
[38] Solomon S B, Silverman S G. Imaging in interventional oncology. Radiology, 2010; 257: 624-640.
[39] Link R E, Permpongkosol S, Gupta A, *et al.* Cost analysis of open, laparoscopic, and percutaneous treatment options for nephron-sparing surgery. J Endourol, 2006; 20: 782-789.
[40] Aarts B M, Klompenhouwer E G, Rice S L, *et al.* Cryoablation and immunotherapy: an overview of evidence on its synergy. Insights Imaging, 2019; 10: 53.
[41] Ito N, Nakatsuka S, Inoue M, *et al*. Computed tomographic appearance of lung tumors treated with percutaneous cryoablation. J Vasc Interv Radiol, 2012; 23: 1043-1052.
[42] Saliken J C, McKinnon J G, Gray R. CT for monitoring cryotherapy. AJR Am J Roentgenol, 1996; 166: 853-855.
[43] Niu L, Xu K, Mu F. Cryosurgery for lung cancer. J Thorac Dis, 2012; 4: 408-419.
[44] Hinshaw J L, Durick N, Leung W, *et al*. Radiology-pathology correlation of pulmonary cryoablation in a porcine model. J Intervent Oncol, 2009; 2: 113-120.
[45] Hinshaw J L, Littrup P J, Durick N, *et al.* Optimizing the protocol for pulmonary cryoablation: a comparison of a dual- and triple-freeze protocol. Cardiovasc Intervent Radiol, 2010; 33(6): 1180-1185.
[46] Maj R, Iacopino S, Stroker E, *et al.* Mid-term outcome following second-generation cryoballoon ablation for atrial fibrillation in heart failure patients: effectiveness of single 3-min freeze cryoablation performed in a cohort of patients with reduced left ventricular systolic function. J Cardiovasc Med (Hagerstown), 2019; 20: 667-675.
[47] Cha C, Lee F T Jr, Rikkers L F, *et al.* Rationale for the combination of cryoablation with surgical resection of hepatic tumors. J Gastrointest Surg, 2001; 5: 206-213.
[48] Glazer D I, Tatli S, Shyn P B, *et al*. Percutaneous image-guided cryoablation of hepatic tumors: single-center experience with intermediate to long-term outcomes. AJR Am J Roentgenol, 2017; 209: 1381-1389.
[49] Deng W, Chen L, Wang Y, *et al.* Cryoablation versus partial nephrectomy for clinical stage T1 renal masses: a systematic review and meta-analysis. J Cancer, 2019; 10: 1226-1236.
[50] Pecoraro A, Palumbo C, Knipper S, *et al.* Cryoablation predisposes to higher cancer specific mortality relative to partial nephrectomy in patients with nonmetastatic pT1b kidney cancer. J Urol, 2019; 202: 1120-1126.
[51] Sohn R L, Carlin A M, Steffes C, *et al.* The extent of cryosurgery increases the complication rate after hepatic cryoablation. Am Surg, 2003; 69: 317-322.
[52] Bouhamama A, Wdowik Q, Grillet F, *et al.* Prognostic factors for local recurrence after cryoablation of desmoid tumors. J Vasc Interv Radiol, 2023; 34(9): 1538-1546.
[53] McDonald D F, Mobley T L, Rudolph J H. Cryotherapy of a heterografted human bladder tumor. Long-term observations. Cryobiology, 1966; 2(5): 280-284.
[54] McDonald D F, Mobley T L, Rudolph J H. Cryotherapy of a heterografted human bladder tumor. J Urol, 1966; 95(4): 526-530.
[55] Cahan W G, Adam Y, Mackenzie R A, *et al.* Intractable bladder

hemorrhage treated by cryosurgery: a preliminary report. J Urol, 1970; 103(5): 606-611.
[56] Mackenzie A R. Cryotherapy of the bladder for cancer. J Urol, 1972; 107(3): 387-388.
[57] McDonald J, Taylor C B, Heckel N J. Rapid freezing of the bladder: an experimental and clinical study. J Urol, 1950; 64: 326-337.
[58] Permpongkosol S, Nicol T L, Kavoussi L R, *et al.* Percutaneous bladder cryoablation in porcine model. J Endourol, 2006; 20: 991-995.
[59] Mou Z, Chen Y, Zhang Z, *et al.* Cryoablation inhibits the recurrence and progression of bladder cancer by enhancing tumour-specific immunity. Clin Transl Med, 2023; 13: 1255.
[60] Littrup P J, Mody A, Sparschu R, *et al.* Prostatic cryotherapy: ultrasonographic and pathologic correlation in the canine model. Urology, 1994; 44: 175-183.
[61] Li J, Chen J, Zhou L, *et al.* Comparison of dual- and triple-freeze protocols for hepatic cryoablation in a Tibet pig model. Cryobiology, 2012; 65: 68-71.
[62] Marjara J, Hilli J, Davis R M, *et al.* Metastatic retro-crural lymph nodes from transitional cell carcinoma of bladder successfully treated with single session cryoablation. Radiol Case Rep, 2020; 15: 1197-1201.
[63] Zhang Q, Zhang S, Zhang S, *et al.* Transperineal cryotherapy for unresectable muscle invasive bladder cancer: preliminary experience with 7 male patients. BMC Urol, 2017; 17: 81.
[64] Sun L, Zhang W, Liu H, *et al.* Computed tomography imaging-guided percutaneous argon-helium cryoablation of muscle-invasive bladder cancer: initial experience in 32 patients. Cryobiology, 2014; 69: 318-322.
[65] Liang Z, Fei Y, Lizhi N, *et al.* Percutaneous cryotherapy for metastatic bladder cancer: experience with 23 patients. Cryobiology, 2014; 68: 79-83.
[66] Xu C, Jiang S, Zou L, *et al.* Endoscopic balloon cryoablation plus transurethral resection for bladder cancer: A phase 2, multicenter, randomized, controlled trial. Cancer, 2023; 129: 415-425.
[67] Gage A A, Baust J. Mechanisms of tissue injury in cryosurgery. Cryobiology, 1998; 37: 171-186.
[68] Yakkala C, Denys A, Kandalaft L, *et al.* Cryoablation and immunotherapy of cancer. Curr Opin Biotechnol, 2020; 65: 60-64.
[69] Srivastava P K. Hypothesis: controlled necrosis as a tool for immunotherapy of human cancer. Cancer Immun, 2003; 18: 4.
[70] Liu S, Zou L, Mao S, *et al.* The safety and efficacy of bladder cryoablation in a beagle model by using a novel balloon cryoprobe. Cryobiology, 2016; 72: 157-160.
[71] Liu S, Zhang L, Zou L, *et al.* The feasibility and safety of cryoablation as an adjuvant therapy with transurethral resection of bladder tumor: a pilot study. Cryobiology, 2016; 73(2): 257-260.