Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergikafkas.org
E-ISSN: 1309-2251

Kafkas Univ Vet Fak Derg
32 (4): 521-528, 2026
DOI: 10.9775/kvfd.2026.36599

RESEARCH ARTICLE

# Sequential Intravesical Instillation of Epirubicin and IL-15/IL-15Rα Enhances Antitumor Efficacy in Orthotopic Bladder Cancer Through Immunogenic Cell Death and CD8+ T Cell-Dependent Immune Activation

Jiawei MA[1], Shichao WEI[1], Feifei PAN[1(*)]

[1] Department of Urology, Nanjing Tongren Hospital, School of Medicine, Southeast University, Nanjing 210000, Jiangsu Province, CHINA

(*) **Corresponding author:**
Feifei PAN
Phone: +86-18952553162
E-mail: panffnth@shu-edu.cn

How to cite this article?

**Ma J, Wei S, Pan F:** Sequential Intravesical Instillation of Epirubicin and IL-15/IL-15Rα Enhances Antitumor Efficacy in Orthotopic Bladder Cancer Through Immunogenic Cell Death and CD8+ T Cell-Dependent Immune Activation. *Kafkas Univ Vet Fak Derg*, 32 (4): 521-528, 2026.
DOI: 10.9775/kvfd.2026.36599

**Article ID:** KVFD-2026-36599
**Received:** 16.03.2026
**Accepted:** 18.07.2026
**Published Online:** 28.07.2026

**Abstract**

This study aimed to investigate whether sequential intravesical instillation of epirubicin (EPI) followed by interleukin-15 (IL-15)/interleukin-15 receptor alpha (IL-15Rα) could integrate cytotoxic and immune-stimulatory effects for improved antitumor efficacy. EPI-induced immunogenic cell death (ICD) was assessed in MB49 cells by measuring calreticulin (CRT) exposure and adenosine triphosphate (ATP) release. Cytotoxic activity of splenocytes against MB49 cells was evaluated by co-culture apoptosis assays and IFN-γ quantification. In vivo efficacy was examined in C57BL/6 mice bearing orthotopic bladder tumors. Tumor volume survival, and immune infiltration were analyzed, and CD8+ T cell depletion was performed to assess the contribution of these cells to therapeutic efficacy. EPI treatment increased surface CRT and ATP release. In co-culture assays, EPI pretreatment enhanced splenocyte-mediated MB49 cell apoptosis and IFN-γ secretion, and these effects were further augmented by IL-15/IL-15Rα. *In vivo*, sequential therapy significantly reduced tumor volume, prolonged survival, and promoted a favorable immune microenvironment, characterized by increased $CD8^+$ T and $NK1.1^+$ cell infiltration, reduced Treg population, an elevated CD8/Treg ratio, and increased IFN-γ level. Importantly, $CD8^+$ T cell depletion attenuated these therapeutic benefits. Sequential intravesical administration of EPI and IL-15/IL-15Rα enhanced antitumor efficacy in an orthotopic bladder cancer model by promoting ICD-associated changes and cytotoxic immune responses. These findings provide preclinical evidence supporting further investigation of EPI-based chemo-immunotherapy for bladder cancer, including BCG-unresponsive or high-risk non-muscle-invasive bladder cancer (NMIBC).

**Keywords:** Chemo-immunotherapy, EPI, IL-15/IL-15Rα, NMIBC

## INTRODUCTION

Bladder cancer (BC) is a common malignancy, with non-muscle invasive bladder cancer (NMIBC) accounting for approximately 75% of all cases [1,2]. Transurethral Resection of Bladder Tumor (TURBT) remains the gold-standard treatment strategy for NMIBC, whereas adjuvant intravesical therapy is selected according to the risk of recurrence and progression [3]. Bacillus Calmette-Guérin (BCG) therapy is widely used for high-risk NMIBC, while radical cystectomy may be considered for selected patients with very high-risk or treatment-refractory disease [4,5]. BCG binds to and is internalized by urothelial tumor cells, thereby triggering a broad immune response involving natural killer (NK) cells and $CD8^+$ T cells that contributes to tumor elimination [6]. Nevertheless, a substantial proportion of patients fails to respond to BCG therapy or experience disease recurrence, and BCG administration may also cause clinically significant adverse effects [7]. Therefore, novel strategies with improved efficacy and acceptable toxicity are urgently needed for NMIBC.

Interleukin-15 (IL-15), recognized as a promising target for cancer immunotherapy, has demonstrated antitumor potential in several malignancies, including ovarian, gastric, and bladder cancers [8-11]. As a common γ-chain cytokine, IL-15 plays an important role in the development, proliferation, and survival of NK cells and $CD8^+$ T cells [12]. Complex formation between IL-15 and IL-15Rα enhances IL-15-mediated stimulation of NK and T cells and has led to the development of IL-15 superagonists and IL-15/IL-15Rα complexes with improved biological

activity [13-15]. ALT-803 (N-803), an IL-15 superagonist, has demonstrated promising clinical activity NMIBC, and its combination with BCG has been developed for BCG-unresponsive carcinoma in situ [16,17]. These findings highlight the therapeutic potential of IL-15/IL-15Rα-based approaches in NMIBC.

Epirubicin (EPI) is a widely used anthracycline chemotherapeutic agent that exerts antitumor activity through DNA intercalation, topoisomerase II inhibition, and induction of apoptosis [18]. Intravesical EPI administered after TURBT effectively can reduce recurrence in NMIBC while limiting systemic exposure, making it suitable for local administration [19]. Beyond its direct cytotoxicity, EPI has also been reported to induce ICD, thereby potentially enhancing anti-tumor immune responses [20]. These properties provide a rationale for sequentially combining EPI with IL-15/IL-15Rα to integrate chemotherapy-induced tumor cell injury with immune stimulation.

In this study, we explored the antitumor effects and potential immune mechanisms of sequential intravesical administration of EPI followed by IL-15/IL-15Rα in an orthotopic bladder cancer model, with the aim of providing preclinical evidence for a novel chemo-immunotherapy strategy.

## Material and Methods

### Ethical Approval

All animal experimental procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals issued by the Ministry of Science and Technology of China, and were approved by the Animal Ethics Committee of Nanjing Tongren Hospital, Southeast University (Approval No.: SHU2023/021).

### Cell Culture and Treatment

The C57BL/6-derived murine BC cell line MB49 (cat. no. iCell-m030; iCell Bioscience Inc) were cultured in DMEM (cat. no. iCell-0001) supplemented with 10% FBS and 1% penicillin-streptomycin. Cells were maintained at 37°C in a humidified atmosphere containing 5% $CO_2$. For EPI treatment, cells were exposed to 0.2 or 1.2 μg/mL EPI for 24 h [21].

### Assessment of ICD

To assess ICD-associated changes, MB49 cells were seeded in 6-well plates and stained with an Alexa Fluor 488-conjugated anti-CRT antibody. Surface CRT expression was subsequently quantified by flow cytometry. To evaluate extracellular ATP release, culture supernatants were collected and analyzed using the ENLITEN ATP Assay System Bioluminescence Detection Kits according to the manufacturer's instructions.

### Animal and Model Establishment

The female C57BL/6 mice (aged 6-8 weeks) were obtained from Beijing Vital River Laboratory Animal Technology Co., Ltd. All mice were acclimatized for 1 week with free access to water and food. The orthotopic bladder cancer model was established as previously described [22]. In brief, mice were anesthetized with isoflurane and placed on a warming pad. Residual urine was expelled by gently pressing the bladder. A 24G x 0.75-inch catheter was then inserted transurethrally, and the bladder was conditioned with 50 μL of poly-L-lysine (Sigma, 1 μg/mL in PBS) for 10 min. The solution was subsequently removed by gently pressing the bladder, which was then rinsed with 50 μL PBS, and instilled with $1x10^5$ MB49 cells. After incubation, the catheter was withdrawn, and the mice were transferred to recovery cages for post-procedure monitoring.

### Experimental Groups and Treatment Protocol

All mice were randomly assigned into four groups (n=8): PBS, EPI, EPI+IL-15/IL-15Rα and EPI+IL-15/IL-15Rα+anti-CD8 antibody. Four days after tumor cell instillation, mice in EPI group received an intravesical instillation of EPI at 5 mg/kg, which was retained in the bladder for approximately 1 h [23]. Mice in EPI+IL-15/IL-15Rα group additionally received an intravesical instillation of 2.5 μg IL-15/IL-15Rα complexes 24 h following EPI administration [24]. To deplete $CD8^+$ T cells, a CD8-specific monoclonal antibody (mAb) was used. Mice in EPI+IL-15/IL-15Rα + anti-CD8 antibody group received an initial intraperitoneal injection of 10 μg anti-CD8 antibody 1 day before intravesical treatment with EPI and IL-15/IL-15Rα [25]. Thereafter, the same dose of anti-CD8 antibody was administered intraperitoneally every four days until sacrifice [26]. Intravesical administration of EPI and/or IL-15/IL-15Rα complexes was performed twice weekly for three consecutive weeks. Mice in the PBS group received 50 μL PBS intravesically as a control. At the experimental endpoint, tumor-bearing bladders were excised and examined macroscopically. Tumor areas were identified as nodular lesions or focal irregular thickening clearly distinguishable from the adjacent normal-appearing bladder wall, with boundaries defined by the visible lesion-normal tissue interface. Identified tumor tissues were collected for subsequent analyses.

### Splenocyte Isolation

Spleens were collected from tumor-free C57BL/6 mice. Splenic tissues were gently dissociated between two frosted microscope slides, and the resulting homogenates were washed with RPMI-1640. After centrifugation at 200 g for 10 min, the splenic homogenates cell pellets were resuspended in 360 μL distilled water for 10 s to induce hemolysis, followed immediately by neutralization with 40 μL of 10× PBS. Pooled splenocytes were cultured in

5 mL RPMI-1640 medium supplemented with 0.05 mM β-mercaptoethanol, 10% FBS, and 1% antibiotics [27]. After 2 h of incubation, the cell-containing supernatant was collected for subsequent co-culture assays.

### Co-culture Assay of MB49 Cells and Splenocytes

Splenocytes were co-cultured with MB49 cells at an effector-to-target (E:T) ratio of 10:1 in complete RPMI-1640 medium for 24 h. Three experimental groups were established: (i) untreated MB49 cells co-cultured with splenocytes, (ii) EPI (1.2 μg/mL)-pretreated MB49 cells co-cultured with splenocytes, and (iii) EPI-pretreated MB49 cells co-cultured with splenocytes in the presence of IL-15/IL-15Rα complexes (7 ng/mL) [24].

### Cell Apoptosis

Following indicated treatment, MB49 cells were washed with PBS and resuspended in 100 μL binding buffer. The cells were then incubated with 2 μL Annexin-V-FITC on ice in the dark, following by PI staining [28]. Cell apoptosis was analyzed using a CytoFLEX flow cytometer according to the manufacturer's protocol.

### Flow Cytometry

Mice were sacrificed and bladder and tumor tissues were harvested. Tumor samples were digested with collagenase (300 U/mL) and hyaluronidase (100 U/mL) [29]. During digestion, the samples were gently pipetted every 15 min and incubated at 37°C for 3 h to generate single-cell suspensions. The suspensions were then filtered through a 200-μm cell strainer and washed with PBS. Cells were subsequently resuspended in FACS buffer and stained with antibodies against CD45, CD3, CD8, NK1.1 and Foxp3. Samples were analyzed using a BD FACSCanto II flow cytometry, and the data were processed using FlowJo software.

### ELISA

Splenocytes were co-cultured with MB49 cells for 24 h, after which the culture supernatants were collected. IFN-γ concentrations in the supernatants were measured using commercially available ELISA kits according to the manufacturer's instructions. For *in vivo* sample collection, 0.5 mL of PBS was instilled into the bladder and subsequently aspirated. The recovered fluid was centrifuged at 2.000 x g to remove cells, and the supernatant was stored at -80°C until analysis. IFN-γ concentrations were measured using the same ELISA procedure and expressed as ng/mL.

### Statistical Analysis

Data from three independent experiments were analyzed using GraphPad Prism 8.0 software. Normally distributed continuous variables are presented as mean ± SD. Comparisons among multiple groups were performed using one-way analysis of variance followed by Tukey's post-hoc test. For non-normally distributed data, the Kruskal-Wallis test was used. Survival analyses were conducted using the Kaplan-Meier method, and differences were evaluated with the log-rank test. P-values <0.05 was considered statistical significance.

## Results

Compared with the Control group, EPI treatment elevated surface CRT level and ATP release in a concentration-dependent manner, suggesting that EPI induced ICD-associated changes in MB49 cells *in vitro* (*Fig. 1-A, B*).

Following the co-culture of MB49 cells with splenocyte, cell apoptosis was assessed by flow cytometry. Compared with the Control group, EPI pre-treatment significantly increased MB49 cell apoptosis, and this effect was further enhanced by the addition of IL-15/IL-15Rα complexes (*Fig. 2-A, B*). ELISA showed that EPI pretreatment significantly increased IFN-γ secretion during co-culture. Following the addition of IL-15/IL-15Rα complexes, IFN-γ levels were further increased (*Fig. 2-C*). These findings indicated that EPI pretreatment enhanced splenocyte-mediated cytotoxicity against MB49 cells and that IL-15/IL-15Rα further strengthened this immune response.

Given that EPI induced ICD-associated changes and IL-15/IL-15Rα enhanced effector immune cell activity, we next

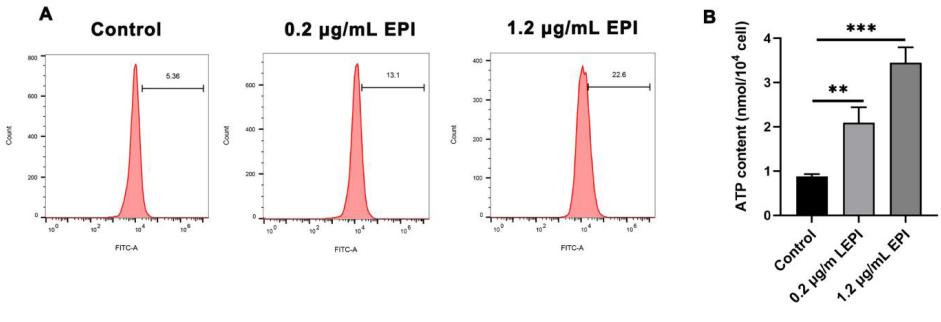


**Fig 1.** EPI contributed to ICD. **(A)** CRT level was detected using flow cytometry. **(B)** The ATP level was detected using ENLITEN ATP Assay System Bioluminescence Detection Kits. **P<0.01 and ***P<0.001

evaluated whether sequential administration of these agents could improve antitumor efficacy *in vivo*. EPI monotherapy significantly reduced tumor volume compared with PBS treatment, whereas sequential administration of EPI followed by IL-15/IL-15Rα resulted in a further reduction in tumor volume *(Fig. 3-A, B)*. Consistent with these findings, survival analysis demonstrated the longest survival in the EPI+IL-15/IL-15Rα group, followed by the EPI monotherapy group, with the PBS group displaying the shortest survival *(Fig. 3-C)*. Importantly, body weights remained generally stable across all groups, and no severe adverse events were observed, suggesting that the treatment regimen was well tolerated under the experimental conditions.

Because EPI-induced ICD may influence the tumor immune microenvironment, we next examined whether sequential combination therapy altered immune cell infiltration *in vivo*. Flow cytometric analysis showed that sequential EPI and IL-15/IL-15Rα therapy significantly increased the infiltration of $CD8^+$ T cells and $NK1.1^+$ cells compared with both the PBS and EPI groups. In contrast, the proportion of regulatory T cells (Tregs) was significantly lower in the EPI+IL-15/IL-15Rα group *(Fig. 4-A)*. The CD8/Treg ratio was increased in the EPI group compared with the PBS group, and was highest in the EPI+IL-15/IL-15Rα group *(Fig. 4-B)*. Consistently, IFN-γ levels were increased after EPI treatment and further elevated after sequential treatment with IL-15/IL-15Rα *(Fig. 4-C)*. Together, these findings indicated that sequential treatment enhanced cytotoxic immune responses while reducing immunosuppressive features of the tumor microenvironment.

Because sequential EPI and IL-15/IL-15Rα therapy was associated with increased $CD8^+$ T cell infiltration, we

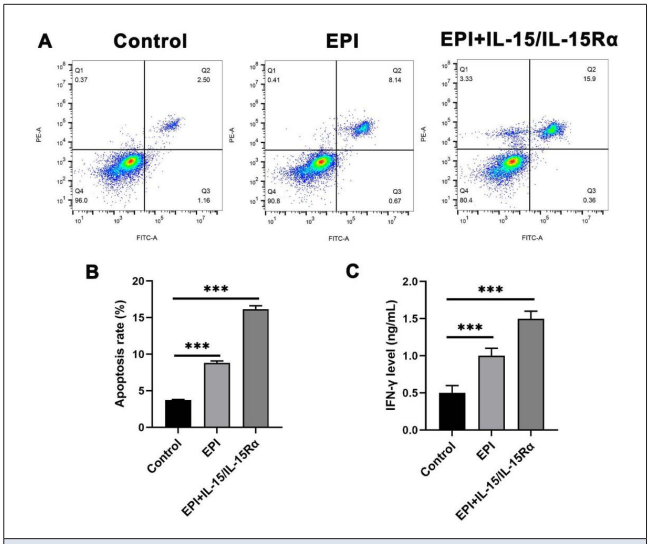


**Fig 2.** IL-15/IL-15Rα augmented splenocyte-mediated cytotoxicity against MB49 cells. **(A-B)** The cell apoptosis was detected using flow cytometry. (C) The level of IFN-γ was detected using ELISA-related IFN-γ assay kits. ***P<0.001

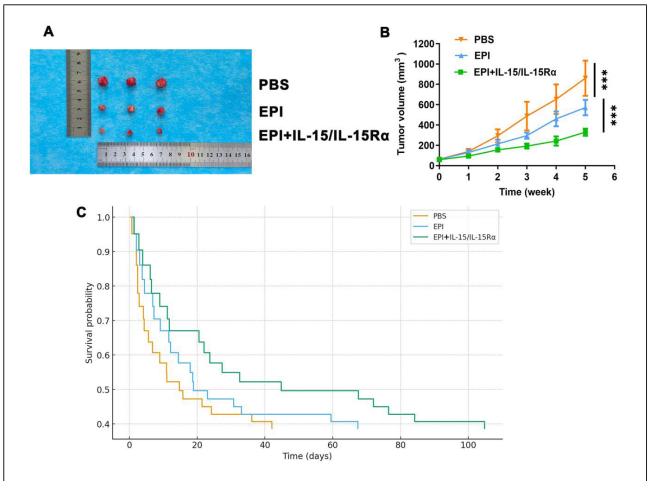


**Fig 3.** Sequential EPI and IL-15/IL-15Rα therapy exhibited superior antitumor efficacy. **(A)** The tumor appearance. **(B)** The tumor size. **(C)** The survival analysis. ***P<0.001

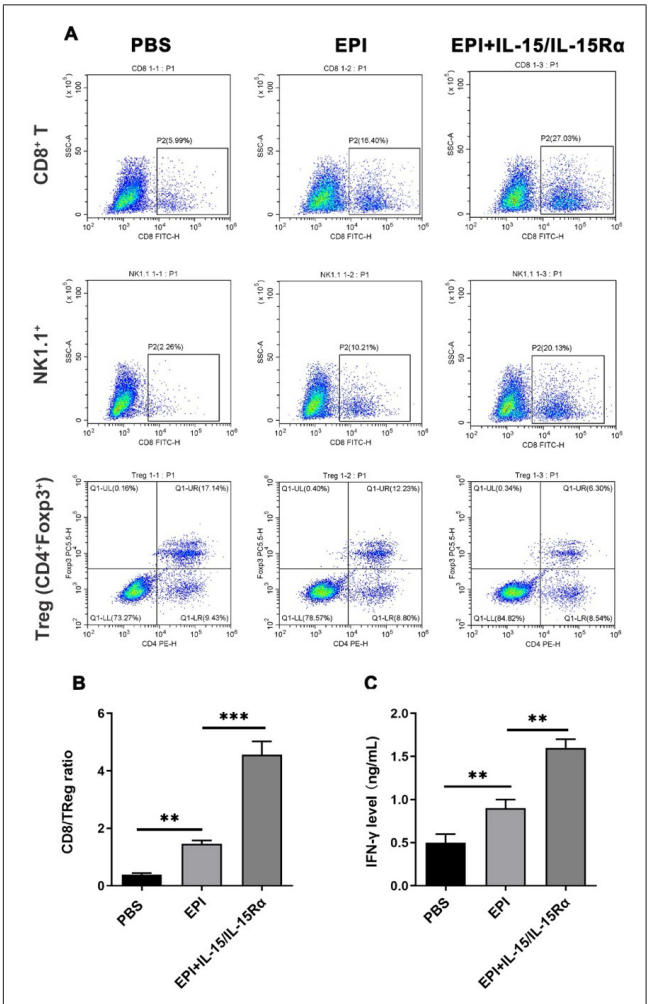


**Fig 4.** Sequential EPI and IL-15/IL-15Rα treatment augments antitumor immunity. **(A)** Flow cytometry was used for the detection of tumor-infiltrating immune cells. **(B)** The CD8/Treg ratio. **(C)** The level of IFN-γ was detected using ELISA-related IFN-γ assay kits. **P<0.01 and ***P<0.001

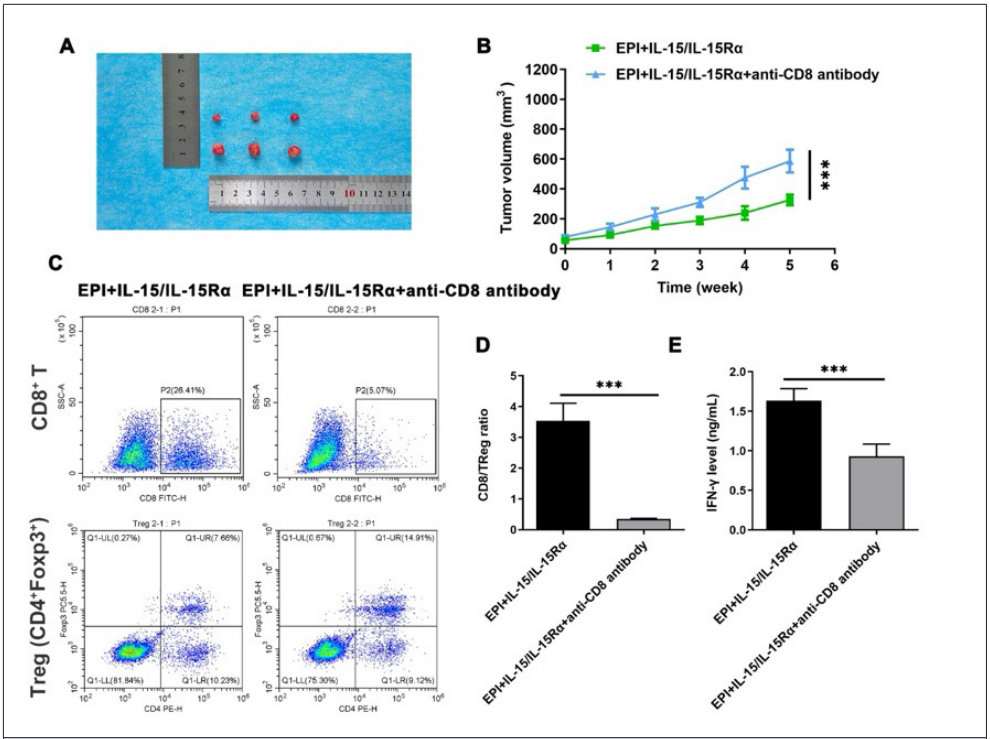


**Fig 5.** $CD8^+$ T cells are essential for the antitumor efficacy of sequential therapy. **(A)** The tumor appearance. **(B)** The tumor size. **(C)** Flow cytometry was used for the detection of tumor-infiltrating immune cells. **(D)** The CD8/Treg ratio. (E) IFN-γlevels measured by ELISA. ***P<0.001

next investigated whether $CD8^+$ T cells contributed to the antitumor effect. Compared with the EPI+IL-15/IL-15Rα group, tumor volume was increased following administration of anti-CD8 antibody *(Fig. 5-A, B)*. Consistent immunological changes were also observed: $CD8^+$ T cell infiltration and IFN-γ levels were reduced, and the CD8/Treg ratio was lower in the EPI+IL-15/IL-15Rα+anti-CD8 antibody group than in the EPI+IL-15/IL-15Rα group *(Fig. 5-C, D, E)*. These findings suggested that $CD8^+$ T cells make an important contribution to the therapeutic activity of sequential treatment.

## Discussion

BC, particularly NMIBC, remains a therapeutic challenge due to high recurrence rates and the substantial proportion of patients who experience recurrence or inadequate responses following BCG therapy [30]. Although intravesical chemotherapy and immunotherapy are widely used, their benefits are often constrained by suboptimal responses or adverse effects [31]. These limitations highlight the need for novel strategies that combine direct cytotoxic effects with immune modulation. In this context, we explored sequential administration of EPI and IL-15/IL-15Rα as a potential approach to integrate chemotherapy-induced tumor cell death with immune activation. Our results showed that EPI induced ICD-associated changes, while IL-15/IL-15Rα further enhanced splenocyte-mediated cytotoxicity and IFN-γ production. *In vivo*, the sequential regimen reduced tumor burden, prolonged survival, and promoted a favorable immune microenvironment. Importantly, $CD8^+$ T cell depletion diminished these effects, indicating their central contribution to the therapeutic activity. Together, these findings support a model in which EPI-induced tumor immunogenicity provides an initial immune stimulus that is subsequently amplified by IL-15/IL-15Rα-mediated activation of antitumor effector cells.

Previous studies have shown that certain chemotherapeutic agents, including anthracyclines, can promote ICD in addition to exerting direct cytotoxic effects, thereby enhancing tumor antigen exposure and immune recognition [32]. Our data are consistent with these findings, as EPI treatment upregulated CRT exposure and ATP release in MB49 cells, both of which are widely recognized features associated with ICD [33]. Surface-exposed CRT can function as a pro-phagocytic signal, whereas extracellular ATP contributes to the recruitment and activation of immune cells, providing a potential link between chemotherapy-induced tumor injury and subsequent immune activation [34,35]. However, induction of ICD-associated signals alone does not necessarily guarantee a durable antitumor immune response.

IL-15 is essential for the proliferation, maintenance, and survival of $CD8^+$ T cells and NK cells [36]. Upon association with IL-15Rα, IL-15 has enhanced stability and biological activity, thereby promoting more sustained stimulation

of cytotoxic lymphocytes [37]. Previous preclinical studies have demonstrated the antitumor activity of IL-15/IL-15Rα-based complexes, although treatment efficacy may vary with the tumor model and delivery strategy [24]. In our study, the addition of IL-15/IL-15Rα complexes after EPI treatment further increased splenocyte-mediated cytotoxicity and IFN-γ secretion. IFN-γ is traditionally regarded as a key mediator of cellular immune responses [38], and its increased production may therefore reflect enhanced activation of antitumor effector cells. These findings suggest that EPI-induced tumor immunogenicity and IL-15/IL-15Rα-mediated effector-cell stimulation may act in a complementary manner, providing a possible explanation for the stronger antitumor effect of the sequential regimen.

The tumor immune microenvironment plays a decisive role in tumor progression and therapeutic response, where immunosuppressive components such as Tregs can facilitate immune evasion, while increased infiltration of effector cells like $CD8^+$ T cells and NK cells is generally linked to favorable prognosis [39,40]. In our study, sequential treatment with EPI and IL-15/IL-15Rα increased the infiltration of $CD8^+$ T cells and NK cells, decreased the Treg population, and increased the CD8/Treg ratio. These changes were accompanied by increased IFN-γ levels, indicating enhanced cytotoxic immune activity. Previous studies have also reported that intravesical chemotherapy can promote $CD8^+$ T cell activity and alter immunosuppressive cell populations in BC [41,42]. However, in our study, EPI alone produced less pronounced immune changes than the sequential regimen. This difference may be related to the chemotherapeutic agent, treatment schedule, tumor model, or timing of immune evaluation. It may also suggest that chemotherapy-induced immune priming can be further reinforced by IL-15/IL-15Rα-mediated activation of cytotoxic lymphocytes. Therefore, the therapeutic benefit of sequential treatment may involve both direct tumor cell injury and favorable modulation of the tumor immune microenvironment.

The functional importance of $CD8^+$ T cells was further supported by the depletion experiments. Administration of anti-CD8 antibody attenuated the tumor-suppressive effect of sequential treatment, accompanied by lower IFN-γ production and a decreased CD8/Treg ratio. These results highlighted $CD8^+$ T cells as indispensable effectors of the sequential therapy. This observation is consistent with prior reports showing that $CD8^+$ T cells are key mediators of response to immune checkpoint inhibitors and cytokine-based therapies [43,44]. Although IL-15 can also activate NK cells, our depletion experiments suggest a particularly important contribution of $CD8^+$ T cells in the present model. Differences in the relative contribution of $CD8^+$ T cells and NK cells across previous studies may reflect differences in tumor type, treatment regimen, and route of cytokine administration. Further studies using selective depletion of additional immune cell populations are needed to define their individual contributions.

The present findings also differ conceptually from previous IL-15-based strategies in NMIBC, which have mainly investigated IL-15 superagonists in combination with BCG [11,45]. In contrast, our strategy used EPI to induce tumor cell injury and immunogenic signals before administration of IL-15/IL-15Rα. This sequential approach may allow cytokine-mediated immune stimulation to build upon chemotherapy-induced tumor immunogenicity. Nevertheless, because simultaneous and sequential administration were not directly compared, further studies are required to determine whether the treatment sequence itself contributes to the improved efficacy.

Despite these promising results, several limitations should be acknowledged. First, our study was limited to a murine orthotopic BC model, and validation in additional models would strengthen the generalizability of our findings. Second, we did not evaluate potential long-term immune memory responses, which could provide important insights into recurrence prevention. Third, although $CD8^+$ T cell depletion supported the involvement of $CD8^+$ T cells, the specific contribution of NK cells and other immune populations was not directly examined. Fourth, CRT exposure and ATP release supported ICD-associated changes, but further studies assessing antigen presentation and tumor-specific T cell responses would provide stronger mechanistic evidence. Finally, while sequential EPI and IL-15/IL-15Rα therapy was well tolerated in mice, further investigation is warranted to assess potential toxicities and optimize dosing regimens for translational applications.

In conclusion, our study provides preclinical evidence that sequential intravesical administration of EPI and IL-15/IL-15Rα complexes enhances antitumor efficacy by inducing ICD-associated changes, strengthening cytotoxic immune responses, and alleviating immunosuppression in the tumor microenvironment. The reduced therapeutic efficacy following $CD8^+$ T cell depletion further supports an important contribution of $CD8^+$ T cell-mediated immunity. These findings provide a basis for further investigation of chemo-immunotherapy strategies combining anthracycline-based chemotherapy with IL-15-based immune stimulation in BC.

## Declarations

**Availability of Data and Materials:** The datasets used and/or analyzed during the current study are available from the corresponding author (FP) on reasonable request.

**Acknowledgements:** None.

**Funding Support:** None.

**Ethical Approval:** All animal experimental procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals issued by the Ministry of Science and Technology of China, and were approved by the Animal Ethics Committee of Nanjing Tongren Hospital, Southeast University (Approval No.: SHU2023/021).

**Competing Interests:** The authors declared that there is no conflict of interest.

**Declaration of Generative Artificial Intelligence (AI):** The article and/or tables and figures were not written/created by AI and AI-assisted technologies.

**Authors' Contributions:** JM and FP designed this study and drafted the manuscript; JM and SW performed this study and analyzed the data; JM designed this study and significantly revised the manuscript. All authors have approved the submission and publication of this manuscript.

## References

1. **Babjuk M, Burger M, Capoun O, Cohen D, Compérat EM, Dominguez Escrig JL, Gontero P, Liedberg F, Masson-Lecomte A, Mostafid AH, Palou J, van Rhijn BWG, Rouprêt M, Shariat SF, Seisen T, Soukup V, Sylvester RJ:** European Association of Urology Guidelines on non-muscle-invasive bladder cancer (Ta, T1, and carcinoma *in situ). Eur Urol,* 81 (1): 75-94, 2022. DOI: 10.1016/j.eururo.2021.08.010
2. **Siegel RL, Miller KD, Wagle NS, Jemal A:** Cancer statistics, 2023. *CA Cancer J Clin,* 73 (1): 17-48, 2023. DOI: 10.3322/caac.21763
3. **Osman Y, Elawdy M, Taha DE, Zahran MH, Abouelkheir RT, Sharaf DE, Mosbah A, Ali-El Dein B:** Bladder perforation as a complication of transurethral resection of bladder tumors: the predictors, management, and its impact in a series of 1570 at a tertiary urology institute. *Int Urol Nephrol,* 55 (9): 2161-2167, 2023. DOI: 10.1007/s11255-023-03638-6
4. **Matulewicz RS, Steinberg GD:** Non-muscle-invasive bladder cancer: Overview and contemporary treatment landscape of neoadjuvant chemoablative therapies. *Rev Urol,* 22 (2): 43-51, 2020.
5. **Lamm DL, Thor DE, Stogdill VD, Radwin HM:** Bladder cancer immunotherapy. *J Urol,* 128 (5): 931-935, 1982. DOI: 10.1016/s0022-5347(17)53283-8
6. **Redelman-Sidi G, Glickman MS, Bochner BH:** The mechanism of action of BCG therapy for bladder cancer - A current perspective. *Nat Rev Urol,* 11 (3): 153-162, 2014. DOI: 10.1038/nrurol.2014.15
7. **Lidagoster S, Ben-David R, De Leon B, Sfakianos JP:** BCG and alternative therapies to BCG therapy for non-muscle-invasive bladder cancer. *Curr Oncol,* 31 (2): 1063-1078, 2024. DOI: 10.3390/curroncol31020079
8. **Robinson TO, Schluns KS:** The potential and promise of IL-15 in immuno-oncogenic therapies. *Immunol Lett,* 190, 159-168, 2017. DOI: 10.1016/j.imlet.2017.08.010
9. **Vidal-Manrique M, Nieuwenstein T, Hooijmaijers L, de Jonge PKJD, Djojoatmo M, Jansen J, van der Waart AB, Brock R, Dolstra H:** IL-15 transpresentation by ovarian cancer cells improves CD34(+) progenitor-derived NK cell's anti-tumor functionality. *Oncoimmunology,* 14 (1):2465010, 2025. DOI: 10.1080/2162402X.2025.2465010
10. **Hu Y, Liu D, Cui P, Zhang W, Chen H, Piao C, Lu Y, Liu X, Wang Y, Liu J, Lu X:** IL-15-induced lymphocytes as adjuvant cellular immunotherapy for gastric cancer. *Invest New Drugs,* 39 (6): 1538-1548, 2021. DOI: 10.1007/s10637-021-01160-z
11. **Chamie K, Chang SS, Kramolowsky E, Gonzalgo ML, Agarwal PK, Bassett JC, Bjurlin M, Cher ML, Clark W, Cowan BE, David R, Goldfischer E, Guru K, Jalkut MW, Kaffenberger SD, Kaminetsky J, Katz AE, Koo AS, Sexton WJ, Tikhonenkov SN, Trabulsi EJ, Trainer AF, Spilman P, Huang M, Bhar P, Taha SA, Sender L, Reddy S, Soon-Shiong P:** IL-15 superagonist NAI in BCG-unresponsive non-muscle-invasive bladder cancer. *NEJM Evid,* 2 (1):EVIDoa2200167, 2023. DOI: 10.1056/EVIDoa2200167
12. **Choi YH, Lim EJ, Kim SW, Moon YW, Park KS, An HJ:** IL-27 enhances IL-15/IL-18-mediated activation of human natural killer cells. *J Immunother Cancer,* 7 (1):211, 2019. DOI: 10.1186/s40425-019-0688-8
13. **Tamzalit F, Barbieux I, Plet A, Heim J, Nedellec S, Morisseau S, Jacques Y, Mortier E:** IL-15.IL-15Rα complex shedding following trans-presentation is essential for the survival of IL-15 responding NK and T cells. *Proc Natl Acad Sci U S A,* 111 (23): 8565-8570, 2014. DOI: 10.1073/pnas.1405514111
14. **Burkett PR, Koka R, Chien M, Chai S, Boone DL, Ma A:** Coordinate expression and transpresentation of interleukin-15Rα and IL-15 supports natural killer cell and memory CD8+ T cell homeostasis. *J Exp Med,* 200 (7): 825-834, 2004. DOI: 10.1084/jem.20041389
15. **Margolin K, Morishima C, Velcheti V, Miller JS, Lee SM, Silk AW, Holtan SG, Lacroix AM, Fling SP, Kaiser JC, Egan JO, Jones M, Rhode PR, Rock AD, Cheever MA, Wong HC, Ernstoff MS:** Phase I trial of ALT-803, a novel recombinant IL-15 complex, in patients with advanced solid tumors. *Clin Cancer Res,* 24 (22): 5552-5561, 2018. DOI: 10.1158/1078-0432.CCR-18-0945
16. **Chen W, Liu N, Yuan Y, Zhu M, Hu X, Hu W, Wang S, Wang C, Huang B, Xing D:** ALT-803 in the treatment of non-muscle-invasive bladder cancer: Preclinical and clinical evidence and translational potential. *Front Immunol,* 13:1040669, 2022. DOI: 10.3389/fimmu.2022.1040669
17. **Chamie K, Chang SS, Rosser CJ, Kramolowski E, Gonzalgo ML, Sexton WJ, Spilman P, Sender L, Reddy S, Soon-Shiong P:** N-803 plus BCG treatment for BCG-naïve or -unresponsive non-muscle invasive bladder cancer: A plain language review. *Future Oncol,* 20 (31): 2307-2317, 2024. DOI: 10.1080/14796694.2024.2363744
18. **Xu K, Zhang Y, Cheng H, Chen W, Chen C, Zhang M, Song H, Wang F:** Triple-negative breast cancer treatment with core-shell Magnetic@Platinium-metal organic framework/epirubicin nano-platforms for chemo-photodynamic combinational therapy: A review. *Medicine (Baltimore),* 103 (39):e39845, 2024. DOI: 10.1097/MD.0000000000039845
19. **Onrust SV, Wiseman LR, Goa KL:** Epirubicin: A review of its intravesical use in superficial bladder cancer. *Drugs Aging,* 15 (4): 307-333, 1999. DOI: 10.2165/00002512-199915040-00006
20. **Li Z, Zhang Q, Li Z, Ren L, Pan D, Gong Q, Gu Z, Cai H, Luo K:** Branched glycopolymer prodrug-derived nanoassembly combined with a STING agonist activates an immuno-supportive status to boost anti-PD-L1 antibody therapy. *Acta Pharm Sin B,* 14 (5): 2194-2209, 2024. DOI: 10.1016/j.apsb.2024.02.006
21. **Chen JF, Yu BX, Yu R, Ma L, Lv XY, Cheng Y, Ma Q:** Monoclonal antibody Zt/g4 targeting RON receptor tyrosine kinase enhances chemosensitivity of bladder cancer cells to epirubicin by promoting G1/S arrest and apoptosis. *Oncol Rep,* 37 (2): 721-728, 2017. DOI: 10.3892/or.2017.5356
22. **Bazargan S, Bunch B, Ojwang' AME, Blauvelt J, Landin A, Ali J, Abrahams D, Cox C, Hall AM, Beatty MS, Poch M, Rejniak KA, Pilon-Thomas S:** Targeting myeloid-derived suppressor cells with gemcitabine to enhance efficacy of adoptive cell therapy in bladder cancer. *Front Immunol,* 14:1275375, 2023. DOI: 10.3389/fimmu.2023.1275375
23. **Hong X, Chen X, Wang H, Xu Q, Xiao K, Zhang Y, Chi Z, Liu Y, Liu G, Li H, Fang J, Lin T, Zhang Y:** A HER2-targeted antibody-drug conjugate, RC48-ADC, exerted promising antitumor efficacy and safety with intravesical instillation in preclinical models of bladder cancer. *Adv Sci (Weinh),* 10 (32):e2302377, 2023. DOI: 10.1002/advs.202302377
24. **Guo S, Smeltz RB, Nanajian A, Heller R:** IL-15/IL-15Rα heterodimeric complex as cancer immunotherapy in murine breast cancer models. *Front Immunol,* 11:614667, 2020. DOI: 10.3389/fimmu.2020.614667
25. **Cross EW, Blain TJ, Mathew D, Kedl RM:** Anti-CD8 monoclonal antibody-mediated depletion alters the phenotype and behavior of surviving CD8+ T cells. *PLoS One,* 14 (2):e0211446, 2019. DOI: 10.1371/journal.pone.0211446

26. **Li J, Huang K, Yang B, Hu X, Mei B, Cheng X, Zhong X, Cao C, Chen Z, Wang H, Zhang J:** TRA upregulates OTUD6B to recruit CD8+ T cells to suppress colorectal liver metastasis by stabilizing DDX5/STAT3/CXCL11 axis. *Cell Death Dis,* 16 (1):521, 2025. DOI: 10.1038/s41419-025-07837-0

27. **Santana JPP, Marcato PD, Massaro TNC, Godoy NL, Anibal FF, Borra RC:** Efficacy of instillation of MB49 cells and thermoreversible polymeric gel in urothelial bladder carcinoma immunization. *Lab Anim Res,* 38 (1):11, 2022. DOI: 10.1186/s42826-022-00122-7

28. **Zhang LC, Wu XY, Yang RB, Chen F, Liu JH, Hu YY, Wu ZD, Wang LF, Sun X:** Recombinant protein *Schistosoma japonicum*-derived molecule attenuates dextran sulfate sodium-induced colitis by inhibiting miRNA-217-5p to alleviate apoptosis. *World J Gastroenterol,* 27(46): 7982-7994, 2021. DOI: 10.3748/wjg.v27.i46.7982

29. **Parikh A, Shin J, Faquin W, Lin DT, Tirosh I, Sunwoo JB, Puram SV:** Malignant cell-specific CXCL14 promotes tumor lymphocyte infiltration in oral cavity squamous cell carcinoma. *J Immunother Cancer,* 8 (2):e001048, 2020. DOI: 10.1136/jitc-2020-001048

30. **Campistol M, Lozano F, Carrion A, Raventós CX, Morote J, Trilla E:** Active surveillance in non-muscle invasive bladder cancer: A systematic review. *Cancers (Basel),* 17 (10):1714, 2025. DOI: 10.3390/cancers17101714

31. **Zhao X, Qi X, Liu D, Che X, Wu G:** A novel approach for bladder cancer treatment: Nanoparticles as a drug delivery system. *Int J Nanomedicine,* 19, 13461-13483, 2024. DOI: 10.2147/IJN.S498729

32. **Showalter A, Limaye A, Oyer JL, Igarashi R, Kittipatarin C, Copik AJ, Khaled AR:** Cytokines in immunogenic cell death: Applications for cancer immunotherapy. *Cytokine,* 97, 123-132, 2017. DOI: 10.1016/j.cyto.2017.05.024

33. **Li X, Zheng J, Chen S, Meng FD, Ning J, Sun SL:** Oleandrin induces immunogenic cell death via the PERK/eIF2α/ATF4/CHOP pathway in breast cancer. *Cell Death Dis,* 12 (4):314, 2021. DOI: 10.1038/s41419-021-03605-y

34. **Obeid M, Tesniere A, Ghiringhelli F, Fimia GM, Apetoh L, Perfettini JL, Castedo M, Mignot G, Panaretakis T, Casares N, Métivier D, Larochette N, van Endert P, Ciccosanti F, Piacentini M, Zitvogel L, Kroemer G:** Calreticulin exposure dictates the immunogenicity of cancer cell death. *Nat Med,* 213 (1): 54-61, 2007. DOI: 10.1038/nm1523

35. **Michaud M, Martins I, Sukkurwala AQ, Adjemian S, Ma Y, Pellegatti P, Shen S, Kepp O, Scoazec M, Mignot G, Rello-Varona S, Tailler M, Menger L, Vacchelli E, Galluzzi L, Ghiringhelli F, di Virgilio F, Zitvogel L, Kroemer G:** Autophagy-dependent anticancer immune responses induced by chemotherapeutic agents in mice. *Science,* 334 (6062): 1573-1577, 2011. DOI: 10.1126/science.1208347

36. **Hangasky JA, Chen W, Dubois SP, Daenthanasanmak A, Müller JR, Reid R, Waldmann TA, Santi DV:** A very long-acting IL-15: implications for the immunotherapy of cancer. *J Immunother Cancer,* 10 (1):e004104, 2022. DOI: 10.1136/jitc-2021-004104

37. **Stoklasek TA, Schluns KS, Lefrançois L:** Combined IL-15/IL-15Rα immunotherapy maximizes IL-15 activity *in vivo. J Immunol,* 177 (9): 6072-6080, 2006. DOI: 10.4049/jimmunol.177.9.6072

38. **Lu Y, Waller EK:** Dichotomous role of interferon-gamma in allogeneic bone marrow transplant. *Biol Blood Marrow Transplant,* 15 (11): 1347-1353, 2009. DOI: 10.1016/j.bbmt.2009.07.015

39. **Zheng Y, Chen Z, Han Y, Han L, Zou X, Zhou B, Hu R, Hao J, Bai S, Xiao H, Li WV, Bueker A, Ma Y, Xie G, Yang J, Chen S, Li H, Cao J, Shen L:** Immune suppressive landscape in the human esophageal squamous cell carcinoma microenvironment. *Nat Commun,* 11 (1):6268, 2020. DOI: 10.1038/s41467-020-20019-0

40. **Nicolini A, Ferrari P:** Involvement of tumor immune microenvironment metabolic reprogramming in colorectal cancer progression, immune escape, and response to immunotherapy. *Front Immunol,* 15:1353787, 2024. DOI: 10.3389/fimmu.2024.1353787

41. **Chen R, Ding S, Fu X, Liu G:** Intravesical chemotherapy enhances anti-tumor immunity in bladder cancer by modulating CD8(+) T cell activation and Treg populations. *Biochem Biophys Res Commun,* 764:151782, 2025. DOI: 10.1016/j.bbrc.2025.151782

42. **Hori S, Miyake M, Tatsumi Y, Onishi S, Morizawa Y, Nakai Y, Tanaka N, Fujimoto K:** Topical and systemic immunoreaction triggered by intravesical chemotherapy in an N-butyl-N-(4-hydroxybutyl) nitorosamine induced bladder cancer mouse model. *PLoS One,* 12 (4):e0175494, 2017. DOI: 10.1371/journal.pone.0175494

43. **Principe N, Kidman J, Goh S, Tilsed CM, Fisher SA, Fear VS, Forbes CA, Zemek RM, Chopra A, Watson M, Dick IM, Boon L, Holt RA, Lake RA, Nowak AK, Lesterhuis WJ, McDonnell AM, Chee J:** Tumor-infiltrating effector memory antigen-specific CD8(+) T cells predict response to immune checkpoint therapy. *Front Immunol,* 11:584423, 2020. DOI: 10.3389/fimmu.2020.584423

44. **Feng B, Bai Z, Zhou X, Zhao Y, Xie YQ, Huang X, Liu Y, Enbar T, Li R, Wang Y, Gao M, Bonati L, Peng MW, Li W, Tao B, Charmoy M, Held W, Melenhorst JJ, Fan R, Guo Y, Tang L:** The type 2 cytokine Fc-IL-4 revitalizes exhausted CD8(+) T cells against cancer. *Nature,* 634 (8034): 712-720, 2024. DOI: 10.1038/s41586-024-07962-4

45. **Gomes-Giacoia E, Miyake M, Goodison S, Sriharan A, Zhang G, You L, Egan JO, Rhode PR, Parker AS, Chai KX, Wong HC, Rosser CJ:** Intravesical ALT-803 and BCG treatment reduces tumor burden in a carcinogen induced bladder cancer rat model; a role for cytokine production and NK cell expansion. *PLoS One,* 9 (6):e96705, 2014. DOI: 10.1371/journal.pone.0096705