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ORIGINAL ARTICLE

Assessment of *ex-vivo* efficacy (oncogram) of immunotherapeutic and chemotherapeutic agents in bladder cancer: A pilot study of personalized treatment

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Summary

Purpose: *To investigate the ex-vivo efficacy of immunotherapeutics and chemotherapeutics in bladder cancer primary cell cultures, to assess the applicability of the method according to the results and to evaluate suitability of the oncogram method for personalized treatment of bladder cancer.*

Methods: *After receiving ethics committee approval, tumor tissue was obtained from patients with transurethral resection performed due to bladder tumor from 2015 to 2017. Primary culture was produced from the obtained fresh tissue. Each culture was divided into 6 groups. The control group had only medium applied, while the other groups had Bacillus Calmette Guerin (BCG), Interferon-a (IFN-a), Gemcitabine, BCG+IFN-a and BCG+Gemcitabine, respectively. Viability tests in the 24th hour were performed on each culture. The results of all cases were compared with their own controls. Also, results of each case were compared between the cases with similar pathologic results.*

Results: *The study assessed 24 bladder cancer cases. Mean patient age was 66.2±11.7 years (34-83), with 19 male (79.5%) and 5 female patients (20.5%). When data were compared between the groups, viability percentages were 31.2%, 30.9%, 27.7%, 32.1% and 29.4% in the BCG, IFN-a, Gemcitabine, BCG+IFN-a and BCG+Gemcitabine groups compared with their own controls (73.1%), respectively ($p<0.001$). In addition, we found that viability results were not similar in all cases.*

Conclusions: *Cell cultures produced from bladder cancer tissue might help to determine sensitivity to treatment. This ex-vivo method (oncogram) is a simple and applicable method that can be used for personalized treatment before intravesical or systemic therapy.*

Key words: *Bacillus Calmette Guerin (BCG), bladder cancer, cell culture, gemcitabine, interferon-a (IFN-a), oncogram*

Introduction

Urothelial carcinoma of the bladder (UC) is a highly morbid and mortal carcinoma among urogenital malignancies [1]. At initial diagnosis, over 70% of patients have non-muscle invasive bladder cancer (NMIBC) and are treated with transurethral resection of the bladder tumor (TUR-BT) with or without additional intravesical chemotherapy or immunotherapy [2]. About 30% of the patients have muscle-invasive bladder cancer (MIBC), and are treated with standard treatment of radical cystectomy (RC) with or without neoadjuvant and adjuvant chemotherapy [1].

For the management of NMIBC, different treatment modalities and follow-up procedures are recommended after TUR-BT according to European Organization for Research and Treatment

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of Cancer (EORTC) risk groups in the European Urology (EAU) Guidelines [2]. According to this, NMIBC patients are divided into 4 risk groups as low-risk, intermediate risk, high-risk and the highest risk groups. An immediate single instillation (ISI) of chemotherapy is recommended for low-risk and some intermediate-risk patients [2]. For intermediate-risk and high-risk tumors, intravesical Bacillus Calmette Guérin (BCG) after TUR-BT is recommended to reduce the risk of tumor recurrence [2]. In addition, using a combination of epirubicin and interferon (IFN) were investigated for intermediate-risk and high-risk tumors in the past [3]. Likewise, in a recent study, combination of BCG and IFN were also investigated [4]. The standard treatment for patients with MIBC is RC. However, RC only provides five-year survival in about 50% of patients [5-7]. To improve these results, platin-based (Cisplatin and Gemcitabine combination) neoadjuvant chemotherapy (NAC) or adjuvant chemotherapy (AC) have been recommended [1,8].

Previous basic research has shown that primary cell culture can be created using specimens obtained from patients with UC of the bladder [9]. Also, these studies showed that primary cell culture growth can be achieved and cultures can be tested for chemosensitivity for drug administration [9]. However, these results have not yet been evaluated as a part of personalized treatment. Therefore, an oncogram should be created with culture using viability results after administering immunotherapeutics and chemotherapeutics to the culture for personalized treatment, similar to an antibiogram for microorganisms.

The aim in this study was to measure the *ex-vivo* efficacy of immunotherapeutics and chemotherapeutics in bladder cancer primary cell cultures, to assess the applicability of the method according to the results and to evaluate the suitability of the oncogram method for personalized treatment of bladder cancer.

Methods

Study approval

The study was approved by Independent Ethics Committee/Institutional Review Boards and performed in accordance with the International Conference for Good Clinical Practice, the national regulations and ethical principles of the Declaration of Helsinki. All patients provided written informed consent.

Methods

After receiving ethics committee approval and obtaining informed consent from each patient, 5 mm fresh tumor tissue was obtained from patients with transurethral resection of bladder tumor (TUR-BT) performed due to bladder cancer from 2015 to 2017.

During all laboratory steps, the standard procedure of our department for primary bladder cancer cell culture mentioned below were performed for each case at different times.

Collection of bladder cancer tissues

The obtained tumor tissue was transferred with medium (Roswell Park Memorial Institute medium (RPMI) and 1% penicillin / streptomycin (P/S)) in cold condition. Subsequently, the tissue was taken into a Petri dish and was separated to small pieces with a lancet and incomplete RPMI was added. Diagnosis of the bladder cancer was microscopically verified by the pathologist. After removal of the pieces of the tissue with transfer medium (RPMI with 1% P/S) they were centrifuged at 3000 rpm for 2 min in 15 ml falcon tubes. The formed supernatant in the falcon tube was poured off and complete RPMI containing 5% dimethyl sulfoxide (DMSO) and 20% fetal bovine serum (FBS) were added into the falcon tube. The bladder cancer cells were thoroughly suspended with complete medium. Then the tube was slowly frozen to -80°C for subsequent experiments.

Primary cell culture of the bladder cancer

After the collection of all tumor tissues, all tissues were defrosted and planted into 24-well plates with 10^5 cells per well. One ml complete RPMI medium containing 20% FBS was added into each well. The wells were incubated in 5% CO_2 at 37°C in an incubator. After the incubation of the wells for 24 h, the cells were approximately in 80% confluence.

Drug and peripheral blood mononuclear cell application to the primary cell cultures

Peripheral blood sample obtained from a 0 Rh (-) patient were centrifuged with 1600 rpm over 2 cc ficoll paque solution. Peripheral blood mononuclear cells were separated from the intermediate layer of the centrifuged blood and obtained by flow-cytometry. The 80% confluent tumor cells in the well plates for each culture were divided into 6 different groups. Drugs were administered to all groups. The control group (Group 1) had only medium (RPMI with 20% FBS) applied, while the other groups had Bacillus Calmette Guerin (BCG, Onco-tice®) (Group 2), Interferon-α (IFN-α, Intron-A®) (Group 3), Gemcitabine (Gemko®) (Group 4), combination of BCG and IFN-α (Group 5) and combination of BCG and Gemcitabine (Group 6) applied, respectively. Each process was applied three times for each case. The obtained peripheral blood mononuclear cells were included in wells (1000 cells in each well) with BCG and IFN-α.

Viability tests with trypan blue and water-soluble tetrazolium salt (WST-1)

The treated cells in the cultures were harvested from the 24-well plates at the end of 24 h and the harvested cells were centrifuged at 1200 g for 7 min. The majority of the supernatant was discarded and the remaining portion was thoroughly suspended by pipette.

Then, the suspended supernatant was filtered to an Eppendorf tube. Then, 10µl of the supernatant and 10µl trypan blue were added to a new Eppendorf tube. The cells were counted in the EVE™ cell counting device. Viability results were checked three times for each drug group in each case. In addition, to check the viability results, WST-1 assay was also used for each culture, like the trypan blue test. Good response rate to the drugs was defined as <30% viability rate. Fibroblasts growth and crisis of plastic exposure were ignored because the value of these effects for all applications of each case was compared by its own control.

Tumor characteristics, pathologic results and follow-up of the patients

Patients were not treated with these drugs. However, patients with NMIBC were treated with routine intravesical therapy and followed-up according to EORTC risk table. Patients with MIBC were treated with RC with or without NAC and/or AC or only chemotherapy. Diagnosis of UC was made by a uropathologist using immunohistochemical staining. Follow-up data of each case were registered. Pathologic and clinical data, intravesical therapy results (for NMIBC), chemotherapy results (for MIBC) and follow-up data of the patients were compared to *ex-vivo* viability results of the drug application to the cell cultures for each case.

Statistics

Data were analyzed using the statistical package for social sciences, version 20.0 (SPSS, Chicago, Ill) software program. Wilcoxon test was used for comparison of drug groups according to their own controls. Also, the Mann-Whitney U test, Kruskal-Wallis test and x^2 test were used for analysis of patient data. Data are given as mean ± SD in the Tables. Statistical significance was set at $p<0.05$.

Results

Twenty-four bladder cancer cases were assessed in this study. The mean patient age was 66.2 ± 11.7 years (34-83), with 19 male (79.5%) and 5 female patients (20.5%). Pathological characteristics of the patients are shown in Table 1. Pathological T stages were Ta for 10 patients, T1 for 10 and T2 for 4 cases. One patient (patient no. 1 in Table 2) who was diagnosed as pathologic T1 stage after TUR-BT was clinically T3-4 stage bladder cancer on radiologic imaging. Twelve cases had low-grade and 12 cases had high-grade tumor. Twenty-three were urothelial carcinoma and one was mucinous carcinoma. Squamous differentiation was present in 5 cases. Also, concomitant carcinoma *in situ* (CIS) was present in 4 cases.

Pathological, clinical (NMIBC/MIBC data) and EORTC risk groups, received additional treatment type (ISI and adjuvant treatment, response status to the treatment in follow-up data) and laboratory results (primary cell culture viability rates after BCG, IFN-α and Gemcitabine administration) for each patient are given in Table 2, separately. Among patients, 19 had NMIBC and 5 had MIBC after pathological and clinical evaluation. In 19 NMIBC patients, 8 were low-risk, 2 were intermediate-risk and 9 were high-risk NMIBC according to the EORTC risk Table. ISI with epirubicin was administered in 8 patients (6 low-risk, 1 intermediate-risk and 1 high-risk patient) after TUR-BT. In addition, 4 high-risk patients received BCG and 3 patients (1 high-risk and 2 intermediate-risk) received epirubicin treatments, whereas 12 of 19 NMIBC cases did not receive any adjuvant intravesical treatment. Recurrence was not shown in 13 of 19 patients; however, 6 of 19 patients had no follow-up data. Platin-based NAC (cisplatin or carboplatin plus gemcitabine combinations) was given to 5 MIBC patients (clinical or pathological T2-4 patients). Partial response was shown after chemotherapy in 3 of 5 patients in follow-up, 1 patient had no follow-up data and one patient had no response after NAC.

Table 1. Pathological characteristics of the cases (n=24)

	n (%)
Pathological stage	
Ta	10 (41.7)
T1	10 (41.7)
T2	4 (16.6)
Tumor grade	
Low-grade	12 (50)
High-grade	12 (50)
Clinical and pathological stage	
NMIBC	19 (79.2)
MIBC	5 (20.8)
EORTC risk table for NMIBC	
Low-risk	8 (42.1)
Intermediate-risk	2 (10.5)
High-risk	9 (47.4)
Tumor type	
Mucinous tumor	1 (4.2)
Urothelial tumor	23 (95.8)
Presence of variant histology (squamous differentiation)	5 (20.8)
Histological pattern	
Solid	5 (20.8)
Inverted	3 (12.5)
Nested	1 (4.2)
Concomittant CIS presence	4 (16.6)

EORTC= European Organization for Research and Treatment of Cancer, NMIBC= Non-muscle invasive bladder cancer, MIBC= Muscle invasive bladder cancer, CIS= Carcinoma *in situ*

Viability rates of primary culture of each case after the application of immunotherapeutics and chemotherapeutics showed various response rates within Ta, within T1 and within T2 tumors as seen in Table 2. For example, in Ta low grade NMIBC (low-risk) cases, some patients (patient no. 11 and 12 in Table 2) showed different response rates to BCG, Gemcitabine and IFN-α administration in culture compared to others (patient no. 8 and 16 had lower viability rates and patients no. 15 and 20 had higher viability rates in Table 2). In 2 intermediate-risk cases, patient no. 13 had different response rates to BCG, Gemcitabine and IFN-α administration in culture compared to patient no. 14. Also, in T1 high grade (high-risk) NMIBC cases, 2 patients (patient no. 2 and 23) had different response rates to BCG (especially), Gemcitabine and IFN-α compared to other patients (patients no. 10 and 24 had higher viability rates and patient no. 4 had lower viability rates). Also, although one patient (patient no. 2) received additional BCG treatment after TURBT, Gemcitabine and IFN-α response rates were higher than the BCG response rate in culture results. In MIBC cases, patient no. 19 had higher viability rates compared to others (patients no. 7 and 9) for BCG, Gemcitabine and IFN-α administration. Also, viability rates for patient no. 1 were different from the other 2 patients (patients no. 7 and 9). Viability distributions of BCG, Gemcitabine and IFN-α administration and Control groups with boxplots for NMIBC/MIBC patients and low-risk, intermediate-risk and high-risk of NMIBC patients are given in Figure 1.

Viability rates and comparative results of groups after the drug application are presented in Table 3. When data were compared between the groups, viability percentages were observed to be low in the BCG (Group 2), IFN-α (Group 3), Gemcitabine (Group 4), combination of BCG and IFN-α (Group 5) and combination of BCG and Gemcitabine (Group 6) groups compared with their own controls (Group 1).

Table 2. Pathological results, clinical stage, received additional treatment type, recurrence status, primary cell culture viability rates after BCG, IFN-α and Gemcitabine applying of each patient

Patient, n	*p stage*	*Histology*	*Grade*	*CIS*	*NMIBC/ MIBC*	*Risk*	*ISI*	*Treatment*	*Response / Recurrence*	*Primary Culture viability rates after drug administration (%)*			
										Control	BCG	G	IFN-α
1	T1	UC	High	+	MIBC	-	-	Car+G	Partial Resp	90.5	41.5	13	20.5
2	T1	UC-Squ	High	-	NMIBC	High	-	BCG	Comp Resp	85.5	31	21	22
3	T1	UC	Low	-	NMIBC	High	-	BCG	Comp Resp	73	23	12	29
4	T1	UC	High	+	NMIBC	High	-	BCG	Comp Resp	89.5	17	30	11
5	T1	UC-Squ	High	-	NMIBC	High	+	-	No data	73.5	12	6	6
6	T1	UC	High	-	NMIBC	High	-	BCG	Comp Resp	85.5	22	19	35
7	T2	UC-Squ	High	-	MIBC	-	-	Cis+G	Partial Resp	82	3	12	16
8	Ta	UC	Low	-	NMIBC	Low	-	-	Comp Resp	77	5	13	5
9	T2	UC-Squ	High	-	MIBC	-	-	Cis+G	No Resp	75	12	3	9
10	T1	UC	High	+	NMIBC	High	-	Ep	Comp Resp	85.5	58	45	12
11	Ta	UC	Low	-	NMIBC	Low	-	-	Comp Resp	70	33	22	42
12	Ta	UC	Low	-	NMIBC	Low	+	-	Comp Resp	67.5	38	39	63
13	Ta	UC	Low	-	NMIBC	Inter	-	Ep	Comp Resp	59	22	27	32
14	Ta	UC	Low	-	NMIBC	Inter	+	Ep	Comp Resp	64.5	51	75	43
15	Ta	UC	Low	-	NMIBC	Low	+	-	No data	57.5	48	18	54
16	Ta	UC	Low	-	NMIBC	Low	+	-	Comp Resp	50.5	4	14	12
17	T2	MU	High	-	MIBC	-	-	Carb+G+pak	Partial Resp	82	22	7	25
18	T1	UC	High	-	NMIBC	High	-	-	No data	75.5	20	40	42
19	T2	UC-Squ	High	-	MIBC	-	-	-	No data	77	58	47	40
20	Ta	UC	Low	-	NMIBC	Low	+	-	Comp Resp	80.5	57	55	78
21	Ta	UC	Low	-	NMIBC	Low	+	-	No data	66.5	22	28	32
22	Ta	UC	Low	-	NMIBC	Low	+	-	Comp Resp	54	23	25	15
23	T1	UC	High	-	NMIBC	High	-	-	Recurrent	41.5	29	13	17
24	T1	UC	Low	+	NMIBC	High	-	-	Exitus	90.5	97	82	80

PN= Patient number, p stage= Pathologic stage, UC= Urothelial carcinoma, Squ= squamous differantiation, MU= Mucinous carcinoma, ISI= Immediate single instilation of chemotherapy, Car= Carboplatin, G= Gemcitabine, Cis= Cisplatin, Pac= paclitaxel, BCG= Bacillus Calmette Guerin, Ep= Epirubicin, Resp= Response, Comp= Complete, IFN-α= Interferon-α

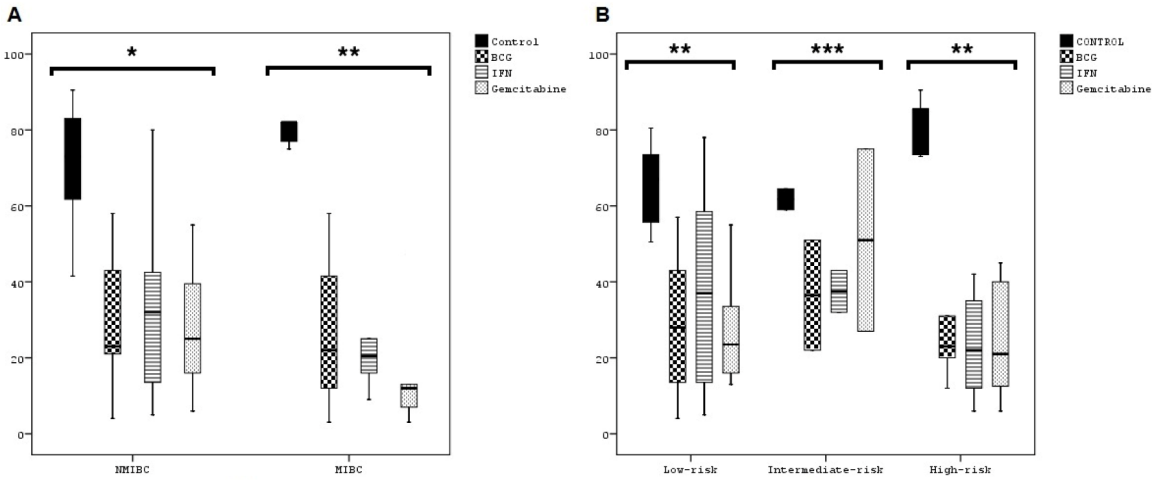

Figure 1. Viability distributions of BCG, Gemcitabine and IFN-α administration and Control groups with boxplots: **A:** for NMIBC / MIBC patients and **B:** for low-risk, intermediate-risk and high-risk of NMIBC patients. *p<0.001, **p<0.05 and ***p>0.05

Table 3. Viability rates and comparative results of groups after the drug administration

	N	*Viability rates (%)*	*p**
Control	24	73.1±13.3 (42-91)	-
BCG	24	31.2±21.9 (3-97)	<0.001
IFN-α	24	30.9±21.3 (5-80)	<0.001
Gemcitabine	24	27.7±20.8 (3-82)	<0.001
BCG + IFN-α	24	32.1±22.2 (0-86)	<0.001
BCG + Gemcitabine	24	29.4±21.4 (0-80)	<0.001

*Analysis of viability rates in other groups compared to the control group using the Wilcoxon test, BCG= Bacillus Calmette Guerin, IFN-α= Interferon-α

Discussion

The treatment of NMIBC remains a challenge because of its tendency for recurrence and progression [10,11]. According to the EAU Guidelines, additional intravesical chemotherapy or immunotherapy are recommended to prevent tumor recurrence and progression in NMIBC [2]. To decrease the risk of recurrence, an ISI of chemotherapy with mitomycin C (MMC) or epirubicin is administered after TUR-BT. The ISI prevents recurrence through eradication of floating tumor cells and residual or overlooked synchronous small tumors [12,13]. For the management of NMIBC, different treatment modalities and follow-up procedures are recommended after TUR-BT according to EORTC risk Table in the EAU Guidelines. ISI are recommended for low-risk and intermediate-risk patients [2]. However, an ISI is not recommended in intermediate-risk patients with a prior recurrence rate of >1/year nor in patients with an EORTC recurrence score ≥5 [10]. Also, in patients with intermediate-risk and high-risk tumors, intravesical BCG after primary TUR-BT or second resection of TUR-BT is recommended to reduce the risk of tumor recurrence [2]. The use of drug combinations like epirubicin + IFN-α or BCG + IFN-α were investigated in the past years [3,4].

However, the risk of toxicity, local irritation, increasing recurrence and progression due to drug resistance can be observed in some patients with intravesical administration of these treatments [10,14]. Therefore, to prevent toxicity and recurrence, different treatment modalities are recommended after TUR-BT according to EORTC risk Table [2]. A recent study evaluated intravesical gemcitabine versus BCG treatment for efficacy and toxicity [15]. In the study, the authors stated that gemcitabine was associated with similar (with a trend toward superior) disease-free survival (DFS) compared to BCG. They also stated that intravesical BCG is the standard first-line adjuvant therapy for NMIBC. However, they reported that gemcitabine could be considered as an alternative for patients who are not suitable for intravesical BCG treatment and for those who have BCG failure, relapsed or refractory disease [15].

In a Cochrane analysis, the instillation of BCG plus IFN-α and BCG alone were assessed in NMIBC and there were no differences in disease recurrence or progression rates between the groups [16]. On the other hand, research about recombinant adenovirus IFN-α with Syn3 (rAd-IFNα/Syn3) using a replication-deficient recombinant adenovirus gene transfer vector is ongoing for patients with high-grade BCG-refractory or relapsed NMIBC [17].

The standard treatment for patients with MIBC is RC. However, RC only provides 5-year survival in about 50% of patients [5-7]. To improve these results, platin-based (cisplatin and gemcitabine combination) neoadjuvant chemotherapy (NAC)

has been recommended [1]. In addition, patients who have high-risk pathologic stage at cystectomy (pT3/T4 and/or pN+) and have not previously received platin-based NAC should be considered for platin-based adjuvant chemotherapy (AC) [8].

Nevertheless, the answer to the question of which drug for which patient is still not clear during decision-making by clinicians. At this step, maybe personalized treatment can be included in the management of bladder cancer. Therefore, the hypothesis in this study was to answer the question of which drug for which patient by *ex-vivo* testing.

In bladder cancer cell culture applications, most studies use animal models, bladder cancer cell lines or short-term culture of tissue specimens. However, findings from animal models included limited evidence from the human UC [18]. Also, most human bladder cancer cell lines have provided more important data for *ex-vivo* cancer research to screen new cancer drugs [19] that are aneuploid and display genetic and molecular alterations [20-23]. Several research groups investigated primary cultures of bladder cancer derived from tumor tissue pieces [24-26] or urinary exfoliated bladder cancer cells (from voided urine of patients or washouts during TUR-BT) [27,28] that provide more information about personal tumor characteristics. To date, some researchers reported that primary cultures of advanced cancers are more easily produced compared to primary culture of low-grade NMIBC. However, a few groups of researchers were able to perform primary culture of low-grade tumors with high grade tumors of NMIBC in times ranging from a few hours to several days [25]. In addition, three-dimensional cell culture systems of bladder cancer have been reported [24] that might be superior to monolayer primary cultures for drug evaluations. However, application of these cell culture systems is difficult, highly expensive and time-consuming. Therefore, we investigated drug response rates using the oncogram method with a primary cell culture model because we demonstrated it is a simple, easy, rapid and applicable method in bladder cancer.

In summary, we observed that some low-risk NMIBC (Ta low grade tumors) were resistant to chemotherapy (patients no. 12 and 20 in Table 2), although some of them were resistant to immunotherapy (patients no. 12, 15 and 20). Some high-risk NMIBC (T1 tumors) (patients no. 2, 10 and 24) were more resistant to BCG than others (patients no. 4 and 5). However, in some high-risk NMIBC (patients no. 3 and 5) tumors were more chemosensitive compared to others. In MIBC (T2 tumors), some were more chemo and immunosensitive (patients no. 7 and 9) than others (patient no. 19). In addition, although viability results of the primary cell cultures after drug administration were significantly lower for drug groups (Group 2-6) compared to their own controls (Group 1), mean viability results of each drug group were found to be similar for BCG, Gemcitabine, IFN-α and their combinations.

According to the results of this study, because bladder tumors can display various behaviors in similar risk groups, pathologic stage and grade of tumors, response to the same drug administration can also be different for these various tumors. Therefore, primary bladder cancer cell culture can be rapidly and easily produced and with the application of *ex-vivo* oncogram of the chemotherapeutics and immunotherapeutics before intravesical treatment for NMIBC or neoadjuvant treatment for MIBC, personalized treatment can be applied according to the oncogram results. In addition, use of combinations of different chemotherapeutics and immunotherapeutics, such as epirubicin-BCG combination or BCG-IFN-α combination or BCG-Gemcitabine combination, can be applied according to the oncogram results.

The major limitation of this study is that some important drugs were not evaluated. For example, epirubicin and mitomycin-C that are used routinely for ISI after TUR-BT in all low-risk and some intermediate-risk NMIBC patients were not assessed in the study. In addition, cisplatin, which is a very important drug for NAC, AC and chemotherapy with or without RC in MIBC, was not also evaluated in the study. Another limitation is that some patients' follow-up data were missed. Also, ISI and additional intravesical treatments could not be applied to some patients with NMIBC. However, the study is a pilot study to create a model oncogram and to evaluate applicability of the model for personalized treatment of NMIBC and MIBC in the routine management of bladder cancer. Therefore, we plan to add these drugs, epirubicin, mitomycin-C, cisplatin and checkpoint inhibitors, to the oncogram in our ongoing study.

In conclusion, primary cancer cell cultures produced from bladder cancer tissue has been shown to be effective for drug sensitivity screening to help treatment decision, and bladder cancer cells are shown to have primary culture rapidly and easily. This *ex-vivo* method (oncogram) is a simple and applicable method that can be used for personalized treatment before intravesical therapy for NMIBC or systemic therapy for MIBC.

Conflict of interests

The authors declare no conflict of interests.

References

1. Witjes JA, Bruins M, Compérat E et al. Muscle-invasive and Metastatic Bladder Cancer. 2018. Uroweb. Available at: http://uroweb.org/guideline/bladder-cancer-muscle-invasive-and-metastatic/
2. Babjuk M, Burger M, Compérat EM et al. EAU Guidelines on Non-Muscle-invasive Bladder Cancer. 2018. Uroweb. Available at: http://uroweb.org/guideline/non-muscle-invasive-bladder-cancer/
3. Duchek M, Johansson R, Jahnson S et al. Bacillus Calmette-Guerin is superior to a combination of epirubicin and interferon-alpha2b in the intravesical treatment of patients with stage T1 urinary bladder cancer. A prospective, randomized, Nordic study. Eur Urol 2010;57:25-31.
4. Marttila T, Järvinen R, Liukkonen T et al. Intravesical Bacillus Calmette-Guerin Versus Combination of Epirubicin and Interferon-alpha2a in Reducing Recurrence of Non-Muscle-invasive Bladder Carcinoma: FinnBladder-6 Study. Eur Urol 2016;70:341-47.
5. Stein JP, Lieskovsky G, Cote R et al. Radical cystectomy in the treatment of invasive bladder cancer: long-term results in 1,054 patients. J Clin Oncol 2001;19:666-75.
6. Stein JP, Skinner DG. Radical cystectomy for invasive bladder cancer: long-term results of a standard procedure. World J Urol 2006;24:296-304.
7. Ghoneim MA, el-Mekresh MM, el-Baz MA, el-Attar IA, Ashamallah A. Radical cystectomy for carcinoma of the bladder: critical evaluation of the results in 1,026 cases. J Urol 1997;158:393-9.
8. Koshkin VS, Grivas P. Perioperative chemotherapy for muscle-invasive bladder cancer: the importance of multidisciplinary management for evidence-based practice and transformative research. Transl Androl Urol 2018;7:504-7.
9. Miura T, Kubota Y, Sakuramoto T, Hosaka M. A trial of the in vitro sensitivity test of anti-cancer drugs using primary cultured urogenital cancer cells. Urol Res 1987;15:187-90.
10. Sylvester RJ, van der Meijden AP, Oosterlinck W et al. Predicting recurrence and progression in individual patients with stage Ta T1 bladder cancer using EORTC risk tables: a combined analysis of 2596 patients from seven EORTC trials. Eur Urol 2006;49:466-77.
11. van Rhijn BW, Burger M, Lotan Y et al. Recurrence and progression of disease in non-muscle-invasive bladder cancer: from epidemiology to treatment strategy. Eur Urol 2009;56:430-42.
12. Pan JS, Slocum HK, Rustum YM, Greco WR, Gaeta JF, Huben RP. Inhibition of implantation of murine bladder tumor by thiotepa in cauterized bladder. J Urol 1989;142:1589-93.
13. Soloway MS, Masters S. Urothelial susceptibility to tumor cell implantation: influence of cauterization. Cancer 1980; 46:1158-1163.
14. Rockenbach L, Bavaresco L, Fernandes Farias P et al. Alterations in the extracellular catabolism of nucleotides are involved in the antiproliferative effect of quercetin in human bladder cancer T24 cells. Urol Oncol 2013;31:1204-11.
15. Prasanna T, Craft P, Balasingam G, Haxhimolla H, Pranavan G. Intravesical Gemcitabine versus Intravesical Bacillus Calmette–Guérin for the Treatment of Non-Muscle Invasive Bladder Cancer: An Evaluation of Efficacy and Toxicity. Front Oncol 2017;7:260.
16. Shepherd AR, Shepherd E, Brook NR. Intravesical Bacillus Calmette-Guérin with interferon-alpha versus intravesical Bacillus Calmette-Guérin for treating non-muscle-invasive bladder cancer. Cochrane Database Syst Rev 2017; 3: CD012112. doi: 10.1002/14651858.CD012112.pub2.
17. Shore ND, Boorjian SA, Canter DJ et al. Intravesical rAd-IFNα/Syn3 for Patients With High-Grade, Bacillus Calmette-Guerin-Refractory or Relapsed Non-Muscle-Invasive Bladder Cancer: A Phase II Randomized Study. J Clin Oncol 2017;35:3410-6.
18. Oyasu R. Epithelial tumours of the lower urinary tract in humans and rodents. Food Chem Toxicol 1995;33:747-55.
19. Okuyama H, Yoshida T, Endo H et al. Involvement of Heregulin/HER3 in the Primary Culture of Human Urothelial Cancer. J Urol 2013;190:302-10.
20. Habuchi T, Ogawa O, Kakehi Y et al. Accumulated allelic losses in the development of urothelial cancer. Int J Cancer 1993;53:579-84.
21. Knowles MA, Elder PA, Williamson M, Cairns JP, Shaw ME, Law MG. Allelotype of human bladder cancer. Cancer Res 1994;54:531-8.
22. Williams SV, Sibley KD, Davies AM et al. Molecular genetic analysis of chromosome 9 candidate tumor-suppressor loci in bladder cancer cell lines. Genes Chromosomes Cancer 2002;34:86-96.
23. Florl AF, Schulz WA. Peculiar structure and location of 9p21 homozygous deletion breakpoint in human cancer cells. Genes Chromosomes Cancer 2003;37:851-7.
24. Burgues JP, Gomez L, Pontones JL, Vera CD, Jiménez-Cruz JF, Ozonas M. A chemosensitivity test for superficial bladder cancer based on three-dimensional culture of tumour spheroids. Eur Urol 2007;51:962-70.
25. Celis A, Rasmussen HH, Celis P et al. Short-term culturing of low-grade superficial bladder transitional cell carcinomas leads to changes in the expression levels of several proteins involved in key cellular activities. Electrophoresis 1999;20:355-61.
26. Reznikoff CA, Gilchrist KW, Norback DH, Cummings KB, Ertürk E, Bryan GT. Altered growth patterns in vitro of human papillary transitional carcinoma cells. Am J Pathol 1983;111:263-72.
27. Okuno H, Kakehi Y, Ozdemir E, Terachi T, Okada Y, Yoshida O. Association of in vitro growth potential of urinary exfoliated cells with tumor localization and intraluminal recurrence rates of urothelial cancers. J Urol 1997;158:1996-9.
28. Herz F, Deitch D, Adler SA, Brijlall D. Short-term cultures of exfoliated cells from the urine of patients with bladder tumours. Urol Res 1993;21:23-6.