In Vitro and *In Vivo* Experimental Models as Tools to Investigate the Efficacy of Antineoplastic Drugs on Urinary Bladder Cancer

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Abstract. *Several drugs have shown in vitro and in vivo pharmacological activity against urinary bladder cancer. This review aims at compiling the different drugs evaluated in in vitro and in vivo models of urinary bladder cancer and to review the advantages and limitations of both types of models, as well as the different methodologies applied for evaluating antineoplastic drug activity.*

Cancer is one of the most important public health issues and the most feared human disease (1). It is the second leading cause of death after coronary heart diseases and one in three persons suffers from cancer throughout their lives and one in four will die from this disease (2). Urinary bladder cancer is a common disease that ranks ninth in worldwide cancer incidence. It is the fourth most common cancer in men and the ninth in women, with a probability of developing in men three-times higher than in women, and with a ratio of 2:1 for caucasians and negros, respectively (3). The risk for developing this disease increases with age, with a peak between 60 and 70 years (4). Remarkable differences can be found in its incidence, it being predominately higher in developed countries such as in North America, Western and Southern Europe (5). In less industrialized countries, such as in Asia, Africa and the Middle East, the incidence of urinary bladder cancer is lower except for regions where *Schistosoma haematobium* is endemic. In these cases, urinary bladder squamous cell carcinoma is common (6). Involving exogenous and endogenous factors, the aetiology of urinary bladder cancer is multifactorial (Figure 1) (7, 8). Cigarette smoking, and environmental and occupational exposure to chemical agents remain the two major risk factors (7).

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Urinary bladder cancer is classified into three main types: transitional cell carcinoma, squamous cell carcinoma and adenocarcinoma. At minor percentages are the small-cell tumours (1%) and sarcomatoid tumours (fewer than 1%) (9). Accounting for more than 90% of all cases, transitional cell carcinoma is the most common form of urinary bladder cancer (10). At diagnosis, nearly 70% of patients with urinary bladder cancer present with non-muscle-invasive lesions. Several clinical factors, such as tumour multiplicity, diameter, concomitant carcinoma *in situ* (CIS) and gender, have been identified as having prognostic significance for recurrence (11). CIS represents a major concern in the treatment of non-muscle-invasive lesions. CIS is a high-grade lesion that is characterized by disorderly proliferation of cells with marked cytological abnormalities (12). Although the European Association of Urology recommends transurethral resection and intra-vesical Bacillus Calmette-Guerin (BCG) immunotherapy for patients with CIS lesions, which achieves a complete response rate, 20% of patients will ultimately die of metastatic disease (12-14). The remaining 30% of patients at diagnosis have muscle-invasive lesions and 10% of these cases has a tendency to metastasize, with a poor prognosis (15).

Urinary Bladder Cancer Treatment

Treatment of non-muscle-invasive lesions. Complete transurethral resection is the standard treatment for non-muscle-invasive lesions (16). Despite good prospects of survival (success rate of 80%), these tumours recur in approximately 70% of patients (10). One of the major challenges in treating non-muscle-invasive tumours is to reduce the high frequency of early recurrences, detected in more than 45% of the patients, three months following transurethral resection (17). In order to reduce the recurrence risk and to delay or prevent progression to a muscle-invasive lesion, after transurethral resection, adjuvant intra-vesical instillations of chemotherapy or

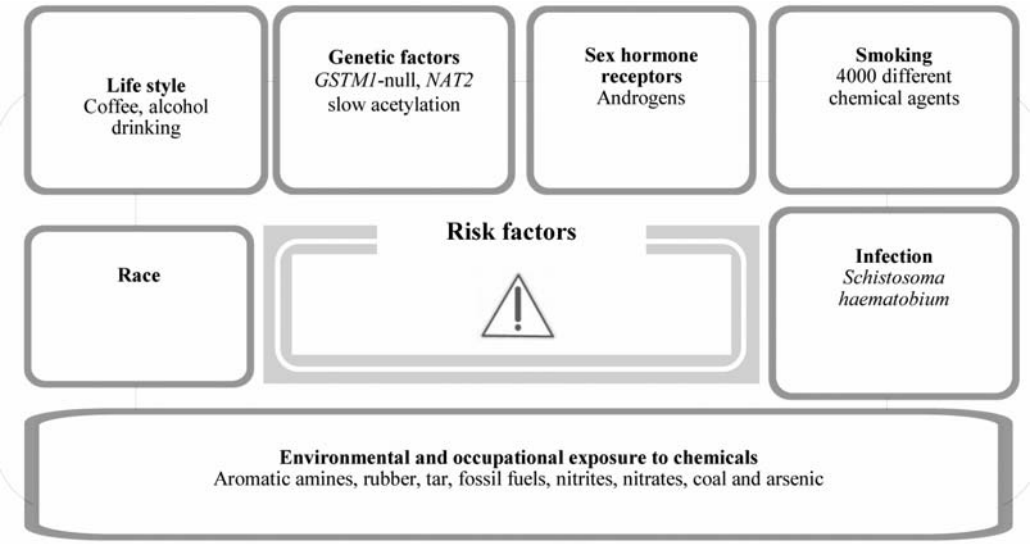

Figure 1. *Risk factors that contribute to the development of urinary bladder cancer. GSTM1: Glutathione S-transferase Mu; NAT2: N-acetyltransferase-2 (arylamine N-acetyltransferase).*

immunotherapy are widely applied with mitomycin C (MMC) and BCG, respectively (16). Use of BCG not only reduces the recurrence rate, but also reduces the risk of a non-muscle-invasive lesion progressing to a muscle-invasive lesion, improving the overall survival (7). The role of BCG in urinary bladder cancer was clinically studied for the first time in 1976, by Morales and collaborators, which found a complete response in seven out of nine patients treated (18). Although treatment with BCG provides better results than transurethral resection without immunotherapy, side-effects arising from its administration are a concern. The development of sepsis, cystitis, dysuria and mild haematuria are frequently reported (19). However, with increased experience in using BCG, the side-effects now appear to be less prominent (20). Intravesical chemotherapy with MMC, epirubicin and doxorubicin have all shown comparable beneficial effects (21).

Treatment of muscle-invasive lesions. The treatment options that are currently available for the management of muscle-invasive urinary bladder cancer include radical cystectomy and chemotherapy-plus-radiation therapy, with the goal of bladder preservation. Combined chemotherapy based on methotrexate, vinblastine, adriamycin and cisplatin (MVAC) was initiated in the 1980s, leading to a disease-free survival rate of 3.7% at six years (22). However, this protocol was highly toxic, with severe side-effects, being associated with a mortality rate of about 4% (23). Thus, new approaches are being continuously investigated to provide superior efficacy with lower toxicity (24).

In Vitro and *In Vivo* Models for the Study of Urinary Bladder Cancer

Experimental models are used to better explain tumour behaviour, to evaluate the effect of chemopreventive agents, and to study the efficacy of antineoplastic drugs (25). Such experimental research can be achieved by means of *in vitro* and *in vivo* models.

In vitro models. To date, cultured urinary bladder cells represent the most frequently used *in vitro* bladder cell model. These models usually consist of isolated urinary bladder cancer cell lines and have been established as a valid *in vitro* model not only to study the mechanism involved in urinary bladder cancer development but also to evaluate anti-neoplastic drug efficacy (26). In 1970, Rigby and Franks established the first human urinary bladder cancer cell line, designated as RT4 (27). Since then, many other human urinary bladder cancer cell lines have been established and characterized according to their origin, grade and stage. A great proportion of these cell lines was established from invasive and metastic tumours, benefiting the investigation of late tumour progression and metastic lesions. On the other hand, few non-muscle-invasive human urinary bladder cancer cell lines are available, which is a disadvantage in the investigation of non-muscle-invasive urinary bladder cancer (26). Urinary bladder cancer cell lines may also be established from rodents exposed to urothelial chemical carcinogens. In 1971, Toyoshima and collaborators established the Nara urinary bladder cancer II (NBT-II) cell line, a rat cell line obtained from a urinary bladder tumour chemically induced by *N*-Butyl-*N*-(-4-hydroxybutyl)

Table I. *Established human and rodent urinary bladder cancer cell lines.*

Urinary bladder cancer cell line	Origin	Established	Reference
Human			
RT4	Recurrent transitional cell carcinoma	1970	(27)
5637	Primary transitional cell carcinoma	1974	(47)
RT112	Primary transitional cell carcinoma	1973	(48)
HT1376	Invasive carcinoma	1977	(49)
HT1197	Recurrent invasive transitional cell carcinoma	1977	(49)
T24	Transitional cell carcinoma	1970	(50)
KU-19-19	Invasive transitional cell carcinoma	1993	(51)
MCR	Subcutaneous metastic lesion	2001	(52)
MGH-U1	Primary transitional cell carcinoma	1972	(53)
U-BLC1	Primary transitional cell carcinoma	1998	(54)
BC-3C	Invasive solid tumour	1998	(55)
CAL-29	Invasive, metastatic transitional cell carcinoma	1985	(56)
TCCSUP	Primary transitional cell carcinoma	1974	(57)
UM-UC-1	Metastic transitional cell carcinoma	1984	(58)
UM-UC-3	Transitional cell carcinoma	1982	(59)
CAT	Transitional cell carcinoma	1997	(60)
647V	Transitional cell carcinoma	1976	(61)
Rodent			
AY-27	Rat (induced by FANFT)	1981	(62)
NBT-II	Rat (induced by BBN)	1971	(28)
BTT-T739	Mouse (induced by BBN)	1996	(63)
MB49	Mouse (induced by FANFT)	1976	(64)
MBT-2	Mouse (induced by FANFT)	1976	(64)

BBN: *N*-Butyl-*N*-(-4-hydroxybutyl) nitrosamine; FANFT: *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide.

nitrosamine (BBN) (28). In the same year, three more urinary bladder cancer cell lines were established from tumours induced by the combined use of *N*-2-fluorenylacetamide and cyclophosphamide in Fischer 344 female rats, two of them epithelial (BC_5 and BC_6) and one fibroblastic (BC7) (29). Five years later, two mouse urinary bladder cancer cell lines were established using the carcinogen *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide (FANFT) and, more recently, seven chemically-induced mouse urinary bladder cancer cell lines (BC13, BC29, BC30, BC46, BC57, BC58 and BC59) were established from tumours developed in C57BL/6 mice exposed to BBN (30). Table I summarizes commercially available human and rodent urinary bladder cancer cell lines.

Under specific culture conditions, normal human urothelial cells may be also established from surgical specimens of urinary bladder (31). These normal cells can be used to evaluate mechanisms by which therapeutic agents interact with normal human urothelial cells and to verify if antineoplastic drugs can induce cell damage. Tumours are, however, three-dimensional complexes in which there is great interaction between tumourous and non-tumourous cells. Thus, cell culture represents an artificial system for investigating features such as vascularization and perfusion (32). Urothelium may be maintained in combination with stroma as a three-dimensional heterotypic or organotypic culture. Such organoids may either be established from intact tissues, or recombined from reconstructed urothelial and stromal compartments prior to culture and may be maintained up to 20 weeks in culture (33). Chang and collaborators were pioneers in using human urinary bladder tumour specimens in three-dimensional histoculture to assess the activity of a new platinum analog (34). The growth of tumour cells as three-dimensional multicellular spheroids *in vitro* has led to important insights in tumour biology, since properties of the *in vivo* tumour, such as proliferation, and nutrient gradients, can be studied under controlled conditions (35). Moreover, three-dimensional culture allows for an understanding of basic paracrine signalling mechanisms that regulate tissue homeostasis, development of new methods for urinary bladder reconstruction and tissue engineering, and generation of models of malignant and benign diseases (33). Furthermore, isolated organs allow an approach towards the assessment of organ physiology and morphology, providing with models that mimic conditions in humans more closely (26). Several years ago, Burgués and collaborators tested several drugs (epirubicin, thiotepa, adriamycin, MMC, verapamil and ciprofloxacin) on *ex vivo* spheroids of non-muscle-invasive urinary bladder cancer. Their study suggests that use of three-dimensional urinary bladder cultures could be

a possible approach in clinical practice to select for the best antineoplastic drug for each patient and to investigate the effect of new antineoplastic drugs or drug combinations (36).

In vivo models. Prediction of drug activity in patients with cancer based only on *in vitro* studies is not reliable, and animal models are widely recognized as being essential to the study of antineoplastic drug efficacy (32). Animal models were defined by Wessler in 1976 as "living organisms with an inherited naturally-acquired or induced pathological process that closely resembles the same phenomenon in man". Their application in biomedical research, and pathophysiological and toxicological studies, allows us to determine aetiological factors, study cancer development and progression, and to develop new medical devices or therapies (37).

For an appropriate and valid animal model for the study of urinary bladder carcinogenesis to exist, several requirements are necessary: the tumour should be of urothelial origin with different stages of disease progression, it should grow intravesically so as to be directly exposed to antineoplastic drugs, it should mimic the pathogenesis of human urinary bladder cancer, and it should present stable molecular and genetic alterations similar to those found in human urinary bladder cancer (38). Several animal species such as dogs, rabbits, guinea pigs, and hamsters may be used. However, rats and mice are the animals most employed, due to their small size, innumerable anatomical, physiological and biochemical similarities to humans, clear genetic background and high reproductive rate. Furthermore, the occurrence of spontaneous tumours in laboratory rodents is uncommon, which is one reason why they are considered as ideal models for the study of chemically-induced urinary bladder cancer (38).

Recently, a study conducted by Palmeira and collaborators described the similarities found between human urinary bladder carcinogenesis and rodent chemically-induced urinary bladder carcinogenesis in regards to the histopathological features and biological alteration profile, namely: DNA aneuploidy, p53 overexpression and high Ki-67 proliferative index. They reported that the spectrum of lesions chemically-induced in rodents, such as hyperplasia, dysplasia, low- and high-grade tumours, CIS and invasive urothelial carcinoma are similar to those observed in humans (39).

Experimentally induced urinary bladder tumours. Three models are currently available for inducing urinary bladder tumours: chemically-induced, genetically-engineered, and transplantable (xenograft and syngeneic animals) (Figure 2) (40). A wide range of compounds are described as urinary bladder carcinogens, but of all, BBN is the most frequently used chemical carcinogen (41). However, it is possible to induce urinary bladder cancer with FANFT and *N*-Methyl-*N*-nitrosourea (MNU). The selection of each of these agents is made according to the animal facilities and aims of the experimental work (42). The xenograft model is the most routinely used transplantable model and BBN is the carcinogen most frequently applied to induce bladder tumours.

Advantages and Disadvantages of *In Vitro* and *In Vivo* Models

As yet, there is not an ideal experimental model for urinary bladder cancer study since both *in vitro* and *in vivo* models have limitations. However, with the information obtained from both models, a better understanding of urothelial bladder carcinogenesis is possible. In pre-clinical studies, the antitumour efficacy of a new drug is first evaluated in *in vitro* models and later in animal models. One of the greatest advantages of *in vitro* models application is that they offer the possibility to maintain cells in completely controlled environmental conditions, allowing the study of specific cellular and molecular pathways in shortened experimental timescales, being less expensive than animal models and less time-consuming. In contrast, the greatest limitation of this model is that cells growing *in vitro* are not the exact dissociated replicates of their *in vivo* counterparts. The use of monolayer cell cultures is usually restricted to a single or at most two cell types. Tumours are composed not only of neoplastic cells but also of stroma and inflammatory cells, which gives a tumour a three-dimensional structure, interacting and influencing its growth (33). The impossibility of tumour angiogenesis and metastasis studies can be considered as limitations of *in vitro* studies, since these are complex processes with many different mechanisms involved. It is, therefore, clearly difficult to perform *in vitro* assays which totally simulate these processes and only a combination of methods will be able to provide a clear picture (43). *In vitro* studies can also provide important information concerning the parameters of pharmacodynamics. To better-understand pharmacokinetics, it is necessary to use *in vivo* models, since these models offer the best approach for effectively combining and interpreting the major determinants of drug kinetics across species (44). Likewise, *in vitro* studies do not predict the adverse effects of drugs (45).

For this reason, *in vivo* models remain important: they preserve the three-dimensional tumour structure with cell-cell interactions and allow for pharmacokinetic and toxicity evaluation of the compounds. Significant limitations of *in vivo* studies include the necessity for animal facilities, they are also more time consuming, and involve high involved (40). The advantages and disadvantages of *in vitro* and *in vivo* models, as well as their possible applications are summarized in Figure 3.

In Vitro Studies to Assess the Efficacy of Antineoplastic and Other Drugs

In the past thirty years, more than 40 studies were conducted in order to evaluate the activity of antineoplastic drugs, making use of several human urinary bladder cancer cell lines, as well as a

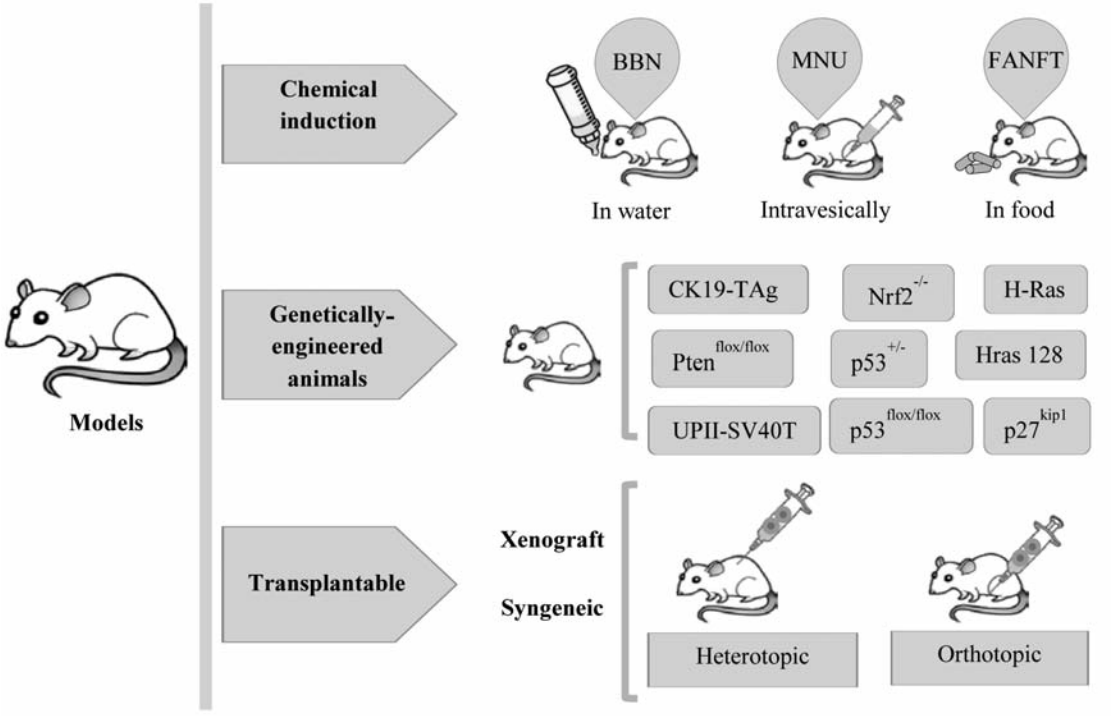

Figure 2. *Possible animal models for inducing urinary bladder cancer development in rodents.*

wide range of methodologies (Table II). It is clearly perceptible that out of the 40 different urinary bladder cancer cell lines used, the T24 muscle-invasive cell line is the one most employed, followed by the HT1376, RT112 and RT4 cell lines. Currently, there is a broad range of methodologies available to assess the *in vitro* efficacy of drugs, but assays such as 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), clonogenic, flow cytometry and western blot are regularly used. *In vitro* models have already provided the basis for study of the activity of many antineoplastic drugs. However, alkylating drugs, namely cisplatin and MMC, as well as anti-metabolite drugs, such as gemcitabine, are among the ones most investigated, whether in isolation or combination therapy. Other antineoplastic drugs with different mechanisms of action have also been analyzed, examples are inhibitors of the mammalian target of rapamycin (mTOR) (rapamycin, everolimus), topoisomerase II (epirubicin, doxorubicin, etoposide) and of mitotic spindle formation (paclitaxel, vincristine, vinorelbine). Many of these drugs already tested *in vitro* (approximately 26) have progressed to clinical studies of urinary bladder cancer. Nevertheless, none of them demonstrated superior efficacy when compared to the drugs currently used in urinary bladder cancer treatment, which is why none of them have been approved as a new therapy.

Through analysing Table III it is clear that non-antineoplastic drugs have also been studied using human urinary bladder cancer cell lines. Similarly to what happens with antineoplastic drugs, T24 and MTT remain the most used urinary bladder cancer cell line and the most frequent methodology applied, respectively. More than 20 compounds classified as non-antineoplastic drugs were tested, being in the majority non-steroidal anti-inflammatory drugs (NSAIDs). However, very few of these drugs have advanced to clinical studies.

Rodent urinary bladder cancer cell lines are used to assess the efficacy of antineoplastic and other drugs. The mouse MBT-2 and the rat AY-27 urinary bladder cancer cell lines are widely employed (Table IV).

In Vivo Studies to Assess the Efficacy of Antineoplastic and Other Drugs

As previously stated, *in vivo* models can be used to evaluate drug efficacy. As shown in Table V, in the last twenty years since chemical carcinogens were discovered, more than 25 studies were carried out with rats and mice to analyze antineoplastic drug efficacy in urinary bladder tumours, chemically-induced or implanted. The chemical induction of urinary bladder tumours is commonly implemented using the carcinogen BBN, and for tumour implantation, rodent (MB49, MBT-2, AY-27) and human (HT1376, KU-19-19, 5637, RT4, T24, UM-UC-3) urinary bladder cancer cell lines may be used. Immunotherapy with BCG, in isolation or combined with other drugs, has been one of the therapeutic approaches most extensively investigated in rodent models of urinary bladder cancer. Similarly to *in vitro* studies, in *in vivo* models, the use of NSAIDs and other drugs are common,

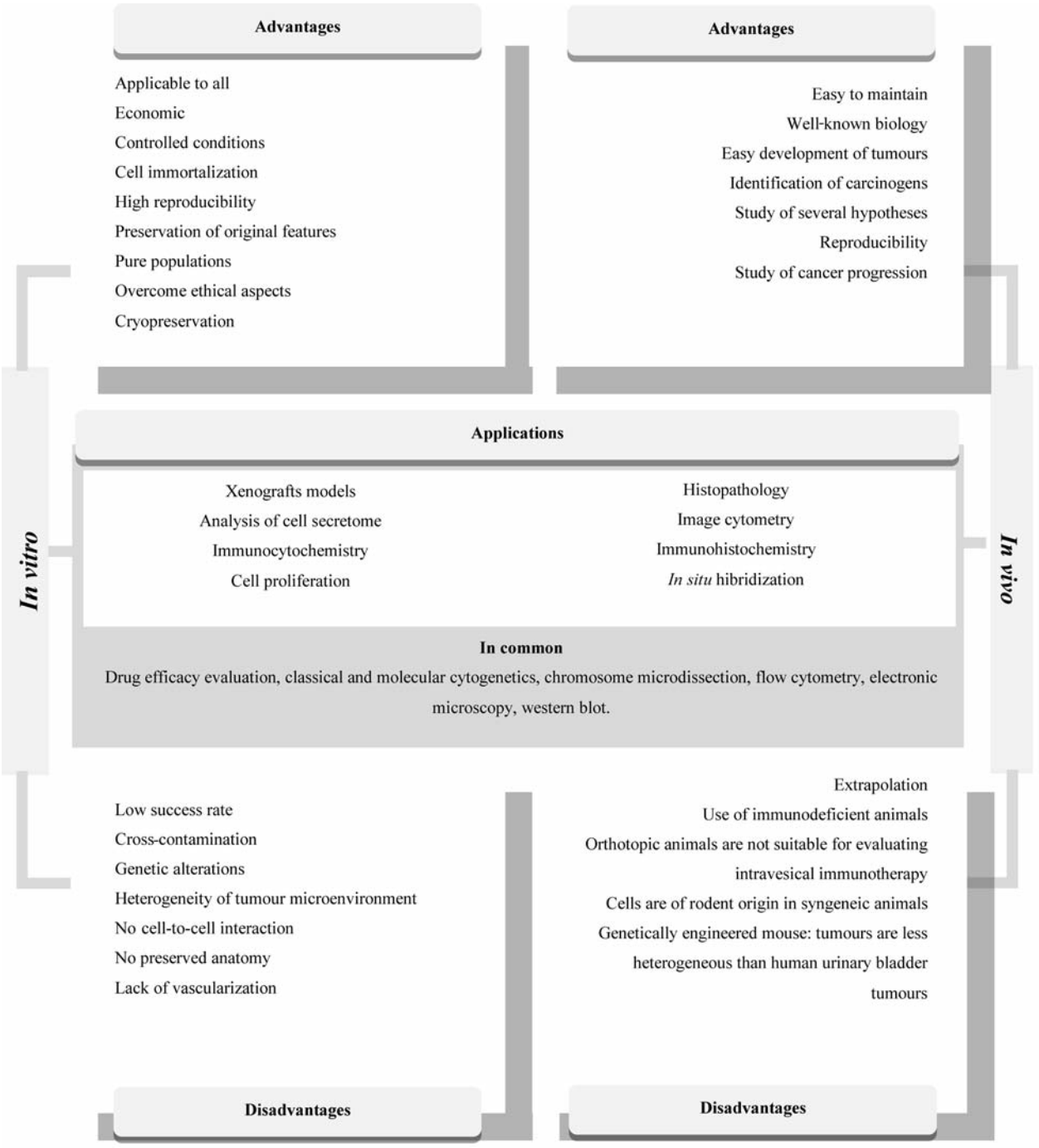

Figure 3. *Advantages, disadvantages and applications of in vitro and in vivo models.*

using both chemically-induced and implanted urinary bladder tumours. We verify that the association of two or more drugs is frequent and histopathology and immunohistochemistry are commonly carried out, the expression of Ki-67, proliferating cell nuclear antigen (PCNA), tumour protein 53 (p53), cyclin, cyclo-oxygenase-2 (COX-2), vascular endothelial growth factor (VEGF) and platelet endothelial cell adhesion molecule (CD31) being widely evaluated on experimental models of urinary bladder cancer. In order to complement the information obtained from the investigation, many studies evaluate the activity of the same drug in both *in vivo* and *in vitro* models (Table V and Table VI). None of the drugs tested *in vivo,* not tested *in vitro* has

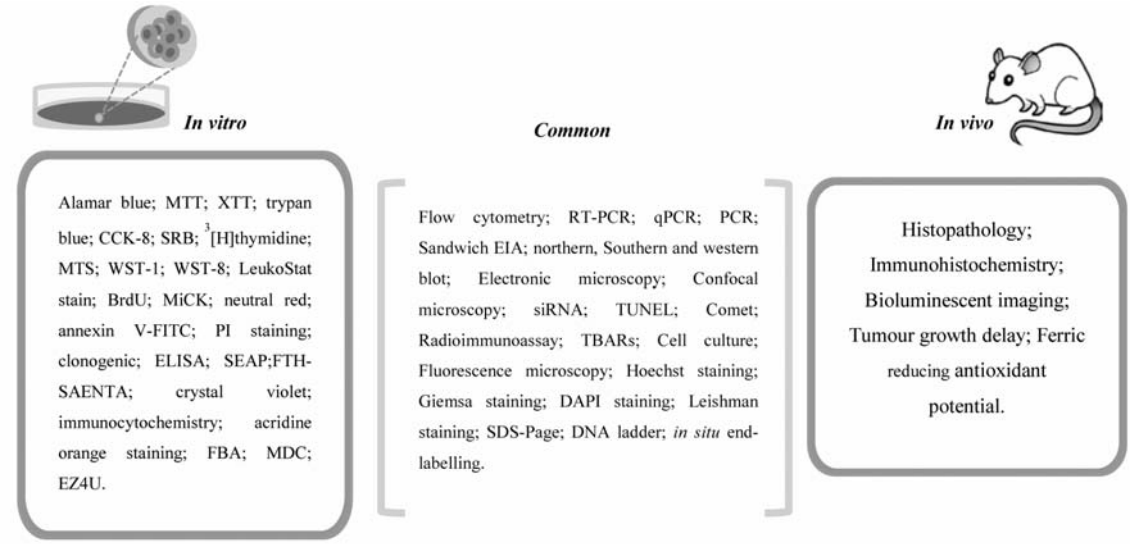

Figure 4. *Different methodologies to assess antineoplastic drug activity. MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; XTT: 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide; CCK-8: Cell Counting Kit-8; SRB: sulforhodamine B; PI staining: propidium iodide; MTS: (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium); BrdU: 5-bromo-2'-deoxyuridine; MiCK: micro-culture kinetic; PCR: polymerase chain reaction; RT-PCR: reverse transcriptase-PCR; qPCR: quantitative-PCR; TBARs: thiobarbituric acid reactive substances; ELISA: enzyme-linked immunosorbent assay; TUNEL: terminal deoxynucleotidyl transferase dUTP nick-end labeling; FTH-SAENTA: 5'-S-{2-(1-[(fluorescein-5-yl)thioureido]-hexanamido)ethyl}-6-N-(4-nitrobenzyl)-5'-thioadenosine; FBA: filter-binding assay; MDC: monodansylcadaverine staining; SEAP: secreted alkaline phosphatase; siRNA: small-interfering RNA; WST: water soluble tetrazolium; SDS-Page: sodium dodecyl sulfate polyacrylamide gel electrophoresis; DAPI: 4',6-diamidino-2-phenylindole.*

advanced to clinical trials, these drugs did not eradicate tumours but only restricted their growth.

Rodent models are particularly valuable for defining the molecular pathways participating in urothelial cell transformation and disease progression, for identifying modifier genes that affect penetrance of the manipulated genes, and for testing various therapeutic and preventive approaches.

Methodologies for Assessing Drug Activity

The use of reliable methodologies to determine and quantify the efficacy of several drugs facilitates the selection of promising candidate drugs for clinical trials (32). As shown in Figure 4, over the years, several methodologies have been developed and used to evaluate the efficacy of different drugs in *in vitro* and *in vivo* models. Tests for cytotoxicity and cellular growth inhibition are the oldest and most commonly used assays (43). For *in vitro* experiments, it should be borne in mind that if a substance leads to a reduced number of viable cells in comparison to the untreated cells after the incubation period, this can be due to the death of some cells, but without affecting growth of another sub-population, or to a general deceleration of growth but survival of all cells. Thus, several assays have been established to measure not only the viable cell number, but also to differentiate between the arrest of growth and cytotoxicity (43). The earliest test methods introduced the use of MTT, and 2,3-*bis*-(2-methoxy-4-nitro-5-sulfophenyl)-2*H*-tetrazolium-5-carboxanilide (XTT), and are based on cellular metabolism. The reduction of these colourless tetrazolium salts into coloured products is correlated with the number of viable cells. However, these assays do not differentiate between the growth arrest and cell death. Thus, it is necessary to additionally scrutinize the number of dead cells, commonly analyzed by microscopic analysis (trypan blue) or flow cytometry (propidium iodide). Other methods commonly applied for measuring cell growth are based on the incorporation of thymidine analogues, such as the ^{3}[H]thymidine and the 5-bromo-2'-deoxyuridine (BrdU) incorporation assays (43). However, even if a drug is effective, this evidence does not necessarily translate into efficacy, we can only see an effect for which our assay was specifically designed. If a substance gives good results when tested, it does not necessarily mean that this substance will be effective (43, 46). Taking this into consideration, it is absolutely necessary to establish guidelines for performing *in vitro* studies in order to create a basic framework with good predictive proprieties (43, 45).

Conclusion

In the present article, we have reviewed experimental data related to the investigation of antineoplastic drugs for urinary bladder cancer, highlighting the use of *in vitro* and *in vivo* models. Used for many decades, well-established *in vitro* and *in vivo* models

Table II. *In vitro studies using human urinary bladder cancer cell lines to assess the efficacy of antineoplastic drugs.*

Drug (Reference)	Cell line (Reference)	Methods used to evaluate drug efficacy (Reference)	Application in models of urinary bladder cancer (Reference)	Clinical studies of urinary bladder cancer (Reference)
Adriamycin, epirubicin, lonidamine (65)	MGH-U1, MGH-U1R, RT112, HT1376, RT4, VM-CUB-III	Clonogenic	No Epirubicin (67)	Adriamycin (66) Lonidamine (68)
BCG, BCG-CWS (69)	T24, HT1376, RT4	MTS, immunoblot, flow cytometry, RT-PCR, PI staining	No	BCG (70)
Candesartan, paclitaxel (71)	KU-19-19	IC (AT1R), alamar blue, ELISA	Yes (71)	Paclitaxel (72)
CDDP (73, 74)	RT4, T24 (73), HT1376, HT1376/P, HT1376/C1, HT1376/F2, HT1376/F4 (74)	MTT (73, 74), western blot, RT-PCR (73), sandwich ELISA (FGF-2), Hoechst staining, DNA ladder (74)	Yes (74)	CDDP (75)
CDDP, ethyl methanesulfonate (76)	RT4, TCCSUP, RT112, RT112-CP	Clonogenic	No	
CDDP, sunitinib malate (77)	TCCSUP, 5637	MTT, annexin V-FITC	Yes (77)	Sunitinib malate (78)
CDDP, carboplatin (79)	T24, J82, HT1197	MTT, Hoechst staining, DNA ladder, IC (BCL-2, BAX)	No	Carboplatin (80)
CDDP, melphalan, MMC, adriamycin, vincristine, 5-Fluorouracil (81)	MGH-U1	Clonogenic.	No	Vincristine (82) 5-Fluorouracil (83)
CDDP, doxorubicin (84)	RT4, RT112, T24, HT1197, HT1376	Clonogenic	No	Doxorubicin (85)
CDDP, bleomycin (86)	T24	Clonogenic	No	Bleomycin (87)
CDDP, sunitinib malate, gemcitabine (88)	HTB5, HTB9, T24, UM-UC-14, SW1710, J82	CCK-8, Chou and Talalay, clonogenic, western blot (cyclin D, cyclin B1, phospho-Akt, total-Akt, BAX, BAD)	No	Gemcitabine (89)
CDDP, everolimus (90)	T24, 5637, HT1376	MTT, IC (Ki-67), TUNEL	No	
CDDP, sodium butyrate (91)	T24, UM-UC-3, 253J	XTT, flow cytometry, western blot	No	
Docetaxel, gefitinib (92)	253J B-V, UM-UC-3, UM-UC-13, KU-7	MTT, western blot, flow cytometry	Yes (92)	Docetaxel (93) Gefitinib (94)
Doxorubicin, vinorelbine (95)	J82, T24	MTT	No	Vinorelbine (96)
Etoposide (97)	RT4, RT112, HT1376	FBA, flow cytometry, western blot, conventional electrophoresis of DNA, field inversion electrophoresis of DNA, alkaline elution	No	Etoposide (98)
Everolimus (99, 100)	UM-UC-3, UM-UC-6, UM-UC-9, UM-UC-14 (99), T24, 5637, HT1376 (100)	MTT (100), crystal violet, ^{3}[H]thymidine (99), flow cytometry, western blot (99, 100), TUNEL(100), radiolabeled protein–uptake (99)	Yes (99, 100)	Everolimus (101)
Exisulind (102)	HT1376	SRB, ELISA, M30 cytokeratin 18, PDE isozyme fractionation, permeabilized cell assay of cyclic nucleotide PDE, enzyme-linked immunoassay, PDE5 immunolabeling	Yes (102)	
Epidoxorubicin, gemcitabine (52)	HT1376, MCR	SRB, Chou and Talalay, flow cytometry, comet, western blot (BAX, BCL-2, p53)	No	Epidoxorubicin, gemcitabine (103)
Epirubicin, ciprofloxacin (104)	HT1197, HT1376	MTT, flow cytometry	No	
Fleroxacin, ciprofloxacin (105)	T24	MTT	Yes (105)	
Fleroxacin, 5-Fluorouracil (106)	T24	MTT	Yes (106)	5-Fluorouracil (83)

Table II. *Continued*

Table II. *Continued*

Drug (Reference)	Cell line (Reference)	Methods used to evaluate drug efficacy (Reference)	Application in models of urinary bladder cancer (Reference)	Clinical studies of urinary bladder cancer (Reference)
Gemcitabine (107, 108)	5637 (107), HT1376, MCR (108)	Trypan blue (107) SRB (108), flow cytometry, annexin V-FITC (107, 108), clonogenic, gel electroforesis, ELISA (caspase 3, 8, 9), RT-PCR (BCL-2, BAX, survivin, FAS, p21, p27, MDR-1, MRP-1, MRP-4), immunofluorescence (p65) (107)	No	Gemcitabine (89)
Gemcitabine (109)	HTB2, HTB3, HTB4	Confocal microscopy, MTS, FTH-SAENTA, nucleoside uptake, DNA isolation, cDNA synthesis and dideoxy sequencing, deoxycytidine and thymidine kinase 2 activities	No	
Gemcitabine, pemetrexed (110)	T24, J82	MTT, flow cytometry, fluorescence microscopy, ELISA (Akt phosphorylation), PCR	No	Pemetrexed (111)
Gemcitabine, paclitaxel (112)	RT112, RT4, T24, TCCSUP	MTT, annexin V-FITC, flow cytometry	No	
Gemcitabine, everolimus (113)	T24, 5637	MTT, flow cytometry, comet, leishman staining	No	
Gemcitabine, ABT-737 (114)	5637, SCABER	MTT, annexin V-FITC, flow cytometry, western blot, RT-PCR, immunoblot, siRNA	No	
Gemcitabine, bortezomib (115)	253JB-V, RT4, KU7, UM-UC3, UM-UC14	MTT, ^{3}[H]thymidine, immunoblot, flow cytometry, ELISA	Yes (115)	Bortezomib (116)
Gemcitabine, CDDP, 1,25D3 (117)	T24, UM-UC-3	MTT, annexin V-FITC, immunoblot (caspases-3, 8, 9), dose-effect analysis, clonogenic, siRNA	Yes (117)	
Lapatinib, gemcitabine, CDDP (118)	RT112, J82	MTT.	No	Lapatinib (119)
MMC (120)	RT112, RT4, 253J, T24	MTT, calculation of synergism	No	MMC (121)
MMC, doxorubicin (122)	RT112, EJ28	BrdU	No	
MMC, epirubicin, gemcitabine, apaziquone (123)	RT4, RT112, 253J, T24	MTT, thermo-chemotherapy, calculation of synergism	No	
MMC, adriamycin, epirubicin, epodyl, thiotepa (124)	MGH-U1	Clonogenic, BrdU	No	Thiotepa (125)
MMC, meglumine eicosapentaenoic acid, epirubicin (126)	MGH-U1, MGH-U1R, RT112	MTT, median effect analysis	No	
Ofloxacin, ciprofloxacin (127)	TCCSUP, T24, J82	MTT, ^{3}[H]thymidine	No	Ofloxacin (128)
Paclitaxel (129)	RT112	MTS, HPLC	No	
Quinazoline (130)	T24	Neutral red, clonogenic, cell death detection ELISAPLUS kit, flow cytometry, morphology, specific inhibition of caspases, oligonucleotide microarrays	No	
Rapamycin (131, 132)	RT112, RT4, T24, SUP (131), T24 (132)	MTT, trypan blue (132), EZ4U (131), ELISA (mTOR, VEGF) (131)	No	
Taurolidine (133)	RT4, RT112	XTT	Yes (133)	
Vinflunine, vinorelbine (134)	ECV304	SRB, flow cytometry	No	

BCG: Bacillus-Calmette Guérin; CDDP: cisplatin; MMC: mitomycin C; IC: immunocytochemistry; ELISA: enzyme-linked immunosorbent assay; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PCR: polymerase chain reaction; RT-PCR: reverse transcriptase-PCR; CCK-8: cell counting kit-8; SRB: sulforhodamine B; FBA: filter-binding assay; TUNEL: terminal deoxynucleotidyl transferase dUTP nick-end labeling; PDE isozyme fractionation: cyclic nucleotide phosphodiesterase isozyme fractionation; MTS: (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium); FTH-SAENTA: 5'-S-{2-(1-[(fluorescein-5-yl)thioureido]-hexanamido)ethyl}-6-N-(4-nitrobenzyl)-5'-thioadenosine; siRNA: small-interfering RNA; BrdU: 5-bromo-2'-deoxyuridine; HPLC: high-performance liquid chromatography; XTT: 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide; PI staining: propidium iodide staining; mTOR: mammalian target of rapamycin; VEGF: vascular endothelial growth factor; BCL-2: B-cell lymphoma-2; BAX: Bcl-2–associated X protein; Akt: protein kinase B; BCG-CWS: BCG-cell wall skeleton; 1,25D3: 1,25 dihydroxyvitamin D3; AT1R: angiotensin II type 1 receptor; BAD: Bcl-2-associated death promoter; FAS: apoptosis antigen 1; p53: tumour protein 53; p21: cyclin-dependent kinase inhibitor 1; p27: cyclin-dependent kinase inhibitor 1B; MDR-1: multidrug resistance protein 1; MRP-1: multidrug resistance- associated protein 1; MRP-4: multidrug resistance protein; annexin V-FITC: fluorescein isothiocyanate-labeled annexin V; FGF-2: basic fibroblast growth factor.

Table III. *In vitro studies using human urinary bladder cancer cell lines to assess the efficacy of other drugs*.

Drug (Reference)	Cell line (Reference)	Methods used to evaluate drug efficacy (Reference)	Application in models of urinary bladder cancer (Reference)	Clinical studies of urinary bladder cancer (Reference)
Aqueous extract of *Magnolia officinalis* (135)	5637	MTT, ^{3}[H]thymidine, flow cytometry, ELISA, immunoblot, zymography	Yes (135)	
Allyl isothiocyanate (136)	UM-UC-3	MTT, western blot, ELISA, flow cytometry	Yes (136)	
Aspirin, NCX 4040, NCX 4042 (137)	HT1376, MCR	SRB, IC, western blot (COX-2, caspases-3, 9, BAX, BCL-2), flow cytometry, TUNEL, annexin V-FITC, DAPI staining, mitochondrial membrane potential depolarization	No	
Aspirin, ibuprofen, acetaminophen, (*R*)-flurbiprofen, indomethacin (138)	T24, RT4, 5637	Cell lysis, immunoblot, transfection, MTT, Hoechst staining	No	
Aspirin, R-Flurbiprofen, sulindac sulfone, celecoxib, nitrosulindac, NCX-4050, NCX-2131(139)	T24	MTT, Hoechst staining, immunoblot (p75NTR, COX-2), RT-PCR, siRNA.	No	
Ascorbic acid, lysine, proline, arginine, green tea extract (140)	T24	MTT, gelatinase zymography, matrigel invasion	No	
Alpha lipoic acid, calcium hydroxycitrate (141)	T24, RT112	Trypan blue	Yes (141)	
Bufalin (142)	T24, EJ	MTT, flow cytometry, DAPI staining, mitochondrial membrane potential, western blot, RT-PCR	No	
Butein (143)	BLS-211, BLX-211	Western blot, electrophoretic mobility shift, immunofluorescence, migration and invasion, RT-PCR	No	
Calphostin C (144)	UM-UC-3, 5637, RT4	MTT, DAPI staining	No	
Catechin, NS-398 (145), catechin, rofecoxib, NS-398 (146)	T24, TCCSUP	MTT, annexin V-FITC	No	
Celecoxib (147, 148)	HT1376, TCCSUP, UM-UC-3 (147), RT4, UM-UC-3 (148)	MTT, clonogenic, poly-HEMA-coated plate, collagen, PGE2 quantitation, overexpression of COX-2, characterization of UM-UC-3COX-2/Tet cells (147), Sorenson's solution, flow cytometry (148), western blot (147, 148)	No	Celecoxib (149)
Celecoxib, NS-398 (150), celecoxib, NS-398, etodolac (151), celecoxib, NS-398, piroxicam (152)	UM-UC-1, UM-UC-3, UM-UC-6 (150), T24, 5637, KK47(151), HT1376, RT4, UM-UC-3 (152)	ELISA, Sorenson's solution, flow cytometry, clonogenic, RT-PCR (150), alamar blue, RT-PCR, flow cytometry, immunocytofluorescence staining, wound healing (151), IC (COX-2), CyQUANT Cell Proliferation assay kit apoptosis (152), western blot (150, 152)	Yes (151, 152)	
Ciglitazone (153)	RT4, T24	Flow cytometry, western blot (caspase-8, 9, p21, p27, p53, cyclin D1, PARP, FABP, cyclin B1, TRAIL, β-actin), enzyme-linked-immunosorbent	Yes (153)	
Combretastatin A-4 (154)	TSGH 8301, BFTC 905	MTT, immunoblot, flow cytometry, clonogenic, Hoechst staining, cell migration.	Yes (154)	
Curcumin (155, 156)	EJ (155, 156), T24, UM-UC-2 (156)	Cell morphology, IC (NF-KB, cyclin D1) (155), clonogenic, fluorescence microscopy, western blot (p53, BCL-2, BAX, survivin protein expression) (156), MTT, flow cytometry (155, 156)	Yes (156)	Curcumin (157)

Table III. *Continued*

Table III. *Continued*

Drug (Reference)	Cell line (Reference)	Methods used to evaluate drug efficacy (Reference)	Application in models of urinary bladder cancer (Reference)	Clinical studies of urinary bladder cancer (Reference)
Curcumin, Ki-67-7 (158)	T24	MTT, qPCR, immunofluorescence, annexin V, flow cytometry, western blot (β-actin, cyclin E, p53, caspase-3, cyclin D1, p21), Micro BCA™ protein assay kit	Yes (158)	
Etodolac (159)	T24, 5637, KK47	Trypan blue, PCR (COX-2, E-cadherin)	Yes (159)	
Furosemide, epirubicin (160)	MGH-u 1R, MGH-u 1	Confocal microscopy, monotetrazolium	No	
Fingolimod (161)	T24, UM-UC-3, HT119	MTT, phase contrast microscopy, DNA fragmentation, western blot	Yes (161)	
Honey (162)	T24, 253J, RT4	MTT, TUNEL, BrdU, flow cytometry	Yes (162)	
Imidazoquinoline (163)	T24	MTT, RT-PCR, western blot, SEAP	Yes (163)	
Meloxicam, sunitinib malate (164)	T24, 5637, HT1376	MTT, Chou and Talalay, MDC, Leishman staining, comet, M30 CytoDEATH antibody	No	
Myricetin (165)	T24	MTT, trypan blue, flow cytometry, DAPI staining, wound healing, western blot, RT-PCR	Yes (165)	
Minodronic acid, CDDP, paclitaxel (166)	253J, 5637, RT4, RT112, TCCSUP, KU-7, UM-UC-3	Western blot, Cell Count Reagent SF, calculation of synergism, Hoechst staining, annexin V-FITC	Yes (166)	
Nitrosulindac (167)	T24, 647V, 1207	MTT, 3HdThd, flow cytometry, Giemsa staining, Hoechst staining, *in situ* end-labelling	No	
NS-398 (168)	T24	MTT, western blot, EIA kit (PGE2), bioluminescence imaging	Yes (168)	
Paeonia lactiflora Pall (169)	TSGH-8301	DAPI staining, flow cytometry, immunoblot	Yes (169)	
Parthenolide (170)	5637	MTT, Hoechst staining, cell invasion, western blot, flow cytometry	No	
Resveratrol (171, 172)	T24 (171), ECV304 (172)	MTT, flow cytometry (171, 172), western blot (171), DNA fragmentation, immunoblot, ELISA, adhesion (172)	Yes (171)	
Shikonin (173)	T24	Trypan blue, phase contrast, flow cytometry, PARP, caspase-3, RT-PCR, western blot (cyclin A, cyclin D, cyclin E, CDK2, CDK4, CDK6, p21)	No	
Silibinin (174)	5637	MTT, annexin V-FITC, caspase activity, western blot, mitochondrial membrane potential, immunofluorescence	Yes (174)	
Scutellariae radix, baicalein, baicalin, wogonin (175)	EJ-1, KU-1	MTT	Yes (175)	
Tachpyr (176)	T24	MTT, clonogenic	Yes (176)	
Triptolide (177)	TSU	^{3}[H]thymidine, clonogenic, DNA fragmentation, western blot	Yes (177)	
Ursolic acid (178)	T24	MTT, trypan blue, western blot, caspase-3, flow cytometry, ELISA, lentiviral transfection, adenoviral-mediated CA-AMPKa1 construct, cellular ceramide levels	No	
Vitamin D (179)	253J, T24	Crystal violet, LeukoStat Stain Kit, TUNEL, annexin V, western blot	Yes (179)	

CDDP: Cisplatin; IC: immunocytochemistry; ELISA: enzyme-linked immunosorbent assay; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; SRB: sulforhodamine B; TUNEL: terminal deoxynucleotidyl transferase dUTP nick-end labeling; annexin V-FITC: fluorescein isothiocyanate-labeled annexin V; siRNA: small interfering RNA; BrdU: 5-bromo-2'-deoxyuridine; 3HdThd: tritiated thymidine incorporation; MDC: monodansylcadaverine; SEAP: secreted alkaline phosphatase; PCR: polymerase chain reaction; RT-PCR: reverse transcriptase-PCR; qPCR: quantitative PCR; COX-2: cyclo-oxigenase-2; BCL-2: B-cell lymphoma-2; BAX: Bcl-2–associated X protein; NF-KB: nuclear factor kappa beta; DAPI: 4',6-diamidino-2-phenylindole; PARP: poly(ADP-ribose)polymerase; BrdU: 5-bromo-2'-deoxyuridine; TRAIL: tumour necrosis factor-related apoptosis inducing ligand; FABP: fatty acid binding protein; p21: cyclin-dependent kinase inhibitor 1; p27: cyclin-dependent kinase inhibitor 1B; PGE2: Prostaglandin E2; CDK-2: cyclin-dependent kinase 2; CDK-4: cyclin-dependent kinase 4; CDK-16: cyclin-dependent kinase 16; p53: tumour protein 53; p75NTR: neurotrophin receptor.

Table IV. *In vitro studies using mouse and rat urinary bladder cancer cell lines to assess the efficacy of antineoplastic and other drugs.*

Drug (Reference)	Cell line (origin)	Methods used to evaluate drug efficacy	Application on human *in vitro/in vivo* models of urinary bladder cancer (Reference)
Allyl isothiocyanate (136)	AY-27 (rat)	MTT, western blot, ELISA, flow cytometry	Yes (136)
Alpha lipoic acid, calcium hydroxycitrate (141)	MBT-2 (mouse)	Trypan blue	Yes (141)
Daporinad (180)	MBT-2 (mouse)	Cell Titer 96 cell proliferation, NAD/NADH quantification	Yes (180)
BCG (181)	MB49 (mouse)	WST-1	Yes (181)
Capsaicin (182)	MBT-2 (mouse)	MTT, DNA fragmentation, flow cytometry, ELISA, western blot, reactive oxygen species, lipid peroxidation	No
CDDP, paclitaxel, gemcitabine, 5-Fluorouracil (183)	MBT-2 (mouse)	MTS	Yes (183)
Curcumin (184)	MB49 (mouse)	Alamar blue	Yes (184)
Curcumin, Ki-67-7 (158)	Y-27 (rat)	siRNA, MTT, qPCR, immunofluorescence, annexin V, flow cytometry, western blot (β-actin, Cyclin E, p53, caspase-3, Cyclin D1, p21), Micro BCA™ protein assay kit	Yes (158)
Epigallocatechin gallate (185)	NBT-II (rat)	MTT, flow cytometry, DNA Ladder Kit, RT-PCR, SDS-page (β-actin, cyclin D1, cdk-4, cdk-6)	No
Hamlet (186)	MB49 (mouse)	Trypan blue, TUNEL, cell morphology	Yes (186)
Fleroxacin, ciprofloxacin (105)	MBT-2 (mouse)	MTT	Yes (105)
Fleroxacin,5-Fluorouracil (106)	MBT-2 (mouse)	MTT	Yes (106)
Honey(162)	MBT-2 (mouse)	MTT	Yes (162)
Imidazoquinoline (163)	MBT-2 (mouse)	MTT, RT-PCR, western blot, SEAP	Yes (163)
MMC, polyphenolic catechins (187)	AY-27 (rat)	Trypan blue, MTT, clonogenic	Yes(187)
N-acetyl-S-(*N*-allylthiocarbamoyl)cysteine (188)	AY-27 (rat)	MTT, flow cytometry, cell death detection ELISA plus kit	Yes (188)
Nordihydroguaiaretic acid, baicalein, AA861, ibuprofen (189)	MBT-2 (mouse)	MTT, acridine orange staining	Yes (189)
Paclitaxel (190)	T50, T5 (mouse)	MiCK	Yes (190)
Resveratrol, rutin, morin, quercetin, gallic acid, tannic acid (191)	MB49 (mouse)	Cell proliferation, expression of urokinase-type plasminogen activator, metalloproteinase-9 expression	No
Sunitinib malate, epirubicin (192)	MBT-2 (mouse)	MTT, microchamber transmembrane migration, flow cytometry, western blot (caspase-3)	Yes
Scutellariae radix, baicalein, baicalin, wogonin (175)	MBT-2 (mouse)	MTT	Yes (175)
Tachpyr (176)	MBT-2 (mouse)	MTT, clonogenic	Yes(176)
Taurolidine (133)	AY-27 (rat)	XTT	Yes(133)
Zinc-Citrate (193)	MBT-2 (mouse)	MTT, Zinc Assay Kit, Bioxytech aconitase-340 assay, DNA ladder, western blot (p53, $p21^{waf1}$, Bcl-2, Bax), ELISA	No

CDDP: Cisplatin; BCG: Bacillus Calmette-Guerin; MMC: mitomycin C; MiCK: micro-culture kinetic; WST-1: water soluble tetrazolium; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; TUNEL: terminal deoxynucleotidyl transferase dUTP nick-end labeling; RT-PCR: reverse transcriptase-polymerase chain reaction; qPCR: quantitative PCR; ELISA: enzyme-linked immunosorbent assay; SEAP: secreted alkaline phosphatase; siRNA: small interfering RNA; MTS: (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium); p53: tumour protein 53; p21: cyclin-dependent kinase inhibitor 1; NAD: nicotinamide adenine dinucleotide; SDS-Page: sodium dodecyl sulfate polyacrylamide gel electrophoresis; Bcl-2: B-cell lymphoma-2; Bax: Bcl-2–associated X protein; XTT: 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-^{2}H-tetrazolium-5-carboxanilide; cdk-4: cyclin-dependent kinase 4; cdk-6: cyclin-dependent kinase 6.

have been available for experimental evaluation of new antineoplastic drugs, providing invaluable pharmacological and toxicological data that may predict for the clinical efficacy of new compounds.

Standard cell culture studies are widely used to delineate the biological, chemical and molecular cues of living cells. Urinary bladder cancer cell lines established from human tumours of different stages and grades allow us to understand

Table V. *In vivo studies to assess the efficacy of antineoplastic drugs.*

Drug (Reference)	Animal model (Reference)	Methods used to induce tumour development and route of treatment to evaluate drug efficacy (Reference)	Application on human *in vitro*/rodent *in vitro* models of urinary bladder cancer (Reference)
BCG (181)	♂C57/BL6 mice	Tumour implantation (MB49), intraperitoneal treatment, Kaplan-Meier method	Yes (181)
BCG(194), BCG, adriamycin (195), BCG, adriamycin, recombinant human tumour necrosis factor (196)	♀Fisher F344 rats	Tumour induction (BBN) (194, 195) (MNU) (196), intravesical instillation, histopathology (194-196), electronic microscopy (195)	No
BCG, MMC (197)	♀Fisher F344 rats	Tumour induction (BBN), intravesical instillations, histopathology, IH (Ki-67, p53), image cytometry	No
BCG, gemcitabine (183)	♀C3H/HeN mice	Tumour implantation (MBT-2), intravesical instillation, histopathology, IH (Ki-67)	Yes (183)
BCG, R8-liposome-BCG-CW (198), BCG, gemcitabine, interferon-α and interleukin-2 (199)	♀Fisher F344 rats	Tumour induction (BBN) (198), tumour implantation (AY-27) (199), intravesical instillation, histopathology (198, 199)	No
BCG, staphylococcal enterotoxin B (200)	♀Fisher 344 rats	Tumour induction (MNU), intravesical instillation, histopathology, TUNEL, Feulgen reaction, IH (VEGF, endostatin, MMP-9, IGFR-1, Ki-67), western blot	No
CDDP(74), CDDP, candesartan (201)	♂Athymic nude BALB/c mice	Tumour implantation (HT1376) (74) (KU-19-19) (201), tumour growth (74), IH (CD34, VEGF), TUNEL (201)	Yes (74)
CDDP, TNP-470 (202)	♂Rat	Tumour induction (BBN), intraperitoneal treatment, histopathology, TUNEL, IH (il-8, Ki-67)	No
CDDP, sunitinib malate (77)	♀Athymic nude mice (Sprague-Dawley)	Tumour implantation (5637), intraperitoneal and oral gavage treatments, tumour growth, IH (VEGFR2, pVEGFR2, caspase-3, CD31, Ki-67)	Yes (77)
Docetaxel, gefitinib (92)	♂Athymic BALB/c nude mice	Tumour implantation (253J B-V), intraperitoneal treatment, tumour volume, tumour kinetics	Yes (92)
Everolimus (99)	♀Nude mice	Tumour implantation (UM-UC-3, UM-UC-6, UM-UC-9), oral gavage treatment, tumour growth, IH (p-p70S6K, CD31, VEGF, p-Akt, p-mTOR)	Yes (99)
Everolimus (100)	♂ICR mice	Tumour induction (BBN), oral gavage treatment, histopathology, IH (mTOR)	Yes (100)
Exisulind (102)	♀Fischer 344 rats	Exisulind diet administration, tumour induction (BBN), histopathology	Yes (102)
Fleroxacin, 5-Fluorouracil (106)	♀C57BL/6 mice	Tumour induction (BBN), oral gavage treatment, histopathology	Yes (106)
Gemcitabine (203, 204), gemcitabine, rapamycin (205)	♀C57/BL6 mice (203), ♀C3H/eb mice(204), ♂ICR mice (205)	Tumour implantation (MB49) (203) (MBT-2)(204), tumour induction (BBN) (205), intravesical instillation (203, 204), histopathology (203-205)	No
Gemcitabine, bortezomib (115), gemcitabine, CDDP, 1,25D3 (117)	♂Athymic nude mice (115), nude mice (117)	Tumour implantation (253JB-V) (115) (T24) (117), intraperitoneal treatment, IH (VEGF, IL-8, CD31), TUNEL (115), excision clonogenic, tumour regrowth delay (117)	Yes (115, 117)
MMC, polyphenolic catechins (187)	♀Fischer 344 rats	Tumour implantation (AY-27), intravesical treatment, histopathology	Yes (187)
MVAC (206)	♂Wistar rats	Tumour induction (BBN), intraperitoneal treatments, histopathology, TUNEL, IH (p53), electronic microscopy	No

Table V. *Continued*

Table V. *Continued*

Drug (Reference)	Animal model (Reference)	Methods used to induce tumour development and route of treatment to evaluate drug efficacy (Reference)	Application on human *in vitro*/rodent *in vitro* models of urinary bladder cancer (Reference)
Paclitaxel (190), candesartan, paclitaxel (71)	♀C3H/eb mice (190), nude athymic Balb/c mice (71)	Tumour implantation (T5, T50) (190) (KU-19-19) (71), intravesical treatment (190), oral gavage and intravenously treatment (71), histopathology(190), tumour volume, IH (CD34, VEGF, AT1R), apoptosis (71)	Yes (71, 190)
Rapamycin (207)	♂ICR mice	Tumour induction (BBN), intraperitoneal treatment, histopathology, IH (Ki-67)	No
Taurolidine (133)	♀Fischer rat (F344)	Tumour implantation (AY-27), intravesical and intravenously treatment, macroscopic analysis	Yes (133)

CDDP: Cisplatin; BCG: Bacillus Calmette-Guerin; MMC: mitomycin C; IH: immunohistochemistry; TUNEL: terminal deoxynucleotidyl transferase dUTP nick-end labeling; BBN: *N*-Butyl-*N*-(-4-hydroxybutyl) nitrosamine; MNU: *N*-Methyl-*N*-nitrosourea; 1,25D_3: 1,25 dihydroxyvitamin D_3; VEGF: vascular endothelial growth factor; AT1R: angiotensin II type 1 receptor; Il-8: interleukin-8; mTOR: mammalian target of rapamycin; p-mTOR: phosphorylated-mTOR; p-AKT: phosphorylated protein kinase B; MVAC: methotrexate, vinblastine, adriamycin and cisplatin; p53: tumour protein 53; VEGFR2: vascular endothelial growth factor receptor-2; pVEGFR2: phosphorylated vascular endothelial growth factor receptor-2; CD31: platelet endothelial cell adhesion molecule; CD34: hematopoietic progenitor cell antigen; MMP-9: metalloproteinase-9; IGFR-1: insulin-like growth factor receptor 1; ♂: male; ♀: female.

the heterogeneous response observed for the same drug between different patients. However, three-dimensional models that recapitulate the tumour microenvironment remain essential. *In vivo* models overcome this *in vitro* drawback. The development and optimization of reliable, sensitive and reproducible methodologies are indispensable tools for assessing the *in vitro* and *in vivo* efficacy of novel antineoplastic drugs.

From this review, we conclude that to date, more than 50 drugs have been tested on urinary bladder cancer, with BCG, cisplatin, gemcitabine and MMC being the most common, in isolation or applied simultaneously with other drugs, in *in vitro* or *in vivo* models. Nowadays, combination therapy is particularly important since the actions of target-based drugs may be supplemented or potentiated by other drugs. This therapeutic approach is increasingly used in experimental models.

Novel targeted-therapies are needed to further improve the chemotherapy efficacy of urinary bladder cancer treatment. Some of the drugs evaluated and described in this article have been shown to be promising in the treatment of this disease. Currently, drugs targeting angiogenesis are promising. Therapeutic investigations should be continued, with the development of new drugs, as well as of targeted therapies to improve treatment results for bladder cancer.

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Table VI. *In vivo studies to assess the efficacy of other drugs.*

Drug (Reference)	Animal model (Reference)	Methods used to induce tumour development and route of treatment to evaluate drug efficacy (Reference)	Application on human in vitro/rodent in vitro models of urinary bladder cancer (Reference)
Aqueous extract of *Magnolia officinalis* (135)	♂C57B1/6 mice	Tumour induction (OH-BBN), treatment in drinking water, histopathology	Yes (135)
Allyl isothiocyanate (136)	♀Fisher 344 rats	Tumour implantation (AY-27), treatment by oral gavage, histopathology, western blot	Yes (136)
Daporinad (180)	♀C3H/HeN mice, ICR mice, NOD.CB17-PRKDC	Tumour implantation (MBT-2), intraperitoneal treatment, histopathology, IH (CD4, CD31)	Yes (180)
Alpha lipoic acid, calcium hydroxycitrate (141)	C3H, C57BL/6 mice	Tumour implantation (MBT-2), tumour growth	Yes (141)
Aspirin (208, 209), aspirin, ketoprofen, sulindac (210)	♂F344 rats, ♂New Zealand rabbits (208), ♂ Wistar rats (209), ♂C57BI/6XDBA/ 2-F1 mice (210)	Tumour induction (FANFT) (208) (BBN) (209) (OH-BBN) (210), treatment in diet (208-210), electronic microscopy, radioimmunoassay (PGE2) (208), histopathology (210)	No
Celecoxib (211-213)	♂B6D2F1 mice, ♀Fischer-344 rats(211), ♂Wistar rats (212, 213)	Tumour induction (OH-BBN) (211) (BBN) (212, 213), treatment by oral gavage (212, 213), treatment in diet (211), histopathology, IH (COX-2) (211-213) Quantikine® ELISA kits, ELISA, TBARs, FRAP (212, 213), IH (COX-2) (213).	No
Celecoxib, NS-398, etodolac (151), celecoxib, NS-398, piroxicam (152)	Athymic BALB/c (*nu/nu*) mice (151), ♂Athymic mice (152)	Tumour implantation (T24) (151), (HT1376, UM-UC-3, RT4) (152), intraperitoneal treatment (151), treatment by oral gavage (152), TUNEL (151), Apoptag Peroxidase *in situ* Apoptosis Detection kit (152)	Yes (151, 152)
Ciglitazone (153)	♀Nu/nu nude mice	Tumour implantation (RT4, T24), intraperitoneal treatment, tumour volume, IH (Ki-67, caspase-3)	Yes (153)
Cimetidine (214)	♂BALB/c mice, ♂Wister rats	Tumour induction (BBN), treatment in drinking water, histopathology, IH (VEGF, E-selectin, PDECGF)	No
Combretastatin A-4 (154)	♀C57BL/6 mice	Tumour implantation (MB49), intravesical treatment, tumour volume, histopathology, biochemical parameters	Yes (154)
Curcumin (156, 184, 215)	♀Wistar rats (156), ♀C57BL/6 mice (184), ♀C57BL/6 mice (215)	Tumour implantation (MB49) (184, 215), tumour induction (MNU) (156), intravesical treatment(184), IH (Bcl-2) (184) (Cyclin D1, Cox-2) (184, 215) (PCNA) (215), histopathology (156, 184, 215), Hoechst staining (156)	Yes (156, 184)
Etodolac (159)	BALB/c *nu/nu* mice	Tumour implantation (T24), intraperitoneal treatment, PCR, IH (E-cadherin, single-strand DNA)	Yes (159)
Fingolimod (161)	♂BALB/c *nu/nu* mice	Tumour implantation (T24, UM-UC-3), intraperitoneal treatment, histopathology, TUNEL, electronic microscope	Yes (161)
Gefitinib, meloxicam (216), gefitinib, naproxen, aspirin, resveratrol (217)	♂Fisher-344 rats (216), ♀Fischer-344 rats (217)	Tumour induction (BBN) (216) (OH-BBN) (217), treatment by oral gavage (216, 217), treatment in diet (217), histopathology (216, 217), serum creatinine levels (216)	No
Green tea (218)	♂♀ICR mice	Tumour induction (BBN), treatment in drinking water, histopathology	No
Hamlet (186)	♀C57BL/6 mice	Tumour implantation (MB49), histopathology, fluorescence imaging, TUNEL	Yes (186)

Table VI. *Continued*

Table VI. *Continued*

Drug (Reference)	Animal model (Reference)	Methods used to induce tumour development and route of treatment to evaluate drug efficacy (Reference)	Application on human *in vitro*/rodent *in vitro* models of urinary bladder cancer (Reference)
Honey (162)	♀C3H/He mice	Tumour induction (FANFT), tumour implantation (MBT-2), intralesional and in drinking water treatments, tumour volume	Yes (162)
Imidazoquinoline (163)	♀C3H/HeJ mice	Tumour implantation (MBT-2), treatment by instillation, histopathology, IH (cyclin D2, PCNA)	Yes (163)
Indomethacin (219, 220)	♂B6D2F1 mice (219), ♀Sprague-Dawley rats (220)	Tumour induction (OH-BBN) (219) (FANFT) (220), treatment in diet, histopathology (219, 220), IH (FHIT, survivin) (219)	No
Minodronic acid, CDDP, paclitaxel (166)	♀SCID mice, BALB/c *nu*/*nu* mice	Tumour implantation (UM-UC-3LUC), tumour growth, immunoblot, histopathology, IH	Yes (166)
Myricetin (165)	♂BALB/c-nude mice	Tumour implantation (T24), intraperitoneal treatment, tumour volume	Yes (165)
N-Acetyl-S-(*N*-allylthiocarbamoyl) cysteine (188)	♀F344 rats	Tumour implantation (AY-27), treatment by oral gavage, histopathology, western blot	Yes (188)
Nordihydroguaiaretic acid, baicalein, AA861, ibuprofen (189)	♂C3H/HeN mice	Tumour implantation (MBT-2), subcutaneous treatment, tumour volume	Yes (189)
Nimesulide (221)	♂F344 rats	Tumour induction (BBN), treatment in diet, histopathology	No
NS-398 (168)	♂C.B-17/IcrCrl-scid mice	Tumour implantation (T24), intraperitoneal treatment, bioluminescent imaging, IH (Ki-67, vWF, CD31, caspase-3)	Yes (168)
Nordihydroguaiaretic acid (222)	♂Fisher 344 rats	Tumour induction (BBN), treatment in diet, histopathology	No
Paeonia lactiflora Pall (169)	♂Sprague-Dawley rats	Tumour induction (BBN), treatment in diet, IH (PCNA, phospho-Chk2)	Yes (169)
Piroxicam, lycopene, beta-carotene (223)	♂Fisher 344 rats	Tumour induction (BBN), treatment in diet (piroxicam) and drinking water (lycopene and beta-carotene), histopathology, IH (PCNA)	No
Resveratrol (171)	♂BALB/c-nude mice	Tumour implantation (T24), intraperitoneal treatment, RT-PCR	Yes (171)
Rofecoxib (224)	♂♀B6/129 F2 mice	Tumour induction (BBN), treatment in diet, histopathology	No
Silibinin (174)	♀Sprague-Dawley rats	Tumour induction (MNU), intravesical treatment, histopathology, TUNEL, IH	Yes (174)
Silibinin (225)	♂Athymic *nu*/*nu* mice	Tumour implantation (RT4), treatment by oral gavage, IH (PCNA, CD31, caspase-3, survivin), TUNEL, western blot (survivin, p53, caspase-3), TiterZyme enzyme immunometric kit, siRNA.	No
Silibinin, silymarin (226)	♂ICR mice	Tumour induction (OH-BBN), treatment by oral gavage, IH (PCNA), TUNEL, immunoblot (PCNA, cyclin D1, ERK-1/2, total ERK1/2, caspase-3, cleaved poly(ADP-ribose) polymerase, survivin, NF-κB p65 (Ser^{276}), phospho–NF-κB p65 (Ser^{536}), total NF-κB p65)	No
Scutellariae radix, baicalein, baicalin, wogonin (175)	♂C3H/HeN mice	Tumour implantation (MBT-2), treatment by oral gavage, tumour volume	Yes (175)

Table VI. *Continued*

Table VI. *Continued*

Drug (Reference)	Animal model	Methods used to induce tumour development and route of treatment to evaluate drug efficacy	Application on human *in vitro*/rodent *in vitro* models of urinary bladder cancer (Author)
Triptolide (177)	BABL/c nude/nude mice	Tumour implantation (TSU), intraperitoneal treatment, tumour weight	Yes (177)
Vitamin D (179)	♀Fischer 344 rats	Tumour induction (MNU), treatment by instillation, histopathology	Yes (179)
Nordihydroguaiaretic acid, baicalein, AA861, ibuprofen (189)	♂C3H/HeN mice	Tumour implantation (MBT-2), subcutaneous treatment, tumour volume	Yes (189)
Nimesulide (221)	♂F344 rats	Tumour induction (BBN), treatment in diet, histopathology	No
NS-398 (168)	♂C.B-17/IcrCrl-scid mice	Tumour implantation (T24), intraperitoneal treatment, bioluminescent imaging, IH (Ki-67, vWF, CD31, caspase-3)	Yes (168)
Nordihydroguaiaretic acid (222)	♂Fisher 344 rats	Tumour induction (BBN), treatment in diet, histopathology	No
Piroxicam, lycopene, beta-carotene (223)	♂Fisher 344 rats	Tumour induction (BBN), treatment in diet (piroxicam) and drinking water (lycopene and beta-carotene), histopathology, IH (PCNA)	No
Resveratrol (171)	♂BALB/c-nude mice	Tumour implantation (T24), intraperitoneal treatment, RT-PCR	Yes (171)
Rofecoxib (224)	♂♀B6/129 F2 mice	Tumour induction (BBN), treatment in diet, histopathology	No

CDDP: Cisplatin; IH: immunohistochemistry; ELISA: enzyme-linked immunosorbent assay; FRAP: ferric reducing antioxidant potential; PCR: polymerase chain reaction; RT-PCR: reverse transcriptase-PCR; BBN: *N*-Butyl-*N*-(-4-hydroxybutyl) nitrosamine; MNU: *N*-Methyl-*N*-nitrosourea; FANFT: *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide; PCNA: proliferating cell nuclear antigen; Bcl-2: B-cell lymphoma-2; Cox-2: cyclo-oxygenase-2; TUNEL: terminal deoxynucleotidyl transferase dUTP nick-end labeling; siRNA: small-interfering RNA; CD4: cluster of differentiation 4; PGE2: Prostaglandin E2; TBARs: thiobarbituric acid reactive substances; OH-BBN: N-Butyl-N-(-4-hydroxybutyl) nitrosamine; FHIT: fragile histidine triad; PDECGF: platelet-derived endothelial cell growth factor; p53: tumour protein 53; VEGF: vascular endothelial growth factor; vWF: von Willebrand factor; phospho-Chk2: phospho-checkpoint kinase 2; CD31: platelet endothelial cell adhesion molecule; ♂: male; ♀: female.

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