Expert Opinion

Strategies to improve drug delivery in bladder cancer therapy

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Bladder cancer is the ninth most common malignancy in the world featuring very high gender variability in occurrence. Current options for bladder cancer therapy include surgery, immunotherapy, chemotherapy and radiotherapy with a trend towards multimodal treatments. However, successful management remains a challenge for urologists and oncologists because of the high risk for recurrence and progression. Particularly in the field of bladder cancer chemotherapy, efficacy of treatment might be improved by advanced drug delivery strategies aimed at prolonged residence time within the bladder cavity and increased permeability of the bladder wall during intravesical instillation. Moreover, a deeper understanding of the biology of bladder carcinogenesis and malignant progression stimulated the development of a new generation of anticancer drugs for targeted therapies that might result in increased treatment specificity together with lower toxic potential and higher therapeutic indices. This review discusses the available strategies for 'targeted therapy', focusing on molecular targets, and for 'controlled delivery', comprising all other approaches towards improved drug delivery.

Keywords: bladder cancer, chemotherapy, controlled delivery, drug targeting, intravesical delivery, targeted therapy, urothelial cell carcinoma

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1. Introduction

Recent literature ranks bladder cancer as the ninth most common malignancy worldwide together with a very high gender variability in occurrence [1]. Interestingly, the incidence of bladder cancer varies significantly among countries, with highest rates in the western world. Particularly high incidence rates were observed for Southern Europe, Northern Africa, North America and Western Europe, whereas occurrence in Asian countries is rather low [2]. In the US, bladder cancer is the fourth most common cancer diagnosed in males, with an estimated 51,230 new cases and 9950 deaths from bladder cancer in 2008 [3]. The two best established risk factors for bladder tumour include cigarette smoking and occupational exposures to urothelial carcinogens. In addition, other factors such as chronic urinary tract infections, cyclophosphamide use, schistosomiasis, and exposure to radiotherapy as well as inadequate consumption of fruits, vegetables and certain vitamins may be associated with higher risk of bladder cancer. Even coffee consumption and artificial sweeteners are discussed to have an impact on tumorigenesis, whereas hereditarily caused bladder cancer is fairly rare compared with other tumour sites [4].

In general, bladder cancer is a rather heterogeneous disease, making classification, staging and grading a challenging task. On average, 70% of bladder urothelial cell carcinomas represent a superficial disease termed non muscle-invasive bladder cancer (NMIBC), while the remaining 30% develop a muscle-invasive disease (muscle-invasive bladder cancer [MIBC]) bearing the risk of metastatic spread of the tumour [5]. Standard treatment of NMIBC is complete transurethral

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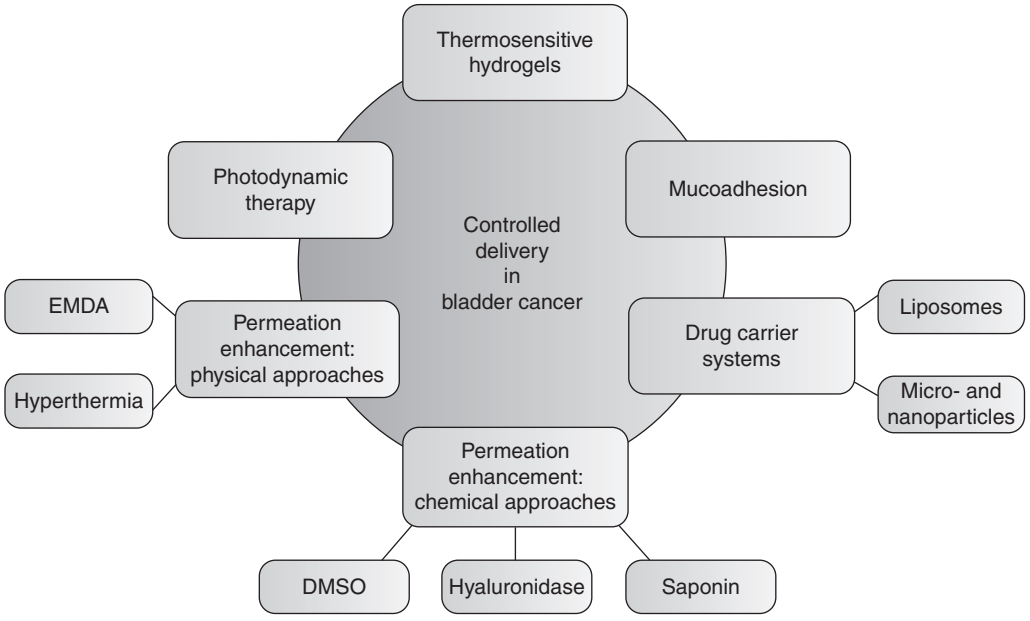

Figure 1. Controlled delivery strategies in the treatment of bladder cancer.
DMSO: Dimethyl sulfoxide; EMDA: Electromotive drug administration.

resection and subsequent cystoscopic surveillance. However, the major challenge in the management of NMIBC is not the removal of a lesion once present, but rather the prevention of tumour recurrence and progression. Thus, intravesical instillation of chemotherapeutic or immunotherapeutic agents is applied to reduce the risk for recurrence and to delay or even prevent progression to a muscle-invasive disease. Independent of the modality of treatment, two-thirds of all patients diagnosed with NMIBC suffer from a recurrence, of which 10 – 20% demonstrate progression to a muscle-invasive disease [6]. A tumour that has grown beyond the lamina propria and invades the muscularis propria is usually treated with radical cystectomy. Radical surgery in the case of MIBC therapy is usually accompanied by perioperative systemic chemotherapy to minimise metastatic dissemination and improve survival of patients. For patients with disseminated disease, aggressive systemic therapy with multiple chemotherapeutics is essential [7].

Particularly in the field of cancer chemotherapy, the urgent need for improved drug delivery by directing the active pharmaceutical ingredient (API) to the diseased tissue has stimulated the research efforts of pharmaceutical technologists for more than a decade. That way, because of their severe side effects, the dose-limited effectiveness of conventional anticancer drugs might be overcome [8]. Improving drug delivery by guiding the drug to the intended site of action is also a relevant issue in anticancer therapy of the bladder. Owing to its unique anatomical and physiological properties, the urinary bladder is ideally suited for minimally invasive intravesical therapy, an option that is widely used in the treatment of urothelial cancer at the superficial stage. However, despite the fairly easy access by instillation, intravesical delivery of effective concentrations remains a challenge. The primary goal in intravesical drug administration is to maximise the exposure of the tumour to the therapeutic agent while limiting toxicity to the host. Owing to the barrier function of the urothelium, absorption of the API into the systemic circulation is limited, avoiding overall toxicity. However, the low residence time of a drug in the bladder, which rarely lasts beyond the first void, requires advanced and intelligent approaches towards sustained delivery in order to achieve efficacious drug concentrations within the bladder cavity. Representing one of the toughest barriers in the human organism [9], transport across the urothelium occurs only by means of passive diffusion. Thus, improving the permeability of the bladder epithelium as well as increasing the concentration gradient between urine and the target site is expected to enhance drug penetration into the diseased tissue. Besides some more general physiological approaches, interesting methods in bladder cancer therapy comprise chemical agents that alter the urothelial permeability as well as device-assisted therapies, such as electromotive drug administration or thermochemotherapy. A prolonged residence time of the API in the bladder is achieved via several sustained drug delivery concepts, including thermosensitive hydrogel formation *in situ*, mucoadhesion, magnetically targeted carriers and drug carriers from the nanotech pipeline, such as liposomes, microparticles, or nanoparticles. Moreover, photodynamic therapy is used in bladder cancer treatment (**Figure 1**).

Recent knowledge of the molecular mechanisms underlying bladder carcinogenesis as well as tumour progression and response has led to a further approach in bladder cancer

therapy that is fundamentally different from the others mentioned and relies on molecular targeting of tumour-relevant signalling pathways, cell cycle regulators, and specific receptors that are overexpressed at the tumour cell surface. Promising molecular targets in bladder cancer therapy include the epithelial growth factor receptor family, the Ras and phosphatidylinositol-3-kinase signalling pathway, cyclin-dependent kinases, and several others. The expected benefits of molecularly targeted therapies rely on a lower overall toxicity due to an increase in selectivity of the therapeutic agent. Moreover, the greater specificity might also show promise for successful therapy of advanced or metastatic disease. Further concepts in targeted bladder cancer treatment are based on gene therapy. Among others, potential targets in this area are the tumour suppressor gene *p53*, telomerase reverse transcriptase and the apoptosis modulator bcl-2. An interesting focus of recent research deals with the prevention of metastatic spreading by the inhibition of neo-angiogenesis, particularly in the management of advanced bladder cancer. In addition, glycotargeting by means of lectins might prove to be a promising alternative approach to selectively target malignant cells of the bladder (**Table 1**).

Below, the term 'targeted' is used primarily for molecular targeting, whereas 'controlled' is used for all other methods of improved drug delivery.

2. Controlled delivery

Bladder tumours at a superficial stage are at present treated by transurethral resection followed by adjuvant instillation of chemotherapeutics or immunotherapeutics to lower the risk of recurrence and progression to a muscle-invasive form of disease. For chemotherapy, usually mitomycin C (MMC) and epirubicin are applied either as a single immediate instillation or as multiple delayed instillations depending on the patient's risk evaluation. Intravesical immunotherapy is predominantly done with bacillus Calmette-Guérin (BCG), an attenuated mycobacterium known as a potent inducer of passive immunity, which is the best choice for high-risk patients even though local and systemic side effects are more frequent and more severe than with intravesical chemotherapy [10]. Gold-standard therapy in the case of MIBC relies on radical cystectomy, usually in conjunction with perioperative systemic chemotherapy using a combination of methotrexate, vinblastine, doxorubicin and cisplatin (M-VAC), which proved to be substantially more effective than any single drug regimen [11,12]. M-VAC therapy is also a typical regimen for the treatment of metastatic carcinoma of the urothelium [13]. Moreover, a multi-drug combination chemotherapy using cisplatin, methotrexate and vinblastine (CMV) is applied in the case of metastatic bladder cancer [14].

Besides approaches relying on new chemotherapeutic agents and regimens [15], improvements in drug delivery have been the focus of recent research in order to enhance the efficacy of bladder cancer therapy. Among the variety of treatment options in this field, which are discussed in detail below (**Figure 1**), there are also some general aspects in terms of the physiological status and function of the kidney and the bladder that should be considered. These parameters include urine-related factors such as the residual volume in the bladder and the continuous urine production rate during intravesical treatment, both resulting in dilution of the drug administered within the instillation fluid. A useful strategy to circumvent these problems is based on complete emptying of the bladder before dose administration as well as limited fluid intake by the patient before and after instillation. In addition, the dosing volume and the dwell time are influencing parameters, but their impact is lower [16]. Moreover, pH might be an issue depending on the chemotherapeutic agent in use. For MMC, rapid degradation in an acidic environment could be avoided by alkalinising the urine by oral administration of sodium bicarbonate. Considering the abovementioned parameters, a decrease of instillation volume together with an increased dosage of cytostatic agent led to a statistically significantly enhanced efficacy of MMC treatment in a randomised Phase III trial [17]. Another crucial point in intravesical chemotherapy is the vehicle used for drug administration. According to Groos and Masters [18], sterile water might be a better diluent than saline solution owing to the fact that osmolality was inversely correlated to the toxicity of chemotherapeutical agents in cell cultures. However, the residence time of the API at the site of action is another decisive parameter that often limits efficient cancer treatment. A simple and reasonable way towards increased drug delivery to the bladder epithelium is prolonged instillation, but more advanced approaches have already been pursued, as follows.

2.1 Thermosensitive hydrogels

To extend the residence time of the API in the bladder beyond the first voiding of urine after instillation, the application of a thermosensitive hydrogel, which forms a drug depot inside the bladder cavity, was proposed by Tyagi *et al.* [19]. Developed by Jeong *et al.* [20], the aqueous solution of the triblock co-polmyer PEG-PLGA-PEG (polyethylene glycol-poly(lactic acid *co*-glycolic acid)-polyethylene glycol) is fluid at room temperature and converts to a gel at body temperature. Using rat bladders and a modified thermogel, Tyagi *et al.* [19] reported a sustained release for up to 24 h provided by the thermosensitive hydrogel, which attaches itself as a smooth layer to the inner surface of the bladder and acts as a drug-loaded matrix. Although representing a promising approach, data for human application are not available yet.

2.2 Mucoadhesion

Another approach to enhancing drug transport is the concept of bioadhesion to the mucosal urothelium: natural or synthetic macromolecules adhere to mucosal surfaces in the body and, when incorporated into pharmaceutical formulations,

Table 1. Molecular targets in bladder cancer therapy.

Target	Ref.
Epidermal growth factor receptor (EGFR) family	[67-95]
Epidermal growth factor receptor (HER-1)	[69-85]
HER2/neu	[86-94]
Dual EGFR/HER2	[95]
Ras signalling pathway	[78,96-99]
Phosphatidylinositol-3-kinase-signalling pathway	[78,100]
Cell cycle regulators	[101-103]
Proteasome	[78,104,105]
DNA hypermethylation	[106-108]
Telomerase	[109,110]
B-cell lymphoma 2 (bcl-2)	[78,111-113]
Clusterin	[114,115]
Survivin	[116,117]
Tumour suppressor genes	[118-125]
p53 gene-based therapy	[119-123]
pRb gene-based therapy	[124,125]
Histone deacetylase	[78,126-129]
Angiogenesis (VEGF, VEGFR; PDGFR; bFGF, FGFR3)	[78,130-157]
Glycocalyx	[158-161]

enhance the absorption of drugs owing to prolonged exposure, shortening of the diffusional pathway and steepening of the concentration gradient from the lumen to the cell. For chitosan and polycarbophil, permeation enhancement across the bladder wall has been assessed in previous studies on porcine bladders [21,22]. For specific postoperative chemotherapy, MMC loaded chitosan and alginate carriers were prepared by Oztürk *et al.* [23], which proved to be successful in mice. Lee *et al.* [24] investigated the effect of formulations with paclitaxel and glyceryl monooleate in a rabbit model of bladder cancer. They observed an increased bioadhesiveness to bladder mucosa, which depicts a step forward towards improved targeting of the urothelial tissue. Current studies that are in progress focus on increased efficiency of drug absorption by API incorporation into liposomes, microparticles and nanoparticles with bioadhesive characteristics, as discussed in detail below.

2.3 Drug carrier systems

2.3.1 Liposomes

Liposomes are artificial vesicles of spherical shape containing an aqueous core surrounded by one or more amphiphilic phospholipid layers. Thus, liposomes can be used as carrier systems for either hydrophilic or lipophilic compounds and can alter pharmacologic and pharmacokinetic parameters of the transported drug. In a pioneering study, Johnson *et al.* [25] assessed whether liposomes can bind to human bladder cancer cells. Binding to tumour cells was saturable and appeared to be specific, as binding of liposomes to normal fetal bladder cells was negligible in comparison. Thus, they might be a suitable vehicle to improve the delivery of anticancer drugs. Vail *et al.* [26] encapsulated doxorubicin in polyethylene glycol-coated liposomes and showed in animal models that increased potency over conventional doxorubicin as well as remission and cure of many cancers was achievable, including bladder tumours. Antiproliferative effects of liposome-encapsulated titanium dioxide in bladder cancer treatment were examined by Chihara *et al.* [27] in nude mice and revealed higher antitumour effects than non-coated titanium oxide. A recent study was performed by Homhuan *et al.* [28], who incorporated the BCG cell wall into cationic liposomes and demonstrated their uptake into murine bladder tumour cells.

2.3.2 Microparticles and nanoparticles

Polymeric microparticles and nanoparticles have become widely applied as effective drug delivery systems. To prolong the residence time of paclitaxel in the bladder and to achieve controlled release, bioadhesive poly(methylidene malonate-2.1.2) microspheres were prepared by Le Visage *et al.* [29], which were applied to Balb/c mice after *N*-butyl-*N* (4-hydroxybutyl)nitrosamine (BBN) induction of bladder cancer and compared with free paclitaxel. After bladder instillation, microspheres adhered to the mucosa, remained in the bladder for up to 48 h and the 9-week survival rate was significantly improved. Lu *et al.* [30] developed paclitaxel-loaded gelatin nanoparticles that rapidly released paclitaxel, yielded significant activity against human bladder cancer cells and showed higher tissue concentration as compared with commercial Cremophor®/EtOH formulations (polyethoxylated castor oil, BASF, Ludwigshafen, Germany). Paclitaxel was also loaded in PLGA nanospheres to increase both efficiency and safety of the drug as well as to improve targeting [31]. Tested on thyroid, breast and bladder cancer cell lines, these nanospheres showed a prolonged drug release and an increase of the cytotoxic effect with respect to free paclitaxel in all cell lines. Recently, paclitaxel was incorporated in nanoparticles made from hydrophobically derivatised hyperbranched polyglycerols. Their evaluation in nude mice with orthotopic KU7-luc tumours [32] revealed that HPG-C10-polyethylenglycol of paclitaxel was significantly more effective in the reduction of tumour growth than paclitaxel formulated in Cremophor. Another recently published study concentrated on the delivery of MMC encapsulated in cationic nanoparticles of chitosan and polycaprolactone [33]. Nanoparticles of poly-ε-caprolactone coated with chitosan turned out to be an efficient formulation for a complete drug release and specific uptake by MB49 bladder carcinoma cells in contrast to normal bladder cells. Recently, carbon nanotubes (CNTs) turned out to be a feasible carrier for carboplatin [34] as cell growth of human bladder cancer cells was inhibited in comparison with unfilled CNTs.

Anchoring of antibodies, lectins, and so on, on the surface of liposomes or microspheres and nanospheres can improve further the site specificity of drug carriers and is discussed in detail in Section 3.

2.4 Permeation enhancement: chemical approaches

Dimethyl sulfoxide (DMSO) is a chemical compound with the distinct capability to penetrate tissues. Co-administration of DMSO proved to enhance the tissue penetration of cisplatin in the urinary bladder of dogs [35], and improved the absorption of doxorubicin [36], pirarubicin [37] and epirubicin [38] in rat models. DMSO also reversed the entrapment of paclitaxel in Cremophor micelles, which is a commercially available formulation (i.e., Taxol®). Summarising all the effects, co-administration with DMSO resulted in increased delivery of paclitaxel to the bladder tissue of dogs [39].

Sasaki *et al.* [40] evaluated the effects of a certain saponin on absorption of 4′-*O*-tetrahydropyranyldoxorubicin (THP) through the bladder mucosa of rats. As the results revealed increased concentrations of THP in bladder tissue without affecting that in plasma, co-administration could be useful for intravesical chemotherapy.

Hyaluronidase, an enzyme that degrades hyaluronic acid as a major constituent of the extracellular matrix, has been administered to improve drug diffusion of cisplatin in 33 patients with superficial transitional bladder cell carcinoma [41]. Higher clinical efficacy over cisplatin alone was not detected, but a further study revealed a decrease in tumour recurrence after co-administration of MMC with hyaluronidase [42] in a randomised trial with 2 groups of 28 patients with highest significance ($p < 0.05$). This finding was verified in comparative studies with 43 patients treated with MMC and hyaluronidase (recurrence rate 7%) and 63 patients with MMC alone (recurrence rate 32%) [43]. Nevertheless, the feasibility of this concept has to be evaluated carefully because hyaluronidase can act either as a tumour suppressor or as a promoter depending on cell type and concentration [44,45].

2.5 Permeation enhancement: physical approaches – device-assisted therapy

2.5.1 Electromotive drug administration

The technique of electromotive drug administration (EMDA) is known to increase penetration of drugs through the urothelial barrier of the bladder after intravesical instillation by the use of an electric source. Based on the active principles of iontophoresis, electroosmosis and electroporation, the transport of water-soluble drugs is enhanced and is most effective for ionised molecules where the rate of drug transport is proportional to the intensity of the applied current [46,47].

In the treatment of bladder cancer, a recent study was conducted by Colombo *et al.* [48] with patients suffering from single, recurrent, low-stage, low-grade superficial bladder tumours. Thirty-six patients were treated with the standard procedure, administering MMC as intravesical instillation, for 15 patients MMC was applied according to the electromotive procedure, and in 29 patients MMC was administered in combination with local microwave-induced hyperthermia. Complete response was identified in 27.7, 40 and 66% of patients, respectively. For high-risk NMIBC Di Stasi *et al.* [49] assessed the efficacy of intravesical electromotive versus passive MMC using BCG as a comparative treatment in 108 patients. After 6 months' follow-up, the complete response for electromotive versus passive MMC was 56% versus 31%, as compared with BCG with 64%. Local and systemic side effects were significantly more frequent in the BCG arm, leading to the suggestion that electromotive MMC may be an alternative therapy. In a randomised trial with 212 patients with stage pT1 bladder cancer, the same authors compared the efficacy of sequential BCG and electromotive MMC with that of BCG alone [50]. After a median follow-up of 88 months, more patients assigned sequential BCG and electromotive MMC were disease-free (57.9% versus 41.9%), higher disease-free intervals were achieved (69 months versus 21 months), progression rate was lower (21.9% versus 9.3%) and the overall (34% versus 21.5%) as well as disease-specific mortality (16.2% versus 5.6%) was lower as compared with BCG alone.

2.5.2 Hyperthermia

Recently, intravesical hyperthermia was applied to improve the efficacy of chemotherapeutics, termed thermochemotherapy. Colombo *et al.* [51] developed the Synergo® system (Medical Enterprises Ltd, Amsterdam, The Netherlands), which consists of a 915 MHz intravesical microwave applicator, inserted through a catheter, which provokes hyperthermia of the bladder wall by means of direct irradiation. The bladder wall temperature is monitored by thermocouples located inside the catheter (goal temperature 42 – 43°C) and a small peristaltic pump circulates the chemotherapeutic solution between the bladder and a drug reservoir. The efficacy of hyperthermia in combination with MMC versus MMC alone was evaluated in several clinical studies [52-54] and was revealed to be more effective than intravesical chemotherapy alone for the treatment of superficial transitional cell carcinoma. A comprehensive study with 90 patients suffering from intermediate or high-risk superficial transitional cell carcinoma was performed by Van der Heijden *et al.* [55]. After 2 years of follow-up the risk of recurrence was 24.6% and no progression in stage and grade was observed. A study from Witjes *et al.* [56] evaluated the potential of thermochemotherapy in 57 patients with mostly BCG refractory carcinoma *in situ*. A complete response rate of 94% was achieved with a recurrence rate of 30% at 1-year follow-up.

2.6 Photodynamic therapy

Photodynamic therapy (PDT) is a potentially selective approach for treating bladder cancer based on the interaction between a photosensitising agent in the target tissue and an intravesical light source to cause tissue necrosis. Using 5-aminolevulinic acid (5-ALA) as a precursor of the potent

photosensitiser protoporphyrin IX, a first clinical trial for treatment of NMIBC was conducted by Kriegmair *et al.* [57]. After 10 – 12 weeks, 4 out of 10 patients had a complete remission and 2 had a partial remission, whereas no change was detected for 3 patients and 1 even suffered from progressive disease. Also, no serious side effects were observed. The same authors evaluated the efficacy of photodynamic therapy after oral administration of 5-ALA in 24 patients with superficial bladder cancer [58]. At a median follow-up of 36 months, 3 of 5 patients with carcinoma *in situ* and 4 of 19 patients with papillary tumours were free of recurrence. Three patients were rendered disease-free by repeated therapy and three underwent cystectomy. However, haemodynamic side effects such as hypotension and tachycardia, especially in patients with cardiovascular co-morbidity, should be taken into account. Thus, efficacy and side effects of PDT with intravesical application of 5-ALA were evaluated again by Berger *et al.* [59] with 31 patients suffering from recurrent NMIBC. Sixteen patients were recurrence-free after an average follow-up of 23.7 months and therapy was well tolerated. The only side effects observed were dysuria and haematuria in four and seven patients, respectively. Evidence for increased susceptibility of bladder cancer cells towards chemotherapeutic agents after sequential administration of MMC and PDT was given by French *et al.* [60] in a preclinical study. In a continuing Phase I study with 24 patients with recurrent NMIBC, Skyrme *et al.* [61] assessed the potential and tolerability of ALA-PDT in association with MMC and found that this procedure is safe, well tolerated and able to manage difficult-to-control superficial transitional cell carcinoma and carcinoma *in situ* of the bladder. Improvements are in progress, on the one hand concentrating on newer generations of photosensitisers such as hypericin [62,63], on the other hand concentrating on new targets such as the transferrin receptor for more specific therapy [64] or p38α MAPK inhibition to avoid tumour survival and recurrence [65].

3. Targeted therapy

Whereas controlled delivery focuses on improving the overall drug exposure to the bladder, targeted therapies are selectively directed to cancer cells or the processes involved in their genesis and metastasis. Tumorigenesis is a multistep process that transforms normal human cells into malignant derivates. Among these steps, self-sufficiency in growth signals, insensitivity to growth-inhibitory signals, evasion of programmed cell death, unlimited replicative potential, sustained neo-angiogenesis, as well as tissue invasion and metastasis are regarded as essential alterations towards malignant growth [66]. The improved understanding of the biology of bladder carcinogenesis and tumour progression has led to the identification of specific genetic lesions as well as biochemical receptors and signal transduction pathways that should improve cancer diagnostics and can also serve as new therapeutic targets (**Table 1**).

3.1 Epidermal growth factor receptor family

The epidermal growth factor receptor (EGFR) family comprises four homologue receptors: ErbB1 (EGFR, HER1), ErbB2 (HER2/neu), ErbB3 (HER3) and ErbB4 (HER4). They are basically composed of an extracellular binding domain, a transmembrane lipophilic segment and an intracellular tyrosine kinase domain. Activation plays a crucial role in many cell regulatory processes, such as proliferation, migration, and modulation of apoptosis leading to the progression of many malignancies including bladder cancer [67,68].

3.1.1 Epidermal growth factor receptor (HER-1)

The EGFR is a 170 kDa transmembrane protein that is normally expressed only in the basal layer of bladder epithelial cells. On malignant transformation, EGFR is highly expressed also in the superficial layers of tumours and overexpression is detected in 31 – 48% of bladder cancer, being associated with progression of the tumour and a poor prognosis [69-72]. To address this potential molecular target, two principal approaches are followed: monoclonal antibodies directed to the extracellular domain of the receptor and inhibition of the receptor's tyrosine kinase.

Regarding monoclonal antibody therapy, a promising candidate is IMC-C225/cetuximab (Erbitux®, Merck Pharma GmbH, Darmstadt, Germany) a human/murine chimeric monoclonal antibody against EGFR. Perrotte *et al.* [73] showed in nude mice with metastatic human transitional bladder cancer that therapy with cetuximab had a significant antitumour effect, which was partially mediated by inhibition of angiogenesis. A study conducted by the same authors [74] investigated further whether the combination with paclitaxel could enhance this therapeutic effect. Again, this treatment downregulated the expression of basic fibroblast growth factor, vascular endothelial cell growth factor, interleukin-8 and matrix metalloproteinase type 9, and inhibited tumour-induced neovascularisation compared with the untreated control ($p < 0.005$). This combination also enhanced apoptosis in tumour and endothelial cells as compared with the drug alone ($p < 0.005$). Current data of clinical studies with cetuximab in combination with chemotherapy are available for treatment of head and neck cancer [75], non-small cell lung cancer [76] and colorectal carcinoma [77]. For bladder cancer, a Phase II randomised trial evaluating the effects of gemcitabine and cisplatin with or without cetuximab is currently recruiting participants [78].

ZD 1839/gefitinib (Iressa®, AstraZeneca, London, UK), is an orally active EGFR tyrosine kinase inhibitor (TKI) that interacts with the intracellular tyrosine kinase domain and subsequently inhibits downstream signalling [79]. A basis for the application in bladder cancer treatment was provided by Nutt *et al.* [80] and Dominguez-Escrig *et al.* [81], who studied the therapeutic potential in preclinical bladder cancer models and evaluated the therapeutic benefit. In a Phase II clinical trial, gefitinib has been tested in combination with cisplatin and gemcitabine in 27 untreated advanced transitional cell carcinoma patients [82]. In summary, the trial showed activity in

advanced transitional cell carcinoma, but the relative contribution of gefitinib was not clearly visible and the regimen was associated with excessive toxicity. At present, a randomised Phase II study is being conducted for further evaluation [78].

In contrast to gefitinib, where the US Food and Drug Administration (FDA) has restricted the use in advanced non-small cell lung cancer only to patients participating in a clinical trial or continuing to benefit from treatment already initiated, OSI-774/erlotinib (Tarceva®, Roche, Basel, Switzerland), another small molecule TKI, has been approved by the FDA for therapy of advanced non-small cell lung cancer and is continuing to be investigated [83]. Expanding this concept on bladder cancer, Yang *et al.* [84] assessed the effects of erlotinib in comparison with interferon-α on bladder cancer cell lines. Both, erlotinib and interferon-α showed significant antiproliferative activity, and combined treatment enhanced the sensitivity of most cell lines. Another study evaluated the epidermal growth factor receptor status of bladder carcinoma cells and the response to erlotinib [85], which demonstrated that even in the absence of receptor mutations erlotinib showed potential as therapeutic agent.

3.1.2 *HER2/neu*

HER2/neu is another transmembrane tyrosine kinase growth factor receptor that is overexpressed in urinary bladder carcinoma. In contrast to breast carcinoma, where HER-2/neu gene amplification and receptor overexpression could be correlated to each other and had prognostic and therapeutic benefit, varying results have been reported for bladder cancer, and the prognostic significance is discussed controversially [86-92]. However, trastuzumab (Herceptin®, Roche, Basel, Switzerland), a recombinant monoclonal antibody against HER2/neu, which gained FDA approval for metastatic breast cancer, is now under investigation in combination with conventional chemotherapy. In a multi-centre Phase II study with 44 patients with HER-2/neu-positive advanced urothelial carcinoma, Hussain *et al.* [93] investigated the safety and efficacy of a combination of trastuzumab, carboplatin, gemcitabine and paclitaxel. This treatment was considered suitable, with a response rate of 70% and a median survival of 14 months, which was favourable as compared with results with gemcitabine/carboplatin. Continuing studies will concentrate on the net contribution of trastuzumab and, in general, on finding the best way to detect HER2/neu in urothelial cancer, as data from immunohistochemistry (IHC) and fluorescent *in situ* hybridisation (FISH) do not correlate to the same extent [94].

3.1.3 *Dual EGFR/HER2*

Dual EGFR/HER2 inhibition can be achieved with GW-572016/lapatinib (Tykerb®, GlaxoSmithKline, London, UK), which is also an orally active small molecule TKI. As tested in a Phase II study in pretreated bladder cancer patients, a median time of progression of 8.6 weeks was assessed, which was comparable to conventional second-line therapies [95].

3.2 Ras signalling pathway

Ras is a signal transduction protein that controls signalling pathways that are key regulators of several aspects of normal cell growth and malignant transformation [96]. As Ras mutations have been found in 30 – 40% of urothelial malignancies [97], blocking the activation of the Ras family oncogenes by farnesyl transferase inhibitors was also tested for bladder cancer treatment. Winquist *et al.* [98] evaluated the antitumour activity of SCH 66336/lonafarnib in a Phase II clinical study with patients with previously treated transitional cell carcinoma (TCC). No single-agent activity was observed, thus a continuing study concentrates on combined therapy with gemcitabine [78].

R115777/tipifarnib (Zarnestra™, Johnson and Johnson Pharmaceutical R&D, Raritan, New Jersey, USA) is another farnesyl transferase inhibitor. As compared with lonafarnib, a Phase II trial in patients with metastatic TCC of the urothelial tract did not show single-agent activity [99]. Studies on this are still being continued. Another approach combines trastuzumab with tipifarnib for bladder cancer treatment [78].

3.3 Phosphatidylinositol-3-kinase signalling pathway

The mammalian target of rapamycin (mTOR) is a protein kinase of the phosphatidylinositol-3-kinase (PI3K)/Akt signalling pathway involved in cell growth, cell motility, cell survival, protein synthesis and transcription [100]. As dysregulation of the mTOR pathway has been found in many human tumours, mTOR inhibitors are under investigation for cancer treatment. At present, RAD001/everolimus (Certican®), which is approved in Europe for drug-eluting coronary stents as an immunosuppressant to prevent restenosis, is under investigation in relation to bladder cancer. In a Phase II clinical study the response rate and the effects of everolimus will be evaluated [78].

3.4 Cell cycle regulators

Cyclin-dependent kinases (CDKs) are one of the main regulators of cell cycle progression, and thus their inhibition results in cell cycle arrest and even in apoptosis. Alvocidib (flavopiridol, HMR 1275, L86-8275) is a semi-synthetic flavon that was the first cyclin-dependent kinase inhibitor in human clinical trials [101]. Evaluation in bladder cancer was performed by Chien *et al.* [102], who elucidated the effects of flavopiridol on normal urothelial cells, immortalised urothelial cell lines and bladder cancer cell lines. They showed that flavopiridol is capable of inducing G2/M arrest, growth inhibition and a modest level of apoptosis. Wirger *et al.* [103] also examined the toxicity and efficacy of flavopiridol in a rat bladder cancer model. According to the results obtained, this agent could be useful for bladder cancer therapy as 7 out of 12 rats were tumour-free and for the remaining tumours a tendency to lower stage and grade was demonstrated.

3.5 Proteasome

The ubiquitin-proteasome system is also involved in the regulation of cellular processes such as cell cycle and division,

regulation of transcription factors, and cellular quality control by degradation of intracellular proteins. Thus, aberrations can play an important role in tumorigenesis and the system has become a target for anticancer strategies [104].

PS-341/bortezomib (Velcade®, Janssen Cilag Pharma GmbH, Titusville, New Jersey, USA), a specific and reversible inhibitor of the proteasome, is FDA approved for use in multiple myeloma. Kamat *et al.* [105] demonstrated for 253JB-V bladder cancer cells that bortezomib is able to inhibit cell growth and augment the growth inhibitory effects of gemcitabine. Besides accumulation of p53, p21 and suppression of cyclin-dependent kinase 2 activity, antiangiogenic effects by inhibiting matrix metalloproteinase-9 (MMP-9), IL-8 and vascular endothelial growth factor (VEGF) were also reported. Recent clinical studies investigate further the efficacy of bortezomib therapy for patients with advanced or metastatic transitional cell cancer of the bladder, the renal pelvis, or the ureter [78].

3.6 DNA hypermethylation

Aberrant DNA methylation has been shown to be a key survival mechanism in cancer. Inhibitors of DNA methylation, such as 5-azacytidine (5-Aza-CR), 5-aza-2-deoxycytidine (5-Aza-CdR) and zebularine, act through reactivation of expression in genes that have undergone epigenetic silencing [106]. For bladder cancer therapy, Bender *et al.* [107] evaluated the effects of 5-Aza-CdR on human cancer cell lines (bladder transitional carcinoma-derived and colon carcinoma-derived cell lines) and human fibroblast cell strains. The results revealed suppression of cell growth in the tumour cell lines, which was associated with reactivation of growth-regulatory genes silenced by *de novo* methylation. Recently, Cheng *et al.* [108] showed an antitumour effect of zebularine on human bladder carcinoma cells transplanted in BALB/c nu/nu mice after oral administration, supporting a potential applicability in cancer treatment.

3.7 Telomerase

Human telomerase reverse transcriptase (hTERT) is an essential component in the telomerase complex, which controls telomerase activity and is highly expressed in bladder cancer cells. Zou *et al.* [109] investigated antiproliferative effects of small hairpin interfering RNA (shRNA)-targeted *hTERT* gene on bladder cancer cells and xenograft mice models. The results revealed a downregulation of hTERT expression and a decrease in telomerase activity, leading to suppression of tumour growth. Gao and Chen [110] also observed enhanced apoptosis of cancer cells on administration of hTERT antisense oligodeoxynucleotides followed by TNF-α application.

3.8 B-cell lymphoma 2

B-cell lymphoma 2 (bcl-2) is an important antiapoptotic factor, whose overexpression has been reported in bladder cancer [111]. Bilim *et al.* [112] evaluated bcl-2 antisense phosphorothioate oligodeoxynucleotide (PODN)-mediated downregulation of bcl-2 expression on human bladder cancer cells. Treatment reduced the bcl-2 protein level, and combined treatment with adriamycin resulted in increased cytotoxicity. Another synergistic effect resulting in significant lower cell survival rates of bladder cancer cells was observed for combined treatment of bcl-2 antisense oligonucleotide and cisplatin [113]. A Phase I/II clinical trial aims to study the effectiveness of bcl-2 antisense oligonucleotide (G3139, oblimersen, Genasense®, Genta, Inc., Berkeley Heights, New Jersey, USA) in treating patients with solid tumours, including bladder tumours [78].

3.9 Clusterin

Clusterin (apolipoprotein J), a glycoprotein that is associated with a variety of physiological functions such as regulation of apoptosis, has been reported to be overexpressed in several human cancers [114]. Miyake *et al.* [115] evaluated whether antisense (As) oligodeoxynucleotide (ODN) targeting the clusterin gene could enhance the cytotoxic effect of gemcitabine in human bladder cancer KoTCC-1 cells. The treatment resulted in significantly enhanced chemosensitivity of KoTCC-1 cells, and administration in a murine intraperitoneal bladder tumour implantation model significantly decreased KoTCC-1 tumour volume. OGX-011/curtisen is a second-generation antisense molecule that blocks production of clusterin and is now under investigation in a Phase I study in combination with docetaxel for therapy of patients suffering from metastatic or locally recurrent solid tumours (bladder, breast, kidney etc.).

3.10 Survivin

Survivin is an apoptosis inhibitor that is upregulated in many malignancies, including bladder cancer. In this context, Fuessel *et al.* [116] and Ning *et al.* [117] evaluated the efficacy of antisurvivin As-ODNs for bladder cancer treatment. According to tests with diverse bladder cancer cell lines, the survivin-directed As-ODNs downregulated the expression of the protein and inhibited proliferation.

3.11 Tumour suppressor genes

Alterations in p53 and pRb, the products of the chromosomes 17p13 TP53 and 13q14 retinoblastoma (Rb) tumour suppressor genes, occur in almost 50 and 33% of all bladder cancers, respectively, and are associated with advanced stages and higher grade of disease [118]. Thus, therapeutic strategies for restoration of the normal function are now under investigation.

3.11.1 p53 gene-based therapy

An encouraging approach is the adenovirus-mediated transfer of wild-type human p53. The intratumoural injection of an adenoviral expression vector encoding wild-type p53 (rAd/p53 or SCH 58500) in patients with invasive bladder cancer [119] revealed biological activity of the treatment without any dose-limited toxicity. In a Phase I study Pagliaro *et al.* [120] investigated the applicability, safety and biological activity of another

adenoviral vector containing the wild-type *p53* gene, namely Ad5CMV-p53 (INGN201, Advexin®, Introgen Therapeutics, Houston, Texas, USA), in patients with locally advanced TCC of the bladder. Repeated installations were conducted without adverse effects, but the efficiency of gene transfer still has to be improved as specific transgene expression was observed in only two out of seven patients. Enhancement of the activity of Ad5CMV-p53 was observed in combination with cisplatin in a study with bladder cell lines [121] as well as in combination with As-ODN-targeting clusterin gene in a human bladder cancer model [122]. Another approach is the use of recombinant vaccinia virus (rVV) as a vector for p53. In an orthotopic murine model, Fodor *et al.* [123] evaluated the efficacy of rVV-TK-53. The treatment resulted in decreased tumour incidence and in a 33% survival rate, whereas the delivery using buffer or empty vector did not show any benefit for survival.

3.11.2 *pRb gene-based therapy*

In preclinical studies with non-small cell lung carcinoma and bladder carcinoma cells, Xu *et al.* [124] evaluated the activity of RB94, which is a more potent tumour growth suppressor than the normal protein (Rb110), in a replication-deficient adenoviral vector (AdCMVpRB94). Gene therapy resulted in regression of the tumour of both Rb-negative and Rb-positive tumour cells and the pRB94, when overexpressed in tumour cells by means of replication-deficient adenovirus vectors, was evidently more potent in tumour suppression than the full-length Rb protein. Generally, in the context of cancer gene therapy, increasing emphasis is put on the development and use of non-viral delivery methods that lack immunogenicity or biohazardous potential. At this, Pirollo *et al.* [125] developed a systemically administrable, nano-sized liposome delivery system with a tumour-targeting moiety, which is either the transferrin (Tf/Lip/RB94) or the antitransferrin receptor single-chain antibody fragment (TfRScFv/Lip/RB94). Using an Rb-negative human bladder carcinoma cell line, transfection with Tf/Lip/RB94 significantly sensitised cells for the chemotherapeutic agent. In mice bearing subcutaneous bladder tumours, the combination of systemically given Tf/Lip/RB94 or TfRScFv/Lip/RB94 plus gemcitabine resulted in significant tumour growth inhibition and/or regression as well as induction of apoptosis. A Phase I study aims to evaluate this system in patients with metastatic Rb-negative bladder cancer.

3.12 Histone deacetylase

Histone deacetylation is associated with transcriptional repression, including a decrease in the expression of tumour suppressor genes. Histone deacetylases (HDACs) are overexpressed in various types of cancer, making them a potential target for cancer treatment. As HDACs also exert influence on the regulation of non-histone proteins such as heat shock protein 90 (Hsp90), p53, tubulin, Rb and E2F1, HDAC inhibitors seem to have a broad anticancer potential [126].

In terms of bladder cancer treatment, a pioneering study was performed by Canes *et al.* [127]. They investigated the effects of sodium butyrate (NaB) and trichostatin A (TSA), two histone deacetylase inhibitors, on bladder carcinoma cells and evaluated the inhibitory activity of TSA on cell growth. In a clinical trial, vorinostat (suberoylanilide hydroxamic acid [SAHA], NSC 701852, Zolinza), a small molecule inhibitor of HDAC with FDA approval for the treatment of cutaneous T-cell lymphoma, was applied to 37 patients with advanced cancer [128]. The results revealed that administration of the agent is safe at doses that inhibit HDAC activity *in vivo*, and four (two lymphoma and two bladder) patients had objective tumour regression with clinical improvement in tumour-related symptoms. At present, a Phase I study of vorinostat in combination with doxetaxel in patients with advanced and relapsed malignancies including bladder cancers is under investigation [78].

Romidepsin (FK228, depsipeptide, FR901228), another HDAC inhibitor, significantly inhibited growth of TCC tumour in xenograft models [129]. A continuing Phase II clinical trial deals with the activity of romidepsin in patients with advanced cancer of the urothelium, including that of the bladder.

3.13 Angiogenesis

Angiogenesis, the process of new blood vessel formation outgoing from pre-existing ones, is one of the fundamental steps in the development of metastatic bladder cancer, and therefore serves as a sound target for cancer therapy. Among the angiogenic factors, the most prominent one involved in bladder cancer is VEGF. Ligand-binding to the receptor results in stimulated cell growth and proliferation, two key features for the development of new blood vessels, and increased vascular permeability, which may also contribute to angiogenesis and tumour growth [130]. The function of VEGF in vessel formation is supported by the platelet-derived growth factor (PDGF) and thus PDGF signalling can indirectly regulate angiogenesis [131]. Other important factors are members of the fibroblast growth factor family, especially the basic fibroblast growth factor (bFGF), which provides endothelial cells and smooth muscle cells with signals for proliferation and survival [132], and FGF3, whose receptor is overexpressed in superficial tumours as well as in many invasive bladder cancers [133]. In addition to the important role of angiogenic factors for diagnosis and prognostic evaluation of bladder cancer [134,135], they, as well as the corresponding receptors, are subject of therapeutic strategies.

As blocking of the VEGF receptors has been shown to decrease Ras signalling and DNA synthesis in bladder cancer cell lines [136], DC101, an anti-VEGFR monoclonal antibody, was applied to mice with metastatic human bladder carcinoma [137]. In this study, a significant antitumour efficacy was observed, especially when combined with paclitaxel. Another study by the same authors dealt with the effects of DC101 on receptor phosphorylation and apoptosis and revealed

that agents targeting a single receptor may not be sufficient to inhibit tumour angiogenesis completely [138]. Bevacizumab (Avastin®, Roche, Basel, Switzerland) is another recombinant humanised monoclonal antibody interacting with VEGFR and is FDA-approved for the first-line treatment of metastatic colorectal cancer in combination with an intravenous 5-fluorouracil-based regimen. At present, a study combining cisplatin, gemcitabine and bevacizumab for therapy of metastatic transitional bladder cancer is under evaluation [78].

The VEGF receptor activity can also be inhibited by catalytic RNA molecules known as ribozymes, which can downregulate VEGF receptor function by specifically cleaving the VEGFR1-mRNA. A study in patients with advanced solid tumours including [139] bladder cancer and administration of RPI.4610 (Angiozyme), a stabilised ribozyme, in combination with carboplatin and paclitaxel revealed a complete response. The focus was set on the safety and the pharmacokinetic profile of RPI.4610 in this combined therapy and the results indicated safe administration without substantial pharmacokinetic interactions.

For targeting VEGF instead of its receptor, VEGF Trap represents a potent angiogenesis inhibitor that consists of partial sequences of human VEGF receptor VEGFR1 and VEGFR2 extracellular domains fused to the Fc portion of human immunoglobulin-γ [131], which binds and inactivates VEGF with a high affinity. A Phase II trial is studying the side effects and the activity of VEGF Trap on the treatment of patients with recurrent, locally, advanced or metastatic bladder cancer [78].

BAY 43-9006/sorafenib (Nexavar®, Bayer AG, Leverkusen, Germany) is an orally adminstrable small molecule tyrosine kinase inhibitor, which is FDA-approved for treatment of metastatic renal cell carcinoma. Inhibiting c-Raf, B-Raf, VEGFR2, VEGFR3 and PDGFR-B [140], it is now under investigation for therapy of advanced and metastic bladder cancer [78]. SU11248/sunitinib (Sutent®) is another orally given, small molecule inhibitor of multiple tyrosine kinases that targets PDGF, VEGFR, stem cell receptor factor (KIT) and fms-like tyrosine kinase 3 (FLT-3) [141]. It is FDA-approved for the treatment of renal cell carcinoma and Imatinib-resistant gastrointestinal stromal tumour. In preclinical bladder carcinoma models, Sonpavde *et al.* [142] assessed the efficacy of sunitinib or cisplatin individually and in combination. Antitumour activity was observed as a single agent and in combination the activity of cisplatin was enhanced. Recently, Bradley *et al.* [143] designed a randomised Phase II trial evaluating the role of sunitinib as maintenance therapy in advanced urothelial cancer.

Endostatin protein is a naturally occurring 20-kDa C-terminal fragment derived from collagen XVIII, which inhibits angiogenesis, and may interfere with the pro-angiogenic action of growth factors such as VEGF and bFGF [144]. Du and Hou [145] investigated the efficacy of recombinant human endostatin at inhibiting tumour growth in bladder cells. In a cell culture model proliferation of endothelial cells was inhibited without any effect on bladder tumour cells. However, in nude mice subcutaneous endostatin blocked angiogenesis and induced apoptosis in bladder cancer cells. Kikuchi *et al.* [146] evaluated lentivirus vector-mediated overexpression of endostatin and reported decreased vascularisation and inhibition of bladder tumour growth. Thus, the application of endostatin seems to be a feasible concept, supported also by Schmidt *et al.* [147], who observed a distinct endostatin-binding pattern in bladder tumours as compared with benign tissue and malignant and benign kidney tissue.

TNP-470, an analogue of fumagillin derived from *Aspergillus fumigatus*, is also a potent inhibitor of angiogenesis [148]. Various preclinical studies evaluated the efficacy for bladder cancer treatment of TNP-470 alone [149-151] or in combination with chemotherapeutics [152,153]. In general, they demonstrated the potential of TNP-470 to inhibit tumour growth and a synergistic antitumour effect with gemcitabine [153]. However, clinical data for bladder cancer are not yet available.

NF-κB is a protein complex that acts as a transcription factor regulating the expression of pro-angiogenic molecules. In a study with human TCC cell lines, Karashima *et al.* [154] showed that NF-κB mediates neo-angiogenesis and proliferation by means of interleukin-8, which renders NF-κB a potential molecular target for bladder cancer therapy.

Targeting bFGF, Inoue *et al.* [155] investigated adenovirus-mediated antisense bFGF gene therapy in mice with human bladder carcinoma. They successfully inhibited tumour growth by downregulating the expression of bFGF and MMP-9, which resulted in decreased tumour cell proliferation and enhanced apoptosis of tumour and endothelial cells.

Specific inhibition of FGFR3 was the subject of preclinical investigations of Martínez-Torrecuadrada *et al.* [156,157]. At first, the potential of six human scFv antibody fragments produced by means of phage display techniques and directed against FGFR3 in RT112 bladder carcinoma cells was studied [156]. As proliferation of RT112 cells was blocked in a dose- and FGF-dependent manner, these human anti-FGFR3 scFv antibodies may have antitumoural potential in bladder cancer. In a continuing study, the ability of the recombinant gelonin toxin (rGel) to enhance this inhibitory effect on bladder cancer cell line RT112 and the corresponding xenografts is investigated further [157]. So far, the results have revealed considerable apoptotic damage of tumours in mice treated with these FGFR3-driven immunotoxins as compared with the control group, and the subcellular location of FGFR3 in immunotoxin-treated tumours indicated a translocation of FGFR3 to the nuclear membrane in contrast to tumours from saline-treated controls. Taken together, these results demonstrate the applicability of FGFR3-driven immunotoxins as effective therapeutic agents against human bladder tumours overexpressing FGFR3.

3.14 Glycocalyx

In common with all eukaryotic cells, the surface of bladder cells is covered by a dense layer of complex carbohydrates

known collectively as the 'glycocalyx'. Biorecognitive proteins such as lectins are capable of detecting and binding to these carbohydrate moieties at the cell surface in a highly specific manner. Owing to this carbohydrate-specific interaction and alteration of the glycosylation pattern of cells on malignant transformation [158], lectin-mediated targeting might be an encouraging approach towards site-specific antitumour therapy. At this, binding to and even uptake in colon cancer cells was demonstrated for prodrug formulations as well as nanoparticulate drug carriers grafted with certain lectins to render these delivery systems targeted [159,160]. Plattner *et al.* [161] evaluated the cytoadhesive and cytoinvasive properties of several plant lectins for a human bladder cancer cell line and prescreened wheat germ agglutinin as a potential target vehicle for functionalised drug delivery systems.

4. Further approaches

4.1 Improved immunotherapeutic strategies

Regarding bladder cancer treatment, improved immunotherapeutic strategies also have to be mentioned. The purpose of immunotherapy is to stimulate the immune system against tumour cells by means of either passive or active immunisation. A promising approach is direct induction of cytokines, such as IL-2, IL-12, and interferons into cancer cells. The feasibility of an intravesical liposome-mediated IL-2 gene transfection was evaluated from Horiguchi *et al.* [162] in an orthotopic mouse bladder cancer model. The results revealed inhibited tumour growth and prolonged survival, which was enhanced by subsequent addition of B-7.1 (a surface immune molecule) gene-modified tumour vaccines [163]. Administration of IL-12 was investigated by O'Donnell *et al.* [164] in mice with transitional cell carcinoma of the bladder. Treatment resulted in prolonged survival and a higher number of cured subjects (75%) at the highest IL-12 dose, and antitumour effects were observed after both systemic and intravesical administration. A preclinical study was conducted by Lee *et al.* [165] to examine the efficacy of murine IL-12 DNA vaccine and recombinant BCG DNA vaccines in a murine bladder tumour model. The combination of both vaccines resulted in a high serum interferon-γ level, which promotes Th1-type immune responses, and was associated with a significantly higher survival rate than that of mice treated with either vaccine alone or that of control mice. High expression of IL-12 can also be induced with AdCD40L gene therapy [166]. CD40L is a type II transmembrane protein belonging to the tumour necrosis factor superfamily. At this, the gene was transferred by adenoviral vectors to mice with orthotopic bladder cancers [166]. The systemic tumour-specific immune response was stimulated and 60% of mice with pre-established tumours were cured. Efficient gene delivery and expression of IFN-α_{2b} protein has been achieved using a recombinant adenovirus gene delivery system (rAd-IFN) together with the small molecule excipient syn3 [167]. The results revealed increasing exposure of IFN and significant tumour reduction in a mouse model with human bladder cancer.

4.2 Radiochemotherapy

The role of radiochemotherapy (RCT) is also now under discussion in the context of bladder cancer treatment. As radiotherapy alone has not been shown to be superior to other conservative treatments [168], several studies have investigated the role of combined radiotherapy with chemotherapy to improve the benefit for patients [169-172] and suggested higher response rates and improved survival. Current approaches also concentrate on improved strategies in combination with hyperthermia [173] or inhibition of oncogene products such as HER2/neu in combination with RCT using paclitaxel and trastuzumab [174].

4.3 Other strategies

The MMP family of extracellular proteinases is regarded as a critical factor assisting tumour cells during metastasis by degrading the extracellular matrix [175]. A key role in this process is featured by MMP-2 and MMP-9, which mainly fragment the basement membrane type IV collagen. Recently, a study with *Magnolia officinalis* was performed [176] to investigate the anticancer activity to urinary bladder cells. A suppressed expression of MMP-2 and MMP-9 was monitored and the treatment of mice with BBN-induced urinary bladder tumours revealed growth inhibition. However, preliminary results from clinical trials aimed at the therapy of various cancers have been disappointing [177], and thus the numerous functions of MMPs should be investigated further to determine effective targeting strategies.

Another target of future interest might be the Hsp90, which has been found to be overexpressed in several tumours, including those of the bladder, and is associated with progression of pathogenic cellular transformation [178,179]. Several Hsp90 inhibitors that showed successful targeting have entered clinical evaluation, but further research is required to expand clinical benefit on a wider range of cancers [180].

An interesting therapeutic approach is the use of magnetic targeted carriers, which are microparticles formed from metallic iron and activated carbon. The iron allows for magnetic manipulation and the carbon component enables adsorption of doxorubicin (MTC-DOX). After intravesical instillation, targeting is achieved by the use of an externally placed magnet. Leakakos *et al.* [181] demonstrated for normal swine bladders that MTC delivery allowed a greater exposure and site-specific deposition of the drug in comparison with doxorubicin alone, but the proof of principle for the treatment of bladder cancer is still an open task.

Moreover, cell-penetrating peptides (CPP) or protein transduction domains have become widely used vehicles for the cellular import of molecules, as they have been shown to be internalised in most cell types and can therefore transport numerous types of drug into cells, including

small-molecule pharmaceuticals, therapeutic proteins and antisense oligonucleotides [182]. Schwarze *et al.* [183] synthesised a 15-oligomer peptide containing an NH_2-terminal 11-amino protein transduction domains from the human immunodeficiency virus TAT protein. The administration resulted in delivery of the biologically active fusion protein to all tissues in mice, including the brain. This lack of specificity might be problematic in the case of systemic use, but nevertheless it could be of further interest for intravesical therapy.

5. Expert opinion

Given the high tendency of bladder cancer to recur together with the ever-present possibility of progression, successful treatment of bladder cancer is still a challenge for urologists and oncologists. Until now the primary goal has been to eradicate the local and any potential micrometastatic disease while maintaining the highest quality of life without compromising survival. Apart from surgical approaches, current treatment modalities comprise primarily nonspecific immunotherapy or chemotherapy, but the response is often insufficient. Owing to the physiology of the urothelium, efficacy of treatment is governed by two crucial factors: the permeability of the bladder wall and the residence time of the API to maintain an adequate concentration gradient for passive diffusion. Improved permeability could be achieved by means of chemical and physical approaches together with good clinical results. However, all of these methodologies rely on nonspecific permeation enhancement, which might provoke some adverse side effects.

As to the residence time, conventional formulations typically stay in the bladder for only a short time of ~ 2 h. As a sufficiently high concentration gradient represents a crucial prerequisite for passive diffusion and thus efficacious treatment, a constantly elevated API level in the bladder for a prolonged period would be desirable. Among the vehicles used for intravesical therapy, colloidal carriers might be of particular importance in future. If such particles are refined with extra bioadhesive properties, appropriate sustained release characteristics together with an often essential improvement in stability of the API might be feasible.

All these modalities, however, lack any clear discrimination between healthy and diseased tissue. Since the time of Paul Ehrlich pharmaceutical technologists all over the world have dreamt of the 'magic bullet', a drug delivery system that has the ability to specifically target diseased cells. Particularly in the field of chemotherapy with its numerous and severe side effects, site-specific delivery and action of the API without compromising non-target cells are profound prerequisites for a more convenient regimen. Molecular targeting might be a step forward in this area. Based on the increased understanding of the biological mechanisms underlying bladder carcinogenesis and tumour progression, various potential molecular targets have been identified and evaluated in preclinical studies, such as the signalling pathways that play a crucial role in the regulation of cell growth. Among others, the receptors of epithelial growth factor, which were found to be overexpressed in bladder cancer cells, have been addressed as attractive targets. Although preclinical data suggested positive aspects, the clinical benefits remain limited. This might also be because EGFR expression is varying and there is often no clear correlation between overexpression of these receptors and tumour prognosis in bladder cancer. Possibly, response rates could be improved by an optimised patient selection using predictive molecular markers. According to the literature, application of specific inhibitors affecting the signalling pathway in a single-agent therapy seems to be generally less effective as compared with combination regimens using either chemotherapeutics or biologic agents that target a different part of the tumorigenetic cascade. At this, further therapeutic improvements might be achieved using sophisticated drug delivery systems instead of a simple parallel application. These systems might consist of nanoparticles that contain the cytotoxic agents in their core and are decorated with a corona of biologicals that guarantee the specific targeting.

Another approach towards targeted therapy relies on strategies acting at the genetic level. Various preclinical *in vitro* and *in vivo* studies suggested the principal feasibility of gene therapy for bladder cancer treatment. At present, several Phase I/II trials using either As-ODNs for suppression of oncogenic genes or tumour suppressor genes for restoration of adequate cell cycle regulation are being performed with promising results. Owing to the rather easy access of the diseased tissue via intravesical instillation, adequate delivery of the therapeutic genes to the cells of interest seems to be a manageable task, making gene therapy a practicable option for treatment of bladder cancer. However, refinement of vector systems remains a challenge for future research in order to increase the transfection efficacy and its specificity. Despite encouraging results, a final decision about the potential of gene therapy in bladder cancer cannot yet be given.

With special emphasis on advanced bladder cancer, antiangiogenic approaches might also hold promise. Prominent targets in this area include either pro-angiogenic factors such as VEGF and bFGF or the corresponding receptors. Some of the strategies directed against angiogenesis of bladder tumours have already revealed their potential in clinical trials and might become even more important in future.

An interesting alternative strategy towards improved selectivity of chemotherapy in bladder cancer might be given by the application of lectins. Offering a clear discrimination between healthy and diseased cells, the advantage of the glycotargeting approach relies not only on the specific enrichment of lectin at the surface of malignant cells, but also on the fact that some lectins are able to actively transport conjugated drugs across the cellular membrane. That way, the chemotherapeutic agent might be accumulated directly in the cytoplasm of the targeted cancer cells if the API and the targeter are linked in a proper way either as a prodrug formulation or as a carrier system.

All in all, pure combination chemotherapy in bladder cancer treatment has reached a plateau. However, new biological therapies appear as a silver lining on the horizon of bladder cancer therapy, and will gain increasing interest in future owing to their targeting facilities. The challenge to be met in this area by investigators all over the world is how to intelligently combine controlled and targeted delivery approaches in order to gain maximal treatment efficacy and thus therapeutic benefit for the patient with minimal physiological stress. Given the current efforts, this might already be achievable within our lifetime.

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