Published in final edited form as:

Target Oncol. 2018 February ; 13(1): 49–60. doi:10.1007/s11523-017-0546-x.

Incorporating DNA Methyltransferase Inhibitors (DNMTis) in the Treatment of Genitourinary Malignancies: A Systematic Review

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Abstract

Inhibition of DNA methyltransferases (DNMTs) has emerged as a novel treatment strategy in solid tumors. Aberrant hypermethylation in promoters of critical tumor suppressor genes is the basis for the idea that treatment with hypomethylating agents may lead to the restoration of a “normal” epigenome and produce clinically meaningful therapeutic outcomes. The aim of this review article is to summarize the current state of knowledge of DNMT inhibitors in the treatment of genitourinary malignancies. The efficacy of these agents in genitourinary malignancies was reported in a number of studies and suggests a role of induced DNA hypomethylation in overcoming resistance to conventional cytotoxic treatments. The clinical significance of these findings should be further investigated.

1 Introduction

The paradigm of a cellular phenotype manifesting as a sole result of the information encoded in the DNA experienced a radical shift over recent years. Epigenetic changes represent a series of mechanisms that interfere with gene expression without altering the base sequence of the coding genes. Both genetic and epigenetic mechanisms cooperate to result in conformational changes in the chromatin, and alter the structure of gene promoters to either aberrantly induce or repress transcriptional gene activity. This may ultimately contribute to carcinogenesis by increasing the expression of oncogenes, or the inhibition of tumor suppressor gene expression [1, 2].

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Conflict of Interest All authors declare no conflict of interest.

Recent studies indicate that epigenetic silencing may be as important as DNA mutations in tumorigenesis and treatment resistance [3]. While mutations represent an irreversible change in the DNA sequence, epigenetic silencing is a reversible process. Known modifications with the ability to influence gene expression without altering the DNA sequence include DNA methylation, histone modifications, nucleosome remodeling induced by ATPases, and regulation via non-coding RNAs [3–6]. This review summarizes the current state of pre-clinical and clinical knowledge in targeting DNA methyltransferases (DNMTs) in genitourinary cancer.

2 Literature Search

We performed a literature search of the PubMed/MEDLINE database and meeting libraries of the American Society of Clinical Oncology (ASCO), ASCO Genitourinary Cancers Symposium, and the American Association for Cancer Research (AACR) for publications with the terms “epigenetics”, “DNMT”, “DNMTi”, “genitourinary”, “testicular cancer”, “germ-cell tumors”, “bladder cancer”, “renal cell carcinoma”, “prostate cancer”, “penile cancer”, “azacitidine”, “decitabine”, “guadecitabine”, “zebularine”, “non-nucleoside”. Combinations of these keywords were used for a comprehensive search, as outlined in Fig. 1. The literature search was last performed on 15 September 2017. Original full-text articles published in English were reviewed and the reference lists of key articles were further evaluated. We did not limit our search by the years of publication. Our search was conducted according to the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) statement. Identified reports were reviewed according to the Consolidated Standards of Reporting Trials (CONSORT) criteria. The search resulted in 4152 publications. One hundred and eleven publications were finally selected for inclusion in our review [88 original papers (79%) and 23 (21%) review articles]. The literature search and the inclusion and exclusion criteria are summarized in Fig. 1 and Table 1.

3 DNA Methylation

The covalent addition of methyl groups ($-\text{CH}_3$) to the carbon-5 position of cytosine represents a non-coding modification of DNA [7]. DNA methylation at the 5'-CG-3' sequence (also known as a CpG dinucleotide) is a unique, heritable mechanism for regulation of gene transcription. Hypermethylation generally acts as a gene silencer by downregulating the transcription of CpG-rich promoter regions (CpG islands) [8]. 5-Methylcytosine may undergo a spontaneous deamination to thymine allowing for changes in the chromatin structure. This represents a change in DNA sequence and creates a significant restraint to initiate transcriptional activity. DNA methylation may be found in distinct locations that may further influence gene expression and results in different biological phenotypes. Promoter region methylation is a repressive mark, while the gene body methylation is usually associated with active gene expression [9, 10]. DNA hypermethylation in the promoter region of tumor suppressor genes in human cancer may represent one of the most important mechanisms in tumorigenesis [11].

DNA methylation was discovered to be present also in the context of non-CpG (CpA, CpT, CpC). Non-CpG methylation coexists with CpG methylation, but its exact role is not clear.

Evidence suggests an involvement in gene expression when non-CpG methylation is found in promoter regions [12]. Unlike CpG methylation, non-CpG methylation is asymmetrical and thus this methylation pattern cannot be maintained after each cell division [13]. As a result, non-CpG methylation is decreased in rapidly proliferating tissues, suggesting a potential role in carcinogenesis and pluripotency [14, 15].

DNA methylation is catalyzed by the enzymatic activity of three DNMTs: DNMT1, DNMT3A, and DNMT3B. The role of DNMT1 is to maintain the existing methylation patterns, while DNMT3A and DNMT3B are responsible for de novo methylation [16].

4 Targeting DNA Methylation with DNA Methyltransferase (DNMT) Inhibitors (DNMTis)

Historical pre-clinical and clinical studies have assessed 5-azacitidine (azacitidine) and 5-aza-2'-deoxycytidine (decitabine), which were among the first agents shown to efficiently downregulate DNA methylation. Azacitidine and decitabine act as inhibitors of DNMTs and are now referred to as “first-generation DNMTis” preceding the “second-generation DNMT inhibitors (DNMTis)” guadecitabine [2'-deoxy-5-azacytidyl-(3'-5')-2'-deoxyguanosine sodium salt] and zebularine (1-[beta]-D-ribofuranosyl]-1, 2-dihydropyrimidin-2-one). These agents act as cytidine antimetabolite-forming covalent complexes with DNMTs and DNA. Trapping the DNMTs in this fashion ultimately alters their function, resulting in downregulation of DNA methylation [17]. This covalent trapping, however, also induces DNA damage that was suggested to be involved in the mutagenic and cytotoxic effects of DNMTis [18]. Azacitidine and decitabine were therefore initially developed as chemotherapeutic agents and were used at cytotoxic doses.

First-generation DNMTis suffer from fast metabolism induced by hydrolytic cleavage and deamination. As a result, their stability in the blood is limited and their use as cytotoxic agents in solid cancers proved unsuccessful [19]. The second-generation DNMTis guadecitabine and zebularine are more stable in the liquid environment and allow for a longer effective half-life [19–21]. While hypomethylation induced by the first-generation DNMTis in in vitro studies [22–24] brings evidence of effectivity of these agents, down-regulating the DNA methylation with guadecitabine in cancer xenograft models [25] provided proof that the unfavorable pharmacokinetic profile of first-generation DNMTis can be overcome. Zebularine was shown to be preferentially incorporated into zebularine-sensitive tumor cells with specific transcriptomic and epigenomic signatures in in vitro and xenograft mouse model experiments with human liver cancer. This could be a promising tool for predicting responses to this second-generation DNMTi [26]. Fluorocyclopentenylcytosine is another novel next-generation nucleoside agent with antimetabolic activity that also acts as a DNMT1 inhibitor. Numerous pre-clinical cell line and xenograft animal models have shown antitumor activity of this drug [27–30].

An alternative mechanism that reduces CpG island methylation is the inhibition of DNMT1 with antisense oligonucleotides, which are not incorporated into genomic DNA. In vitro experiments in bladder cancer cells have shown that MG88 targeting the 3'-untranslated

region of *DNMT1* resulted in suppressed DNMT1 expression, thus allowing for reexpression of the tumor suppressor gene *a-CDKN2A* [31].

Human organic cation and nucleoside transporters may both mediate the intake and/or efflux of azacitidine, decitabine, and zebularine, and these transporters may thus contribute to chemoresistance or chemosensitivity to DNMTis in cancer therapy [32].

Other non-nucleoside targeting DNMTi agents are procaine [33], N-acetylprocainamide, procainamide (perturbing the interactions between the protein and its target sites), hydralazine (decreases the expression of DNMT1 and 3A) [34], epigallocatechin-3-gallate (EGCG, a catalytic pocket blocker of DNMT1 found in green tea) [35], and RG108 (the first rationally designed inhibitor of DNMTs) [36]. Non-nucleoside agents have shown considerably less demethylating activity in bladder and prostate cancer cell lines compared to decitabine [34]. Ongoing clinical trials using DNMTi agents are summarized in Table 2.

5 Proposed Mechanism of Action of DNMTis in Solid Tumors

An in-depth understanding of the mechanisms underlying the effects of agents that target DNA methylation, DNMTis, is virtually non-existent. Thus, hypotheses and emerging evidence are outlined in this review article in an attempt to summarize the current state of knowledge. DNMTis induce global hypomethylation, which results in the (re)expression of certain tumor suppressor genes [31, 37]. On the other hand, global hypomethylation in tumors has been linked to an aberrant activation of cancer germ-line oncogenes that promote cell proliferation, angiogenesis, and metastasis [38]. It is therefore unclear whether the intra-tumoral hypomethylation-activating oncogenes can be overcome by treatment effects of DNMTis, which alter the entire methylome, including of tissues other than the tumor. This non-specificity of DNMTis may raise doubts about the safety of such drugs with the potential to induce the expression of both tumor-suppressor genes and oncogenes. Available data from a phase II clinical trial in ovarian cancer did not show any secondary malignancies in patients treated with decitabine for 2–30 months [39]. The primary target population for clinical trials evaluating the efficacy of these novel agents are patients with refractory solid tumors pretreated with conventional treatments. Described evidence suggests that survival benefit of treatment with DNMTis in this patient population may outweigh the risk of potential secondary malignancies. The most common toxicities are nausea, constipation, allergic reactions, and bone marrow suppression. Grade 3–4 neutropenia and thrombocytopenia were seen in 22% and 11% of patients, respectively [39].

Most of the initial clinical trials using a single-agent DNMTi to treat solid tumors were unsuccessful in improving outcomes. DNMTis were used at high doses with the intent to deliver a non-specific cytotoxic effect; however, delivery at low doses is optimized to inhibit DNA methylation [39–41]. More recent trials are testing whether DNMTis could sensitize refractory tumors to chemotherapy, and whether they can improve immune responses and boost cancer immunotherapy.

5.1 DNMTis to Overcome Chemo-Resistance

Multiple hypotheses have been proposed to explain the role of DNMTis in overcoming resistance to chemotherapy, in particular of DNA-targeted drugs (DTDs). Lethal DNA damage is the main mechanism of cell death induced by platinum compounds and doxorubicin [42]. Cisplatin induces DNA breaks by creating inter-strand adducts. Doxorubicin also creates DNA damage after being incorporated into DNA. DNMTis may potentiate the DNA damage induced by cisplatin and doxorubicin by increasing the accessibility of these drugs through loosening chromatin globally, which is required for DTD incorporation and DNA damage [43]. Furthermore, epigenetic synergy of decitabine and platinum agents, but no other cytotoxic drugs, was observed by Qin et al. [18] in a colon cancer cell line. Treating the cell line with 16 cytotoxic drugs including platinum agents did not result in the activation of a hypermethylated cytomegalovirus promoter. However, the addition of the DNMTi decitabine to platinum compounds achieved a striking synergy in activating the promoter. Experiments also resulted in significantly better reactivation of hypermethylated tumor suppressor genes (*MHLMI* and *PDLIM4*) with the combination of decitabine and carboplatin, compared to each drug alone, therefore offering evidence that platinum resistance can be overcome by the addition of decitabine [18].

5.2 DNMTis and Immune Responses

DNMTis have the potential to reactivate silenced tumor suppressor genes by DNMT inhibition, and are capable of upregulating the genes encoding major histocompatibility complex (MHC) I molecules, tumor antigens [44–46], and interferon (IFN) response proteins [47]. Decitabine upregulated chemokine expression in vitro in ovarian tumor cells and increased the number of natural killer (NK) and CD8+ cells in malignant ascites in an orthotopic mouse model [48].

Recent reports suggest immune modulation as a possible mechanism of action for azacitidine. The expression of endogenous retroviruses (ERVs) is silenced by DNA methylation [49, 50], and treatment with low doses of azacitidine reactivated ERVs in ovarian and colon cancer cells [51, 52]. While most ERVs have lost the ability to mature and infect other cells, they can still expand within their host genome [46, 53]. Upon the expression of ERVs, a double-strand RNA (dsRNA) is expressed and induces a type I IFN response [51, 52]. As a result, azacitidine may induce tumor cells to mimic virally infected cells, resulting in an antitumor immune response [54]. Several clinical trials with azacitidine or guadecitabine in combination with immune checkpoint inhibitors are ongoing in various solid tumors.

6 Targeting DNMTs in Genitourinary Cancer

The following chapters discuss the current state of evidence in the pre-clinical and clinical use of DNMTis in genitourinary malignancies.

6.1 Germ Cell Tumors

Germ cell tumors (GCTs) exhibit global DNA hypomethylation that may explain an exceptional sensitivity to platinum-based chemotherapy [55–57] associated with the highest

de novo DNMT expression (DNMT3A/3B) among solid cancers (Fig. 2) [58, 59]. However, numerous promoters in GCT cell lines were discovered to be hypermethylated in non-seminomas [60] and promoter hypermethylation of *RASSF1A* and *HIC1* genes was linked to cisplatin resistance in embryonal carcinoma cell lines [61]. Beyrouthy et al. [62] have shown that overexpression of DNMT3B is associated with hypersensitivity to decitabine. Treatment with decitabine resulted in a re-sensitization of testicular cancer cells to cisplatin. Furthermore, the demethylation resulted in a reactivation of tumor suppressor genes [62]. Similar observations were made by Wermann et al. [57], who observed an increased sensitivity of platinum-resistant GCT cell lines to cisplatin after treatment with 5-azacitidine.

We evaluated guadecitabine in vitro and in an in vivo mouse model of cisplatin-refractory GCT and found that testicular cancer cells were sensitive to single-agent exposure. Guadecitabine completely abolished progression and induced complete regression of cisplatin-resistant testicular cancer xenografts even at doses well below those required to impact somatic solid tumors [41]. A phase I study of guadecitabine in combination with cisplatin in refractory GCT subjects who relapsed after multiple lines of chemotherapy including high-dose chemotherapy (HDCT) is currently ongoing at Indiana University (NCT02429466). Anecdotal evidence from a phase II study evaluating hydralazine and magnesium valproate showed stable disease in one patient with refractory non-seminoma [63].

6.2 Bladder Cancer

Aberrant DNA methylation was initially found to be relevant to carcinogenesis in human bladder tumors and cell lines. Later, comprehensive genomic and promoter assessments have shown characteristic DNA methylation patterns in bladder cancer [64, 65]. Maruyama et al. [66] assessed the promoter methylation status of several cancer-related genes including *CDH1*, *RASSF1A*, *APC*, *CDH13*, *FHIT*, *RARβ*, *GSTP1*, *p16INK4a*, *DAPK*, and *MGMT* in 98 bladder tumors. High methylation frequency in *RASSF1A*, *APC*, *CDH1*, *CDH13*, and *FHIT* correlated with poor prognostic clinical–pathological features and shorter overall survival [66]. Hypermethylation of *RASSF1A*, *APC*, and *MGMT* has been linked to high-grade and invasive tumors in 76 patients with urothelial bladder cancer [67]. In pre-clinical studies, azacitidine inhibited the proliferation of bladder cancer cells and arrested cells at the G0/G1 phase. Wang et al. performed in vivo and in vitro experiments showing that azacitidine markedly downregulated the expression of DNMT3A/3B, reactivated expression of *hepaCAM*, and inhibited bladder cancer growth in nude mice [68]. Treatment with decitabine and zebularine induced growth inhibition in urothelial bladder cancer and renal cell cancer cells resulting in a 17–132% prolongation of cell doubling time [69]. A pre-clinical study evaluating single-agent azacitidine in 19 dogs with spontaneous urothelial bladder cancer confirmed a myelosuppressive, but relatively safe, toxicity profile. Partial remission was seen in 22%, stable disease in 50%, and progressive disease in 22% of dogs evaluable for tumor response [70]. Another pre-clinical study showed that sensitization of cisplatin-resistant bladder cancer cell lines can be achieved in vitro by decitabine pre-treatment alone and in combination with the histone deacetylase (HDAC) inhibitor vorinostat [71]. Chuang et al. [72] conducted a pre-clinical in vivo assessment of single-agent decitabine and guadecitabine in murine xenograft models derived from bladder cancer

cell lines. Intraperitoneal delivery of both drugs was effective in reducing the level of DNA methylation at the P16 promoter. Murine tumors also exhibited growth inhibition, although this treatment was insufficient to reduce the size of the tumors. Subcutaneous administration showed similar results, while the toxicity profile was more favorable with this route [72]. While zebularine effectively induced hypomethylation [73] and reactivated silenced genes [74] in T24 bladder cell lines, another study showed its complex metabolism and limited incorporation into the DNA in bladder cancer cell lines. This may be the reason for lower efficacy of zebularine than of azacitidine and decitabine in bladder cancer in vitro [75]. Interestingly, zebularine increased radiation-induced DNA damage in bladder cancer cells and increased the radiation-induced tumor growth delay in a xenograft mouse model [76]. The function of DNMT1 in bladder cancer cell lines was recently discovered to be mediated by the long non-coding (lnc) RNA *DBCCR1-003* derived from the locus of *DBCCR1* tumor suppressor gene [77]. Habuchi et al. previously demonstrated a role of the loss of *DBCCR1* expression in transitional-cell bladder cancer [78]. Qi et al. [77] reported that *DBCCR1-003* normally binds to DNMT1 and prevents the hypermethylation of *DBCCR1*, thus allowing its expression. Treatment with decitabine or overexpression of *DBCCR1-003* resulted into increased expression of *DBCCR1* via reversed promoter hypermethylation and DNMT1 binding to *DBCCR1-003* and promoter *DBCCR1* in the T24 bladder cancer cell line. This process led to significant growth inhibition of the cell line, suggesting *DBCCR1-003* as a novel biomarker and potential treatment target [77]. In vivo experiments in patient-derived bladder cancer xenografts in mice showed that guadecitabine reduced DNA methylation at the *p16* promoter region and reduced tumor growth [79].

A combination of azacitidine with sodium phenylbutyrate was used in a phase I study of patients with refractory solid tumors. Two of 28 patients had bladder cancer; however, no objective responses were observed within this trial [80]. Another phase I study used azacitidine with valproic acid in 55 patients with refractory malignancies, of whom three had advanced urothelial cancer. The combination did not produce any objective responses. Stable disease was achieved in 25% of patients with various malignancies, but not urothelial carcinoma [81].

Two clinical trials evaluating treatment with decitabine (NCT00030615) and CC-486 (NCT01478685), an oral formulation of azacitidine, in solid tumors including bladder cancer were conducted, but to the best of our knowledge, the results have not been reported yet. Another clinical study (NCT02223052) with CC-486 in solid tumors including genitourinary cancer is currently underway. Additionally, a genomic-based assignment of treatment including azacitidine and decitabine is currently ongoing in advanced urothelial carcinoma (NCT02788201).

A phase I study of MG98, an antisense oligonucleotide inhibitor of DNMT1, assessed the safety and efficacy of the treatment given in an infusion over 7 days to 33 patients with solid tumors, including bladder, upper urinary tract, and prostate cancer. Treatment-related toxicities were generally mild, most commonly being fatigue, headache, and myalgia of grade 2. Dose-limiting toxicities were grade 3 transaminitis and grade 3 thrombocytopenia. Evidence of activity was observed in this study; however, none of the patients that responded had genitourinary cancers [82].

Fluorocyclo-pentenylcytosine (RX-3117) has shown antitumor activity in gemcitabine-resistant pre-clinical models and is currently being evaluated in a phase I/II study in pancreatic and urothelial cancer (NCT02030067) [27–30].

The insufficient clinical activity of most of the DNMTi in urothelial cancer could be explained by the short half-life of the first-generation DNMTi agents. From a mechanistic standpoint, DNMTi may be better used in combination with cytotoxic drugs such as cisplatin or with immune checkpoint inhibitors.

6.3 Renal Cell Carcinoma

Abnormal hypermethylation as well as hypomethylation of DNA may occur in renal cell carcinoma (RCC), resulting in chromosomal instability and tumorigenesis [83, 84]. Numerous tumor suppressor genes have been reported to be partially or completely silenced due to the hypermethylation of their enhancer and promoter regions leading to increased tumor cell proliferation, invasion, and metastasis [85]. Deep DNA methylation and transcriptome profiling of diverse histological RCC subtypes uncovered that clear cell (ccRCC), papillary, and translocation RCC as well as mucinous and spindle cell carcinomas, are 3-fold more hypermethylated than oncocytoma and chromophobe RCC [86]. Morris et al. [87] described nine genes that showed frequent promoter region methylation in primary RCC tumor samples. The methylation of *SCUBE3* was associated with a significantly increased risk of cancer death or relapse [87]. Li et al. [88] found that the DNMT1 protein was expressed significantly higher in ccRCC than in normal tissues (56.2% and 27.3%, respectively). The expression of DNMT1 was positively correlated with tumor size, highly malignant phenotype, lymph node metastasis, vascular invasion, recurrence, and poor prognosis. This observation was confirmed in vitro in cell lines, where the knock-down of DNMT1 significantly inhibited ccRCC cell viability, induced apoptosis, and decreased colony formation and invasion [88]. A German group recently proposed a metastasis-associated methylome signature obtained from genome-wide The Cancer Genome Atlas (TCGA) datasets. The authors predicted metastatic disease with 93% sensitivity and 89% specificity and proposed the prospective validation of this tool [89].

Treatment with azacitidine suppressed cell proliferation in all 15 RCC cell lines evaluated by Ricketts et al. [90]. Interestingly, the response correlated with alterations in *VHL* promoter methylation; however some cell lines without *VHL* tumor suppressor gene methylation responded to the treatment as well. This finding is suggestive of other hypermethylated suppressor genes activated by DNMTi treatment, with several candidate genes identified (*RGS7*, *NEFM*, *TMEM74*, *GCM2*, and *AEBP1*). Methylation of *GCM2*, *NEFM*, and *RGS7* also strongly correlated with poor prognosis [90]. Treatment of A-498 RCC cells with low-dose zebularine resulted in limited cell inhibition; however, authors observed an upregulation and downregulation of 308 and 253 gene transcripts, respectively. Many of the re-expressed genes belong to the metallothionein family, potent protectors against oxidative stress that were discovered to be downregulated in RCC tumors [91]. Decitabine, but not zebularine, was able to re-express a hypermethylated *VHL* gene in RCC cell lines and caused tumor shrinkage in an RCC xenograft mouse model. Only tumors with hypermethylated *VHL* responded to the treatment. *VHL*-mutated mice did not show any

response [92]. Guadecitabine showed an interesting potential to increase the immunogenicity of RCC and other tumor cell lines. Treatment with guadecitabine induced de novo expression or reexpression of cancer testis antigen-related genes (MAGE-A1, MAGE-A2, MAGE-A3, MAGE-A4, MAGE-A10, GAGE 1–2, GAGE 1–6, NY-ESO-1, and *SSX1–5*) and human leukocyte antigen (HLA) class I antigens of intercellular adhesion molecule (ICAM)-1, an important factor for improved recognition of cancer cells by gp-100 specific cytotoxic T lymphocytes [93].

Reu and colleagues [94] have shown a synergistic potential of cytokine therapy combined with decitabine. Resistance to the antiproliferative and apoptotic effects of IFNs was postulated to result from silencing of IFN response genes by promoter hypermethylation. Treatment of human RCC cells with decitabine overcame IFN resistance, while normal epithelial kidney cells remained unaffected. IFN response gene expression was augmented greater than ten times by decitabine [94].

A phase I study conducted by Lin et al. [80] aimed to find the safe and effective dose of azacitidine in combination with sodium phenylbutyrate in refractory solid tumors. Three of the patients included in this study had RCC, but clinical effects in these patients were disappointing [80]. An ongoing phase I/II clinical trial is assessing the feasibility, safety, and efficacy of an anti-programmed cell death protein 1 (PD-1) antibody in combination with low-dose decitabine in patients with relapsed or refractory malignancies including RCC (NCT02961101). Another phase I/II trial of azacitidine in combination with bevacizumab for advanced RCC previously treated with vascular endothelial growth factor (VEGF)- or mammalian target of rapamycin (mTOR)-targeted therapy with or without prior immunotherapy was recently concluded and the results are pending (NCT00934440).

6.4 Prostate Cancer

Among all solid tumors evaluated in TCGA, prostate cancer has the lowest level of expression of *DNMT3A/B* (Fig. 2). *GSTP1* is, however, a frequently methylated gene in prostate cancer [95]. Functional epigenetic analyses in prostate cancer cell lines showed the re-expression of genes regulated by promoter hypermethylation after treatment with zebularine or decitabine (IFI6, *GSTP1*) [95–97]. While zebularine failed to achieve the re-expression of *GSTP1*, it effectively reexpressed two glutathione-S-transferase (GST)-detoxifying enzymes (GST-pi and GST-mu) in mice [96, 97]. Another commonly hypermethylated promoter in the *ASC* gene (apoptosis-associated speck-like protein) was demethylated and re-expressed by decitabine and zebularine in five prostate cancer cell lines [98]. Gertych et al. demonstrated that treatment with azacitidine and zebularine changed the DNA topology status in terms of DNA–histone complex decondensation, along with demethylating effects in prostate and liver cancer cells [99]. A study by Kim et al. showed that the overexpression of lysine-specific demethylase 4A (JMJD2A) resulted in the initiation of prostate cancer development in mice mediated by the JMJD2A/ETV1/YAP1 pathway [100]. In further pre-clinical studies, azacitidine demonstrated synergistic effects with docetaxel and cisplatin in androgen receptor (AR)-positive 22RV1 and in AR-negative PC3 cells [101]. Decitabine also exhibited synergy with cisplatin and cyclophosphamide in non-prostate cancer cell lines, although the relationship to induced DNA demethylation was

unclear [102]. Prostate cancer cell lines that were treated with decitabine showed partial demethylation of the *TMS1/ASC* locus (a frequently hypermethylated gene in prostate cancer) and a subset of alleles remained unmethylated for over 3 months while others were remethylated within 1 week [103]. Azacitidine prevented de novo development of cancer in all 14 mice used in a model of transgenic adenocarcinoma of the mouse prostate [104].

Azacitidine and sodium phenylbutyrate failed to induce responses in a phase I trial including five prostate cancer patients, perhaps due to the undetectable DNMT activity before treatment [80]. Another phase I trial evaluating azacitidine and valproic acid, which included two patients with advanced prostate cancer, reported stable disease in one patient [81]. In a phase II trial, subcutaneous azacitidine did not resensitize tumors to androgen-deprivation therapy in 36 patients with progressive castration-resistant prostate cancer (CRPC) [105]. However, the combination of azacitidine with docetaxel was active in metastatic CRPC patients [106]. In a phase I/II study, azacitidine was given daily for 5 days followed by docetaxel on day 6. PSA response was seen in ten of 19 evaluable patients and an objective response was observed in three of ten evaluable patients. Significant demethylation of *GADD45A* was observed with azacitidine treatment [106].

Thus far, the clinical efficacy of azacitidine in prostate cancer trials has only been modest. Possible reasons may be the low level of DNMT3 expression or instability of DNA methylation inhibitors due to their short half-life [107]. Wong et al. provided strong evidence for DNA methylation recovery and found that histone H3K9 trimethylation and H3K27 trimethylation were closely associated with DNA methylation recovery [108]. Overall, the efficacy of DNMTi in prostate cancer treatment is yet to be determined [2].

6.5 Penile Cancer

Several studies summarized by Kuasne et al. attempted to describe epigenetic alterations in penile carcinoma (PeCa). Hypermethylation of the *CDK2A* gene promoter was found to be present in 15–42% of PeCa samples [109]. Feber et al. [110] evaluated the methylation profile of 38 PeCa samples using high-density genome-wide methylation arrays. The authors identified a clear hypermethylation profile associated with the cancer phenotype and identified novel epigenetic signatures associated with human papillomavirus (HPV) infection and loco-regional spread. Interestingly, epigenetic signatures that were predictive of metastases in lymph nodes suggested a lower metastatic potential of hypermethylated tumors. In addition, HPV infection showed significant correlation with DNA methylation. The majority of HPV-positive samples were hypomethylated and showed better clinical outcomes [110]. Distinct methylome and transcriptome patterns were described in a more recent genomewide methylation and transcriptome analysis. Aberrant DNA methylation was linked to the expression of specific genes connected to higher tumor aggressiveness and a shorter duration of survival [111]. These studies suggest a role for DNA methylation in PeCa. Nevertheless, to our knowledge pre-clinical and clinical trials with DNMTis have not been reported.

7 Conclusions

Epigenetic targeting is an exciting new field in cancer research. Pre-clinical efforts to elucidate the underlying mechanisms of treatment resistance have resulted in the initiation of several clinical trials using epigenetic modulation in genitourinary malignancies. Current knowledge does not yet robustly support the incorporation of DNMTis into the treatment of genitourinary cancer. However, DNA methylation appears to be an important mechanism of treatment resistance, which may be overcome by incorporating DNMTis into therapy regimens. Initial studies evaluating older generations of DNMTis have shown insufficient activity due to unfavorable pharmacokinetics. Nevertheless, the discovery of new-generation DNMTis, coupled with a better understanding of their mechanisms of action, have provided the rationale for combination therapies, which may lead to more favorable clinical outcomes. Large clinical studies are needed to provide a better understanding of whether these agents will find a place in the treatment of genitourinary cancer.

Acknowledgments

Funding The preparation of this article was supported by the Slovak Development and Research Agency under contract no. APVV-15-0086 for Michal Chovanec.

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Key points:

Increasing knowledge of the epigenetic landscape in cancer has led to the discovery of promising novel drugs that target hypermethylation of tumor DNA.

Evidence from pre-clinical and clinical studies suggests that DNA methyltransferase inhibitors provide anticancer activity in a number of tumors.

DNA methyltransferase inhibitors are able to overcome resistance to cytotoxic chemotherapies in several genitourinary malignancies.

Fig.1.

Schematic overview of the literature search. Keywords used were “epigenetics”, “DNMT”, “DNMTi”, “genitourinary”, “testicular cancer”, “germ-cell tumors”, “bladder cancer”, “renal cell carcinoma”, “prostate cancer”, “penile cancer”, “azacitidine”, “decitabine”, “guadecitabine”, “zebularine”, “non-nucleoside”. AACR American Association for Cancer Research, ASCO American Society of Clinical Oncology, ASCO GU ASCO Genitourinary Cancers Symposium, GCTs germ cell tumors, RCC renal cell carcinoma

Fig. 2.

The expression of DNA methyltransferase (DNMT) 3A/B in different types of tumors [58, 59]. ACC adrenocortical carcinomas, adeno adenocarcinoma, AML acute myeloid leukemia, ccRCC clear cell renal cell carcinoma, chRCC chromophobe renal cell cancer, DLBCL diffuse large B cell lymphoma, GBM glioblastoma, PCPG pheochromocytoma and paraganglioma, pRCC papillary renal cell carcinoma, squ squamous, TCGA The Cancer Genome Atlas, CS carcinosarcoma

Table 1
Inclusion and exclusion criteria and the selection process for including publications in the review article

Inclusion criteria	Exclusion criteria
1. Pre-clinical in vitro studies evaluating DNMTis in GU tumors including germ-cell tumors, bladder cancer, renal cell carcinoma, prostate cancer, and penile cancer	1. Title including diagnoses, cell lines, and animal models for cancers different than GU (e.g., hematologic malignancies, other solid tumors)
2. Pre-clinical in vivo studies evaluating DNMTis in GU tumors including germ-cell tumors, bladder cancer, renal cell carcinoma, prostate cancer, and penile cancer	2. Abstract including cell lines, animal models, and clinical trials for cancers different than GU
3. Clinical studies evaluating DNMTis in GU tumors including germ-cell tumors, bladder cancer, renal cell carcinoma, prostate cancer, and penile cancer	3. Full-text articles including cell lines, animal models, and clinical trials for cancers other than GU tumors
The selection process of published works was conducted in several steps. First, review of publication titles retrieved from the literature search was performed. If the title met the inclusion criteria, the abstract was reviewed. If abstract met the inclusion criteria, the full-text article was reviewed. If inclusion criteria for a full-text article were met, the article was included in our review manuscript. If a title of general nature was found (e.g., DNMTis in cancer, solid tumors, etc.), the abstract was reviewed. Subsequently, if the abstract did not clearly specify the cancer types, the full-text article was reviewed and included or excluded according to the inclusion/exclusion criteria	

DNMTis DNA methyltransferase inhibitors, GU genitourinary

Table 2
Ongoing clinical studies with DNA methyltransferase inhibitors listed on [ClinicalTrials.gov](https://clinicaltrials.gov)

Study ID	Phase	Treatments	Indication	Planned patients (N)/th	Date started	Planned completion date
NCT02429466	I	SGL-110 plus cisplatin	Relapsed platinum-refractory germ cell tumors	15	April 2015	May 2018
NCT022223052	I	Oral azacitidine	Adult cancer subjects including genitourinary cancers	60	October 2017	January 2018
NCT02788201	I/II	75 approved agents	Advanced urothelial carcinoma	20	20 May 2016	29 June 2019
NCT02961101	I/II	Anti-PD-1 antibody plus decitabine	Relapsed or refractory malignancies including RCC	100	May 2016	May 2020
NCT01799083	I/II	Decitabine alone and/or in combination with chemotherapy and/or cytokine-induced killer cell transfusion	Relapsed or refractory solid tumors and B cell lymphomas	100	December 2012	December 2017
NCT02423057	I	4'-Thio-2'-deoxycytidine	Advanced solid tumors	46	13 April 2015	18 April 2018
NCT00978250	II	5-Fluoro-2'-deoxycytidine with tetrahydrotidine	Solid tumors including urinary bladder neoplasms	165	20 August 2009	27 May 2017
NCT02959437	I/II	Azacitidine plus pembrolizumab and epacadostat	Advanced solid tumors	142	24 January 2017	October 2021
NCT02998567	I	Guadecitabine plus pembrolizumab	Castration-resistant prostatic cancer and non-small cell lung cancer	35	December 2016	November 2018
NCT02030067	I/II	Single-agent RX-3117	Metastatic bladder and pancreatic cancer	72	December 2013	December 2017

PD-1 programmed cell death protein 1, RCC renal cell carcinoma