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TOPIC PAPER

Forty years of cisplatin-based chemotherapy in muscle-invasive bladder cancer: are we understanding how, who and when?

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Abstract

Purpose For 40 years cisplatin-based chemotherapy has been administered to patients with muscle-invasive bladder cancer (MIBC). The best evidence of its efficacy is found in the context of neoadjuvant chemotherapy (NAC). However, the benefit to the patient is modest, with an improvement in 5-year overall survival of only 5–8%. Approximately 60% of patients still have muscle-invasive disease at cystectomy despite NAC. Selecting patients based on the likelihood of response appears to be a promising strategy to improve on this modest benefit. To realize this promise, researchers are investigating biomarkers for identifying responders and non-responders prior to NAC.

Methods In this review, we discuss a number of tissue- and liquid-based biomarkers associated with the response to NAC.

Results and conclusions We elaborate biomarkers at the methylation, DNA, RNA and protein levels and give their current status in clinical trials and/or their implementation in daily clinical practice. In particular, detection of alterations in DNA damage repair pathways as well as molecular subtypes seems to be a promising method for identifying responders to NAC. Furthermore, we illustrate liquid-based biomarkers. Circulating tumor DNA (ctDNA) in patient blood and urine appear to offer an elegant way for biological characterization of MIBC. Recent data show that the presence of ctDNA is limited in patients with localized MIBC being considered for NAC. At this disease stage, ctDNA in patient urine may be more promising for the genomic characterization of MIBC. However, ctDNA in blood or urine has not yet been rigorously investigated in this clinical context.

Keywords Muscle-invasive bladder cancer · Neoadjuvant chemotherapy · Cisplatin resistance · Second-line treatment · Molecular subtypes · Gene expression analysis

Introduction

Forty years ago this year, the FDA approved cisplatin for the systemic treatment of patients with cancers of the testes and the urinary bladder. In patients with testicular cancer, this approval revolutionized treatment outcomes and survival. Response rates of 90% and 100% were observed in stage II and stage I disease, respectively. Unfortunately, for patients with muscle-invasive bladder cancer (MIBC), treatment response and survival rates were only modest [1–3]. The most rigorous data on response to cisplatin in MIBC are available from the neoadjuvant setting. Across several randomized trials, response rates were between 15 and 20% and patient outcomes improved by 5–8% at 5 years [1, 2, 4]. These findings accord with the benefit shown for chemotherapy in the adjuvant setting for other solid tumors like breast cancer or lung cancer. Despite this modest survival benefit, clinical guidelines recommend neoadjuvant cisplatin-based chemotherapy (NAC) for all patients with MIBC [5]. However, approximately 60% of patients receiving NAC still have muscle-invasive disease at cystectomy and are, therefore, non-responders [6]. Moreover, they suffer from unnecessary side effects; cystectomy is delayed for several weeks, during which time their tumors may progress and non-responders may even experience a worsening of prognosis.

Clinicians are faced with an ambiguous situation: Based on the evidence, NAC should be recommended even though a considerable percentage of patients will not benefit from it. There is, therefore, a high unmet need to improve clinical

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decision making, by guiding patients for example towards NAC according to their likelihood of response. Researchers are aware of this dilemma and have recently generated evidence that may facilitate the identification of such patients.

In this review, we discuss mechanisms of resistance to chemotherapeutic agents and biomarkers present in patients' tumors, blood and urine that are associated with the likelihood of response to NAC.

Mechanisms of action of agents used in cisplatin-based regimens

Cisplatin

Cisplatin interferes with DNA replication and destroys proliferating cells [7]. In the human body, chloride is generally replaced by water in a process called "aquation". This process particularly happens intracellularly because the concentration of chloride is even lower compared to the extracellular compartment. Cisplatin has two chloride units which upon entering into a cell seek replacement by water which is achieved by binding to N-heterocyclic bases of the DNA, preferentially guanine. Crosslinking of the DNA can occur, which interferes with cell division by mitosis. This activates apoptosis provided the DNA repair mechanism fails. Several mechanisms have been proposed to explain cisplatin resistance, such as changes in cellular uptake, drug efflux, inhibition of apoptosis, and improved DNA repair. In metastatic bladder cancer patients, initial responsiveness is usually high but rarely durable.

Cisplatin-toxicity

A major obstacle in the use of cisplatin is the persistence of severe side effects. The major dose-limiting effect consists in nephrotoxicity. It is dose dependent and irreversible in some patients and primarily affects the proximal tubules. An estimated 20% of patients receiving high-dose cisplatin develop severe renal dysfunction [8]. Cisplatin nephrotoxicity can present with various types of symptoms such as acute kidney injury (AKI), hypomagnesemia, distal renal tubular acidosis, hypocalcemian, hyperuricemia and renal salt wasting [9].

Renal damage caused by cisplatin is cumulative and can be classified into four types: tubular toxicity (cell death by necrosis or apoptosis), vascular damage (renal vasoconstriction), interstitial injury (damages by inflammatory response) and glomerular injury (damages affecting the glomerular compartments encompassing capillaries, basement membrane, podocytes, mesangial and parietal cells). The cascade that can result in renal damage is caused by the accumulation of potentially toxic compounds in the tubular fluid, which then diffuse into the highly permeable tubular cells.

Active hydration with saline and simultaneous administration of mannitol before, during and after cisplatin administration, significantly reduce cisplatin nephrotoxicity and this strategy has been accepted as the standard of care as a preventive measurement.

Risk factors for acute kidney injury consists of administering higher doses of cisplatin that results in high peak plasma-free platinum concentrations, previous exposure to cisplatin, preexisting kidney damage, and the concomitant use of nephrotoxic agents. In most centers, cisplatin is avoided among patients with an estimated GFR of < 50 mL/min with the exception of situations in which cisplatin has a proven curative role, such as patients with testicular cancer.

Another dose-dependent, cumulative and often irreversible side effect of cisplatin is ototoxicity (neurosensorial hearing loss). Hearing loss at least to some degree may develop with an average of 60% in patients treated with cisplatin [10]. Early toxic effects cause hearing impairment in the high frequencies (6–8 kHz and above), while multiple doses of cisplatin affect the speech frequencies (500–4000 Hz). Risk factors for cisplatin-associated hearing loss consist of preexisting noise-induced-, or age-related hearing impairment, renal failure, concomitant therapy with ototoxic medications (e.g., aminoglycoside antibiotics, loop diuretics), duration and dose schedule of cisplatin infusion. Until today, otoprotective therapies like intratympanic dexamethasone or systemic administration of a phosphorylated aminothiol (amifostine) failed to prove any protective effect against cisplatin-induced hearing loss [10, 11].

The other toxic manifestations of cisplatin are common among antitumor drugs. Since most of these drugs exert their effect by inhibition of DNA synthesis during the cell cycle, normal tissues with a high rate of cellular proliferation will also be affected adversely: bone marrow (myelosuppression), gastrointestinal epithelial cells (e.g., diarrhea), hair follicles (hair loss) and skin (e.g., exfoliative dermatitis).

Gemcitabine

A frequently used combination in the systemic treatment of bladder cancer is cisplatin plus gemcitabine (GC). Gemcitabine is an antimetabolite and administered as a prodrug [12]. In human cells, gemcitabine is activated by phosphorylation. Subsequently, it is used for DNA synthesis instead of cytidine, thus interrupting DNA synthesis, blocking the progression of the cell cycle in the G1/S-phase and initiating apoptosis. Mechanisms of resistance to gemcitabine are best studied in pancreatic cancer and can be caused by drug transporters, the activating and inactivating of enzymes, and competitive substrates to active metabolites.

Methotrexate

Methotrexate is used in combination with vinblastine, adriamycin and cisplatin (MVAC) in the second standard regimen after GC. Like gemcitabine, methotrexate is an antimetabolite and appears to have two different mechanisms of action. It inhibits the enzyme dihydrofolate reductase that catalyzes the conversion of dihydrofolate to tetrahydrofolate. This active folic acid is needed for the DNA synthesis that is essential for purine and pyrimidine synthesis. Therefore, methotrexate adversely interferes with DNA, RNA and protein synthesis. This pathway is especially important for the treatment of cancer. The use of methotrexate to treat rheumatoid arthritis is based on its inhibition of enzymes of the purine metabolism. This inhibits T cell activation and causes selective downregulation of B cells and other activities relevant to immune system function such as an inhibition of interleukin 1β. Although not examined yet, the interaction of methotrexate with the immune system might be interesting in view to the recent successful treatment of bladder cancer patients with checkpoint inhibitors.

Vinblastine

Vinblastine is used as part of the MVAC regimen. It inhibits mitosis by binding to the protein tubulin. Consequently, microtubules responsible for the separation of the two chromosomal pairs are not generated, resulting in a blockade of mitosis. Similar to the other agents described here, it is thought to mainly affect fast proliferating cells. Although only limited research has been done, upregulation of multidrug resistance mechanisms such as overexpression of MDR1 is thought to be the initial step in resistance to vinblastine [13].

Adriamycin

Adriamycin (also called doxorubicin) is an anthracycline that blocks the enzyme topoisomerase 2. This enzyme modifies the topology of DNA by cutting the double strands of the DNA, unwinding its coils and splicing the DNA back together. Underwinding and overwinding of DNA happen during all processes where DNA movement is required such as replication and protein synthesis. Consequently, adriamycin is most effective in fast proliferating cells and cells with a high protein turnover. The glutathione-*S*-transferase pathway has been linked to resistance to adriamycin in breast cancer cell lines [14]. However, no data exist with regard to bladder cancer.

In sum, different combinations of chemotherapeutic agents plus cisplatin are recommended for NAC in MIBC; all the agents are primarily targeting fast proliferating cells, DNA replication and protein synthesis. While some knowledge exists about in vitro resistance to individual agents, more recent data have revealed mechanisms of tumor sensitivity and resistance.

Investigations in the primary tumor and relation to responsiveness

Epigenetic biomarkers

Using an in vitro drug screen, analysis of bladder cancer cell lines and patient tumors, Xylinas et al. [15] found that higher methylation levels of the CpG islands in the HOXA9 promoter are associated with cisplatin resistance. Manipulation of epigenetics with DNA methylation inhibitors could recover drug sensitivity in cisplatin-resistant cell lines. With a similar approach, Shindo et al. [16] were able to associate the epigenetic downregulation of miR-200b with cisplatin resistance and to rescue this phenomenon using demethylating agents. Loss of the miR-200 family is also known to promote epithelial-to-mesenchymal transition (EMT) [17]. Interestingly, this finding is in line with recent gene expression data (see "Gene expression analysis").

Genomic DNA analysis

Several groups have investigated genomic DNA analysis as a method for assessing the response to NAC. Van Allen et al. [18] reported that truncating mutations in excision repair cross-complementation group 2 (ERCC2), a protein that is heavily involved in DNA damage repair (DDR), are associated with increased sensitivity to NAC. They could not only confirm these findings in vitro but also validate them in an independent cohort [19]. Although the difference was not significant, in the dataset of Groenendijk et al. [20] ERCC2 mutations were more frequent in responders; in their dataset, ERBB2 mutations were only present in complete responders, a finding that could not be validated by others [21, 22]. Plimack et al. [21] discovered and validated mutations in panels of genes (ATM, FANCC and RB1) that predicted response to NAC. ATM is a DDR gene, a cell cycle checkpoint that is required for the cell to respond to DNA damage. FANCC mutations are present in less than 3% of MIBC [23], FANCC is implicated in interstrand DNA cross-link repair, a type of DNA damage caused by cisplatin. A loss of RB1 function is not directly related to DDR. RB1 is a negative regulator of the cell cycle and its loss results in fast proliferating tumors, probably increasing their susceptibility to NAC (see "Mechanisms of action of agents used in cisplatin-based regimens"). The relation between deficient DDR and sensitivity to NAC is underscored by the most recent publication of Iyer et al. [24]. In their prospective Phase II trial investigating neoadjuvant dose-dense GC, deleterious

DDR alterations yielded a positive predictive value of 89% for treatment response; none of the patients with an alteration in one member of this gene panel showed cancer recurrence. Currently, disruption of DDR seems to be one of the most promising biomarkers predicting response to NAC. It must be borne in mind, however, that in the same trial of Iyer et al. most responders (14/21) did not harbor deleterious DDR gene alterations, suggesting that additional factors affect chemosensitivity.

Gene expression analysis

In addition to investigation of individual genes, the discovery and clinical investigation of molecular subtypes have gained increasing interest with regard to responsiveness to NAC. Choi et al. [25] were the first to mention the concept of relating molecular subtypes to NAC response and identified "p53-like" tumors that are associated with resistance to NAC. In a subsequent analysis, the same group identified that although the pathologic response to NAC was comparable between subtypes, MIBC presenting a basal-like phenotype had the best prognosis [26]. Importantly, basal-like MIBC was identified as having the worst prognosis in cystectomy only series [23, 25, 27, 28]. This hypothesis is solidified by a direct comparison between MIBC patients receiving NAC followed by cystectomy and MIBC patients undergoing cystectomy alone [29]. Patients with basal-like MIBC showed a dramatic improvement in survival when treated with NAC. The rapid proliferation of basal-like MIBC and, therefore, their particular susceptibility to NAC may explain this repeated observation. However, pathological downstaging was not related to molecular subtypes [26, 29]. It is noteworthy that in line with findings on epigenetic biomarkers described in "Epigenetic biomarkers" of this review, in the dataset of MD Anderson [25, 26] and in our own dataset [29] the outcome of MIBC with high expression of EMT markers was worst in patients treated with NAC.

The first single-sample classifier to determine the molecular subtype of an individual patient tumor was published in 2017 [29]; new and better classifiers will certainly follow. Importantly, these classifiers are not yet ready for prime time because prospective clinical validation is lacking. Nevertheless, from the scientific perspective molecular subtypes definitely merit being taken into account when thinking about responsiveness to treatment and thus should be tested in clinical trials.

Protein expression analysis

Several groups have used immunohistochemistry (IHC) to identify biomarkers related to NAC response [30–33]. Despite being cheap, fast and universally available, determination of protein expression by IHC harbors many limitations that prevent clinical implementation of these biomarkers. Nevertheless, the Lund group was pioneers in establishing protocols and strategies to determine molecular subtypes using IHC [34, 35]. There is good reason to believe that IHC will also identify that patients with basal-like MIBC are more likely to benefit from NAC. However, this hypothesis remains to be validated in prospective clinical trials.

Histomorphology of the primary tumor

Our assumption suggests that the faster proliferating MIBC is more susceptible to NAC. Indeed, in a small analysis we could show that patients with a higher proliferation rate show more regressive changes at cystectomy [36]. However, this relation could not be confirmed by Shen et al. [37].

In a secondary analysis of SWOG8710 (a prospective randomized trial that showed a survival benefit of 5–7% at 5 years for MICB patients receiving NAC prior to cystectomy), mixed histological features were investigated [38]. MICB patients with a non-urothelial component (mixed), urothelial carcinoma with either squamous or adenocarcinoma variants showed a superior survival benefit. Interestingly, urothelial MIBC with squamous differentiation frequently shows a basal-like phenotype [25] (see "Protein expression analysis").

The recommendations for NAC in clinical guidelines are based on trials that did not include histologies other than squamous and adenomatous differentiation. However, a total of 13 histological variants have been described. Several authors recommend administering NAC in patients with the micropapillary variant [39, 40], a suggestion, however, that is based on retrospective observations and not on biological investigations. Only the high aggressiveness of this variant was used to support this conclusion. Vetterlein et al. [41] drew a similar conclusion that the presence of a variant histology, such as a micropapillary, sarcomatoid or adenocarcinoma histology, is more likely to benefit from NAC. However, this retrospective database analysis lacks a central pathologic review and does not allow for clinical implementation. Ultimately, a robust evaluation of variant histology in bladder cancer and their responsiveness to NAC remains to be published.

Analysis of blood and urine to assess response to chemotherapy in MIBC

To date, information regarding putative predictive biomarkers for NAC has been gained mainly from studying the TUR-biopsy of the primary tumor with subsequent molecular or histological analysis (see previous sections). However, the heterogenous clinical course of bladder cancer is also

reflected in its biological heterogeneity, not only inter-individually but also intra-individually. This heterogeneity precludes the use of a one-size fits all strategy not only in terms of treatment but also in terms of tissue investigation. Studying only a single biopsy from a single tumor site is likely to miss important information.

More recent reports indicate that analysis of circulating tumor DNA (ctDNA) in a patient's blood or urine may be a method for a more comprehensive genomic characterization of this heterogeneous disease. In addition, the simple extraction enables repeated sampling during the course of NAC and may, therefore, allow for monitoring of treatment response without invasive re-biopsies. Nevertheless, these new approaches have to deal with several limitations (e.g., only a proportion of patients provides enough ctDNA for genomic analysis). Importantly, moreover, the proportion of the ctDNA fraction originating from micrometastases is thought to be low compared to the total ctDNA in patients with localized MIBC who are guided towards NAC to target the micrometastatic compartment. Besides NAC, several studies show that the targeting of specific mutations (e.g., FGFR3 mutations, TSC1 somatic mutations) can achieve impressive responses in metastatic bladder cancer patients [42]. These specific mutations, however, are rare and generalizations cannot be made until prospective trial data for patient selection are available.

Despite several methodological constraints, the analysis of tumor-associated genomic alterations in ctDNA is rapidly developing as a promising platform for biomarker discovery in cancer patients. Advances in digital droplet polymerase chain reaction and highly sensitive next-generation sequencing (NGS) allow capture and analysis of ctDNA even when highly diluted with non-malignant cell-free DNA [43, 44]. For MIBC, only very few studies have investigated the presence of ctDNA in the plasma and/or urine, especially in the neoadjuvant setting.

Vandekerkhove et al. [45] used a targeted sequencing strategy to capture the exonic regions of a panel of bladder cancer-relevant genes in 51 patients. Fourteen patients had localized, 37 patients metastatic (nodal and/or distant) MIBC. While two-thirds of metastatic patients had ctDNA proportions above 2% of total ctDNA, only 14% of patients with localized disease provided enough ctDNA for analysis. Across the 26 patients with quantifiable ctDNA, the authors detected 281 somatic mutations including 121 protein-altering mutations. The most common detected mutations affected the TP53 gene (65%) and disrupting rearrangements in chromatin modifier genes (73%) [including truncating mutations within ARID1A and in KMT2D (MLL2)]. Furthermore, alterations to the PI3K/mTOR pathway, including hotspot missense mutations in PIK3CA (23%), truncating mutations in TSC1 (15%, mutually exclusive with PIK3CA hotspot mutations), PIK3R1 stop-gain mutations (4%), PTEN stop-gain mutations (4%), and TSC2 truncating rearrangements (4%) could be verified. Alterations in MAPK signaling, including activating ERBB2 and RAS mutations, were detected in 62%. All in all, they reported on a wide spectrum of somatic and potentially actionable alterations.

Interestingly, they collected plasma before, during, and after platinum-based chemotherapy in three patients with metastatic and one with localized MIBC. Although the sample size was limited and the abundance of ctDNA particularly in patients with localized MIBC was low, they showed that patient-unique somatic mutations can be detected during treatment. They also demonstrated the feasibility of analyzing ctDNA in the plasma of patients with both localized and metastatic MIBC. With their approach, putative actionable mutations could be detected but no attempt was made to correlate a specific mutation with a response to systemic treatment.

In a second report, Patel et al. [46] analyzed 248 liquid biopsy samples including plasma, urinary cell pellets, and urinary supernatants of spun urine from 17 MIBC patients treated with NAC. Interestingly, they extended the analysis to patient's urine which may improve overall detection of DNA mutation, particularly in patients with localized MIBC undergoing NAC. Indeed, DNA alterations were more frequently detected in urine (both urinary cell pellet [27.5%] and urinary supernatant USN [34.5%]) than in plasma samples (9.9%). Unfortunately, the detection of mutated ctDNA in pre-NAC samples (plasma, urine cell pellet, urine supernatant) did not correlate with the early response to NAC. However, the persistence of mutated ctDNA in peripheral samples taken just prior to the 2nd NAC cycle constituted a strong predictor of recurrence (100% positive predictive value and 85.7% negative predictive value). In addition, of the single nucleotide variants (SNVs) detected in the pre-NAC TUR specimens, one or more SNVs could also be detected in pre-NAC plasma (30.8%), urine cell pellets (46.2%), and urine supernatants (46.2%).

All in all, ctDNA seems to be a promising technique for non-invasive biomarker assessment in patients with MIBC. While sampling of patient blood allows for monitoring of patients with metastatic MIBC, sampling of urine appears to be more promising in patients with localized MIBC and, therefore, also for monitoring of treatment response to NAC. Although the data presented here is encouraging, more rigorous investigations in the context of NAC as well as larger datasets are definitely required before clinical implementation.

Discussion

The modest 5–8% benefit in overall survival from cisplatin-based NAC corresponds to a number needed to treat of about 15, which indicates that very few patients benefit from

cisplatin-based NAC [1, 2, 47]. There are, however, currently no prospectively verified predictive biomarkers of response to NAC in MIBC, though some studies have shown initial promise using gene expression, mutation status of DNA repair genes or detecting proteins identified by gene expression analysis [15, 29, 33, 38]. In addition to evaluating transurethral resection of bladder cancer specimens, the non-invasive liquid biopsy approach analyzing ctDNA in plasma and/or urine could represent a valuable complement: repeated genomic evaluation during the NAC treatment phase is feasible and could guide treatment decisions towards a more personalized approach in non-responders to conventional NAC [45, 46].

Molecular stratification will be critical for optimal use of NAC in the future. Even if we could one day reliably select for patients who will benefit most from neoadjuvant cisplatin-based chemotherapy, the question would remain which systemic treatment options could be offered to non-responders who are still at increased risk of recurrence after definitive local treatment. Such a selection could be based, e.g., on the cancer genome atlas (TCGA) which elucidates putatively targetable recurrent mutations in genes affecting several key pathways of tumorigenesis [23]. New targeted treatment approaches in bladder cancer (anti-nectin-4 antibody drug conjugates, pan-FGFR-receptor inhibitors, PI3K-/mTOR pathway inhibitors) as well as a new class of immunotherapeutics, so-called checkpoint inhibitors (anti-CTLA4; anti-PD-L1/anti-PD1) have entered the arena and will in future increase treatment opportunities. As ever more basic and clinical data emerge from ongoing trials, it is just a matter of time until molecular profiling will allow selection of the best systemic treatment for the individual patient.

Practical molecular stratification of patient tumors will be critical to guiding the use of emerging targeted therapies and immunotherapies not only for metastatic bladder cancer but also for localized MIBC.

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Compliance with ethical standards

Conflict of interest The authors have no direct or indirect commercial financial incentive associated with publishing this review article.

Experimental protocol and ethics For this review, no tissue was analyzed or patient data collected. Therefore, no ethical approval was obtained.

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