Review

Photodynamic Diagnosis and Therapy in Non-Muscle Invasive Bladder Cancer

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Review

Photodynamic Diagnosis and Therapy in Non-Muscle Invasive Bladder Cancer

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Simple Summary: Bladder cancer (BC) possesses distinct molecular profiles that influence progression depending on its biological nature and delivered treatment intensity. Muscle-invasive BC (MIBC) and non-MIBC (NMIBC) demonstrate great intrinsic heterogeneity regarding different prognoses, survival, progression, and treatment outcomes. Transurethral resection of bladder tumor (TURBT) is the standard treatment for NMIBC. The high risks of disease recurrence from residual tumor and progression after TURBT in NMIBC are well known. A new-generation photosensitizer, 5-Aminolevulinic acid (5-ALA), with high tumor specificity, has been studied for detecting precise tumor areas. Moreover, it has been applied for treatment by producing its cytotoxic reactive oxygen species, as well as screening for urological carcinomas by excreting porphyrin in the blood and urine. Thus, 5-ALA may contribute to the inclusive treatment of NMIBC.

Abstract: Bladder cancer (BC) possesses distinct molecular profiles that influence progression depending on its biological nature and delivered treatment intensity. Muscle-invasive BC (MIBC) and non-MIBC (NMIBC) demonstrate great intrinsic heterogeneity regarding different prognoses, survival, progression, and treatment outcomes. Transurethral resection of bladder tumor (TURBT) is the standard of care in treating NMIBC and serves both diagnostic and therapeutic purposes, despite the prevalent recurrence and progression among many patients. In particular, flat urothelial carcinoma in situ and urothelial carcinoma with lamina propria invasion are the major precursors of MIBC. A new-generation photosensitizer, 5-Aminolevulinic acid (5-ALA), demonstrates high tumor specificity by illuminating the tumor lesion with a specific wavelength of light to produce fluorescence, and has been studied for photodynamic diagnosis to detect precise tumor areas by TURBT. Additionally, it has been applied for treatment by producing its cytotoxic reactive oxygen species, as well as screening for urological carcinomas by excreting porphyrin in the blood and urine. Moreover, 5-ALA may contribute to screening before and after TURBT in NMIBC. Here, we summarize the updated evidence and ongoing research on photodynamic technology for NMIBC, providing insight into the potential for improving patient outcomes.

Keywords: non-muscle invasive bladder cancer; transurethral resection of bladder tumor; 5-Aminolevulinic acid; photodynamic diagnosis; photodynamic therapy

1. Introduction

Bladder cancer (BC) is one of the prevalent tumors that cause health issues globally [1,2]. Cigarette smoking is the most prominent risk factor for developing BC in most countries [3,4]. Overall, BC, with urothelial carcinoma (UC) as the prevalent histology, is comprised of two distinct molecular subtypes based on each molecular heterogeneity and clinical staging: muscle-invasive BC

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(MIBC), including stages T2 (muscularis propria invasion), T3 (perivesical fat invasion), and T4 (adjacent organ involvement), and non-MIBS (NMIBC), including stages Tis (flat urothelial carcinoma in situ [CIS]), Ta (NMIBC occurs as papillary lesion), and T1 (NMIBC with lamina propria invasion), consisting of 80% BC. NMIBC region of clonally related bladder tumors is frequently treatable [5]. The 5-year survival rate for NMIBC demonstrated no marked changes despite advancements in research and care, and the frequent NMIBC recurrence has burdened public health systems [6–8].

Understanding molecular principles of NMIBC tumorigenesis is crucial in developing more effective preventive measures. The sequencing and gene expression studies have guided the discovery of numerous genetic alterations and revealed several distinct molecular signatures and subtypes, thereby providing a more accurate prediction of disease progression in BC.

Transurethral resection of bladder tumor (TURBT) demonstrates both diagnostic and therapeutic functions in effective NMIBC management. TURBT, followed by intravesical Bacillus Calmette–Guérin (BCG) administration, is the standard of care in flat-lesion CIS, despite prevalent recurrence and progression among many patients. The use of photodynamic technology for future management of challenging cases enables the safe and effective performance of TURBT. CIS is usually difficult to detect and differentiate from inflammatory lesions under the cystoscopic examination [9]. Advanced cystoscopy technologies, such as narrow-band imaging, photodynamic diagnosis, and image 1S, are required to improve BC detection. Novel techniques significantly improve the precision of transurethral surgery and lower the risk of complications.

This review aims to summarize the current evidence and recent developments in the molecular and translational aspects of BC biology and discuss their current or potential future clinical applications in managing NMIBC.

2. Common Genetic Alterations in NMIBC

Deletions of chromosome 9, frequently found in NMIBC, include the CDKN2A locus (9p21) that encodes p16 and p14ARF, which are RB and p53 pathway regulators, respectively. Loss of TSC1 (9q34), which is an mTOR signaling regulator, associated with upregulated expression of mTOR targets [10], such as telomerase reverse transcriptase (TERT) activity, has been observed in NMIBC. Overexpressed TERT upregulates oncogenic signaling pathways [11], which are crucial in maintaining tumor immortality and contribute to tumor progression in BC, to maintain telomere integrity [12–15]. The promoter mutations of TERT are chief genetic alterations occurring with a frequency of 70%–80% in patients with BC [16–19]. Other copy number alterations in NMIBC (8%–22%), particularly in stage T1 tumors, include gains of 1q, 5p, 18q, 20p, and 20q and losses of 8p, 11p, 17p, and 18q [20], which are more commonly detected in MIBC [21].

FGFR3 point mutations (S249C) were predicted to result from Apolipoprotein B mRNA Editing Catalytic Polypeptide-like(APOBEC) activity [22] as an early event is the most prevalent genetic alteration associated with NMIBC low tumor grade and stage [10,23–26]. APOBEC targets PIK3CA mutations that are present in <30% of NMIBC [27]. RAS or FGFR3 mutation, found in Ta urothelial carcinoma (UC), are mutually exclusive and associated with low tumor grade and stage [10,27]. These data indicate that both PI3K and RAS–MAPK signaling pathways are usually activated in NMBC. ERBB2 and ERBB3 mutations that provide PI3K activation [28] are detected in <15% of T1 tumors. Micropapillary and plasmacytoid UCs are biologically aggressive subtypes [5]; Micropapillary, UC has a disproportionately higher rate of ERBB2 amplification than conventional UC [29–31], and plasmacytoid UC is defined by distinct CDH1 mutations [32]. Inactivating mutations and loss of expression are observed in <30% of low-grade Ta, but in fewer in T1 tumors, and often with FGFR3, PIK3CA, and/or KDM6A (chromatin regulator) mutations [27,33,34]. The exact roles of FGFR3 mutations in NMIBC tumorigenesis remain unknown, but mutant FGFR3 induces overgrowth of the cultured normal human urothelial cells at a confluence, indicating a potential contribution to urothelial hyperplasia in vivo [35].

Moreover, BC demonstrates significant epigenetic dysregulation such as changes in DNA methylation [36]. Identifying frequent mutations and epigenetic modifications may benefit novel

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therapeutic approaches, as well as urine and blood surveillance for both early detection and disease monitoring after treatments.

3. Tumorigenesis Associated with NMIBC

Relative to treating NMIBC, Ta tumors above all have demonstrated good-prognosis compared to T1 and Tis tumors, and their treatment plan is markedly different [1]. The use of tissue to predict progression from T1to MIBC has been a subject of interest for some time. The American Joint Committee on Cancer (AJCC) indicated that T1 UCs showed that tumor in the lamina propria related to a higher rate of the progress based on a large number of studies [37]. Leivo et al. adopted a cutoff of ≥2.3 mm as the best predictor of muscle-invasive disease progression, acknowledging that additional nonhistopathologic methods may be required to increase broad applicability and further reduce the false-positive threshold [38]. Additional molecular biological approaches (e.g., molecular and/or protein biomarkers) to predicting MIBC progression have been assessed in numerous studies [39,40]. These studies indicated that improved sequencing techniques have contributed to the understanding of BC as a heterogeneous disease and enabled the development of several biomarkers with the potential to help predict treatment response and appropriate patient selection.

T1 tumors frequently share molecular characteristics with MIBC, and these tumors usually differ substantially from low-grade Ta tumors [41–43]. High-grade UCs are either NMIBC or MIBC, of which T1 UCs prevalently progress to MIBC, requiring more aggressive clinical treatment and follow-up. No obligate pathway exists from NMIBC to MIBC, and these tumor categories have largely non-overlapping pathogenesis pathways associated with different genetic features [44,45]. Histopathological and molecular data indicate the CIS as the major MIBC precursor, whereas most papillary NMIBC originate from normal-appearing urothelium. However, some patients with NMIBC, particularly those with tumors that invade the lamina propria, demonstrate progression from initially non-invasive to invasive disease. Figure 1 summarizes the correlation between pathological findings of NMIBC and the progress to MIBC.

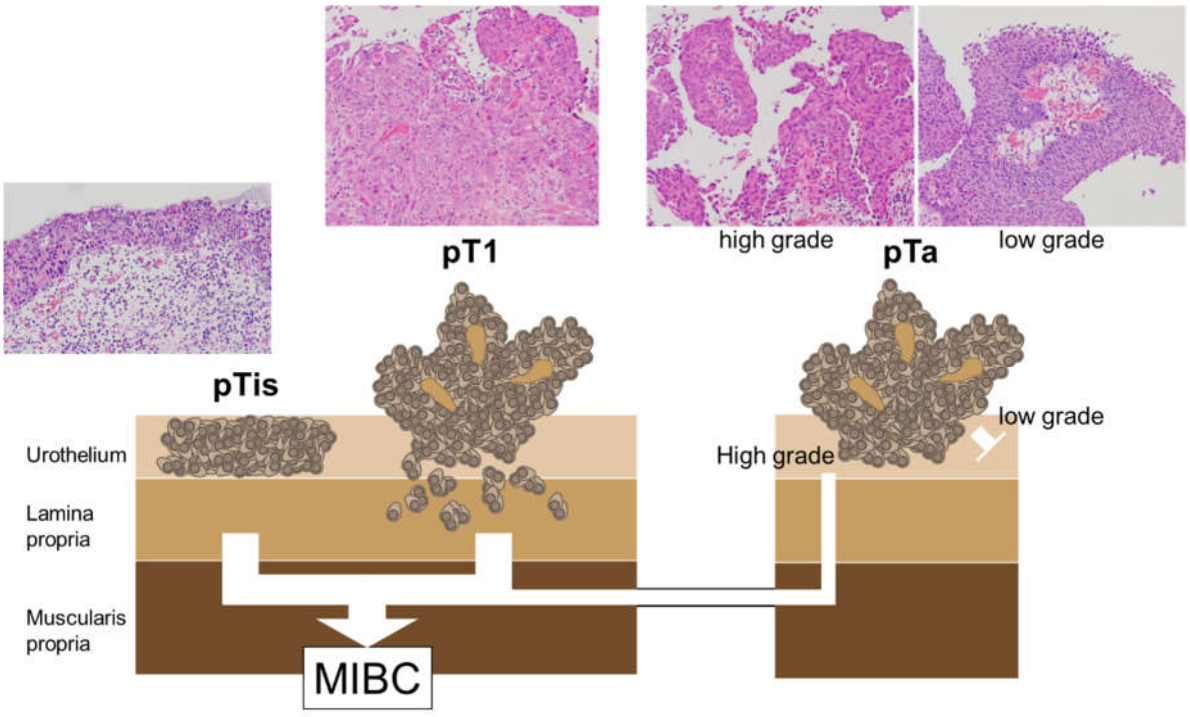

Figure 1. The correlation between pathological findings and MIBC progression in NMIBC. The CIS and pT1 UCs are the major MIBC precursors. pTis: flat urothelial CIS; pT1a: non-invasive papillary lesion; pT1: UCs with invasion of the lamina propria.

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4. Pathological Diagnosis and Screening of NMIBC

4.1. Urine-based Diagnosis

A prospective observational study revealed that approximately 75% of patients with BC present with non-symptomatic macrohematuria, with increasing incidence alongside aging [46,47]. Diagnostic evaluation of patients with hematuria should involve a response to urine-based examination. Urine cytology has been credited as an easy, safe, and inexpensive test with high specificity for diagnosing UC [48]. The Paris System for Reporting Urinary Cytology published in 2016 is utilized as a standard classification system in urine cytology specimens [49]. The sensitivity of this analysis is not best, but its specificity is high, especially for high-grade UC; thus, urine cytology remains the well-established technique in BC diagnosis compared with marker-based studies, such as protein- and molecular-based urine tests [49–55].

4.2. Blood-based Diagnosis

Circulating cell-free DNA is considered as one of the major breakthroughs in the field of innovative diagnosis, used as a liquid biopsy. Cell-free DNA with tumor-specific alterations is derived from dying (i.e., apoptotic) cells and released into the blood flow (Circulating tumor DNA: ctDNA) [56]. The half-life of ctDNA is ~2 h [57]. It is useful for real-time tracking of tumor burden postoperatively and during oncological treatment. Analysis of ctDNA revealed promising results for early risk stratification of patients, prediction of treatment response, and early detection of metastatic relapse in BC. [58].

4.3. Cystoscopic Diagnosis

Cystoscopy is considered the gold standard for diagnosing BC. White-light (WL) imaging cystoscopy is the conventional method to detect UC but may overlook small and flat lesions, such as CIS. If a lesion is seen on cystoscopy, this is followed by examination under anesthesia at the time of TURBT, although in spite of this thorough diagnostic and staging process, discrepancy between clinical and pathologic stage is a common finding [59]. Pathological examination of patients includes urinary cytology and biopsy analysis or TURBT samples of visible lesions.

4.4. Tissue-based Diagnosis

Pathological analysis of tissue samples by bladder biopsy or TURBT during cystoscopy is the most prevalent and useful procedure in initially diagnosing the presence of cancer, histological type, and stage. BC is categorized by grade into low-grade and high-grade [5]. UC is the most predominant histological subtype of all BC, with the remainder of minor subtypes including squamous cell carcinoma, adenocarcinoma, and neuroendocrine carcinoma [5,60]. UC occurred as a broad array of variants or subtypes. The World Health Organization Classification of Tumors of the Urinary System and Male Genital Organs defined the several subtypes of UC categories, such as micropapillary, plasmacytoid, nested, and lymphoepithelioma-like carcinomas, which have been associated with unique molecular and/or therapeutic considerations [5,29–32,61,62]. The UROMOL study categorized NMIBC into classes 1 (luminal-like signature), 2 (luminal-like, epithelial–mesenchymal transition [EMT] and cancer stem cell signatures), and 3 (basal-like signature) [63]. Class 2 tumors are associated with poor prognosis compared with class 1 or 3 tumors [63]. Pathological diagnosis identifies the tumor staging based on the AJCC, currently in its eighth edition [37]. NMIBC occurs as either papillary (pTa) or flat urothelial CIS (pTis). Cancer patients with similar prognosis are grouped by using prognostic stage tables. Lamina propria invasion (pT1), muscularis propria invasion (pT2), perivesical fat (pT3), and adjacent organ involvement (pT4) are associated with a progressive reduction in survival [37].

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5. Management of NMIBC

5.1. TURBT and en bloc Resection

Determining pathological diagnosis and staging on TURBT samples is challenging due to the extent of sampling, interpretation artifact caused by cautery or crush degeneration, and lack of objective markers to conclusively determine the presence of muscularis propria. En block resection of Bladder tumor (ERBT) is removal technique of BC in one piece. Dyrskjøt et al. reviewed results from three randomized trial comparing ERBT and TURBT in ≤ 3cm BT as follows [64]. At first, Gellioli et al. reported that the rate of detrusor muscle presence for ERBT was noninferior to that for TURBT (94% vs 95%; n = 248), and T1 substaging was feasible in 80% of TURBT cases versus 100% of ERBT cases (p = 0.02) [65]. In the second trial, D'Andrea et al. reported that ERBT was superior to TURBT in retrieval of detrusor muscle (80.7% vs 71.1%; p = 0.01; n = 452) [66]. Finally, Teoh et al. reported ERBT reduced the 1-year recurrence rates and progression rate compared to TURBT (28.5 % vs 38.1%; p = 0.007 and 0% vs 2.6%; 0.065, respectively; n = 276) [67].

Furthermore, intravesical chemotherapy (CT), along with transurethral resection of the bladder, is quite effective in reducing disease recurrence [68,69].

Either way, increasing the detection rate of tumors may contribute the better prognosis in BC by these procedure.

6. Photodynamic Diagnosis, Photodynamic Therapy, and Photodynamic Screening with 5-aminolaevulinic Acid

CIS is difficult to detect and differentiate from inflammation. The prognosis of the patients is improved if the expanse of the lesion in the BC, especially in T1 UC, and the flat lesion (CIS), which is the major precursor of MIBC, can be detected and resected exactly. Photodynamic diagnosis (PDD) is a technique that improves the detection of occult bladder tumors during cystoscopy. Photodynamic therapy (PDT) for BC involves either systemic infusion or bladder instillation of a photosensitizer, which undergoes a photochemical reaction when activated by visible light of an appropriate wavelength to produce reactive and singlet oxygen species in situ that are frequently lethal. The amino acid, 5-aminolaevulinic acid (5-ALA), is a new-generation photosensitizer with high tumor specificity.

Generally, 5-ALA is generated in plants and animals from glycine and succinyl CoA. The cytoplasmic 5-ALA precursor is transported to the mitochondria via the ATP-binding cassette (ABC) subfamily B member 6 to produce fluorescent endogenous porphyrins, including protoporphyrin IX (PpIX). Ferrochelatase catalyzes ferrous iron insertion into PpIX to form heme and bilirubin. Ferrochelatase contributes to PpIX catabolism in addition to heme production. However, ferrochelatase is inactive in various cancer types, including BC. Thus, PpIX accumulates more in tumor cells than in healthy cells due to decreased ferrochelatase activity [70–72]. The activation of the 5-ALA synthetic enzyme and 5-ALA influx transporter (peptide transporter 1) promotes PpIX production in tumor cells [3]. Additionally, ABC superfamily G member 2, which excretes PpIX, is inactivated in tumor cells, thereby downregulating PpIX excretion from cells [72–74].

PpIX possesses photoactivity and emits red fluorescent light of 600–700 nm when excited by light irradiation at a specific wavelength, mainly visible blue light (375–445 nm). Thus, cancer cells were accurately identified via PDD by detecting PpIX fluorescence using ALA. PpIX is excited at low excitation wavelengths in the red (600–740 nm) and green (450–580 nm) visible ranges. PpIX returns from the excited state to the ground state after absorbing the light, while releasing energy, which produces cytotoxic reactive oxygen species (ROS) in tumor cells. ROS damages mitochondria and induces apoptosis of tumor cells, causing cell death. This mechanism emphasizes the effects of PDT with ALA [72].

PpIX accumulates in the mitochondria in the cancer cells, as mentioned above. This excessive PpIX accumulation causes saturation of porphyrin precursors of PpIX (i.e., uroporphyrin and coproporphyrin) and promotes the excretion of these precursors of PpIX in blood and urine. Thus, their amounts in the urine and blood may be increased, particularly in patients with cancer.

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Photodynamic screening (PDS) aims to measure the amount of porphyrin excreted in urine after oral 5-ALA administration [75]. Figure 2 shows the mechanisms of ALA-PDD, ALA-PDT, and ALA-PDS.

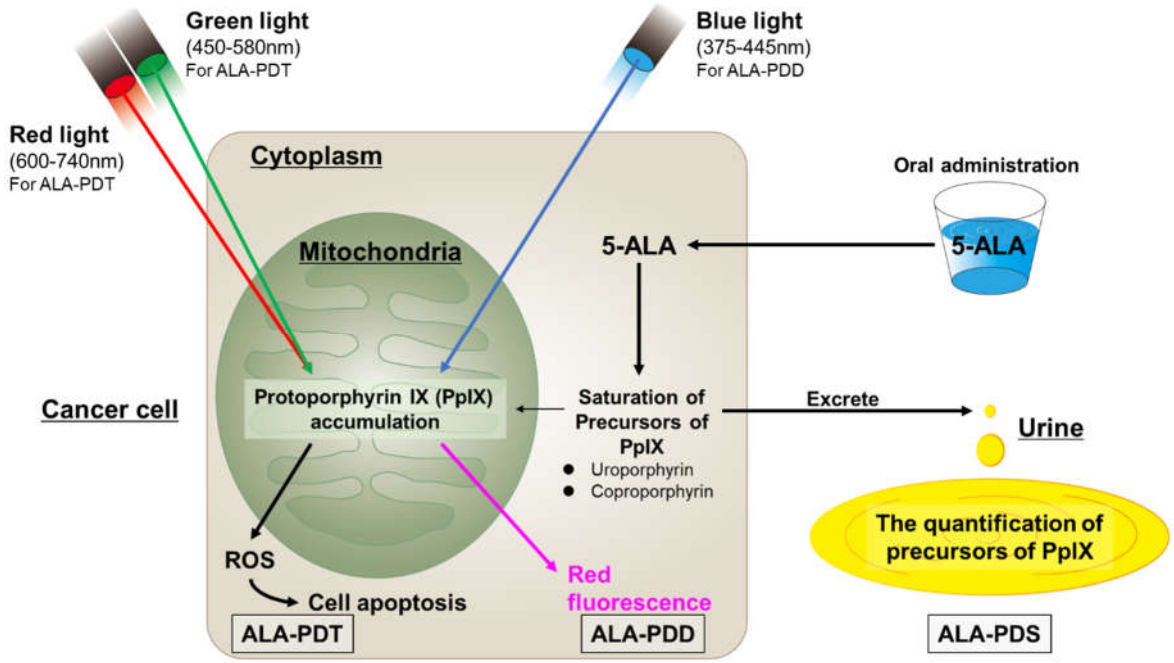

Figure 2. The mechanisms of ALA-PDD, ALA-PDT, and ALA-PDS.

The 5-ALA is administered via oral and transurethral routes. Japan was the first country to approve oral 5-ALA administration as diagnostic agents. The clinical treatment of NMIBC by the Pharmaceuticals and Medical Devices Agency approved ALAGRIO® which was covered by insurance in 2017. ALAGRIO® was approved for visualizing NMIBC during TURBT. The recommended dose of ALA is 20 mg/kg body weight, and ALA-PDD should be initiated approximately 3 h (range: 2–4 h) after oral ALA administration. ALAGRIO® was used in approximately 13,000 cases across approximately 370 institutions in the first 3 years after approval [72].

Transient adverse events for oral ALA include nausea, vomiting, photosensitivity, hypotension, and liver enzyme dysfunction. Our group revealed that among the 76 patients included, 7 (9.2%) experienced hypotension (systolic blood pressure (SBP) of <80 mmHg or mean arterial pressure of <60 mmHg), with a median onset time of 9 (range: 3–28) min after inducing anesthesia [76]. Severe hypotension that requires intensive care management after ALAGRIO® administration has been reported in Japan [77–79]. Our group revealed that the multiple factors in the prediction model for ALA (ALAGRIO®)-induced hypotension using the detection tree analysis include the following. First, general anesthesia, age of ≥74 years, and American Society of Anesthesiologists physical status of ≥2; second, SBG of ≤115 mmHg at the beginning of anesthesia under spinal anesthesia; and third, SBP of ≥115 mmHg at the beginning of spinal anesthesia and an estimated glomerular filtration rate of ≤42 ml/min/1.73 cm^2 [79]. Liver dysfunction in response to ALAGRIO® was transient and gradually normalized despite no treatment [80].

Adding PDD and narrow-band imaging (NBI) to WL increases the cancer detection rate for flat lesions, which are difficult to detect with conventional WL [81–84]. A systematic review by Chen et al. indicated that NBI is a more sensitive diagnostic intervention for patients with NMIBC compared with either 5-ALA or hexylaminolevlinate (5-ALA derivative), based on diagnostic test accuracy assessment using WL cystoscopy as the reference standard [85]. However, Hagimoto et al. revealed that ALA-PDD was more sensitive than NBI for CIS lesions (94.6% vs. 54.1%) [86]. NBI improves the cancer detection rate, but its effects on reducing the recurrence rate of BC are unclear, except in patients at low risks (pTa, grade 1, <30 mm, and no CIS) [87]. The Clinical Practice Guidelines for

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Bladder Cancer in Japan (2019 revision) recommended PDD because it lowers the recurrence rate of BC more than NBI [88].

7. PDD with 5-ALA for TURBT in NMIBCs

ALA-PDD is an effective method for diagnosing NMIBC. Fluorescence light from 5-ALA and WL was previously reported to detect CIS lesions with sensitivities of 92.1% and 47.4%, respectively, and ALA-PDD was significantly more sensitive [76]. However, false-positive lesions increased due to the tangent effect in some distal bladder lesion locations, including the bladder neck, trigone, and prostatic urethra [89]. Thus, ALA-PDD demonstrated lower specificity compared with WL. However, the higher sensitivity of ALA-PDD compared with WL enables more tumor and CIS lesion detection [88]. ALA-PDD may detect more imperceptibly small tumors and CIS lesions compared with WL.

TURBT is the standard surgical treatment for NMIBC. However, conventional TURBT with WL may not detect 4%–41% of small papillary tumors, CIS, multifocal growth, and microscopic lesions [90,91]. Several systematic reviews revealed that PDD-assisted TURBT with 5-ALA or its derivative (hexaminolevulinic acid) increased the detection rate of tumors, particularly for CIS. Additionally, PDD-assisted TURBT with 5-ALA reduced the risk of disease recurrence more than WL-TURBT in patients with NMIBC [92]. Figure 3 shows these concepts.

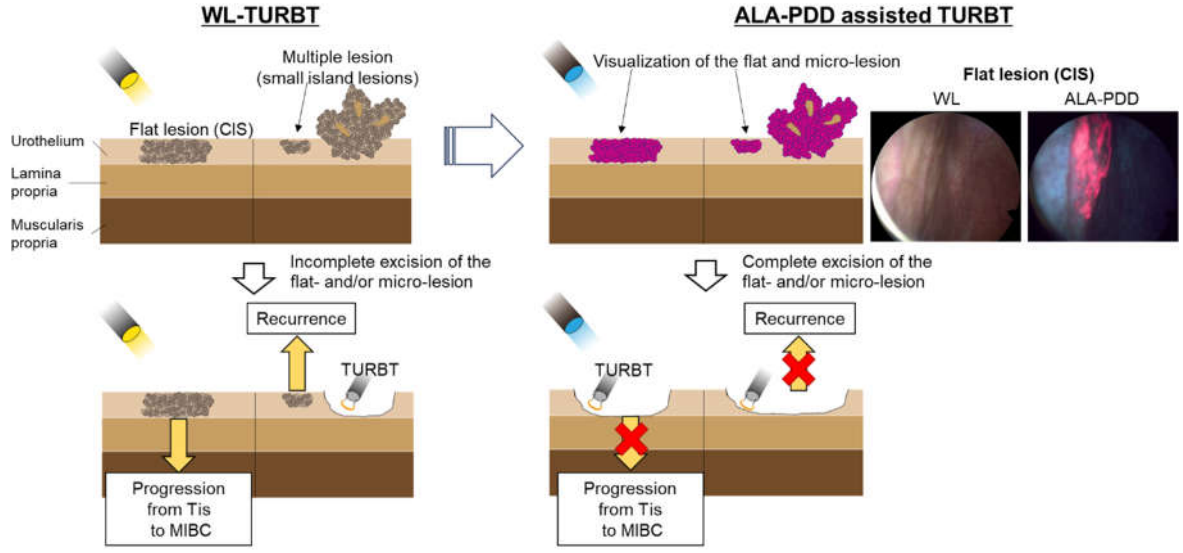

Figure 3. Comparison between WL and 5-ALA for TURBT. PDD-assisted TURBT with 5-ALA increased the detection rate of tumors, particularly for CIS. Additionally, PDD-assisted TURBT with 5-ALA reduced the risk of disease recurrence more than WL-TURBT in patients with NMIBC.

A recent meta-analysis reported 1- and 2-year recurrence-free survival (RFS) rates after ALA-PDD of 50.4%–89.6% (vs. 39.0%–85.0% for WL) and 40.0%–89.6% (vs. 28.0%–72.0% for WL), respectively. A meta-analysis of nine trials (1782 patients) revealed better 1-year RFS after PDD compared with WL (hazard ratio [HR]: 1.14; 95% confidence interval [CI]: 1.05–1.23, I2 = 70%, p = 0.002), indicating that PDD improved RFS. A meta-analysis of five trials (925 patients) revealed improved 2-year RFS following PDD (HR: 1.25; 95% CI: 1.15–1.35, $p \leq 0.001$) [92]. Matsushita et al. reported significantly longer RFS in the PDD group in all subgroups except for tumor size compared with the WL group after 1:1 propensity score matching of 383 patients for age, sex, concomitant history of upper urinary tract UC, preoperative cytology, tumor multiplicity, and tumor size [94]. Moreover, in a study of 1578 consecutive patients with primary NMIBC, Miyake et al. revealed that PDD-TURBT decreased the risk of high- and low-grade tumor recurrences compared with WL-TURBT. PDD significantly reduced the risk of International Bladder Cancer Group-defined progression in patients with NMIBC [95]. However, PDD-TURBT did not reduce recurrence in patients with NMIBC of ≥30 mm [95,96]. The 5-ALA-induced stability of PpIX is dependent on the wavelength and intensity of the right side. PpIX elimination, called photobleaching, is accelerated

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during WL cystoscopy [90]. Thus, PDD-TURBT did not reduce recurrences in patients with large tumors that require long-term use of WL cystoscopy. Yamashita et al. revealed that a spectrometer with a liquid crystal tunable filter, the 5-ALA-PDD using the average fluorescence altitude of 660–700 nm instead of the peak altitude at 630 nm, is more effective in distinguishing between tumorous and non-tumorous tissues in the gastrointestinal tract, because of the lower photobleaching effect at this specific spectral range [97]. This technique may improve the photobleaching effect in ALA-PDD for NIMBC.

Intravesical BCG instillation induces bladder wall inflammation. This immune response activation induces and supports antitumor mechanisms [98]. BCG therapy prevents intravesical recurrence and progression, thereby prolonging survival in patients with high-grade NMIBC [99]. However, BCG-induced inflammation may cause false-positive fluorescence in PDD. Draga et al. indicated that >3 months of BCG instillation before fluorescence cystoscopy decreases PDD specificity [100]. However, a study of 99 patients by Nakagawa et al. revealed that the combined use of PDD and BCG improved the identification and resection of tumors, especially those that could not be identified by WL alone and may prolong RFS, although the PDD + BCG (PDD-TURBT followed by BCG treatment) group demonstrated higher risk and a high CIS detection rate [101]. Nakagawa et al. proposed several factors that may influence RFS. First, BCG uptake is improved at the tumor resection site. Second, the use of PDD exhibits a photodynamic therapeutic effect [101–103]. No significant difference in the 2-year prognosis-free survival (PFS) was detected between the PDD + BCG and WL + BCG groups. However, the PDD + BCG group demonstrated worse PFS [101]. Makino et al. revealed that prior BCG instillation history, particularly BCG unresponsive disease, caused poor cancer recurrence and prognostic factor progression for patients with NMIBC undergoing PDD-TURBT, and special attention should be given to postoperative follow-up [104].

8. PDT with 5-ALA for TURBT in NMIBCs

Rahman et al. reviewed as follows. Bladder tissue is more translucent and readily accessible with a thin fiber optic cable compared with other human tissues, making BC an excellent choice for PDT. Increasing wavelength improves the penetration depth while elevating the chance of muscle damage. Photofrin is given intravenously and red (630 nm) light is used for whole bladder illumination in clinically approved PDT for BC in Canada. However, such treatment conditions caused collateral damage to the whole bladder, particularly the muscle layer, resulting in functional damage to the bladder. In contrast, 5-ALA- or HAL-generating PpIX is excited by a green light that does not penetrate >1 mm into the bladder, thereby sparing the muscular layer [105]. Hence, NMIBC is especially an excellent imminent target for ALA-PDT.

Flat UC demonstrates high recurrence rates. Thus, most patients required additional treatment (e.g., CT and BCG) to prolong recurrence-free intervals and prevent recurrence and disease progression. However, additional intensive care is often necessary after combined modality treatments (such as TURBT + BCG and TURBT + CT) to treat complications associated with chemotherapeutic agent toxicity or BCG-induced severe inflammation [106,107]. ALA-PDT is a painless procedure, and the antitumor effects are induced by low-energy excitation. Thus, patients do not require anesthesia, and the procedure can be repeated. Furthermore, ALA-PDT specifically targets PpIX that has accumulated in tumor cells, like ALA-PDD [108]. In 1996, Kriegmair et al. first reported the use of 5-ALA in PDT for BC treatment, resulting in subsequent clinical studies [103,109–114]. These trials were primarily performed in patients with treatment-resistant BC and bladder CIS. Additionally, excitation wavelengths in the green (514 nm), red (630–635 nm), and white (380–700 nm) ranges were used because they can be delivered at a low heat density of 15–100 J/cm^2. A study of 45 patients by Filonenko et al. revealed that intraoperative ALA-PDT after TURBT significantly reduced the 1-year recurrence rate for superficial BC (pT stage: Ta-T1) compared with the recurrence rate after TURBT monotherapy (22% vs. 40%–80%). These results were at least equivalent to convenient adjuvant treatments in patients with bladder tumors (1-year recurrence rates were 36%–44% after TURBT + CT and 20%–59% after TURBT + BCG) [115]. ALA-PDT acts on a neoplastic cell attributively and is highly accurate, minimally invasive, and widely applicable. Further

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improvements, such as a wavelength or the luminescence sensitizer, may be necessary, but ALA-PDT may replace additional treatments for BC, especially CIS. Moreover, large trials with long-term follow-ups should confirm the beneficial effects of ALA-PDT for BC.

9. Screening Using 5-ALA After TURBT in NMIBCs

Urine cytology is frequently performed to screen for BC. Generally, urine cytology is frequently problematic because of its sensitivity in low-grade UC in clinical practice. Interestingly, Yamamichi et al. revealed that 5-ALA-induced fluorescent-selective upper tract urinary cytology, by which the urine sample was centrifuged and the pellets suspended in minimum essential media using 5-ALA hydrochloride was more sensitive than conventional cytology for diagnosing upper urinary tract UC (90.4% vs. 66.3%, $p < 0.001$) regardless of pT and tumor grade [116]. Therefore, cystoscopy remains necessary as a followup procedure, but the use of ALA-induced cytology together with PDS may be an effective technique for following up after TURBT in NMIBC.

10. Conclusion and Future Perspective

NMIBC and MIBC exhibit great intrinsic heterogeneity regarding different prognoses, survival, progression, and treatment outcomes, but decreasing tumor recurrence in BC (especially in CIS and pT1 UC) that detects the range of the exact lesion and removes them completely is crucial.

A new-generation photosensitizer, 5-ALA, demonstrated high tumor specificity. Moreover, 5-ALA is naturally generated from glycine and succinyl CoA. ALA-PDD and ALA-PDT use 5-ALA. However, ALA-PDD is influenced by the tangent effect and photobleaching, but ALA-PDD has a significantly higher sensitivity for detecting tumors than WL-PDD and NBI, especially in CIS. TURBT, assisted by ALA-PDD, substantially reduces recurrence risk and significantly decreases progression compared with WL-TURBT in patients with NMIBC. Hypotension is well known as a transient adverse event for oral ALA, but our group revealed the multiple factors that are mutually related in the prediction model for ALA-induced hypotension, as mentioned above, and showed the possibility of the conquest of this side effect.

Additionally, ALA-PDT demonstrated limited efficacy for advanced-stage BCs, but it can affect the superficial lesion and prevent muscle damage-induced bladder dysfunction. Therefore, NMIBC is an excellent imminent target for ALA-PDT. In Japan, ALA-PDT has not been recommended yet. ALA-PDT is highly accurate, minimally invasive, and widely applicable and may replace additional BC treatments such as CT and BCG. A clinically successful ALA-PDT for NMIBC may be developed by clinical experiences and a deeper understanding of pathobiology of NMIBC, similar to ALA-PDD.

Moreover, in the future, patients with NMIBC will be treated by PDD-TURBT together with ALA-PDT, and then received followup-up using ALA-PDS and ALA-induced fluorescent urinary cytology. Furthermore, BC diagnosis, treatment, and follow-up may be performed using 5-ALA. Techniques using 5-ALA are based on the Warburg effect, which is a basic property across all cancers. The techniques will be used as a therapeutic strategy of various cancer types and can be beneficial for patients.

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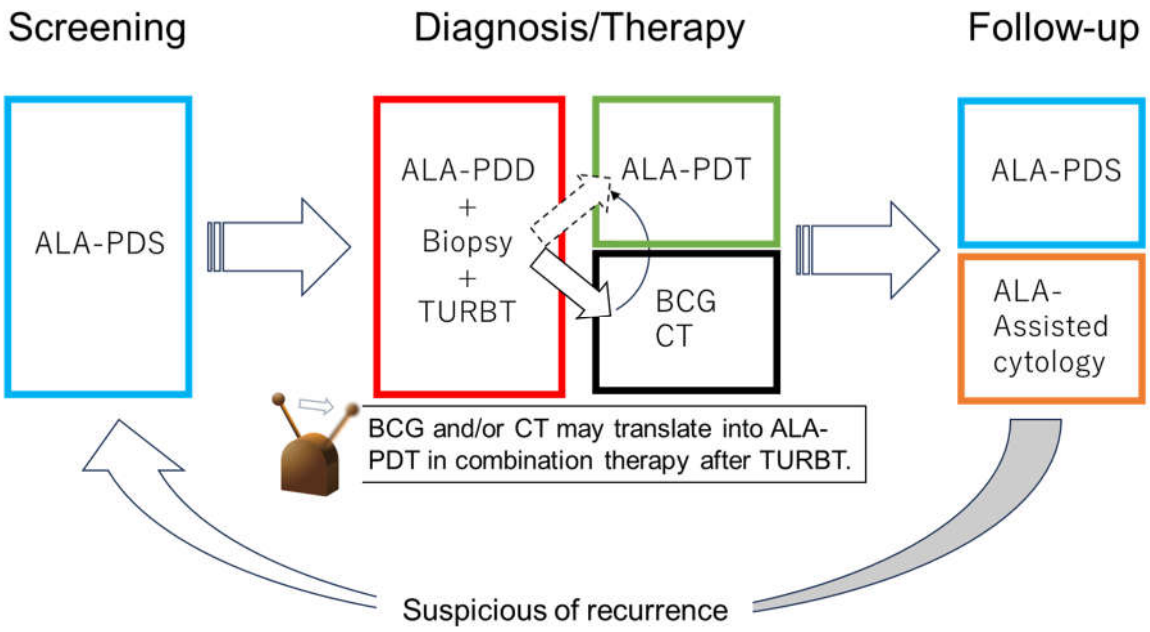

Figure 4. Future perspective of 5-ALA in NMIB. In the future, bladder cancer diagnosis, treatment, and followup-up may be performed with 5-ALA.

11. Author Contributions:

Conceptualization, A.K. and M.F.; writing—original draft preparation, A.K., M.F and K.I.; writing—review and editing, A.K., H.F., K.F., W.I., M.F.and K.I. All authors have read and agreed to the published version of the manuscript.

12. Conflicts of Interest:

The authors declare no conflicts of interest.

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