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Hexyl Aminolevulinate
In the Detection of Bladder Cancer

James E. Frampton and *Greg L. Plosker*

Adis International Limited, Auckland, New Zealand

Contents

Abstract

▲ Hexyl aminolevulinate, the hexyl ester of 5-aminolevulinic acid, is a photosensitising agent designed to enhance the detection of bladder cancer tumours, in particular highly malignant carcinoma in situ (CIS).

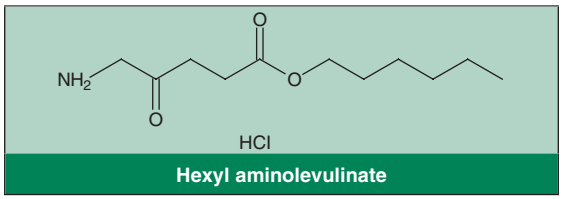
▲ After cellular uptake, hexyl aminolevulinate and/or hydrolysed 5-aminolevulinic acid enter the haem biosynthetic pathway and induce accumulation of the photoactive compound protoporphyrin IX (PpIX) in malignant as opposed to nonmalignant cells. PpIX emits red fluorescence when illuminated under blue light.

▲ Blue-light fluorescence cystoscopy with hexyl aminolevulinate (hexyl aminolevulinate cystoscopy) was more effective than standard (white-light) cystoscopy for detecting non-muscle-invasive ('superficial') disease in two European, multicentre, phase III trials, which evaluated hexyl aminolevulinate cystoscopy as an adjunct to standard cystoscopy in patients with known or suspected bladder cancer.

▲ In one trial, hexyl aminolevulinate cystoscopy detected 96% of the patients with CIS; it identified 28% more patients with CIS than standard cystoscopy. In the other trial, 17% of patients were selected to receive more complete treatment following hexyl aminolevulinate cystoscopy than standard cystoscopy, because of the improved tumour detection rate.

▲ Hexyl aminolevulinate cystoscopy is well tolerated as an adjunct to standard cystoscopy; adverse events were those typically associated with standard cystoscopy/biopsy (e.g. postoperative pain).

Features and properties of hexyl aminolevulinate (Hexvix®, hexaminolevulinate, 5-aminolevulinic acid hexylester, hexyl 5-aminolevulinate, hexylester aminolevulinate)	
Indication	
Detection of bladder cancer (e.g. carcinoma in situ)	
Mechanism of action	
Photosensitiser (hexyl ester of 5-aminolevulinic acid)	
Dosage and administration	
Route of administration	Intravesical
Instillate concentration	8 mmol/L
Instillation volume	50mL
Instillation time	≈1h
Pharmacokinetic profile	
After cellular uptake, hexyl aminolevulinate either directly or after hydrolysis into 5-aminolevulinic acid (by nonspecific esterases) enters the haem biosynthetic pathway, where it is metabolised into the photoactive intermediate compound protoporphyrin IX	
Tolerability profile (as an adjunct to standard cystoscopy)	
Similar to that of standard cystoscopy/biopsy; most adverse reactions (e.g. postoperative pain) are transient and mild or moderate in intensity	



Urinary bladder cancer, the fourth most common malignancy among men in the Western world, is 3–4 times more common in men than in women.[1] In the US, for example, there were an estimated 47 010 new cases diagnosed in men and 16 200 new cases diagnosed in women in 2005.[2] Bladder cancer is primarily a condition of advanced age; the median age at diagnosis is 65–70 years.[1]

Urothelial (transitional cell) carcinomas account for >90% of all bladder cancers; non-muscle-invasive (or 'superficial') transitional cell carcinomas account for 70% of all newly diagnosed bladder cancer cases.[3] Non-muscle-invasive cancers comprise mostly exophytic papillary tumours confined to the mucosa (pathological stage pTa) or submucosa (pT1), but also include nonexophytic flat lesions confined to the mucosa (i.e. carcinoma in situ; CIS).[3,4] Whereas pTa tumours are mostly low grade (minimal to moderate risk of progression to potentially life-threatening muscle-invasive disease), pT1 tumours are generally more aggressive (moderate to high risk of progression) and CIS, by definition, is highly malignant (high risk of progression). In addition, all non-muscle-invasive cancers are characterised by a high rate of recurrence (50–90%[4]) despite treatment, which essentially consists of transurethral resection (TUR) of the bladder followed by intravesical immunotherapy with bacillus Calmette-Guerin for all CIS, pT1 and high-grade pTa lesions.[4,5]

Muscle-invasive tumours are staged pT2–4 according to the degree of muscle penetration and have an associated 5-year mortality rate of 50%.[6] Treatment consists of radical cystectomy with pelvic lymphadenectomy (with or without neoadjuvant chemotherapy).[4,5]

Typically used in combination, cystoscopy and urinary cytology are the standard methods for diagnosing (and monitoring) bladder cancer.[7,8] Cystoscopy allows direct visual inspection of the urothelium and mucosa; cystoscopy-guided biopsy (i.e. TUR of suspicious lesions/areas) remains the 'gold standard' procedure for verification of bladder cancer, including CIS.[5,8,9] However, conventional cystoscopy is limited in its ability to detect flat lesions, such as CIS, which may be diffuse and indistinguishable from normal or nonspecific inflammatory-appearing mucosa,[1] while the use of random biopsies to detect and confirm CIS is of questionable benefit, as the detection rate is low and the risk of tumour seeding may be increased.[10] There is also a significant risk of overlooking papillary tumours with conventional cystoscopy.[1,11]

Cytology has both a high specificity and sensitivity for high-grade lesions; it remains the noninvasive diagnostic tool of choice for bladder cancer detection (including CIS) in preference to urinary markers, which appear to have better sensitivity (especially for low-grade, non-muscle-invasive tumours), but less specificity than cytology.[7,9] Nonetheless, cytology results are not immediately available, are highly dependent on the interpreter and provide no information on the location and extent of disease.[7,12,13]

Missed diagnosis leading to delayed or incomplete treatment has significant prognostic implications for patients with potentially aggressive tumours, such as CIS, and is also a factor contributing to the high rate of recurrence of non-muscle-invasive cancers after TUR. Improved methods of detecting bladder lesions are therefore desirable.

Intravesical porphyrin-based fluorescence cystoscopy has been developed to enhance the early diagnosis of bladder cancer. Briefly, this technique involves instilling into the bladder via a catheter a photosensitising agent that, by mechanisms not fully understood, induces preferential intracellular accumulation of photoactive (fluorescent) endogenous porphyrins, mainly protoporphyrin IX (PpIX), in malignant cells as opposed to nonmalignant cells of urothelial origin. Under subsequent blue-light illumination, the neoplastic lesions emit red fluores-

cence (and thus can be visualised) in contrast to the non-fluorescing normal mucosa.[8]

5-Aminolevulinic acid, a precursor of PpIX in the haem biosynthesis cycle, was initially investigated as a topical photosensitising agent.[8] 'Blue-light' fluorescence cystoscopy with 5-aminolevulinic acid (5-aminolevulinic acid cystoscopy) significantly improved the tumour and premalignant lesion detection rate relative to standard (white-light) cystoscopy.[14-16] Additionally, TUR of bladder tumours guided by 5-aminolevulinic acid cystoscopy was superior to TUR guided by standard cystoscopy in terms of the residual tumour detection rate and recurrence-free survival in patients with non-muscle-invasive disease.[16,17]

Since cellular uptake of 5-aminolevulinic acid (a charged molecule) is limited, it has been chemically modified (esterified) to increase its lipophilicity and penetration into tissues, and hence photosensitising effect. Hexyl aminolevulinate (Hexvix®),[1] the hexyl ester of 5-aminolevulinic acid, provides brighter fluorescence after a shorter instillation time.[13] It has been investigated in the detection of bladder cancer, and is the subject of this profile.

1. Pharmacodynamic Properties

- Once located in the intracellular environment, hexyl aminolevulinate and/or 5-aminolevulinic acid (see section 2) bypass the regulatory step of the haem biosynthetic pathway (negative feedback control from haem to 5-aminolevulinic acid synthase), causing a temporary accumulation of PpIX in situ, particularly in malignant cells.[18,19]
- Hexyl aminolevulinate represented a good compromise between lipophilicity, solubility and activity in an *in vitro* study in which porcine or human bladder mucosae were incubated with 5-aminolevulinic acid or one of its esters, including the ethyl-, butyl-, or octyl-esters.[18] Compared with 5-aminolevulinic acid, the optimum concentration of hexyl aminolevulinate was 45 times lower, but induced approximately 2.5 times greater fluorescence (i.e. PpIX accumulation). Hexyl aminolevulinate also accelerated urothelial accumulation of PpIX relative to 5-aminolevulinic acid and resulted in a more homogeneous distribution of PpIX accumulation within the urothelium.[18]
- Similarly, hexyl aminolevulinate at an ≈20-fold lower concentration than 5-aminolevulinic acid (8 vs 180 mmol/L) produced an approximately 2-fold greater fluorescence signal in a clinical pilot study in which six patients had 50mL of hexyl aminolevulinate (n = 2) or 5-aminolevulinic acid (n = 4) solution instilled for 4 hours followed by a 2-hour (solution-free) rest period before fluorescence cystoscopy.[19] The fluorescence level was markedly enhanced by reducing the instillation time of 8 mmol/L hexyl aminolevulinate solution from 4 to 2 hours (while retaining the 2-hour rest period) in this trial[19] and another.[20]
- Use of a short instillation time (≈0.5 hours) without a rest period did not compromise the diagnostic efficacy of the technique;[21] typically, a 1-hour instillation time was used in preregistration studies (section 2).

2. Pharmacokinetic Properties

Many investigators assume that after cellular uptake hexyl aminolevulinate is hydrolysed (by non-specific esterases) into 5-aminolevulinic acid, which then enters the haem biosynthetic pathway.[19,20,22] However, it remains unclear whether the metabolism of hexyl aminolevulinate in the bladder is dominated by (i) hydrolysis to 5-aminolevulinic acid; (ii) direct entry of this ester into the haem biosynthetic pathway; or (iii) other decomposition reactions. In the case of experimental (hairless mouse) skin models, results suggest that metabolic processes other than hydrolysis are involved in the degradation of 5-aminolevulinic acid esters (e.g. hexyl aminolevulinate).[23]

Additional information regarding the pharmacokinetics of hexyl aminolevulinate is very limited and currently available only from the manufacturer's prescribing information.[24]

1 The use of trade names is for product identification purposes only and does not imply endorsement.

- *In vivo* autoradiography studies in rats showed that high (unspecified) concentrations of hexyl aminolevulinate were present in the bladder wall following intravesicular administration of the drug.[24]
- The systemic bioavailability of total radioactivity was approximately 5–10% after intravesical instillation of radiolabelled hexyl aminolevulinate in healthy volunteers.[24]

3. Clinical Use

The diagnostic efficacy of blue-light fluorescence cystoscopy with hexyl aminolevulinate (hexyl aminolevulinate cystoscopy) versus that of standard cystoscopy in bladder cancer has been evaluated in several multicentre studies, including two phase III trials conducted in Europe,[13,25] one phase III trial performed in the US/Canada[26,27] and one phase II trial carried out in Europe.[28] The three European studies, which included 211,[13] 146[25] and 52[28] efficacy-evaluable patients, have been published in full. In contrast, preliminary reports from the (completed) North American phase III study are available as abstracts only.[26,27]

The European studies[13,25,28] enrolled adult male and female patients (mean age 67–72 years) with known or suspected bladder cancer based on cystoscopy findings or abnormal cytology; the largest trial[13] aimed to recruit a high proportion of patients at high risk for CIS lesions. Ineligible patients were those with gross haematuria, porphyria or allergy to hexyl aminolevulinate. Patients who had received topical treatment with intravesical immunotherapy or chemotherapy within 3 months prior to the study were also excluded[13,25,28] (except those who had received only a single course of chemotherapy immediately following TUR of the bladder[25]).

Similar to the largest European trial,[13] the North American phase III trial[26,27] aimed to recruit a large percentage of patients at high risk for CIS lesions.[29] All studies shared a similar prospective, open-label, within-patient controlled design[13,25-28] in which 50mL of hexyl aminolevulinate 8mmol/L solution (where reported[13,25,28]) was instilled into the empty bladder and retained for 1–3 hours[13,25-28] (typically 1 hour[13,25,28]) prior to planned cystoscopy and TUR of the bladder under locoregional or general anaethesia.

The bladder was inspected with standard cystoscopy followed by hexyl aminolevulinate cystoscopy in a nonrandomised order, since the latter was used as an adjunct to the former.[13,25-28] The number, location and type of tumours and suspicious areas seen with each method were documented on the same bladder chart. Thereafter, all lesions mapped during standard cystoscopy and[13,28]/or[25] hexyl aminolevulinate cystoscopy were biopsied or resected for histological confirmation by a local and[25]/or[13,26-28] central pathologist blinded to the identity[13,28] or mode of identification[25] of the lesion.

Primary endpoints in the European studies were the sensitivity and specificity of hexyl aminolevulinate cystoscopy based on biopsy findings,[28] the proportion of patients who had more histologically confirmed CIS lesions detected by hexyl aminolevulinate cystoscopy than by standard cystoscopy[13] and the difference in intended patient treatment (more or less aggressive) following diagnosis with hexyl aminolevulinate cystoscopy or standard cystoscopy.[25] The latter was assessed by rating the severity of treatment (none to cystectomy) and comparing two treatment plans for each patient, one based on the results of hexyl aminolevulinate cystoscopy and the other based on the results of standard cystoscopy, each established by an independent urologist blinded to the diagnostic technique. In the event that the plans were identical, but more lesions were detected on hexyl aminolevulinate cystoscopy, the patient was deemed to undergo more complete treatment (i.e. more extensive resection).[25]

In the European phase III studies,[13,25] the lesion detection rate was defined as the number of lesions detected by hexyl aminolevulinate or standard cystoscopy divided by the total number of lesions detected by hexyl aminolevulinate and/or standard cystoscopy (excluding duplicates). The false-positive detection rate was defined as the number of lesions mistakenly identified (negative histology) by hexyl aminolevulinate or standard cystoscopy divided by the total number of lesions identified by,[13] or

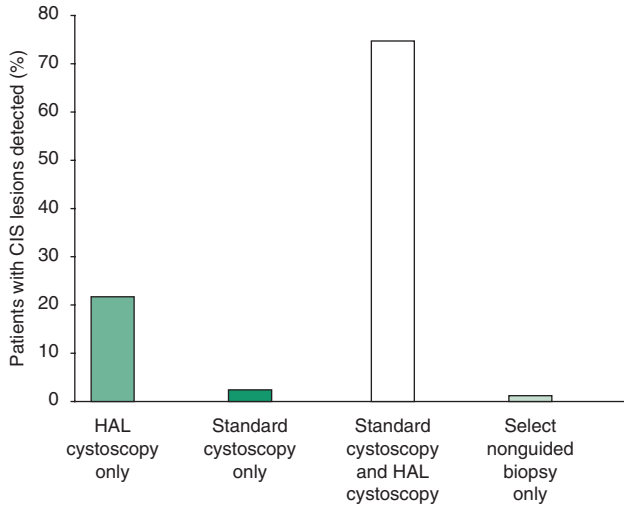


Fig. 1. Efficacy of blue-light fluorescence cystoscopy with hexyl aminolevulinate (HAL) [HAL cystoscopy] for detection of carcinoma in situ (CIS) in patients with bladder cancer. Shown are the proportions of patients with biopsy-confirmed CIS (n = 83 total) who had their CIS lesions identified by HAL cystoscopy only, by standard cystoscopy only or by both HAL and standard cystoscopy in a large, within-patient controlled, multicentre, phase III trial conducted in Europe.[13]

areas biopsied under,[25] hexyl aminolevulinate and standard cystoscopy, respectively.

Using methods similar to those employed in the afore-mentioned trials, two small studies (n = 20[30] and 45[31]) conducted in single centres in Europe compared hexyl aminolevulinate cystoscopy with standard cystoscopy performed using a flexible and[31]/or[30] rigid cystoscope.

- Hexyl aminolevulinate cystoscopy was superior to standard cystoscopy for detecting CIS lesions in the larger of the two European phase III studies.[13] Of the 211 efficacy-evaluable patients, 83 (39%) had biopsy-confirmed CIS; of these, 18 had CIS alone and 65 had CIS in conjunction with pTa or pT1-4 tumours. Eighty (96%) patients with CIS were detected by hexyl aminolevulinate cystoscopy compared with 64 (77%) detected by standard cystoscopy (figure 1).

- Eighteen (22%) of the 83 patients with CIS lesions had their CIS lesions detected by hexyl aminolevulinate cystoscopy alone (figure 1); accordingly, this procedure identified 28% more patients with CIS lesions than standard cystoscopy (i.e. an additional 18 patients vs 64 diagnosed by standard cystoscopy).[13]

- Of the 83 patients with CIS, 46 (55%) had more CIS lesions detected by hexyl aminolevulinate cystoscopy compared with 3 (4%) who had more CIS lesions detected by standard cystoscopy ($p < 0.0001$). Of the remaining patients, 33 (40%) had the same number of CIS lesions detected by hexyl aminolevulinate and standard cystoscopy; one had a CIS lesion detected by nonguided biopsy only.[13]

- All 18 patients with CIS-only lesions were identified by hexyl aminolevulinate cystoscopy compared with 12 detected by standard cystoscopy.[13]

- Seventy-two patients with CIS had bladder barbotage or voided-urine cytology results, of whom 61 (85%) showed positive cytology according to standard definitions.[13] All 11 cytology-negative CIS cases were detected by hexyl aminolevulinate cystoscopy compared with only 7 identified by standard cystoscopy.

- The lesion detection rate pattern was similar in both European phase III studies.[13,25] In particular, the proportion of dysplasia, CIS and pTa lesions identified by hexyl aminolevulinate cystoscopy relative to standard cystoscopy was significantly higher in the larger trial[13] (94% vs 53%, 97% vs 58% and

97% vs 88%, respectively; p-values not provided) and numerically higher in the smaller trial[25] (93% vs 48%, 95% vs 68% and 96% vs 85%, respectively; statistical tests not reported).

- Overall, hexyl aminolevulinate cystoscopy detected 96%[25] and 97%[13] of all lesions (including CIS) in the smaller[25] and larger[13] European phase III trials, respectively, compared with 77% and 78% identified by standard cystoscopy. The overall false-positive detection rate for hexyl aminolevulinate cystoscopy was numerically higher than that for standard cystoscopy, although the absolute difference between the two imaging techniques was only 3% in the larger study[13] (13% vs 10%) compared with 11% in the smaller study[25] (37% vs 26%).
- The improvement in the overall lesion detection rate with hexyl aminolevulinate cystoscopy relative to that with standard cystoscopy led to more complete treatment in 25 (17%) of 146 efficacy-evaluable patients in the smaller European phase III study ($p < 0.0001$).[25] Specifically, 15 patients had additional postoperative procedures recommended and 10 had more extensive resection as a result of hexyl aminolevulinate cystoscopy. As such, hexyl aminolevulinate cystoscopy improved treatment in 21.7% (i.e. ≈1 in 5) of those patients with confirmed lesions (n = 115).
- Preliminary results from the North American phase III trial suggest that hexyl aminolevulinate cystoscopy may complement standard cystoscopy in the diagnosis of non-muscle-invasive bladder cancer.[26,27] For example, whereas all 41 patients with biopsy-confirmed CIS were identified by hexyl aminolevulinate and/or standard cystoscopy, individually, these imaging techniques failed to identify CIS in two different sets of seven patients (both 83% per-patient sensitivity).[26]
- Hexyl aminolevulinate cystoscopy had 89% and 94% per-patient sensitivity for pTa and pT1 lesions, respectively, in this trial; the corresponding results for standard cystoscopy were 92% and 90%, respectively.[27] Similar to experience in the smaller European phase III study (in which 13 [20%] of 66 patients with pTa lesions had more lesions detected by hexyl aminolevulinate cystoscopy),[25] 17 (26%) of the 65 patients with pTa lesions had more lesions detected by hexyl aminolevulinate cystoscopy.[27]
- Hexyl aminolevulinate and standard cystoscopy had, respectively, a 96% and 73% per-patient sensitivity, a 76% and 46% per-lesion sensitivity and a 79% and 93% per-lesion specificity in the European phase II study (n = 52).[28]
- Results from the two small studies,[30,31] which compared hexyl aminolevulinate cystoscopy with standard cystoscopy performed using a flexible and[31]/or[30] rigid cystoscope, indicated that flexible hexyl aminolevulinate cystoscopy was feasible and generally similar in diagnostic efficacy to the other imaging techniques. In relative terms, however, flexible hexyl aminolevulinate cystoscopy increased the CIS lesion detection rate by 35% and 24% compared with flexible standard and rigid standard cystoscopy, respectively, in the larger of the two studies.[31]

4. Tolerability

- Hexyl aminolevulinate cystoscopy (including instillation of hexyl aminolevulinate into the bladder) was well tolerated as an adjunctive procedure to standard cystoscopy.[13,25,28] Adverse events reported by patients in clinical trials were those typically associated with standard cystoscopy/TUR of the bladder (e.g. postoperative pain); <3% of negative experiences were considered to be (clearly) related to hexyl aminolevulinate.[13,25,28] Most reported adverse reactions were transient and mild or moderate in intensity, according to the manufacturer's prescribing information.[24]
- Postoperative pain (13% of patients), haematuria (5%), abdominal pain (3.6%), insomnia (3.6%), urinary tract infection (3.2%), urinary retention (2.9%), dysuria (2.5%) and pyrexia (2.5%) were the most commonly reported adverse events in the largest European phase III trial (n = 279 safety-evaluable patients).[13] Overall, 47% of patients in this study reported one or more adverse event; 6% reported one or more serious adverse event (none of which were considered definitely related to hexyl aminolevulinate instillation). No unexpected adverse events were reported in this study.

- No clinically significant changes in laboratory safety parameters or vital signs were observed during hexyl aminolevulinate instillation/cystoscopy.[13,28]

5. Dosage and Administration

Hexyl aminolevulinate is indicated for the detection of bladder cancer, such as CIS, in patients with known disease or suspected disease based on screening cystoscopy and/or positive urine cytology.[24]

Adults (including the elderly) should have 50mL of hexyl aminolevulinate 8 mmol/L solution instilled into their bladder for ≈1 hour; cystoscopy should commence within 1 hour following evacuation of the bladder. Hexyl aminolevulinate cystoscopy should be used as an adjunct to standard (white-light) cystoscopy to guide the taking of biopsies (which is normally carried out under white light). Hexyl aminolevulinate cystoscopy has not been evaluated in children and adolescents under 18 years of age.[24]

Hexyl aminolevulinate is contraindicated in patients with porphyria and women of child-bearing potential.[24] Local prescribing information should be consulted for details of special warnings and precautions regarding the use of hexyl aminolevulinate.

6. Hexyl Aminolevulinate: Current Status in the Detection of Bladder Cancer

Hexyl aminolevulinate was approved for use as a diagnostic tool for bladder cancer in the EU in March 2005; a New Drug Application was submitted to the US FDA in June 2005.[32] Hexyl aminolevulinate cystoscopy improves the detection of bladder tumours, particularly CIS, compared with standard cystoscopy;[13,25,28] this led to improved treatment in a significant number of patients in a pivotal phase III study.[25] Of note, current European Association of Urology Guidelines recommend fluorescence cystoscopy (e.g. with hexyl aminolevulinate) for diagnosis of CIS.[9]

Hexyl aminolevulinate cystoscopy is a simple and well tolerated procedure that can easily be implemented as an adjunct to standard cystoscopy, without increasing the risk of complications.[13,25,28] Ongoing studies are assessing whether diagnosis and treatment guided by hexyl aminolevulinate cystoscopy, as opposed to standard cystoscopy, reduces recurrence rates (e.g. as a result of greater detection and/or more complete resection of lesions) or improves cure rates (e.g. as a result of greater identification of appropriate candidates for cystectomy).[25]

Disclosure

During the peer review process, the manufacturer of the agent under review was offered an opportunity to comment on this article; changes based on any comments received were made on the basis of scientific and editorial merit.

References

1. Kirkali Z, Chan T, Manoharan M. Bladder cancer: epidemiology, staging and grading, and diagnosis. Urology 2005 Dec; 66 (6 Suppl. 1): 4-34
2. Jemal A, Murray T, Ward E, et al. Cancer statistics, 2005 [published erratum appears in CA Cancer J Clin 2005; 55: 259]. CA Cancer J Clin 2005; 55: 10-30
3. Sawzcuk IS. Overview of superficial bladder cancer. In: Diagnostic and treatment issues in superficial bladder cancer monograph [online]. Available from URL: http://www.projectsinknow ledge.com [Accessed 2006 Jan 31]
4. National Comprehensive Cancer Network. Clinical practice guidelines in oncolology: bladder cancer (version 1.2006) [online]. Available from URL: http://www.nccn.org/professionals/physicians_gls/PDF/bladder.pdf [Accessed 2006 Jan 19]
5. Sonpavde G. Bladder cancer update: current evaluation methods and standard of care [online]. Postgrad Med 2005; 118 (4). Available from URL: http://www.postgradmed.com [Accessed 2006 Jan 31]
6. van der Meijden APM. Bladder cancer. BMJ 1998 Nov 14; 317: 1366-9
7. Bassi P, De Marco V, De Lisa A, et al. Non-invasive diagnostic tests for bladder cancer: a review of the literature. Urol Int 2005; 75 (3): 193-200
8. Zaak D, Karl A, Knuchel R, et al. Diagnosis of urothelial carcinoma of the bladder using fluorescence endoscopy. BJU Int 2005 Aug; 96 (2): 217-22
9. van der Meijden, Sylvester R, Oosterlinck W, et al. EAU guidelines on the diagnosis and treatment of urothelial carcinoma in situ. Eur Urol 2005; 48: 363-71
10. van der Meijden A, Oosterlinck W, Brausi M, et al. Significance of bladder biopsies in Ta, T1 bladder tumors: a report from the EORTC Genito-Urinary Tract Cancer Cooperative Group. EORTC-GU Group Superficial Bladder Committee. Eur Urol 1999 Apr; 35 (4): 267-71
11. Brausi M, Collette L, Kurth K, et al. Variability in the recurrence rate at first follow-up cystoscopy after TUR in stages

TaT1 transitional cell carcinoma of the bladder: a combined analysis of 7 EORTC studies. Eur Urol 2002; 41: 523-31

12. Pirtskalaishvili G, Konety BR, Getzenberg RH. Update on urine-based markers for bladder cancer. Postgrad Med 1999 Nov; 106 (6): 85-94
13. Schmidbauer J, Witjes F, Schmeller N, et al. Improved detection of urothelial carcinoma in situ with hexaminolevulinate fluorescence cystoscopy. J Urol 2004 Jan; 171 (1): 135-8
14. Koenig F, McGovern FJ, Larne R, et al. Diagnosis of bladder carcinoma using protoporphyrin IX fluoresence induced by 5-aminolaevulinic acid. BJU Int 1999 Jan; 83 (1): 129-35
15. Zaak D, Hungerhuber E, Schneede P, et al. Role of 5-aminolevulinic acid in the detection of urothelial premalignant lesions. Cancer 2002; 95: 1234-8
16. Riedl CR, Daniltchenko D, Koening F, et al. Fluorescence endoscopy with 5-aminolevulinic acid reduces early recurrence rate in superficial bladder cancer. J Urol 2001 Apr; 165 (4): 1121-3
17. Filbeck T, Pichlmeier U, Knuechel R, et al. Clinically relevant improvement of recurrence-free survival with 5-aminolevulinic acid induced fluorescence diagnosis in patients with superficial bladder tumours. J Urol 2002 Jul; 168 (1): 67-71
18. Marti A, Lange N, van den Bergh H, et al. Optimisation of the formation and distribution of protoporphyrin IX in the urothelium: an in vitro approach. J Urol 1999 Aug; 162 (2): 546-52
19. Lange N, Jichlinski P, Zellweger M, et al. Photodetection of early human bladder cancer based on the fluorescence of 5-aminolaevulinic acid hexylester-induced protoporphyrin IX: a pilot study. Br J Cancer 1999 Apr; 80 (1-2): 185-93
20. Marti A, Jichlinski P, Lange N, et al. Comparison of aminolevulinic acid and hexylester aminolevulinate induced protoporphyrin IX distribution in human bladder cancer. J Urol 2003 Aug; 170 (2 Pt 1): 428-32
21. Jichlinksi P, Marti A, Aymon D, et al. First experience of hexylester aminolevulinic acid induced fluorescence cystoscopy in patients with superficial bladder cancer [abstract no. 644]. J Urol 2002; 167 (4 Suppl.): 160
22. Kloek J, Akkermans W, Beijersbergen van Henegouwen GM. Derivatives of 5-aminolevulinic acid for photodynamic therapy: enzymatic conversion into protoporphyrin. Photochem Photobiol 1998 Jan; 67 (1): 150-4
23. De Rosa FS, Lopez RFV, Thomazine JA, et al. In vitro metabolism of 5-ALA esters derivatives in hairless mice skin homogenate and in vivo PpIX accumulation studies. Pharm Res 2004; 21 (12): 2247-52
24. Hexvix (hexaminolevulinate): summary of product characteristics. Oslo: Photocure ASA, 2005 Mar 1
25. Jocham D, Witjes F, Wagner S, et al. Improved detection and treatment of bladder cancer using hexaminolevulinate imaging: a prospective, phase III multicenter study. J Urol 2005 Sep; 174 (3): 862-6; discussion 866
26. Gomella LG, Barton Grossman H, Presti JC, et al. The use of Hexvix and fluorescence cystoscopy as an adjunct in the diagnosis of carcinoma in situ of the urinary bladder [abstract]. 99th Annual Meeting of the American Urological Society; 2004 May 8-13; San Francisco (CA)
27. Barton Grossman H, Gomella LG, Fradet Y, et al. The use of Hexvix and fluorescence cystoscopy as an adjunct in the diagnosis of stage Ta/T1 urothelial cancer in the urinary bladder [abstract]. 99th Annual Meeting of the American Urological Society; 2004 May 8-13; San Francisco (CA)
28. Jichlinski P, Guillou L, Karlsen SJ, et al. Hexyl aminolevulinate fluorescence cystoscopy: a new diagnostic tool for the photodiagnosis of superficial bladder cancer. A multicenter study. J Urol 2003 Jul; 170 (1): 226-9
29. Data on file. Photocure ASA, 2006
30. Witjes JA, Moonen PM, van der Heijden AG. Comparison of hexaminolevulinate based flexible and rigid fluorescence cystoscopy with rigid white light cystoscopy in bladder cancer: results of a prospective phase II study. Eur Urol 2005 Mar; 47 (3): 319-22
31. Loidl W, Schmidbauer J, Susani M, et al. Flexible cystoscopy assisted by hexaminolevulinate induced fluorescence: a new approach for bladder cancer detection and surveillance? Eur Urol 2005 Mar; 47 (3): 323-6
32. Photocure ASA. Hexvix® NDA submitted to FDA [media release]. 2005

Correspondence: *James E. Frampton*, Adis International Limited, 41 Centorian Drive, Private Bag 65901, Mairangi Bay, Auckland 1311, New Zealand.
E-mail: demail@adis.co.nz