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Intracutaneous and Intravesical Immunotherapy With Keyhole Limpet Hemocyanin Compared With Intravesical Mitomycin in Patients With Non–Muscle-Invasive Bladder Cancer: Results From a Prospective Randomized Phase III Trial

Rianne J.M. Lammers, Wim P.J. Witjes, Maria H.D. Janzing-Pastors, Christien T.M. Caris, and J. Alfred Witjes

Rianne J.M. Lammers, Wim P.J. Witjes, and J. Alfred Witjes, Radboud University Nijmegen Medical Centre, Nijmegen; Wim P.J. Witjes, Maria H.D. Janzing-Pastors, and Christien T.M. Caris, CuraTrial Site Management Organisation & Research, Arnhem, the Netherlands.

All authors had access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. This trial was not officially registered, because registration was not required when this trial started (2002).

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

Corresponding author: J. Alfred Witjes, MD, PhD, Radboud University Nijmegen Medical Centre, Department of Urology, Geert Grooteplein South 10 (659), PO Box 9101, 6500 HB Nijmegen, the Netherlands; e-mail: f.witjes@uro.umcn.nl.

ABSTRACT

Purpose
Despite current treatment after transurethral resection of a bladder tumor, recurrences and progression remain a problem. Keyhole limpet hemocyanin (KLH) was beneficial in earlier studies. In this study, safety and efficacy of KLH were compared with that of mitomycin (MM).

Patients and Methods
Patients with intermediate- and high-risk non–muscle-invasive bladder cancer (NMIBC) without carcinoma in situ were enrolled in a randomized phase III trial. In all, 283 patients were randomly assigned for 16 adjuvant intravesical instillations with KLH after preimmunization, and 270 patients were randomly assigned for 11 adjuvant intravesical instillations with MM. Primary outcome measurement was recurrence-free survival (RFS). Secondary outcome measurements were progression-free survival, adverse events (AEs), and the effect of delayed-type hypersensitivity (DTH) response on clinical outcome.

Results
There were significantly more pT1 tumors in the MM group ($P = .01$). In a log-rank test, univariate and multivariate Cox regression analysis, KLH was less effective than MM regarding RFS (all $P < .001$). Progression was uncommon (n = 20). In univariate Cox regression analyses, KLH tended to prevent progression more effectively than MM, but in multivariate Cox regression analyses, this could not be shown. AEs were common but mild. Fever, flu-like symptoms, and fatigue occurred significantly more after KLH treatment. Allergic reactions and other skin disorders occurred significantly more after MM treatment. Significantly more DTH-positive patients developed a recurrence than DTH-negative patients.

Conclusion
KLH had a different safety profile and was inferior to MM in preventing NMIBC recurrences. KLH tended to be more effective than MM in preventing progression. More research is needed to clarify the immunologic effects of KLH and the effects of KLH on progression.

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INTRODUCTION

Worldwide, about 386,300 new cases and 150,200 deaths from bladder urothelial cell carcinoma occurred in 2008.[1] Approximately 70% is non–muscle-invasive bladder cancer (NMIBC).[2] The risk of recurrence and progression after treatment varies. Low-grade Ta NMIBC has a high chance of recurrence and a low chance of progression, whereas high-grade tumors have a high potential for malignancy. Treatment consists of transurethral resection of the bladder tumor (TURBT), followed by intravesical treatment. The choice of adjuvant treatment with either chemotherapy (eg, mitomycin [MM]) or immunotherapy (eg, *Bacillus* Calmette-Guérin [BCG]) depends on the risk group to which the patient belongs.[3-6] Unfortunately, recurrences and adverse events (AEs) are common.[7]

Keyhole limpet hemocyanin (KLH), a glycoprotein from the gastropod mollusk *Megathura crenulata* is an immunocyanin that contributes to the uptake, transport, and release of oxygen during respiration. It also activates both the humoral and cellular immune systems, but the precise mechanism of action of KLH remains unknown.[8] One commercially available intravesical immunocyanin is Immucothel (biosyn, Fellbach, Germany).

Olsson et al[9] were the first to report on KLH in 1974, with promising results on tumor recurrence. Results of other small phase II studies suggested a potential benefit of KLH over known treatments and a favorable safety profile.[10-12] Our goal was to compare the efficacy and safety of KLH with that of MM in a prospective, randomized phase III trial.

PATIENTS AND METHODS

Eighteen Dutch institutions participated in this multicenter, prospective, randomized phase III trial. The study was conducted in compliance with Good Clinical Practice, the Declaration of Helsinki (version 1996), and with permission of all local ethical committees.

Patient Selection

Patients with a histologically proven pTa/1 grade 2 to 3 tumor, or with multiple or recurrent pTa grade 1 tumors, in whom complete TURBT was possible, were included after obtaining informed consent. Patients who received intravesical therapy within 6 months before entry or more than one instillation of MM within 2 years before inclusion were excluded. Patients with primary solitary pTa grade 1 tumors, carcinoma in situ (CIS), or tumors ≥ pT2 were also excluded. Other exclusion criteria were concurrent malignancy (except basal cell or squamous cell carcinoma of the skin), history of another malignancy with a disease-free interval ≤ 5 years, expected poor compliance, WHO performance status more than 2, uncontrollable urinary tract infection, any previous systemic cancer therapy or radiotherapy within 5 years, localization of tumor in prostatic urethra or upper urinary tract, pregnancy or lactation for women of reproductive age who refused to take adequate contraceptive measures, congenital or acquired immune deficiency syndromes, known hypersensitivity to MM, and previous (≤ 1 month) or current treatment with an investigational drug.

Treatment Schedule

After TURBT was complete and informed consent was signed, patients were randomly assigned for treatment with either KLH (Immucothel) or MM. The restricted blockwise procedure was used for random assignment.

In the KLH arm, patients started with preimmunization: intracutaneous (IC) injection of 1 mg KLH at intervals of 2 to 7 days until a delayed-type hypersensitivity (DTH) response was obtained. A real DTH response was defined as a palpable infiltrate in the skin. In case no DTH response developed, up to four IC injections were given. Intravesical treatment was started within 2 weeks after the TURBT, independent of the preimmunization result. If there was no DTH response after four injections, this was not a reason for discontinuing the treatment. Patients received a total of 16 intravesical instillations (20 mg KLH in 20 mL solvent) given once per week for 6 weeks and then once per month for 10 months (months 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12).

In the MM arm, patients received a total of 11 instillations (40 mg MM in 50 mg saline) given once per week for 4 weeks (weeks 1, 2, 3, and 4) and once per month for 5 months (months 2, 3, 4, 5, and 6) and then at 9 and 12 months.

Follow-Up and Evaluation of Therapy

Patients received urinary cytology and cystoscopy every 3 months for the first year, every 4 months during the second and third years, and every 6 months thereafter. All visible lesions were completely resected and confirmed by histologic examination. If a recurrence without progression was observed during the treatment period, the therapy was continued after complete TURBT. Patients went off-study in case of withdrawal of informed consent, second recurrence during treatment, first recurrence after completion of therapy, 5-year disease-free survival, occurrence of CIS, tumor of upper tract or prostatic urethra, or distant metastasis. Patients in whom a lesion was coagulated, not biopsied, or not treated at all continued in follow-up and were censored at the time of last follow-up.

Objectives and Statistical Analysis

The primary end point was the duration of recurrence-free survival (RFS). Secondary objectives were recurrence rate, number of recurrent tumors, duration of progression-free survival (PFS), AEs, and the effect of DTH response on clinical outcome. RFS was defined as the time interval between TURBT and time to first recurrence. Recurrence was defined as positive cystoscopy and histologically confirmed positive biopsies. Recurrence rate was defined as the number of cystoscopies at which recurrences were observed divided by the duration of follow-up in months multiplied by 100. PFS was defined as the time interval between TURBT and time to progression. Progression was defined as recurrence with histologic pathology stage ≥ pT2, distant metastases, or death related to bladder cancer.

KLH is registered for prevention of bladder cancer after TURBT and after failure of established therapies. Therefore, a subgroup analysis was done in which RFS after KLH treatment was compared with MM treatment in patients with recurrence disease and a history of intravesical treatments.

Power analysis showed that 251 evaluable patients were needed in each treatment group to reject the hypothesis of noninferiority, at error rates of $\alpha/2 = .025$ and $\beta = .20$. With an expected dropout rate of 10%, a total of 552 patients were needed in this study. The study was designed as a noninferiority study regarding RFS. Inferiority was defined as a ratio of median RFS (t_{KLH}/t_{MM}) more than 0.75 (eg, 1.31/1.75 years), assuming proportional hazards.

The Kaplan-Meier method and one-sided log-rank test were used to compare for differences in RFS and PFS between the two treatments. Crude hazard ratios (HRs) with univariate Cox regression were used to analyze the influence of patient and tumor characteristics separately on RFS and PFS. Adjusted HRs with multivariate Cox regression were used to correct for confounding factors. Patient and tumor characteristics were compared by using a two-sided χ^2 test. Number of patients with a recurrence was compared by using a one-sided Fisher's exact test. Number of recurrent tumors was compared by using the one-sided Mann-Whitney U test, and toxicity was compared by using the two-sided χ^2 test. Effect of DTH response on outcome was analyzed with two-sided χ^2 tests and log-rank tests. All two-sided analyses were performed at a 5% level of significance; all one-sided analyses were performed at a 2.5% level of significance. Statistical analyses were done with SPSS version 16.0 software (SPSS, Chicago, IL).

RESULTS

Between July 2003 and November 2007, 553 patients were randomly assigned: 283 patients to treatment with KLH and 270 to treatment with MM. For safety analysis, 537 patients were available: 273 patients in the KLH group and 264 patients in the MM group. A total of 272 patients received at least one KLH instillation, and 263 patients received at least one MM instillation. One patient was randomly assigned for KLH but received MM and was included in the MM group. One patient did not receive KLH treatment but experienced an AE before instillation, so he was included in the KLH group (Fig 1).

For the efficacy analyses, 267 patients in the KLH arm and 256 patients in the MM arm were included. In the KLH arm, 16 patients were excluded: 10 did not receive any instillation, one patient was treated with MM, and five patients were ineligible (concurrent malignancy, n = 3; incomplete TURBT, n = 2). In the MM arm, 14 patients were excluded: seven did not receive any instillation, and seven were ineligible (concurrent malignancy, n = 6; tumor in prostatic urethra, n = 1; Fig 1).

Patient and Tumor Characteristics

Patient and tumor characteristics were comparable in both efficacy groups (Table 1), with the exception of tumor stage. Patients in the MM group had significantly more pT1 tumors (two-sided χ^2 test $P = .015$) but not significantly more grade 3 tumors (two-sided χ^2 test $P = .235$). DTH response was positive in 61.8% of patients treated

CONSORT Diagram

Assessed
(N = 553)

Enrollment

Random allocation — Excluded (n = 0)

Allocation

Allocated to KLH (n = 283)
Received allocated intervention (n = 272)
Did not receive allocated intervention (n = 11)
    Allocated to KLH but received MM (n = 1)
    Consent withdrawn (n = 2)
    Ineligible (n = 8)

Allocated to MM (n = 270)
Received allocated intervention (n = 263)
Did not receive allocated intervention; ineligible (n = 7)

Follow-Up

Lost to follow-up (n = 1)
Reason: unknown

Discontinued intervention (n = 103)
    Second recurrence during treatment (n = 45)
    Administrative error (n = 13)
    Adverse events (n = 11)
    CIS recurrence (n = 11)
    First recurrence during treatment (n = 11)

Lost to follow-up (n = 0)
Reason: unknown

Discontinued intervention (n = 61)
    Adverse events (n = 30)
    Administrative error (n = 7)
    Consent withdrawn (n = 6)
    Progression (n = 6)

Analysis

Safety analysis (n = 273): received at least one instillation (n = 272) + patient with AE prior to treatment (n = 1)

Efficacy analysis (n = 267): 283 – no treatment received (n = 10) – treated with MM (n = 1) – ineligible (n = 5)

Safety analysis (n = 264): received at least one instillation (n = 263) + patient was randomized for KLH but received MM (n = 1)

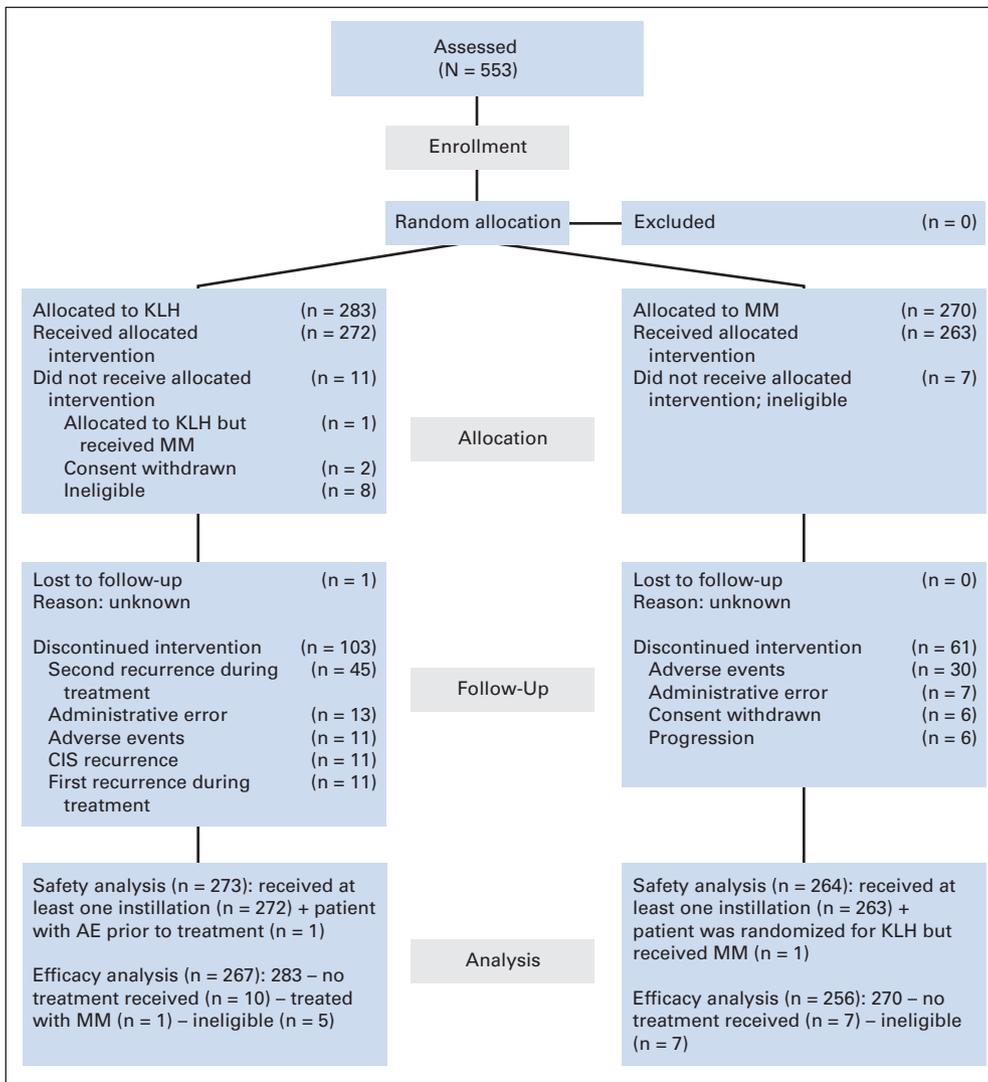Efficacy analysis (n = 256): 270 – no treatment received (n = 7) – ineligible (n = 7)

Fig 1. CONSORT diagram. AE, adverse event; CIS, carcinoma in situ; KLH, keyhole limpet hemocyanin; MM, mitomycin.

with KLH (165 of 267), negative in 97 patients (36.3%), and unknown in five patients (1.9%).

Safety

AEs were common (72% of all patients) but generally mild (56%), with no differences between the KLH and MM arms (557 AEs v 514 AEs). No differences in frequency of AEs grouped in system classes were found except for two AE system classes. General disorders such as fever, flu-like symptoms, and fatigue occurred significantly more frequently in the KLH group (51 AEs v 28 AEs; two-sided χ^2 test $P = .02$). Allergic reactions and skin disorders such as eczema, rash, and itch occurred significantly more frequently in the MM group (53 AEs v 9 AEs; two-sided χ^2 test $P < .001$). Significantly more mild AEs occurred in patients with a positive DTH response (two-sided χ^2 test $P = .004$; Table 2).

Efficacy

In total, 163 patients (61%) developed a recurrence after KLH instillations compared with 87 (34%) after MM instillations (one-sided Fisher's exact test $P < .001$). Median RFS in the KLH group was significantly shorter than that in the MM group (106 weeks [95% CI, 86 to 126 weeks] v 297 weeks [95% CI, 202 to 392 weeks]; log-rank test $P < .001$; Fig 2). Some recurrences occurred outside the bladder: three recurrences in the MM group (once in the prostatic urethra; twice in the upper urinary tract) and four in the KLH group (all in the prostatic urethra).

Univariate Cox regression for RFS showed a crude HR of 2.26 for KLH (95% CI, 1.74 to 2.94; $P < .001$; Table 3). Multivariate Cox regression showed an adjusted HR of 2.32 for KLH (95% CI, 1.79 to 3.02; $P < .001$). Ratio of median RFS was 106 of 297 or 0.36 (95% CI, 0.321 to 0.426). Because 0.426 is far below the threshold of 0.75, KLH is inferior to MM regarding RFS at a 0.001 level of significance.

Significantly more patients developed progression after MM (n = 15) than after KLH (n = 5; one-sided Fisher's exact test; $P = .015$). Univariate analyses showed a possible relationship between treatment and progression chances in favor of KLH (log-rank test $P = .049$; univariate Cox regression HR, 0.38 [95% CI, 0.14 to 1.04]; $P = .059$; Table 3). In the multivariate Cox regression analysis, this relationship was less obvious (adjusted HR, 0.43 [95% CI, 0.15 to 1.17]; $P = .099$; Table 4).

Table 1. Patient Demographics and Clinical Characteristics at Study Entry for All Patients in the Efficacy Analyses

Characteristic	KLH			MM			Total			P
	No.	%	SD	No.	%	SD	No.	%	SD	
No. of patients	267			256			523			
Sex										.910
Male	218			210			428			
Female	49			46			95			
Mean age, years	66.9		10.3	67.7		9.3	67.3		9.8	.428
History of CIS	3			4			7			.502
Tumor status										.476
Primary	144	54		146	57		290	55		
Recurrent	123	46		110	43		233	45		
Previous treatment in patients with recurrent tumors at baseline (n = 233)										.086
Current single-post TURBT intravesical chemotherapy	5	4		2	2		7	3		
Previous single-post TURBT intravesical chemotherapy	10	8		3	3		13	6		
Local adjuvant intravesical therapy	31	25		27	25		58	25		
Other therapy	3	2		0	0		3	1		
No therapy other than TURBT	74	60		78	71		152	65		
Tumor stage										.015
pTa	216	81		184	72		400	76		
pT1	51	19		72	28		123	24		
Tumor grade										.235
1	88	33		79	31		167	32		
2	148	55		134	52		282	54		
3	31	12		43	17		74	14		
No. of tumors										.849
Single	89	33		83	32		172	33		
Multiple	178	66		172	67		350	67		
Unknown	0			1	0		1	0		
Largest diameter, mm	20		29	19		16	39		24	.496

NOTE. Patients in the mitomycin (MM) group had significantly more pT1 tumors than patients in the keyhole limpet hemocyanin (KLH) group (two-sided χ^2 test $P = .015$).
Abbreviations: CIS, carcinoma in situ; SD, standard deviation; TURBT, transurethral resection of bladder tumor.

The mean number of recurrent tumors after KLH was 3.1 (standard deviation, 3.1) and 2.4 after MM (standard deviation, 2.2; one-sided Mann-Whitney U test $P = .154$). Recurrence rate was 2.32 after KLH and 1.05 after MM. Significantly more DTH-positive patients than DTH-negative patients developed a recurrence (two-sided χ^2 test $P = .031$; Table 5), but RFS and number of recurrences were not significantly different (log-rank test $P = .163$ and two-sided t test $P = .108$, respectively). Significantly more patients with a negative DTH response (n = 4) than DTH-positive patients

(n = 1) showed progression (two-sided χ^2 test $P = .044$), and time to progression tended to be shorter for DTH-negative patients (log-rank test $P = .051$).

For the subgroup analysis in patients with recurrent disease and a history of intravesical treatments, a total of 81 patients were identified (49 in the KLH group and 32 in the MM group). The number of patients with recurrences and time to recurrence was not statistically significantly different for the two treatment groups (one-sided Fisher's exact test $P = .194$ and log-rank test $P = .558$, respectively).

DISCUSSION

In this prospective, randomized phase III trial, significantly more recurrences occurred after treatment with KLH compared with MM ($P < .001$). Literature reporting on KLH and recurrence chances is diverse, and the number of patients is low. Jurincic et al[10] treated 44 patients with either KLH (single IC preimmunization followed by an unknown number of monthly instillations with 10 mg KLH) or MM (unknown number of monthly instillations with 20 mg MM in 30 mL saline) and found significantly fewer recurrences after KLH treatment than after MM treatment ($P < .05$). Kälble et al[11] compared KLH (IC preimmunization followed by instillations [10 mg KLH in 20 mL saline] given once per week for

Table 2. Severity and Number of Adverse Events Per DTH Response

	DTH Response						
	Positive		Negative		Unknown		
Severity	No.	%	No.	%	No.	%	Total
Mild	207	64	111	50	1	12.5	319
Moderate	96	30	89	39	6	75	189
Severe	22	7	25	11	1	12.5	48
Unknown	0	0	1	0	0	0	1
Total	325		224		8		557

NOTE. Patients with a positive delayed-type hypersensitivity (DTH) response had significantly more adverse events (two-sided χ^2 test $P = .004$).
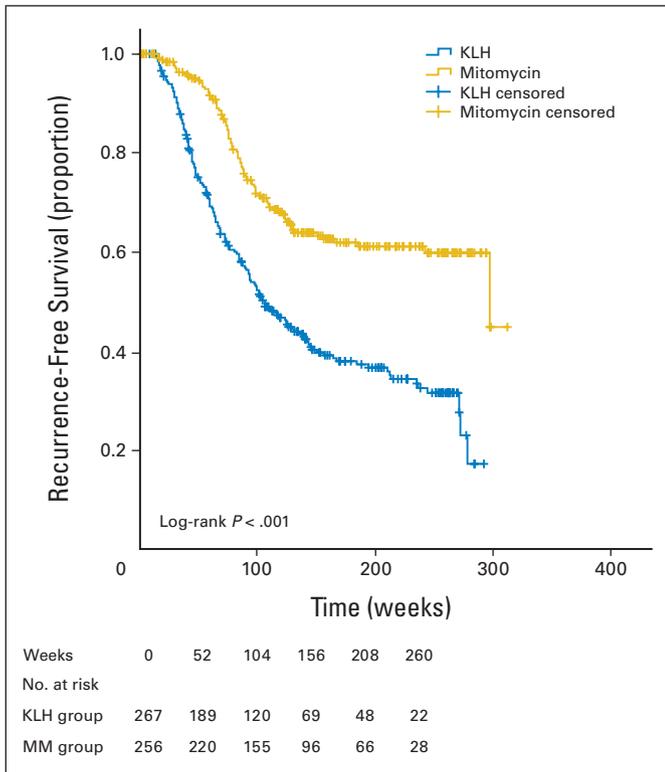
Fig 2. Kaplan-Meier curve for recurrence-free survival. KLH, keyhole limpet hemocyanin; MM, mitomycin.

6 weeks, once per month for the next 4 months, followed by two more instillations given at months 7 and 10) to BCG (instillations [120 mg BCG in 50 mL saline] given once per week for 6 weeks, once per month for the next 4 months, followed by two more instillations at months 7 and 10) in 38 patients and reported more recurrences after treatment with KLH (41.2% v 14.3% after BCG instillations). Jurincic-Winkler et al[13] found that 11 (52%) of 21 patients with CIS achieved a complete response after preimmunization and instillation with 20 mg KLH in 20 mg saline once per week for 2 weeks, of which five patients (24%) remained CIS free during follow-up (mean follow-up, 31.7 months). The long-term outcome showed long-term tumor remission in only two pa-

tients.[14] Echarti et al[15] found a significantly longer RFS in 37 patients after KLH treatment compared with several other intravesical treatments ($P = .0012$). However, treatment schedule and KLH dosage varied among these patients. Lamm et al[6] treated 64 patients with CIS and/or residual tumor with KLH (IC preimmunization followed by instillations once per week for 6 weeks with KLH in increasing dosages: 0.4, 2.0, 10, and 50 mg). Complete response was seen in 50% of patients with CIS and in 20% of patients with residual tumor. Linn et al[16] reviewed KLH efficacy and concluded that it appeared to be similar or better than intravesical chemotherapy. However, best dosage, treatment interval, and manner of application (intravesical, intralesional, or systemic) remains uncertain. In all, studies on KLH are small with a wide variety of inclusion criteria and treatment schedules and therefore diverse results.

In our study, progression was uncommon (3.8%; n = 20) since patients were observed until they developed a first recurrence, not until progression. Nevertheless, progression occurred significantly more often after MM treatment than after KLH treatment. Univariate Cox regression analysis for PFS showed no statistically significant difference between KLH and MM (HR, 0.38; 95% CI, 0.14 to 1.04; $P = .059$). When correcting for confounding factors by including more pT1 tumors in the MM group, the statistical significance was even less obvious (adjusted HR, 0.43; 95% CI, 0.15 to 1.17; $P = .099$). This suggests that the larger number of patients with pT1 tumors in the MM group might have introduced a bias. Because our results on progression are conflicting and paradoxical compared with the results of other studies, further research should focus on clarifying this.

There is little literature on progression after KLH. Jurincic et al[10] found progression in three patients treated with MM versus in one patient treated with KLH. However, progression was defined as upgrading. No progression was found after KLH treatment in patients with CIS, but mean follow-up was only 23.5 months.[11] Kälble et al[11] found an increase in grade or stage in two patients (11.8%) after KLH treatment versus no progression after BCG. As in other studies,[6,10,11,13,15,16] we found generally mild AEs after KLH instillations.

In this study, the MM schedule was instillations once per week for 4 weeks and then once per month for 7 months, which was the schedule commonly in use when this trial was conducted. Currently,

Table 3. Individual Crude HRs Calculated with Univariate Cox Regression for RFS and PFS

Variable	RFS			PFS		
	HR	95% CI	P	HR	95% CI	P
Treatment (reference group, MM)	2.26	1.74 to 2.94	< .001	0.38	0.14 to 1.04	.059
Sex (reference group, male)	0.94	0.68 to 1.31	.720	1.22	0.41 to 3.66	.719
Prior recurrence rate (reference group, primary)*	1.43	1.12 to 1.84	.005	0.60	0.23 to 1.57	.297
Tumor stage (reference group, Ta)	0.99	0.73 to 1.35	.967	3.85	1.59 to 9.33	.003
Grade 2 tumor (reference group, grade 1)	1.26	0.96 to 1.66	.097	7.16	0.92 to 55.45	.060
Grade 3 tumor (reference group, grade 1)	0.88	0.57 to 1.36	.562	19.89	2.49 to 159.03	.005
Tumor size (reference group, ≤ 15 mm)	0.82	0.62 to 1.08	.152	0.65	0.24 to 1.75	.392
No. of tumors (reference group, single)†	1.94	1.45 to 2.58	< .001	1.39	0.53 to 3.61	.505

Abbreviations: HR, hazard ratio; MM, mitomycin; PFS, progression-free survival; RFS, recurrence-free survival.
*Prior recurrence rate: primary v recurrent.
†No. of tumors: single v multiple tumors.

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Table 4. Adjusted HRs Calculated With Multivariate Cox Regression for RFS and PFS

	RFS			PFS		
Variable	HR	95% CI	P	HR	95% CI	P
Treatment (reference group, MM	2.32	1.79 to 3.02	< .001	0.43	0.15 to 1.17	.099
Prior recurrence rate (reference group, primary)*	1.36	1.06 to 1.75	.015	N/A		N/A
Tumor stage (reference group, Ta)	N/A		N/A	2.13	0.82 to 5.56	.121
Grade 2 tumor (reference group, grade 1)	N/A			N/A	5.66	0.71 to 44.90	.101
Grade 3 tumor (reference group, grade 1)	N/A			N/A	11.76	1.34 to 103.53	.026
No. of tumors (reference group, single)†	1.88	1.41 to 2.51	< .001	N/A		N/A

Abbreviations: HR, hazard ratio; MM, mitomycin; N/A, not applicable; PFS, progression-free survival; RFS, recurrence-free survival.
*Prior recurrence rate: primary v recurrent.
†No. of tumors: single v multiple tumors.

many different schedules are being used for MM, and guidelines do not recommend any particular schedule.[5,17,18]

Patients in this study had mainly intermediate or high risk of recurrences. The current guidelines advise BCG treatment for these patients,[5,18] because randomized controlled trials and meta-analyses have shown BCG maintenance to be superior to MM in preventing recurrences.[19,20] However, literature on superiority of BCG over MM for progression is less clear, as shown by Mälmstrom et al.[20] This suggests that KLH is also inferior to BCG. Only Kälble et al[11] compared BCG with KLH, and they indeed found that KLH is inferior to BCG.

Our results are contradictory on the need for preimmunization: a positive skin test appeared to be a prognostic factor for recurrences, but a negative DTH response was associated with progression. However, preimmunization was adequate in only 62% of the patients.

The exact mechanism behind KLH is not known. The most likely hypothesis is that KLH enhances the cellular immune system,[21-25] which is the rationale behind preimmunization. Several studies reported on the need for preimmunization: Swerdlow et al[26] found fewer tumors in mice preimmunized with KLH than in mice without preimmunization. However, the effect also depended on the intravesical KLH dose. Lamm et al[27] found significantly reduced tumor incidence and tumor volume (P < .001) and

significantly increased survival (P = .001) of mice after immunization before tumor transplantation compared with immunization after tumor transplantation. Jurincic-Winkler et al[25] found a relationship between positive skin test and fewer recurrences in only nine patients with primary NMIBC treated with intravesical KLH. As suggested by Oyelaran et al,[28] differences in clinical responses with KLH may be due to differences in antibody profiles, which vary considerably from person to person. In all, the immunology behind KLH has not been clarified and needs further testing.

KLH is registered for prevention of bladder cancer after TURBT and after failure of established therapies. In a subgroup analysis with these patients (n = 81), RFS was not significantly different between the KLH and MM arms. However, these results should be interpreted with care because subgroup analyses are prone to selection bias and, because of the small sample size, they are often underpowered. Therefore, no firm conclusions can be drawn from these subgroup analyses.

In daily urologic practice, NMIBC is sometimes coagulated instead of resected, but this means a lack of histologic confirmation. Therefore, additional analyses were done in which coagulated lesions, lack of biopsies, or lack of any resection were considered to be a protocol violation. Then, 210 patients developed a recurrence, with significantly more recurrences and significantly shorter RFS for KLH patients (both P < .001).

Sometimes in daily urologic practice, a recurrence is seen at cystoscopy but is not immediately resected. When considering the date at which the recurrence was first seen to be the date of recurrence, RFS was significantly shorter after KLH treatment than after MM treatment (P < .001). When considering all lesions, whether pathologically confirmed, coagulated, or not resected at all, to be a recurrence, RFS was once more significantly shorter for patients treated with KLH (P < .001).

In conclusion, this prospective, randomized phase III trial shows that KLH had a safety profile different from that of MM. Patients with a positive skin test as a result of preimmunization with intracutaneous KLH had significantly more recurrences. KLH was inferior to MM in preventing NMIBC recurrences. KLH tended to prevent progression more effectively than MM, but when correcting for the higher number of patients with pT1 tumors, this could not be shown. More research is needed to clarify the immunologic effects of KLH and the effects of KLH on progression.

Table 5. Relationship Between DTH Response and Clinical Outcome

	DTH Response						
	Positive		Negative		Unknown		
Response	No.	%	No.	%	No.	%	Total
Recurrence							
Yes	109	66	51	53	3	60	163
No	56	34	46	47	2	40	104
Total	165	100	97	100	5	100	267
Progression							
Yes	1	1	4	4	0	0	5
No	164	99	93	96	5	100	262
Total	165	100	97	100	5	100	267

NOTE. A positive delayed-type hypersensitivity (DTH) is associated with a higher chance of recurrence (two-sided Pearson χ^2 test P = .031). A negative DTH is associated with a higher chance of progression (two-sided Pearson χ^2 test P = .044).

AUTHOR CONTRIBUTIONS

Conception and design: Wim P.J. Witjes, J. Alfred Witjes
Administrative support: Wim P.J. Witjes
Collection and assembly of data: Wim P.J. Witjes, Maria H.D. Janzing-Pastors, Christien T.M. Caris
Data analysis and interpretation: Rianne J.M. Lammers, Wim P.J. Witjes, Christien T.M. Caris, J. Alfred Witjes
Manuscript writing: All authors
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