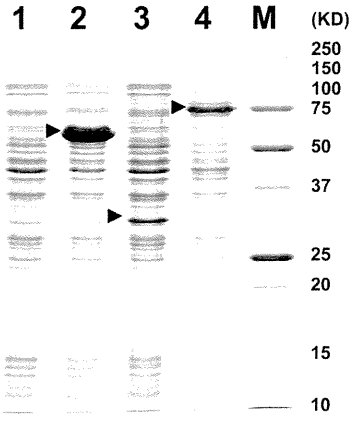

FIG 7 SDS-PAGE (12.5%) analysis of cell extracts from recombinant enzymes. 1, cell extract from pQE30Xa/*E. coli* JM109; 2, cell extract from pQESL-1/*E. coli* JM109; 3, cell extract from pQESL-2/*E. coli* JM109; 4, cell extract from pQESL-3/*E. coli* JM109; M, Precision Plus protein standard (Bio-Rad, Richmond, CA). Recombinant enzymes are indicated by arrows.

sumed to be a cytoplasmic protein, suggesting that their locations are different. *Lactococcus* and *Slackia* are bacteria belonging to different phyla; the former is found mainly in fish and animal milk and is rarely isolated from human (7, 24, 27, 28), whereas the latter inhabits the human intestines (11, 19, 22, 26). At present, although the phyletic evolution of these enzyme genes is unknown, the interbacterium propagation of the genes and their physiological roles in the bacteria are of interest.

This is the first report of the identification of 2 enzymes (ORF-1 and ORF-2) that catalyze the *cis/trans*-THD-to-equol and DHD-to-*cis/trans*-THD conversion reactions and their genes. Through a homology search of amino acid sequences, ORF-1 of *Slackia* sp. strain NATTS was shown to have a primary structure similar to that of the succinate dehydrogenase and fumarate reductase/succinate dehydrogenase flavoprotein domain protein derived from several bacteria, such as *Desulfovibrio fructosovorans* JJ and *Geobacter lovleyi* SZ (Fig. 4) However, these previously reported enzymes were among those found in a recent genomic sequencing, and only their functions have so far been inferred (8, 15). In addition, since the alignment analyses of the amino acid sequences of these enzymes and ORF-1 identified no consensus sequences, it is likely that the ORF-1 identified is a novel one involved in the metabolism of *cis/trans*-THD. On the other hand, our homology search and analysis of the primary structure of amino acids suggested that ORF-2 belongs to the short-chain dehydrogenase/reductase (SDR) superfamily (Fig. 5). The SDR family is a group of enzymes that catalyzes the oxidation-reduction reactions of steroids, cofactors, carbohydrates, lipids, aromatic compounds, and amino acids, using NADPH as an electron donor or acceptor (12). Until now, it was not known that the enzymes belonging to this family are involved in *cis/trans*-THD production; we have therefore suggested for the first time that this family is responsible for *cis/trans*-THD production.

Our analysis of the genes in *Slackia* sp. NATTS strain encoding the daidzein-to-equol conversion enzymes, and their genes, showed that the daidzein-to-equol conversion reaction proceeds by the action of three independent enzymes. These find-

TABLE 2 Daidzein- or DHD-metabolizing properties of clones

Plasmid	Substrate (100 μM)	Isoflavone concn (μM)[a]				Daidzein-to-DHD or DHD-to-equol % conversion
		Daidzein	DHD	*cis/trans*-THD	Equol	
pQESL-1	DHD	ND	97.4 ± 4.1	ND	ND	
pQESL-2	DHD	ND	43.2 ± 5.7	53.6 ± 1.4	ND	53.5
pQESL-1 and pQESL-2	DHD	ND	38.7 ± 1.6	7.3 ± 1.9	53.0 ± 1.2	
pQESL-3	Daidzein	1.4 ± 2.4	94.5 ± 1.0	ND	ND	98.6
pQE30Xa	Daidzein	100.5 ± 3.6	ND	ND	ND	
	DHD	ND	122 ± 3.3	ND	ND	

[a] Data are expressed as means and standard deviations. ND, not detected.

ings will lead to progress in enzymological studies of the daidzein-to-equol conversion reaction in enteric bacteria and in research on the ecology of equol-producing bacteria.

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Prostate Cancer Chemoprevention Study: An investigative randomized control study using purified isoflavones in men with rising prostate-specific antigen

Naoto Miyanaga,[1,13,14] Hideyuki Akaza,[2] Shiro Hinotsu,[3] Tomoaki Fujioka,[4] Seiji Naito,[5] Mikio Namiki,[6] Satoru Takahashi,[7] Yoshihiko Hirao,[8] Shigeo Horie,[9] Taiji Tsukamoto,[10] Mitsuru Mori[11] and Hirokazu Tsuji[12]

[1]Department of Urology and Andrology, Graduate School of Comprehensive Human Science, University of Tsukuba, Tsukuba; [2]Department of Strategic Investigation on Comprehensive Cancer Network, Research Center for Advanced Science and Technology, The University of Tokyo, Tokyo; [3]Department of Pharmacoepidemiology, Graduate School of Medicine and Public Health, Kyoto University, Kyoto; [4]Department of Urology, Iwate Medical University School of Medicine, Morioka; [5]Department of Urology, Graduate School of Medical Sciences, University of Kyushu, Fukuoka; [6]Department of Urology, Kanazawa University Graduate School of Medical Science, Kanazawa; [7]Department of Urology, Nihon University School of Medicine, Tokyo; [8]Department of Urology, Nara Medical University, Kashihara; [9]Department of Urology, Teikyo University School of Medicine, Tokyo; Departments of [10]Urology and [11]Epidemiology, Sapporo Medical University School of Medicine, Sapporo; [12]Yakult Central Institute for Microbiological Research, Kunitachi, Tokyo, Japan

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Our previous case–control study suggested that equol, a metabolite of isoflavone, has a preventive effect on prostate cancer. To examine the prostate cancer risk based on isoflavone intake and equol production, we carried out a phase II, randomized, double-blind, placebo-controlled trial of oral isoflavone (60 mg/day) for 12 months. The inclusion criteria were Japanese men between 50 and 75 years of age, a serum prostate-specific antigen level of 2.5–10.0 ng/mL, and a single, negative prostate biopsy within 12 months prior to enrollment. The study included 158 men in eight Japanese centers. Their median age was 66.0 years, and the numbers of equol producers and non-producers were 76 (48%) and 82 (52%), respectively. The majority of adverse events were mild or moderate in severity, and the scheduled intake of tablets was completed by 153 patients (96.8%). The prostate-specific antigen value showed no significant difference before and after treatment. Of the 89 patients evaluated by central pathological review, the incidence of biopsy-detectable prostate cancer in the isoflavone and placebo groups showed no significant difference (21.4% *vs* 34.0%, $P = 0.140$). However, for the 53 patients aged 65 years or more, the incidence of cancer in the isoflavone group was significantly lower than that in the placebo group (28.0% *vs* 57.1%, $P = 0.031$). These results support the value of isoflavone for prostate cancer risk reduction. A large-scale phase III randomized study of isoflavone tablets in men with different hereditary factors and living environments is warranted. Registered with the UMIN Clinical Trials Registry (UMIN-CTR) for clinical trials in Japan (C000000446). (*Cancer Sci* 2012; 103: 125–130)

Prostate cancer is the second most common cancer in men and the third most common cause of male cancer death worldwide.[1] The incidence of clinical cancer in Japan is low, however, the incidence of total clinical and latent prostate cancer is the same between Japanese and American populations.[2] Diet is thought to play an important role in the progression from microscopic to clinical cancer.[1,3] Fat and calcium have been reported to be risk factors for prostate cancer. Conversely, lycopene, selenium, soy isoflavone, and vitamin E were reported to be preventive factors.[4–6] However, the SELECT (Selenium and Vitamin E Cancer Prevention Trial) study,[7] a recent large-scale, double-blind study, was unable to show a preventive effect for selenium or vitamin E on prostate cancer.

Basic research, including epidemiologic studies, suggested that soy isoflavone exerts an anticarcinogenic effect on prostate cancer.[8,9] Our case–control study of the serum isoflavone levels in patients with prostate cancer and healthy volunteers[8] found that some individuals were able to degrade daidzein into equol (equol producers) whereas others were not (non-producers).

In another case–control study involving residents in Japan, Korea, and the USA,[9] we found that the percentage of equol producers in patients with prostate cancer was significantly lower than in the healthy controls (30.3% *vs* 49.5%; $P = 0.013$). The percentage of equol producers in patients and controls was 29% and 46% in Japan ($P = 0.004$) and 30% and 59% in Korea ($P = 0.001$), respectively. The serum isoflavone level was markedly lower and the percentage of equol producers was also lower (17% for patients and 14% for controls) for Americans as compared to the Japanese and Koreans.

In another study,[10] we carried out an age-stratified dietary survey of soybean food consumption and measured the serum isoflavone levels in healthy Japanese and Korean men. The daily intake of genistein and daidzein in the teenage group was significantly lower than in the age group ≥30 years ($P < 0.05$). In the Japanese cohort, the proportion of equol producers in the teenage group was only 10%, which was significantly the lowest among all age-strata. Those results suggest that equol or equol-producing ability may be deeply involved in prostate cancer risk, and decreased intake of isoflavones in the young generation may lead to an increase in the prostate cancer incidence in Japan and Korea.

Recently, we clarified the mechanism of biodegradation from daidzein into equol by two kinds of intestinal bacteria.[11] As a strategy for chemoprevention of prostate cancer, clinical intervention by changing equol non-producers to producers, as well as by ingesting equol-containing supplements, are anticipated.

Considering this background, we carried out a phase II, randomized, double-blind, placebo-controlled trial of oral isoflavone (60 mg/day) for 12 months.

Patients and Methods

Patients. This was a phase II, randomized, double-blind, placebo-controlled trial of isoflavone (60 mg/day), given orally for

[13]To whom correspondence should be addressed.
E-mail: nmiyanaga@mito-saisei.jp
[14]Present address: Department of Urology, Mitosaiseikai General Hospital, Mito, Ibaraki, Japan.

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12 months. The inclusion criteria were Japanese men between 50 and 75 years of age, a serum PSA level of 2.5–10.0 ng/mL (50–60 years) or 3.0–10.0 ng/mL (>60 years), and a single, negative prostate biopsy (6–12 cores) within 12 months prior to enrollment. Men with HG-PIN or ASAP in the baseline biopsy, or a history of prostate cancer, were excluded from the study. None of the patients were using a steroidal or non-steroidal antiandrogen. The protocol was approved by the Institutional Review Board of each study site. The study was carried out in accordance with the Helsinki Declaration, and all participants signed informed consent forms.

Study design. The ingredients in the soy isoflavone tablet are shown in Table 1. Eligible patients were randomized to receive 10 isoflavone tablets (isoflavone 6 mg/tablet, totally 60 mg/day) or 10 placebo tablets for 12 months. Six-core transrectal ultrasound-guided biopsies were planned to be carried out at 12 months. The investigator was allowed to carry out "protocol-independent" (for-cause) biopsies whenever deemed clinically necessary. Protocol-mandated biopsies and for-cause biopsies were to be submitted for confirmation by central pathological review (University of Kyushu, Fukuoka, Japan). However, central pathological review was not the essential requirement for registration. No biopsy samples were collected at baseline. The negative biopsy prior to enrolment was confirmed by local pathological review only.

Because this study was planned as a pilot phase II study of the large-scale clinical trial, the primary endpoints were the tolerability of the soy isoflavone tablet and the changes in PSA and sex hormones including testosterone, DHT, SHBG, and estradiol. The secondary endpoints were the incidences of biopsy-detectable prostate cancer, HG-PIN, and ASAP at 12 months.

Measurement of serum isoflavone levels. Blood samples were drawn at 0, 3, and 12 months (serum isoflavones and sex hormones) or 0, 3, 6, 9, and 12 months (PSA). They were drawn before breakfast, and the sera were separated and stored at −10°C or less. The frozen samples were transported to the laboratory of SRL (Tokyo, Japan). The details of the measurement of serum isoflavone levels have been described elsewhere.[8] The assayed isoflavones were genistein, daidzein, and equol. Equol producers were defined as having a baseline serum equol concentration above the lower limit of detection of the present assay system, that is, 0.5 ng/mL.[8]

Statistical analyses. The efficacy analysis population consisted of patients who ingested the study medication for 12 months, in accordance with the study protocol. The safety analysis population included all randomized patients.

Table 1. Ingredients of soy isoflavone tablet given to study participants for 12 months

Component	mg/10 tablets (%)
Daidzin	19.1 (31.9)
Genistin	3.5 (5.8)
Glycitin	10.4 (17.3)
Malonyl daidzin	8.1 (13.5)
Malonyl genistin	2.2 (3.7)
Malonyl glycitin	3.4 (5.7)
Acetyl daidzin	7.3 (12.2)
Acetyl genistin	1.9 (3.2)
Acetyl glycitin	3.6 (6.0)
Daidzein	0.2 (0.3)
Genistein	0.1 (0.1)
Glycitein	0.2 (0.3)
Total	60.0 (100.0)

Ten isoflavone tablets (6 mg/tablet, totally 60 mg/day) are divided twice.

Statistical analyses were carried out using Wilcoxon's test (non-parametric), the chi-square-test and Fisher's exact test. For the individual changes of laboratory tests we used the paired *t*-test. A *P*-value of <0.05 was defined as representing a statistically significant difference. Data were analyzed using SAS version 8 software (SAS Institute, Cary, NC, USA).

Results

Baseline patient characteristics. The study enrolled 158 men in eight Japanese centers. Their median age at the first blood collection was 66.0 years (range, 50–75 years). The number of equol producers and non-producers was 76 (48%) and 82 (52%), respectively. These patients were randomized into an isoflavone group (n = 78) and a placebo group (n = 80).

Table 2 shows data on the baseline patient characteristics. These baseline characteristics, age, family history of prostate cancer, total PSA, prostate volume, and equol production, were generally similar in each treatment group to those of the overall study population.

Safety and tolerability. The planned intake of tablets was completed in 153 of 158 patients (96.8%). Of the five patients who did not complete the treatment course, three decided for themselves to quit taking the tablets. The other two patients had grade 3 adverse events: one in the isoflavone group suffered iliac artery stenosis, and the other in the placebo group suffered ileus. However, the majority of adverse events were mild in severity. The completion rates in the isoflavone and placebo group were 96.2% (75/78) and 97.5% (78/80), respectively. No significant changes in laboratory data were observed during the study (data not shown).

Serum isoflavone levels. Figures 1 and 2 show the median serum levels of daidzein and equol, stratified by treatment and baseline equol production. Daidzein was significantly increased in the isoflavone groups, and its level was lower in the isoflavone/producer group compared with the isoflavone/non-producer group (Fig. 1).

In Figure 2, equol producers who received isoflavone greatly increased equol production and showed the highest equol level. Equol producers given the placebo showed no change in the serum equol level from before treatment. The two groups of non-producers, given either isoflavone or placebo, showed the lowest equol levels.

Table 2. Baseline patient characteristics

	Total (n = 158)	Isoflavone (n = 78)	Placebo (n = 80)	*P*-value
Age (years)				
Median	66.0	66.5	65.0	0.974†
Range	50.0–75.0	52.0–75.0	50.0–75.0	
Family history, no. (%)	3 (2)	1 (1)	2 (3)	0.873‡
Total PSA (ng/mL)				
Median	5.75	5.83	5.73	0.784†
Range	2.76–10.20	2.76–9.77	3.00–10.20	
Prostate volume (mL)				
Median	37.7	37.6	37.6	0.349†
Range	0.5–93.5	12.3–93.5	0.5–84.0	
Equol production, no. (%)				
Producer	76 (48)	38 (49)	38 (48)	0.502‡
Non-producer	82 (52)	40 (51)	42 (53)	

Non-producers, individuals unable to degrade daidzein into equol; producers, individuals able to degrade daidzein into equol.
†Wilcoxon's test; ‡Fisher's exact test.

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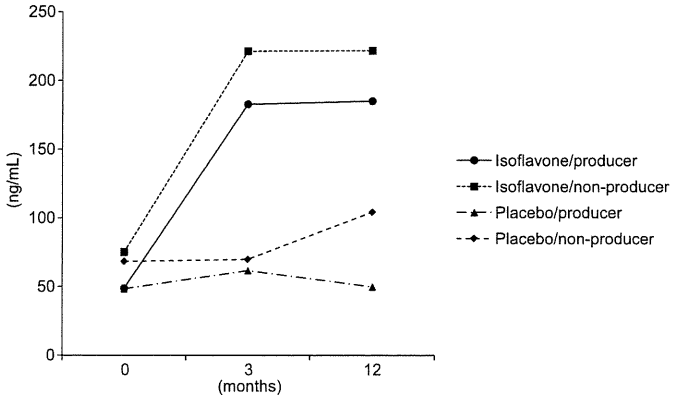
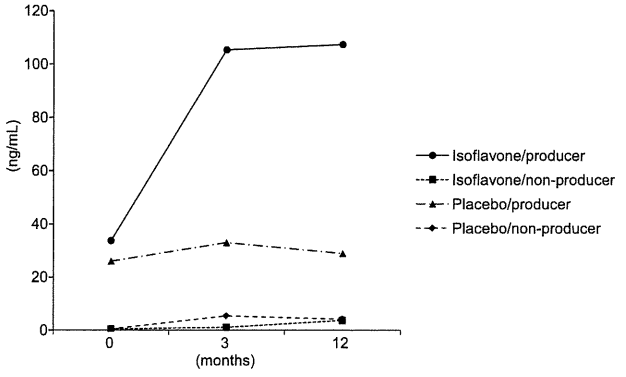


	0 month	3 months	12 months	*P*-value†
Isoflavone/producer	48.69	182.68	185.04	<0.0001*
Isoflavone/non-producer	75.08	221.27	221.72	0.0005*
Placebo/producer	48.37	61.66	49.84	0.4140
Placebo/non-producer	68.27	69.78	104.20	0.1240

† Paired *t* test (0 month *vs.* 12 months)
* $P < 0.05$

Fig. 1. Median serum levels of daidzein in Japanese men given oral isoflavone (60 mg/day) or placebo for 12 months (n = 158). Daidzein was significantly increased in the isoflavone groups, and its level was lower in individuals who could also degrade daidzein into equol (isoflavone/producer group) compared with those who could not (isoflavone/non-producer group).



	0 month	3 months	12 months	*P*-value†
Isoflavone/producer	33.68	105.35	107.34	0.0007*
Isoflavone/non-producer	0.50	1.12	3.70	0.0830
Placebo/producer	25.97	32.99	28.88	0.6080
Placebo/non-producer	0.50	5.39	4.13	0.1270

† Paired *t* test (0 month *vs.* 12 months)
* $P < 0.05$

Fig. 2. Median serum levels of equol in Japanese men given oral isoflavone (60 mg/day) or placebo for 12 months (n = 158). Individuals who could degrade daidzein into equol (equol producers), who received isoflavone, greatly increased equol production and showed the highest equol level. Equol producers given the placebo showed no change in the serum equol level from before treatment. The two groups of non-producers (those unable to degrade daidzein into equol), treated with either isoflavone or placebo, showed the lowest equol levels.

Prostate-specific antigen and sex hormones. The serum PSA value showed no significant change during the study (Fig. 3). There were also no differences among the treatment groups.

The interval changes in testosterone, DHT, and SHBG were not significant. In the non-producer groups, estradiol decreased significantly, independent of treatment (Table 3).

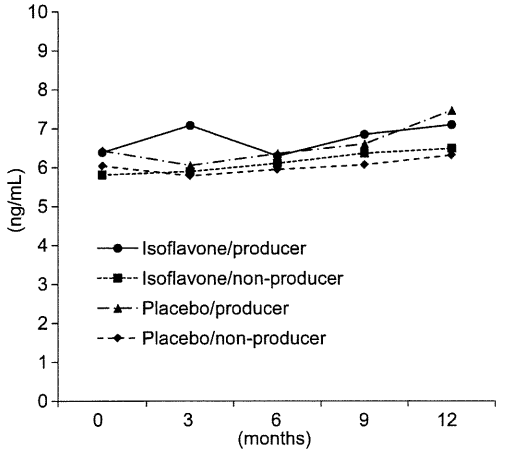
Pathologic endpoints. Of the 153 patients who completed the planned intake of tablets, 121 underwent needle biopsy of the prostate at 12 months. The remaining 32 patients did not agree to undergo the needle biopsy.

Of the 121 patients, 89, consisting of 42 in the isoflavone group and 47 in the placebo group, were evaluated by central pathological review, and 112 patients, consisting of 55 in the isoflavone group and 57 in the placebo group, were evaluated by local pathological review (Table 4). The specimens for 23 of the 112 patients were not approved to be sent to the central pathology laboratory by the Institutional Review Board. Thus, 89 were evaluated by both central and local pathological reviews. There were no significant differences between the results of central pathology and local pathology (data not shown). The following discussion focuses on the central pathology results.

The incidence of biopsy-detectable prostate cancer in the isoflavone and placebo groups was 21.4% (9/42) and 34.0% (16/47), respectively. These incidences of cancer detection were not statistically significantly different. However, for the patient stratum aged 65 years or more, the incidence of cancer in the isoflavone group was significantly lower than that in the placebo group (28.0% [7/25] *vs* 57.1% [16/28], $P = 0.031$).

The incidence of a Gleason score of 6 or less and HG-PIN were numerically lower in the isoflavone group compared with the placebo group, but the difference was not statistically significant.

Table 5 presents the central pathology results for prostate cancer incidence based on isoflavone intake and equol production. The incidence of prostate cancer in the isoflavone group was significantly lower than that in the placebo group for the



	0 month	3 months	6 months	9 months	12 months
Isoflavone/producer	6.39	7.08	6.29	6.85	7.10
Isoflavone/non-producer	5.81	5.89	6.10	6.37	6.49
Placebo/producer	6.43	6.05	6.36	6.60	7.46
Placebo/non-producer	6.04	5.79	5.95	6.07	6.32

Fig. 3. Serum prostate-specific antigen (PSA) levels in Japanese men given oral isoflavone (60 mg/day) or placebo for 12 months (n = 158). The PSA value showed no significant change during the study. There were also no differences among the treatment groups. Non-producers, individuals unable to degrade daidzein into equol; producers, individuals able to degrade daidzein into equol.

Table 3. Changes in hormone levels in Japanese men given oral isoflavone (60 mg/day) or placebo for 12 months (n = 158)

	0 month (pre)	3 months	12 months	P-value†
Testosterone				
Isoflavone	5.45	5.12	5.31	0.131
Producer	5.33	4.92	5.28	0.405
Non-producer	5.57	5.33	5.34	0.200
Placebo	5.12	5.36	5.07	0.286
Producer	5.25	5.22	5.19	0.508
Non-producer	5.01	5.48	4.99	0.411
DHT				
Isoflavone	0.94	0.95	1.01	0.533
Producer	0.92	0.89	1.00	0.708
Non-producer	0.96	1.01	1.02	0.619
Placebo	0.91	0.99	0.98	0.337
Producer	1.02	1.09	1.04	0.869
Non-producer	0.83	0.91	0.94	0.207
Estradiol				
Isoflavone	28.56	27.41	26.53	0.027*
Producer	27.44	26.39	26.29	0.416
Non-producer	29.59	28.46	26.76	0.023*
Placebo	27.20	26.65	25.27	0.0002*
Producer	26.71	26.97	25.70	0.122
Non-producer	27.61	26.36	24.94	0.007*
SHBG				
Isoflavone	52.00	55.43	55.97	0.064
Producer	52.76	52.70	57.00	0.261
Non-producer	51.31	58.17	54.95	0.138
Placebo	50.11	48.82	51.71	0.321
Producer	53.98	50.11	53.48	0.511
Non-producer	46.89	47.64	50.32	0.468

DHT, dihydrotestosterone; non-producers, individuals unable to degrade daidzein into equol; producers, individuals able to degrade daidzein into equol. SHBG, sex hormone binding protein. †Paired t-test (0 *vs* 12 M); *P < 0.05.

patient stratum aged 65 years or more and equol non-production.

Discussion

Because of the high incidence of microscopic prostate cancer and the long latency period from microscopic lesions to clinical disease, development of strategies for reducing the risk of prostate cancer is a reasonable and promising approach. Clinical research has been carried out regarding the preventive effect of isoflavones on prostate cancer, including dietary supplement intervention trials for prostate cancer patients.[12,13] In those studies, patients were given isoflavone-containing drugs and the serum PSA was examined as a surrogate marker. Kumar *et al.*[12] gave a soy isoflavone beverage for 12 weeks to men with prostate cancer on watchful waiting, randomizing 59 patients to a soy group and a placebo group. Serum total PSA decreased or was unchanged in 69% of the subjects in the isoflavone-treated group compared to 55% in the placebo group. Schröder *et al.*[13] gave an isoflavone-containing drug to men with prostate cancer with increasing PSA after primary treatment. Forty-two patients were examined in a placebo-controlled, double-blind, crossover study that involved 10 weeks of intervention, followed by a 4-week washout period prior to crossover. Although no statistically significant difference was found in either total (P = 0.076) or free (P = 0.988) PSA between the two groups, the free PSA doubling-time was significantly increased in the supplement group compared with the control group: 1150 *vs* 445 days (2.6-fold, P = 0.041). These studies establish the need to further explore the effects of prolonged and consistent soy consumption.

In the present study, pure isoflavone was administered to 158 patients over a comparatively long period of 12 months. In an interventional study, if some patients more aggressively consume foods containing isoflavones, such as tofu, miso, and natto, the influence on intervention was supposed. In the present study, however, the plasma isoflavone concentrations in the isoflavone groups were clearly higher than in the placebo control groups. Therefore, such influence to the results is able to be excluded.

The incidence of prostate cancer was lower in the isoflavone group, but not significantly. However, for the patient stratum aged 65 years or more, the incidence of cancer in the isoflavone group was significantly lower than that in the placebo group (28.0% *vs* 57.1%, P = 0.031). The reason why a significant difference was not shown in the total patient cohort might be related to the fact that the incidence of prostate cancer was small.

One of the primary endpoints, the effect on PSA level, was not proven. However, one of the secondary endpoints, the incidence of biopsy-detectable prostate cancer, was confirmed to have been significantly reduced in the group aged ≥65 years. Serum PSA is well-established as a biomarker of prostate cancer, but it is not specific to neoplasia, and the data do not suggest that the level is related directly to the extent of neoplastic progression.

The incidence of Gleason scores of 6 or less and HG-PIN were numerically lower in the isoflavone group compared with the placebo group, but not significantly. Based on this result, isoflavone may suppress small and low-grade cancers. This may be one of the reasons why an effect of isoflavone on the serum PSA level was unable to be proven.

We are also interested in prostate cancer risk reduction based on isoflavone administration and equol production. Unfortunately, because of the insufficient number of enrolled men, we were unable to analyze for a relationship between isoflavone intake and equol production.

Although intake of isoflavone suppressed the incidence of prostate cancer, the hormonal data did not show significant changes.

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Table 4. Numbers and proportions of men with prostate cancer and high-grade prostatic intraepithelial neoplasia (HG-PIN) who participated in this study (n = 158)

	Central pathology			Local pathology		
	Isoflavone (n = 42)	Placebo (n = 47)	P-value†	Isoflavone (n = 55)	Placebo (n = 57)	P-value†
No. of patients with tumors	9/42 (21.4%)	16/47 (34.0%)	0.140	8/55 (14.6%)	14/57 (24.6%)	0.137
Age (years)						
<64	2/17 (11.8%)	0/19 (0.0%)	0.220	2/27 (7.4%)	0/26 (0.0%)	0.255
≥65	7/25 (28.0%)	16/28 (57.1%)	0.031*	7/31 (22.6%)	14/31 (47.1%)	0.035*
Equol production						
Producer	5/22 (22.7%)	8/22 (36.4%)	0.255	5/29 (17.2%)	6/19 (24.0%)	0.390
Non-producer	4/20 (20.0%)	8/25 (32.0%)	0.288	3/26 (11.5%)	8/32 (25.0%)	0.168
No. of positive cores						
1	6	11	0.713	5	10	0.510
2–4	3	5	(1 *vs* 2–4)	3	4	(1 *vs* 2–4)
Gleason score						
5	0	0		0	1	
6	5	12		4	8	
7	3	4		3	2	
8	0	0		1	3	
9	1	0		0	0	
5–6	5/9 (55.6%)	12/16 (75.0%)	0.287	4/8 (50.0%)	9/14 (64.3%)	0.416
7–9	4/9 (44.4%)	4/16 (25.0%)	(G5–6 *vs* 7–9)	4/8 (50.0%)	5/14 (35.7%)	(G5–6 *vs* 7–9)
HG-PIN	2/42 (4.8%)	8/47 (17.0%)	0.660	NE	NE	NE

The Gleason score is the sum of the two most common histological patterns. NE, not evaluated; non-producers, individuals unable to degrade daidzein into equol; producers, individuals able to degrade daidzein into equol. †Fisher's exact test; *P < 0.05.

Table 5. Numbers and proportions of men with prostate cancer and high-grade prostatic intraepithelial neoplasia (HG-PIN), based on an isoflavone intake administration and equol production (central pathology)

	Equal producer			Equal non-producer		
	Isoflavone (n = 22)	Placebo (n = 22)	P-value†	Isoflavone (n = 20)	Placebo (n = 25)	P-value†
No. of patients with tumors	5/22 (22.7%)	8/22 (36.4%)	0.255	4/20 (20.0%)	8/25 (32.0%)	0.288
Age (years)						
<64	1/9 (11.1%)	0/6 (0.0%)	0.600	1/8 (12.5%)	0/13 (0.0%)	0.381
≥65	4/13 (30.8%)	8/16 (50.0%)	0.293	3/12 (25.0%)	8/12 (66.7%)	0.049*
No. of positive cores						
1	4	7	0.641	2	2	0.727
2–4	1	1	(1 *vs* 2–4)	4	4	(1 *vs* 2–4)
Gleason score						
6	2	6		3	6	
7	2	2		1	2	
8	0	0		0	0	
9	1	1		0	0	
5–6	2/5 (40.0%)	6/8 (75.0%)	0.250	3/4 (75.0%)	6/8 (75.0%)	0.764
7–9	3/5 (60.0%)	2/8 (25.0%)	(G6 *vs* 7–9)	1/4 (25.0%)	2/8 (25.0%)	(G6 *vs* 7–9)
HG-PIN	1/22 (4.6%)	4/22 (18.2%)	0.172	1/20 (5.0%)	4/25 (16.0%)	0.251

The Gleason score is the sum of the two most common histological patterns. †Fisher's exact test; *P < 0.05.

These results suggest that isoflavone exerts a cancer chemoprevention effect through an action other than hormonal. In published reports, effects such as apoptosis induction, tyrosine kinase inhibition, anti-angiogenic action, anti-oxygenation, and antipromotion have been reported for isoflavones based on *in vitro* and *in vivo* studies.[14,15] In the non-producer groups, estradiol decreased significantly, independent of treatment. We are not able to definitely interpret the phenomenon. Estrogen may decrease if the body weight tends to increase in non-producer groups. In this study, the change in body weight was not measured.

This study was carried out in Japanese patients. Isoflavone intake showed an effect on prostate cancer, even though Japanese ordinarily ingest considerable isoflavone in daily life. It can be expected that this effect would become even greater in Europe and America, who ordinarily ingest little isoflavone. A global study including Europe and America is recommended.

In the PCPT[16] and REDUCE[17] studies, which used 5AR inhibitors, the incidence of prostate cancer was reduced by 25%.[18] However, this treatment strategy is high in cost and leads to complications such as sexual dysfunction.[18] Therefore, its suitability can be considered to be limited to men at very high risk of prostate cancer. However, foods and supplements including isoflavones would be suitable for men in general, because of

their safety, low cost, and high feasibility. In addition, isoflavones are effective not only against prostate cancer but also cardiovascular diseases, osteoporosis, and hyperlipidemia.

The isoflavone tablets used in the present study showed no specific safety problems and were well tolerated, with 96.2% (75/78) of the patients in the isoflavone group completing the treatment regimen. Equol binds specifically with 5a-DHT and sequesters it from the androgen receptor.[19] The end result is similar to that achieved with 5AR inhibitors. We are able to obtain a similar effect to 5AR inhibitors, and safely, using isoflavone.

In conclusion, the incidence of prostate cancer in the isoflavone group was lower than that in the placebo group. In addition, the safety and tolerability of isoflavone intake were proven. These results support the value of isoflavone treatment for prostate cancer risk reduction. A large-scale phase III randomized study, preferably international, that takes into account different hereditary factors and living environments is warranted.

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Disclosure Statement

The authors have no conflicts of interest.

Abbreviations

5AR	5-α-reductase
ASAP	atypical small acinar proliferation
DHT	dihydrotestosterone
HG-PIN	high-grade prostatic intraepithelial neoplasia
PSA	prostate-specific antigen
SHBG	sex hormone binding protein

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BJUI
BJU INTERNATIONAL

Overall survival and good tolerability of long-term use of sorafenib after cytokine treatment: final results of a phase II trial of sorafenib in Japanese patients with metastatic renal cell carcinoma

Seiji Naito, Taiji Tsukamoto*, Masaru Murai†, Koichi Fukino‡ and Hideyuki Akaza§[1]

*Department of Urology, Graduate School of Medical Sciences, Kyushu University, Fukuoka *Department of Urology, Sapporo Medical University School of Medicine, Sapporo, †International Goodwill Hospital, Yokohama, ‡Bayer Yakuhin, Ltd, Osaka, and §Department of Urology and Andrology, Graduate School of Comprehensive Human Science, University of Tsukuba, Tsukuba, Japan*

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[1]Present affiliation: Research Center for Advanced Science and Technology, University of Tokyo, Tokyo, Japan.

Study Type – Therapy (Phase II non-randomized trial)
Level of Evidence 2b

What's known on the subject? and What does the study add?

Interim result of this study had shown promising efficacy, with response rate of 14.7% and median PFS of 7.4 months, and good tolerability of sorafenib in previously-treated Japanese patients with metastatic RCC. Final result of the study adds: (1) the median overall survival of 25.3 months, which is longer than that in the global phase III study TARGET; (2) the response rate which elevated to 19.4% because of 6 late responders achieved after 9.2 months or longer of SD period; (3) lack of either unknown adverse events nor cumulative toxicity in the long-term use of sorafenib.

OBJECTIVE

- To explore the long-term efficacy and safety of sorafenib in Japanese patients with metastatic renal cell carcinoma (RCC) in a phase II trial.

PATIENTS AND METHODS

- In all, 131 Japanese patients with metastatic RCC who had received nephrectomy and failed at least one cytokine-containing systemic therapy received continuous sorafenib 400 mg twice daily, and the efficacy and safety parameters were evaluated in these patients, including objective response rate, progression-free survival and overall survival.

RESULTS

- Of the total, 129 patients were valid for intention-to-treat analyses and 131 patients were valid for safety analyses.
- Twenty-five patients (19.4%) had confirmed partial response and 87 patients (67.4%) had stable disease as best overall response. The 25 patients included six late-responders who achieved response after 9.2 months or longer of stable disease. The objective response rate and disease control rate were 19.4% and 73.6%, respectively.
- The median overall survival and median progression-free survival were 25.3 and 7.9 months, respectively.
- Safety profile was consistent with those previously reported, with hand–foot skin reaction (58.0%), lipase elevation (57.3%) and diarrhoea (42.7%) as the most frequently observed drug-related adverse events. Neither unknown adverse event nor cumulative toxicity was observed over the long-term use of sorafenib.
- Despite the dose discontinuation/interruption/reduction, the mean and median relative dose intensities were 86.4% and 97.4%, respectively.

CONCLUSION

- The final results of this trial showed that long-term use of sorafenib after cytokine treatment was well tolerated and provided new efficacy data, including late-response events and favourable overall survival in Japanese patients with metastatic RCC.

KEYWORDS

sorafenib, renal cell carcinoma, efficacy, overall survival, safety

INTRODUCTION

RCC comprises 90–95% of kidney-derived tumours [1]. Although early-stage RCC may be cured with some treatments, including nephrectomy, patients with advanced RCC usually show poor prognosis despite application of cytokine therapies [1–3].

Sorafenib (Nexavar®; Bayer HealthCare, Montville, NJ, USA; Onyx Pharmaceuticals, Emeryville, CA, USA) is an orally available, multikinase inhibitor that blocks the activities

of multiple kinases, including Raf kinases as well as receptor tyrosine kinases, which are involved in tumour cell proliferation and angiogenesis, respectively. RCC is known to be rich in angiogenesis, and vascular endothelial growth factor may function as a tumour-associated angiogenic factor in RCC [4]. Moreover, Oka *et al.* [5] reported activation of a mitogen-activated protein kinase signalling transduction pathway in RCC, where Raf kinase may be involved. Therefore, sorafenib was a very strong candidate drug for treatment of advanced RCC. In fact, the results of the phase III Treatment Approaches in Renal Cancer Global Evaluation Trial (TARGET), a randomized, double-blind, placebo-controlled study, indicated that sorafenib is effective and safe for patients with advanced RCC [6,7]. In Japanese patients with advanced RCC, the phase II clinical trial of sorafenib showed encouraging efficacy and tolerability [8]. Those Japanese patients who did not complete the study protocol at the cut-off date for the previous report [8] continued the treatment and/or follow-up observation as defined by the protocol, and this is the final report of the Japanese phase II clinical trial. So far, according to a Japan-specific post-marketing survey, sorafenib has been administered to more than 3000 Japanese patients, thereby establishing its firm position in the treatment of Japanese patients with RCC. The results of the all-patient survey and the long-term effects of sorafenib are awaited. We present here the final results of the phase II study that examined the long-term efficacy with long overall survival (OS) and safety of sorafenib in Japanese patients with metastatic RCC.

PATIENTS AND METHODS

PATIENT SELECTION AND STUDY DESIGN

Details of study design and patient inclusion/exclusion criteria of this non-randomized, open-label, phase II trial in Japan have been described previously [8]. In short, Japanese patients with histologically or cytologically confirmed metastatic RCC who had undergone nephrectomy and failed at least one cytokine-containing regimen were regarded as eligible and entered this study. Other main inclusion criteria included Eastern Cooperative Oncology Group performance status (ECOG-PS) of 0 or 1, intermediate or low risk of the Motzer score and adequately preserved organ function. To compare the results of the present study with those in TARGET [6,7], the Motzer score used in this study protocol was the same version as used in the TARGET protocol [9]. Any patient with metastasis in the central nervous system was excluded. Patients received continuous sorafenib 400 mg twice daily until the occurrence of disease progression, unacceptable toxicity or death. Dose interruption of less than 30 days or one or two levels of dose reduction (first to 400 mg once daily, then to 400 mg every 2 days) were permitted in cases of adverse events.

PATIENT OUTCOMES

The primary objective of the current study was to determine the best objective response rate in Japanese patients with metastatic RCC treated with sorafenib. The secondary objectives included assessment of OS, progression-free survival (PFS), time to response, duration of response and safety.

Antitumour efficacy

Throughout the treatment period, lesions identical to those identified and measured at baseline were evaluated using the same technique, and preferably by the same investigator/radiologist to maintain consistency of tumour evaluation, according to Response Evaluation Criteria in Solid Tumours (RECIST). Tumour evaluation presented in this report was done by investigators' assessment. Efficacy variables included best objective response rate (the proportion of patients with confirmed partial remission [PR] and complete remission according to RECIST), overall disease control rate (the proportion of patients who had a best response rate of complete remission, PR or stable disease [SD] according to RECIST that was maintained for at least 28 days from the first demonstration of that rating), OS (the time from initiation of treatment to death), PFS (the time from initiation of treatment to disease progression or death), time to response and duration of response.

Safety

All patients who received at least one dose of sorafenib were evaluated for safety. All the examinations and observations for safety of sorafenib included results of physical examinations, vital signs, adverse events, concomitant medications and abnormal laboratory tests. Patients were monitored at every visit, and as needed for adverse events. The incidence, grade and causal relationship of adverse events to sorafenib were evaluated according to the National Cancer Institute's Common Terminology Criteria for Adverse Events, version 3.0. To evaluate the tolerability of sorafenib in Japanese patients with metastatic RCC, duration of sorafenib treatment and actual daily dose of sorafenib were calculated.

Statistical analysis

The primary population for efficacy analysis was the intent-to-treat population, and all patients who had received at least one dose of sorafenib and had any safety data after initiation of sorafenib dosing were evaluated for safety. Demographic and other baseline characteristics were summarized in a descriptive manner. Best objective response rate was defined as the proportion of patients who had a best response rating of complete remission or PR according to RECIST. For best objective response rate and overall disease control rate, point estimates and their 95% confidence intervals were calculated. OS, PFS and duration of response were analysed using the Kaplan–Meier method. Adverse events were summarized according to the terms and grades of the National Cancer Institute's Common Terminology Criteria for Adverse Events, version 3.0. Other safety parameters, including laboratory data, were summarized in a descriptive manner.

ETHICS

The protocol was approved by the appropriate institutional review boards and/or ethical committee. This study was conducted in accordance with the ethical principles of the Declaration of Helsinki, and the protocol complied with the Good Clinical Practice issued on 1 April 1997. All patients provided written informed consent before participating in the study.

RESULTS

PATIENT CHARACTERISTICS

As previously described [8], a total of 131 patients entered into the current study and received at least one dose of sorafenib. Two patients were excluded from the intent-to-treat population for efficacy analyses because of violation of inclusion criteria. The baseline

TABLE 1 Baseline demographic and disease characteristics in patients valid for intent-to-treat analysis (N = 129)

	Number of patients (%)
Sex	
Male	100 (77.5)
Female	29 (22.5)
Age (years)	
Median (range)	63.0 (30.0–83.0)
Age group	
<65 years	74 (57.4)
≥65 years	55 (42.6)
Time since initial diagnosis (years)	
Median (range)	2.6 (0.26–17.02)
Time since first progression/relapse (years)	
Median (range)	1.32 (0.09–8.81)
Motzer risk	
Low	52 (40.3)
Intermediate	77 (59.7)
ECOG-PS	
0	101 (78.3)
1	28 (21.7)
Number of tumour sites	
1	38 (29.5)
2	41 (31.8)
>2	50 (38.8)
Stage at study entry (TNM classification)	
Stage IV	129 (100)
Histology subtype	
Clear cell	122 (94.6)*
Papillary	5 (3.9)
Chromophobe	1 (0.8)
Other	1 (0.8)
Previous anticancer therapy	
Radiotherapy	17 (13.2)
Nephrectomy	129 (100)
Interferons	128 (99.2)
Interleukins	60 (46.5)
Number of previous systemic anticancer therapy	
1	46 (35.7)
2	47 (36.4)
3	34 (26.4)
6	2 (1.6)

*Expressed as patient number (%) except for age, time since initial diagnosis and time since first progression/relapse. *Including 10 (7.8%) patients who had been diagnosed as 'granular' subtype by the time of study entry.*

demographic and disease characteristics of the 129 patients valid for intent-to-treat analysis are summarized in Table 1: 100 male patients (77.5%), 29 female (22.5%); Motzer score low and intermediate risk was 52 patients (40.3%) and 77 patients (59.7%), respectively; the great majority of the patients (122; 94.6%) had clear-cell type RCC; 128 patients (99.2%) had received interferon treatment, and 60 patients (46.5%) had received interleukin treatment. The number of previous systemic regimens ranged from one to six.

ANTITUMOUR EFFICACY

Table 2 summarizes the results of best overall response according to RECIST. Twenty-five patients (19.4%) had confirmed PR and 87 patients (67.4%) had SD as their best overall response. The objective response rate and the overall disease control rate with 95% confidence intervals were 19.4% (13.0, 27.3) and 73.6% (65.2, 81.0), respectively. Although only 25 patients (19.4%) showed PR by RECIST, 102 of the 129 patients (79.1%) valid for efficacy analyses showed some degree of target-lesion shrinkage according to RECIST, based on investigators' assessment (Fig. 1A). In the 25 patients showing PR, median time to response and median duration of response were 2.8 and 13.8 months, respectively. In addition to the 19 PR patients reported previously [8], an additional six patients achieved PR after 9.2–18.3 months of SD (Fig. 1B). Five of these six patients (83.3%) were Motzer score low-risk patients. Duration of disease control (period for SD and PR) exceeded 36 months in four of the 25 patients with PR (Fig. 1B). Based on investigators' assessment, median OS and median PFS with confidence intervals were 25.3 (19.0, 32.0) and 7.9 (6.4, 10.8) months, respectively (Fig. 2).

SAFETY

All of the 131 patients who had at least one dose of sorafenib experienced at least one treatment-emergent adverse event, and 127 of the 131 patients (96.9%) developed at least one drug-related adverse event (Table 3). One patient (0.8%) showed a grade 5 event (dyspnoea), as previously described [8]. Fifteen types of drug-related adverse events in six categories occurred in more than 10% of the patients (Table 3). The most frequently observed drug-related adverse events were hand–foot skin reaction (58.0%), elevation of serum lipase level (57.3%) and diarrhoea (42.7%). In addition to hand–foot skin reaction, alopecia and rash/desquamation occurred relatively often (41.2% each), suggesting that skin toxicities were characteristic adverse events of sorafenib. Similarly to the level of serum lipase, that of amylase was frequently elevated (39.7%). These elevations of serum pancreatic enzymes were asymptomatic. The frequently reported adverse events were generally considered to be mild and manageable.

EXPOSURE TO SORAFENIB

As an indicator of tolerability, we evaluated the exposure to sorafenib in all 131 patients. Adverse events resulting in permanent

discontinuation of study treatment occurred in 29 patients (22.1%), while dose interruption and reduction occurred in 55 (42.0%) and 45 (34.4%) patients, respectively. Dose changes due to adverse events, including discontinuation, interruption or reduction of sorafenib dosing, were observed in 73 patients (55.7%), and the great majority of the first dose changes occurred in the early stage of treatment, i.e. for 39 patients (53.4%) within the first 3 months (Table 4). Although more than half of the patients experienced dose changes due to adverse events, the relative dose intensity was as high as 86.4% as the mean value and 97.4% as the median value (Table 4). The mean and the median durations of treatment were 12.0 and 7.7 months, respectively. At the end of the current study, 10 patients transferred to commercial sorafenib treatment without showing either intolerable toxicity or disease progression.

TABLE 2 Summary of best response based on investigator assessment in patients valid for intent-to-treat analysis (N = 129)

	Number of patients (%)	95% CI
Complete response	0 (0)	0.0, 2.8
Partial response	25 (19.4)	13.0, 27.3
Stable disease	87 (67.4)	58.6, 75.4
Disease progression*	13 (10.1)	5.5, 16.6
Not evaluated	4 (3.1)	0.9, 7.7
Objective response	25 (19.4)	13.0, 27.3
Disease control†	95 (73.6)	65.2, 81.0

**Radiological or clinical disease progression. †Patients having complete response, PR or SD maintained for at least 28 days.*

TABLE 3 Overall and grade 3/4 drug-related adverse events occurring in >10% of all patients valid for safety analysis (N = 131)

	Grade		
Category and event	Any	3	4
Any category			
Any event	127 (96.9)*	70 (53.4)	20 (15.3)
Cardiac general			
Hypertension	43 (32.8)	22 (16.8)	–
Constitutional symptoms			
Fatigue	22 (16.8)	3 (2.3)	–
Weight loss	23 (17.6)	4 (3.1)	–
Dermatology/skin			
Alopecia	54 (41.2)	–	–
Hand–foot skin reaction†	76 (58.0)	12 (9.2)	NA
Pruritus	15 (11.5)	–	–
Rash/desquamation	54 (41.2)	5 (3.8)	–
Gastrointestinal			
Anorexia	27 (20.6)	4 (3.1)	–
Diarrhoea	56 (42.7)	7 (5.3)	–
Metabolic/laboratory			
ALT	16 (12.2)	3 (2.3)	3 (2.3)
Amylase	52 (39.7)	8 (6.1)	–
AST	16 (12.2)	2 (1.5)	2 (1.5)
Lipase	75 (57.3)	33 (25.2)	9 (6.9)
Metabolic/lab – other	42 (32.1)	19 (14.5)	2 (1.5)
Pulmonary/upper respiratory			
Voice changes	17 (13.0)	–	–

*Expressed as patient number (%). NA, not applicable; ALT, alanine aminotransferase; AST, aspartate aminotransferase. *Includes one (0.8%) grade 5 event (dyspnoea). †Severity of hand–foot skin reaction was graded from 1 to 3.*

DISCUSSION

The previous report on this phase II trial revealed that treatment with sorafenib showed an encouraging antitumour activity in Japanese patients with histologically or cytologically confirmed metastatic RCC who had undergone nephrectomy and failed one or more cytokine-containing regimens [8]: favourable efficacy results were comparable with those in the pivotal TARGET phase III trial [6,7]. This Japanese phase II study was extended to investigate the long-term efficacy and safety of sorafenib in Japanese patients with RCC. In addition to the 19 patients showing PR in the previous report, six patients newly achieved PR after a 9.2-month SD period, after the cut-off date for the previous report [8], suggesting that sorafenib might bring a long-term benefit to patients with RCC by not only maintaining SD status but also obtaining PR afterwards. Five of these six late-responders were Motzer score low-risk patients while the other 19 PR patients included six low-risk and 13 intermediate-risk patients. The mechanisms behind such late-onset tumour shrinkage and the patient subsets involved are not known. In RCC xenograft models, sorafenib was shown to induce tumour stasis or stabilization mainly due to inhibition of angiogenesis [10]. However, sorafenib exerts inhibitory effects on several kinases [11], and variety among the patients with RCC in combinatorial inhibition of multiple pathways may affect the clinical course under sorafenib treatment, such as degree and/or timing of response to antitumour effects of sorafenib and so on. To find out what kinds of characteristic features are typical in early- or late-responders, we need more PR patients to be analysed statistically.

Recently, the final results of TARGET were published, showing 17.8 months of median OS in patients receiving sorafenib [7]. Although no median OS was available at the time of the previous publication, this final analysis obtained the median OS value with

*FIG. 1. Tumour response to sorafenib. **A**, Waterfall plot of target-lesion shrinkage in patients valid for intent-to-treat analysis (N = 129). Of the 129 patients, 102 patients (79.1%) showed some degree of target-lesion shrinkage according to RECIST, based on investigators' assessment. (Data missing for two patients.) **B**, Time to response and duration of response in patients with PR (N = 25). Median time to response and median duration of response were 2.8 and 13.8 months, respectively. Six patients achieved PR after approximately 10 months of SD. Duration of disease control (period for SD and PR) exceeded 32 months in six of the 25 patients showing PR. Two patients (*) discontinued the study treatment because of cholecystitis and hyperbilirubinaemia, respectively; four patients (†) were transferred to commercial sorafenib without showing disease progression; and the remaining 19 patients discontinued the study treatment due to progressive disease.*

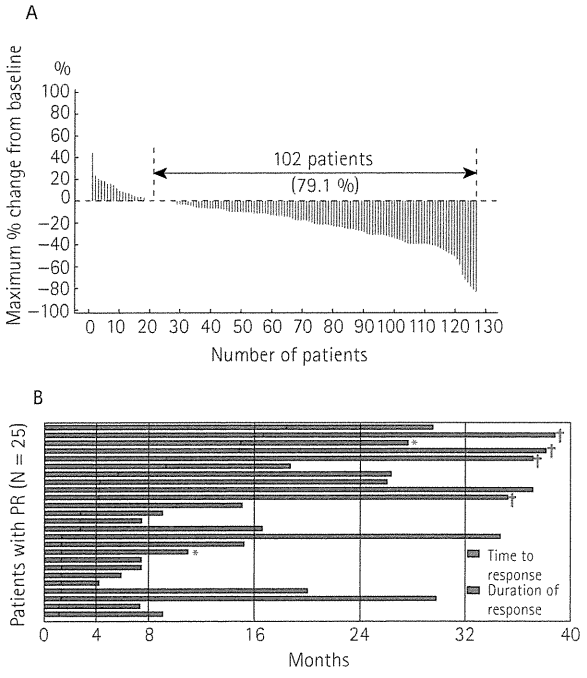
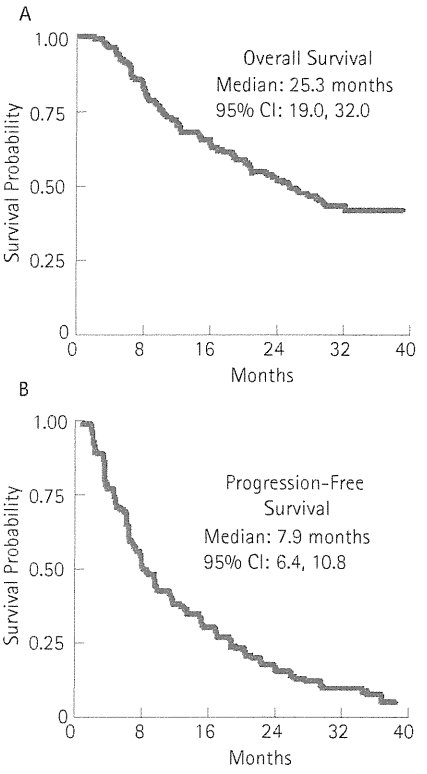


*FIG. 2. OS (**A**) and PFS (**B**) in patients valid for intent-to-treat analysis (N = 129). Median OS (95% CI) and median PFS (95% CI) were 25.3 (19.0, 32.0) and 7.9 (6.4, 10.8) months, respectively.*



71 failed patients. The median OS of 25.3 months obtained in the present study is superior to that of sorafenib-treated patients in TARGET and comparable with the median OS of 26.4 months of sunitinib-treated patients with RCC in the phase III trial, in which all of the enrolled patients were systemic anticancer therapy naïve [12].

Both this Japanese trial and TARGET evaluated the efficacy and safety of sorafenib monotherapy in patients with advanced RCC who had received systemic anticancer treatments. Although we cannot directly compare the results of two independent trials, sorafenib seemed to provide better efficacy with longer OS in this Japanese phase II trial than in TARGET [7]. Naito *et al.* [13] found in a retrospective manner that several factors seemed to improve the prognosis of 1463 Japanese patients with RCC, including early diagnosis of metastasis, nephrectomy, metastasectomy and cytokine-based therapy. For the numbers of metastatic sites at baseline, 62 (14%) and 131 (29%) had one and two sites, respectively, in TARGET [6]. On the other hand, in the Japanese phase II trial 38 patients (29.5%) and 41 patients (31.8%) had one and two tumour sites, respectively, at the baseline, suggesting that this trial contained more patients whose metastases were diagnosed earlier than in TARGET. With regard to the ECOG-PS, 101 patients (78.3%) had a score of 0 and 28 patients (21.7%) had a score of 1 in the present Japanese trial whereas 219 patients (49%) had 0 and 223 patients (49%) had 1 in TARGET [6]. Based on these baseline data on tumour sites and ECOG-PS, the Japanese trial might contain fewer advanced patients than TARGET. However, with regard to the other prognostic factors in Naito *et al.* [13], there was no clear difference between the present Japanese trial and TARGET in the baseline data on nephrectomy (100% vs 94%) and cytokine-based therapy (100% vs 83%) [6]. With regard to the Motzer risk score, the present study included 40% low-risk patients whereas there were 52% low-risk patients in TARGET. We cannot deny the possibility of ethnic differences in response to sorafenib treatment between Japanese and Western patients with RCC. These results of PFS and OS in the present trial strongly suggest the clinical benefit of sorafenib in Japanese patients with RCC, although the present study was conducted as a one-arm, uncontrolled study.

The updated safety profile in the present study was consistent with that of the previous report [8], suggesting that there was no major adverse event specific to a late stage of long-term treatment with sorafenib and that sorafenib had no cumulative toxicity. A median relative dose intensity as high as 97.4% was obtained, despite 73 patients (55.7%) experiencing dose changes (Table 4). In fact, adverse events resulting in permanent discontinuation were observed in 29 patients (22.1%), while dose interruption and reduction were found in 55 (42.0%) and 45 (34.4%), respectively. These results meant that

many of the treatment-emergent adverse events, including hand–foot skin reaction and lipase elevation, which occurred very frequently, were not serious and were manageable without permanently discontinuing sorafenib. The first dose changes due to adverse events occurred within the first 3 months in 39 of the 73 patients (53.4%) who experienced at least one dose change (Table 4), indicating the importance of adverse event management in the early stage of sorafenib treatment. In the randomized phase II trial of first-line treatment with sorafenib vs interferon alpha-2a in patients with metastatic RCC, sorafenib-treated patients experienced better quality of life than interferon-treated patients [14]. The favourable safety and quality of life profiles of sorafenib may allow long-term and high-dose-intensity sorafenib treatment, in turn leading to its long-term efficacy.

TABLE 4 Summaries of dose changes due to adverse events and drug exposure

Time (month)	Number of patients (%) Interval	Cumulative
Time to first dose change (discontinuation/interruption/reduction) due to adverse events (N = 73)		
<3	39 (53.4)	39 (53.4)
3 to <6	13 (17.8)	52 (71.2)
6 to <9	5 (6.8)	57 (78.1)
9 to <12	2 (2.7)	59 (80.8)
12 to <18	6 (8.2)	65 (89.0)
18 to <24	5 (6.8)	70 (95.9)
24 to <30	2 (2.7)	72 (98.6)
30 to <36	1 (1.4)	73 (100)
Summary of drug exposure of all patients (N = 131)		
Duration of treatment (month)		
Mean ± SD	12.0 ± 10.8	
Median (range)	7.7 (0.1–38.6)	
Actual daily dose (mg)		
Mean ± SD	691.2 ± 151.6	
Median (range)	779.4 (210.9–800.0)	
Relative dose intensity (%)		
Mean ± SD	86.4 ± 19.0	
Median (range)	97.4 (26.4–100)	

It is worth mentioning again that the patients included in this trial had received treatment for RCC before starting sorafenib treatment. The efficacy and safety of long-term therapy with sorafenib in such patients suggest that a comprehensive treatment approach, including sorafenib, interferon alpha, interleukin-2 and cytoreductive surgery, may provide improved clinical benefit for patients with advanced RCC.

In conclusion, the updated and final results of the phase II trial of sorafenib monotherapy after cytokine treatment in Japanese patients with metastatic RCC demonstrated that sorafenib provided favourable clinical data from the viewpoints of both efficacy and safety, in particular including late-response events and preferred OS.

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CONFLICT OF INTEREST

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Correspondence: Seiji Naito, Department of Urology, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan.
e-mail: naito@uro.med.kyushu-u.ac.jp

Abbreviations: **OS**, overall survival; **TARGET**, Treatment Approaches in Renal Cancer Global Evaluation Trial; **ECOG-PS**, Eastern Cooperative Oncology Group performance status; **PFS**, progression-free survival; **RECIST**, Response Evaluation Criteria in Solid Tumours; **PR**, partial remission; **SD**, stable disease.

Review Article

A Review of Current Guidelines and Best Practice Recommendations for the Management of Nonmuscle Invasive Bladder Cancer by the International Bladder Cancer Group

Maurizio Brausi,*,† J. Alfred Witjes,‡ Donald Lamm,§ Raj Persad,† Joan Palou,‖ Marc Colombel,§ Roger Buckley,† Mark Soloway,¶ Hideyuki Akaza† and Andreas Böhle†

From the Department of Urology, AUSL Modena, Modena, Italy (MB), Department of Urology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands (JAW), Department of Surgery, University of Arizona and BCG Oncology, Phoenix, Arizona (DL), Department of Urology/Surgery, Bristol Royal Infirmary & Bristol Urological Institute, Bristol, United Kingdom (RP), Department of Urology, Fundació Puigvert, Universitat Autònoma de Barcelona, Barcelona, Spain (JP), Department of Urology, Claude Bernard University, Hôpital Edouard Herriot, Lyon, France (MC), Department of Urology, North York General Hospital, Toronto, Ontario, Canada (RB), Department of Urology, University of Miami School of Medicine, Miami, Florida (MS), Department of Urology, University of Tsukuba, Tsukuba, Japan (HA), and Department of Urology, HELIOS Agnes Karll Hospital, Bad Schwartau, Germany (AB)

Abbreviations and Acronyms

AUA = American Urological Association

BCG = bacillus Calmette-Guérin

CIS = carcinoma in situ

EAU = European Association of Urology

EORTC = European Organization for the Research and Treatment of Cancer

FICBT = First International Consultation on Bladder Tumors

IBCG = International Bladder Cancer Group

IPD = individual patient data

MMC = mitomycin C

NCCN = National Comprehensive Cancer Network

NMIBC = nonmuscle invasive bladder cancer

TURBT = transurethral resection of the bladder tumor

Purpose: Although the European Association of Urology, First International Consultation on Bladder Tumors, National Comprehensive Cancer Network and American Urological Association guidelines all provide an excellent evidence-based framework for the management of nonmuscle invasive bladder cancer, these guidelines vary with respect to important issues such as risk level definitions and management strategies for these risk categories. Therefore, we built on the existing framework provided by current guidelines, and provide consensus on the definitions of low, intermediate and high risk nonmuscle invasive bladder cancer, as well as practical recommendations for the treatment of patients in each of these risk categories.

Materials and Methods: An international committee of experts on bladder cancer management identified and analyzed the European Association of Urology, First International Consultation on Bladder Tumors, National Comprehensive Cancer Network and American Urological Association guidelines as well as the published English language literature related to the treatment and management of nonmuscle invasive bladder cancer available as of April 2010.

Results: Based on review of the current guidelines and literature, the International Bladder Cancer Group developed practical recommendations for the management of nonmuscle invasive bladder cancer.

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* Correspondence: AUSL Modena, Via G. Molinari, 2, 41012 Carpi, Modena, Italy (telephone: +39 059 65 9310; FAX: +39 059 65 9310; e-mail: brausi@interfree.it).

† Nothing to disclose.

‡ Financial interest and/or other relationship with MEL Amsterdam, Spectrum Pharm, Sanofi Pasteur, TEVA, Telormedix, ENDO Pharmaceuticals, GE Healthcare, Photocure, Astra Tech Sweden and Astellas.

§ Financial interest and/or other relationship with Sanofi Pasteur.

‖ Financial interest and/or other relationship with Sanofi Pasteur, Lilly, Amgen and Zambon Group.

¶ Financial interest and/or other relationship with Spectrum and GE Medical.

Editor's Note: **This article is the first of 5 published in this issue for which category 1 CME credits can be earned. Instructions for obtaining credits are given with the questions on pages 2512 and 2513.**

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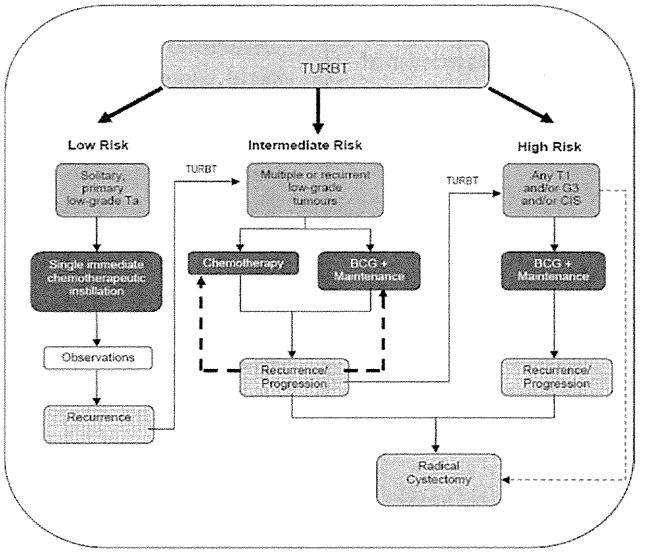
Conclusions: Complete transurethral bladder tumor resection is recommended for all patients with nonmuscle invasive bladder cancer. For low risk disease a single, immediate chemotherapeutic instillation after transurethral bladder tumor resection is recommended. For intermediate or high risk disease there is no significant benefit from an immediate, postoperative chemotherapeutic instillation. For intermediate risk disease intravesical bacillus Calmette-Guérin with maintenance or intravesical chemotherapy is recommended. For high risk disease bacillus Calmette-Guérin induction plus maintenance is recommended. The appropriate management of recurrence depends on the patient level of risk as well as previous treatment, while the management of treatment failure depends on the type of failure as well as the level of risk for recurrence and disease progression.

Key Words: urinary bladder neoplasms; clinical protocols; administration, intravesical; drug therapy; mycobacterium bovis

CURRENTLY there is wide variation regarding the management of NMIBC in the community urological setting. Although the EAU, FICBT, NCCN and AUA guidelines contribute to an excellent evidence-based framework for the management of NMIBC,[1–5] there are differences in the recommendations proposed in these guidelines, and contentious areas and gaps in evidence-based knowledge that need to be addressed through expert consensus. Therefore, an international committee of experts on bladder cancer management known as the IBCG was convened to compare these guidelines. Based on this comparison, they developed practical recommendations that would be relevant to community urologists and encourage a more uniform approach to NMIBC management. These recommendations were originally published in *European Urology Supplements* in October 2008.[6] This review provides an update to this publication, taking into account the most recent EAU and NCCN guidelines as well as recent findings regarding the value of BCG maintenance therapy and immediate chemotherapeutic instillations after TURBT for intermediate and high risk NMIBC. In this review we build on the existing framework provided by the 4 guidelines, and provide consensus on the definitions of low, intermediate and high risk NMIBC, as well as practical recommendations for the management of patients in each of these risk categories (see figure). Note that the IBCG considered the EAU guidelines to represent best practice with regard to TURBT and the use of intravesical therapy and, as such, these were adopted as appropriate recommendations for community urologists (with some minor modifications).[1]

METHODS

The IBCG met in closed sessions on 6 occasions between October 2006 and April 2010. The EAU, FICBT, NCCN and AUA guidelines were presented, and the quality of evidence and strength of recommendations made in these guidelines were reviewed. Although the rigorous methodology applied in the completion of the 4 cited guidelines was not used, a MEDLINE® search was conducted to identify the published English language literature related to NMIBC management. Keywords included "non-muscle invasive bladder cancer," "bacillus Calmette-Guerin," "intravesical chemotherapy" and "transurethral resection of the bladder tumor". Reference lists of review and original articles were reviewed to identify additional applicable literature. Articles with the highest level of evidence were identified with the consensus of all collaborative authors and were critically reviewed. Final preparation and modifications of the recommendations presented in



Algorithm for treatment and management of primary NMIBC proposed by IBCG. Single immediate chemotherapeutic instillation after TURBT is recommended for patients with primary, small, solitary low grade tumors, except those with obvious or suspected bladder wall perforation. Recent evidence suggests no statistically significant benefit from early postoperative chemotherapeutic instillation in patients with large or recurrent tumors (ie intermediate risk) or in those with high risk NMIBC. Recommendations have been simplified for ease of use and will need to be customized to each individual patient. For example, high risk patient with low grade Ta recurrence on maintenance BCG would be candidate for intravesical chemotherapy rather than cystectomy as shown in algorithm. Adapted from Lamm et al.[6]

this article were made by electronic communication. Recommendations are based on consensus interpretation of the current guidelines and literature.

RESULTS

Definition of Risk Levels

Guideline comparison. Although the 4 guidelines agree on the importance of risk stratification for NMIBC management based on patient risk of recurrence and/or progression, there are differences in the definitions of risk as well as in the proposed treatments for each risk category (Appendix 1).[1,3–5,7–10]

IBCG recommendations. Upon review of the 4 guidelines the IBCG proposed the practical definitions of low, intermediate and high risk disease based on risk of recurrence and disease progression (see figure). Low risk was defined as solitary, primary low grade Ta; intermediate risk as multiple or recurrent low grade tumors; and high risk as any T1 and/or G3 and/or CIS.

Transurethral Bladder Tumor Resection

Guideline comparison and supporting evidence. All guidelines recommend TURBT as the gold standard for the initial diagnosis and treatment of NMIBC.[1,3–5,7–10] After TURBT the 10-year disease specific survival is 85% for Ta tumors and 70% for T1 tumors.[11] Appropriate resection techniques should be used. Brausi et al found wide variability in recurrence rates in patients receiving and in those not receiving intravesical treatment, which was attributed to the quality of the TURBT performed by the individual surgeons.[12] A recent retrospective study (sample size of 47) demonstrated that 70% of included patients had an incomplete initial resection.[13] Of these patients 30% had macroscopic residual tumor at the resection site and 70% had at least 1 unresected tumor away from the previous resection site.

The EAU outlined resection techniques depending on the size of the lesion.[1] Small tumors (less than 1 cm) can be resected en bloc and the specimen should contain complete tumor plus part of the underlying bladder wall. Larger tumors should be resected separately in fractions, including the exophytic part of the tumor, the underlying bladder wall with the detrusor muscle and the edges of the resection area. Specimens from different fractions must be referred to the pathologist in separate containers to allow for a correct diagnosis. Cauterization should be avoided as much as possible to prevent tissue destruction.

Based on expert experience, repeat TURBT 2 to 6 weeks after the initial procedure is recommended in patients with high grade T1 tumors, incomplete initial resection or when the specimen contained no muscle tissue.[1] Repeat TURBT should also be considered in referred patients. A recent study of referred patients with bladder cancer revealed substantial differences between initial pathological reports and subsequent review and reinterpretation of TURBT slides that were sufficiently significant to alter management in almost 30% of subjects.[14]

IBCG recommendations. Based on a review of the 4 guidelines the IBCG recommends complete TURBT for all patients with NMIBC using appropriate/accepted TURBT techniques. A bladder diagram is recommended. Repeat TURBT is recommended for high grade tumors if initial resection is incomplete and/or when no muscle is present in the specimen. Repeat TURBT should be considered in referred patients. Pathology slides should be reviewed directly with the pathologist when possible.

Management of Low Risk Disease

Guideline comparison. The EAU, FICBT and AUA recommend TURBT plus an immediate postoperative chemotherapeutic instillation for low risk disease.[1,4,5,7] The NCCN considers TURBT alone the standard for this patient population, and an immediate postoperative chemotherapeutic dose and/or induction intravesical chemotherapy (based on risk of recurrence and progression) should be considered.[3] Guideline recommendations for low risk disease are summarized in Appendix 2.

Additional supporting evidence. An EORTC meta-analysis showed that a single, immediate instillation of intravesical chemotherapy after TURBT results in a 12% absolute reduction in tumor recurrence (a decrease of 39% in the odds of recurrence).[15] No significant differences in efficacy were noted among the chemotherapeutic agents studied, indicating that the choice of chemotherapeutic drug is optional. In an AUA meta-analysis TURBT and single dose MMC resulted in a 17% absolute reduction in recurrence compared to TURBT alone when all patient risk groups were considered.[4]

The timing of the instillation is important. In 1 study there was a doubling in the risk of recurrence if the first of 5 weekly MMC instillations was not given within 24 hours of TURBT.[16] In 2 EORTC trials in which patients received 9 instillations of epirubicin or MMC during 6 months, starting treatment on the day of TURBT was more effective than starting 7 to 15 days later in patients who did not receive further maintenance after 6 months.[17] In another study in which patients received 15 instillations of doxorubicin or MMC during a 1-year period, fewer patients randomized to start treatment within 6 hours had recurrence than did those randomized to start treatment after 7 to 14 days, particularly in the MMC arm.[18] Note that in the major-

ity of these studies the instillations were given within 24 hours, generally immediately or within 6 hours after TURBT. Despite the benefits of an immediate, chemotherapeutic instillation, it should be avoided in cases of overt or suspected intraperitoneal or extraperitoneal perforation because complications have been noted in these cases.[19]

Recent evidence suggests that the benefit of a single, immediate chemotherapeutic instillation after TURBT is limited to patients with low risk disease.[20] In patients with primary, small (less than 5 mm), solitary, low grade tumors, a single early chemotherapeutic instillation after TURBT significantly reduces the risk of recurrence, which is seen in the first 2 years of followup. There appears to be no advantage to an immediate postoperative instillation in recurrent, large (greater than 5 mm) or high grade tumors, or in high risk patients receiving BCG therapy.[21–28] Therefore, the IBCG recommends that a single, immediate postoperative chemotherapeutic instillation be reserved for patients with low risk NMIBC. Although some experts have questioned the ability to accurately diagnose low grade papillary tumors at cystoscopy, evidence suggests that cystoscopic expertise combined with a negative urine cytology accurately identifies low grade tumors in more than 90% of cases.[29,30] Herr et al examined the correlation between cystoscopic appearance and histopathology in 125 patients with 144 recurrent papillary tumors.[29] Of 97 tumors the 90 (93%) considered TaG1 at cystoscopy were confirmed to be low grade papillary lesions at biopsy. Of 86 TaG1s associated with negative cytology, 85 (99%) were low grade papillary tumors histologically. Only 1 of 97 (1%) tumors deemed TaG1 proved to be invasive on biopsy. An EORTC study reported that 5.6% of 501 tumors believed to be noninfiltrating at cystoscopy were under staged compared to histopathology.[30] Tumors appearing superficial that were less than 3 cm in diameter were correctly staged in 96% of cases. No data were available regarding the accuracy of cystoscopy in predicting tumor grade.

IBCG recommendations. Based on comparison of the 4 guidelines and the current literature, for low risk disease the IBCG recommends complete TURBT plus an immediate, single, postoperative chemotherapeutic instillation, except in those patients with obvious or suspected bladder wall perforation (see figure).

Management of Intermediate Risk Disease

Guideline comparison. All guidelines recommend that adjuvant therapy with BCG or chemotherapy is necessary for intermediate risk disease. However, the strength of this recommendation varies and controversy exists about whether induction plus maintenance or induction alone should be used (Appendix 2). The EAU recommends adjuvant BCG with maintenance (1 year or more) or further instillations of chemotherapy (6 to 12 months) for intermediate risk disease.[1] The FICBT recommends intravesical chemotherapy (less than 6 months) as first line and BCG as second line therapy in patients with recurrent, multiple, low grade Ta tumors, or when high risk factors for recurrence are present.[7]

According to the NCCN, options for intermediate risk disease include observation, or treatment with intravesical BCG (preferred) or MMC.[3] The AUA recommends an induction course of BCG or MMC for patients at high risk for recurrence but at low risk for progression (intermediate risk). Although maintenance BCG or MMC is considered optional in these patients, the AUA acknowledges that maintenance is more effective in decreasing recurrence than induction alone.[4,5]

Additional supporting evidence. An EORTC meta-analysis showed that compared to TURBT alone, adjuvant chemotherapy after TURBT significantly improves disease-free survival but has no effect on progression.[31] In a review of controlled trials of intravesical chemotherapy Lamm et al reported an absolute 14% decrease in tumor recurrence but also found no effect on tumor progression.[32]

To our knowledge no consensus currently exists on the optimal chemotherapeutic schedule. Results from a systematic review of intravesical chemotherapy trials suggest that a short, intensive schedule of instillations (3 to 4 months) after an immediate instillation may be as effective as longer term schedules.[33] The investigators concluded that long-term instillations of 1 year or more should only be considered when an immediate instillation has not been provided.

Recent data suggest that BCG with maintenance may be superior to chemotherapy for intermediate risk disease and may be the preferred treatment option for this patient population. The EORTC 30911 trial compared the long-term efficacy of 6 weekly intravesical instillations of epirubicin, BCG and BCG plus isoniazid followed by 3 weekly maintenance instillations at months 3, 6, 12, 18, 24, 30 and 36 after TURBT in patients with intermediate (497) and high risk (323) NMIBC.[34] Median followup was 9.2 years. Time to first recurrence ($p < 0.001$), time to distant metastases ($p = 0.046$), and overall ($p = 0.023$) and disease specific survival ($p = 0.026$) were all significantly prolonged in the 2 BCG arms combined compared to the epirubicin arm. However, no difference in progression was noted between the treatment arms. The investigators concluded that intermediate and high risk patients benefit from BCG therapy. The observed treatment benefit was

at least as large, if not larger, in the intermediate vs high risk patients.

Results from a recent IPD meta-analysis of 9 trials (2,820) further confirm the superiority of BCG when maintenance therapy is provided.[35] This meta-analysis revealed no overall difference in time to first recurrence ($p = 0.09$) between BCG and MMC. However, in the trials using BCG maintenance a 32% reduction in the risk of recurrence with BCG vs MMC was found ($p < 0.0001$), while there was a 28% risk increase ($p = 0.006$) with BCG in the trials without maintenance. BCG with maintenance was more effective than MMC in patients previously treated and in those not previously treated with chemotherapy. No significant differences in progression, overall survival and cancer specific survival were noted between the 2 groups. Note that the majority of patients included in this meta-analysis were intermediate risk (74%), further confirming the value of BCG maintenance in this patient population.[35]

IBCG recommendations. Based on guidelines and supporting evidence, for intermediate risk disease the IBCG recommends the initiation of BCG induction plus maintenance or intravesical chemotherapy after complete TURBT (see figure). Adjuvant chemotherapy should not exceed 12 months.

Management of High Risk Disease

Guideline comparison. All guidelines regard BCG as the standard adjuvant treatment for high risk patients (Appendix 2). The EAU recommends a second TURBT 2 to 6 weeks after the initial resection and adjuvant intravesical BCG for at least 1 year for high risk disease. Immediate radical cystectomy may be offered to the highest risk patients such as those with multiple, recurrent high grade tumors, high grade T1 tumors or high grade tumors with CIS.[1] For patients with CIS the EAU recommends intravesical BCG plus maintenance for at least 1 year.[36]

For high grade Ta the FICBT recommends a 6-week induction course of BCG plus 1 to 3 years of maintenance.[9] In patients with completely resected T1 tumors (based on negative repeat resection) initial intravesical BCG therapy should be considered.[8] According to the FICBT, radical cystectomy at CIS diagnosis constitutes overtreatment in up to 50% of patients. Therefore, intravesical BCG with at least 1 year of maintenance is recommended because it is associated with the highest rate of complete response and the highest long-term disease-free rate among intravesical treatments.[9]

The NCCN advises re-resection for T1 disease. If residual disease is present, BCG (category 1) or cystectomy is advised. If there is no residual disease, intravesical BCG (preferred, category 1) or MMC is recommended.[3] According to the AUA, repeat resection followed by BCG induction plus maintenance is recommended for patients with initially histologically confirmed high grade Ta, T1 and/or CIS with lamina propria invasion (T1) but without muscularis propria in the specimen. Cystectomy is considered an option for initial therapy in select patients due to the risk of initially under staged disease or progression to muscle invasive disease.[4,5]

Note that high grade Ta tumors represent a relatively small subgroup of cases and the histological diagnosis is subject to considerable misclassification. The FICBT recommends repeat TURBT and bladder mapping 2 to 4 weeks later for Ta tumors.[2] Given that the pathologist cannot confirm if muscle is uninvolved unless it is present in the specimen, all guidelines suggest repeat TURBT when there is no muscle in the specimen.[1–5] However, general guidelines and recommendations cannot dictate each individual management decision. Therefore, some experts indicate that re-resection is unnecessary if muscle is not present in the specimen but the lamina propria is clearly uninvolved and the diagnosis of Ta disease is secure.

Additional supporting evidence. EORTC 30911 and the IPD meta-analysis by Malmström et al (discussed previously) highlight the importance of maintenance BCG in intermediate and high risk NMIBC.[34,35] EORTC 30911 showed that time to first recurrence, time to distant metastases, and overall and disease specific survival were all significantly prolonged in the 2 BCG arms combined compared to the epirubicin arm, and the IPD meta-analysis showed a 32% reduction in the risk of recurrence with BCG maintenance vs MMC.[34,35] Although neither study demonstrated a beneficial effect of BCG on disease progression, an EORTC meta-analysis of 24 trials (4,863) showed that BCG maintenance therapy was associated with a 37% reduction in the risk of tumor progression compared to the control groups (TURBT alone, TURBT plus intravesical chemotherapy, TURBT plus another immunotherapy).[37] Another meta-analysis of 9 trials comparing BCG to MMC showed that BCG maintenance was significantly superior to MMC for the prevention of tumor progression.[38]

A meta-analysis of 11 clinical trials demonstrated that BCG was superior to MMC in decreasing tumor recurrence (OR 0.56, 95% CI 0.38 to 0.84, $p = 0.005$).[39] In the subgroup treated with BCG maintenance all 6 individual studies showed a significant superiority of BCG compared to MMC (OR 0.43, 95% CI 0.35 to 0.53, $p < 0.001$). In a single arm AUA meta-analysis of randomized trials in high risk patients the 5-year recurrence rate was 34% in those receiving TURBT and BCG mainte-