European Journal of Cancer 69 (2016) 9–18

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejcancer.com

Original Research

Phase III randomised chemoprevention study with selenium on the recurrence of non-invasive urothelial carcinoma. The SELEnium and BLAdder cancer Trial

Maria E. Goossens [a,*], Maurice P. Zeegers [b,c,d], Hendrik van Poppel [e], Steven Joniau [e], Koen Ackaert [f], Filip Ameye [g], Ignace Billiet [h], Johan Braeckman [i], Alex Breugelmans [j], Jochen Darras [k], Kurt Dilen [l], Lieven Goeman [m], Bertrand Tombal [n], Siska Van Bruwaene [h], Ben Van Cleyenbreugel [e], Frank Van der Aa [e], Kris Vekemans [l], Frank Buntinx [a,c]

[a] *Department of General Practice, University of Leuven, ACHG-KU Leuven, Kapucijnenvoer 33, Blok J, Bus 7001, 3000, Leuven, Belgium*
[b] *University of Maastricht, NUTRIM School for Nutrition and Translational Research in Metabolism, Maastricht, The Netherlands*
[c] *CAPHRI School for Public Health and Primary Care, University of Maastricht, The Netherlands*
[d] *School of Cancer Sciences, University of Birmingham, Birmingham, UK*
[e] *Department of Urology, UZ Leuven, Leuven, Belgium*
[f] *Department of Urology, Sint-Elisabethziekenhuis, Turnhout, Belgium*
[g] *Department of Urology, AZ Maria Middelares, Gent, Belgium*
[h] *Department of Urology, AZ Groeninge, Kortrijk, Belgium*
[i] *Department of Urology, UZ Brussel, Brussel, Belgium*
[j] *Department of Urology, Heilig Hart ziekenhuis, Leuven, Belgium*
[k] *Department of Urology, AZ Damiaan, Oostende, Belgium*
[l] *Department of Urology, Jessa ziekenhuis, Hasselt, Belgium*
[m] *Department of Urology, Heilig Hartziekenhuis, Roeselaere, Belgium*
[n] *Department of Urology, Cliniques universitaires Saint-Luc, Brussel, Belgium*

Received 26 June 2016; received in revised form 8 September 2016; accepted 16 September 2016
Available online 29 October 2016

KEYWORDS
Selenium;
Bladder cancer;

Abstract ***Background:*** In Belgium, bladder cancer (BC) is the fifth most common cancer in men. The per-patient lifetime cost is high. Previous epidemiological studies have consistently reported that selenium concentrations were inversely associated with the risk of BC. We therefore hypothesised that selenium may be suitable for chemoprevention of recurrence of BC.

* *Corresponding author*: ACHG-KU Leuven, Kapucijnenvoer 33, Blok J, Bus 7001, 3000, Leuven, Belgium.
E-mail address: Mieke.Goossens@kuleuven.be (M.E. Goossens).

http://dx.doi.org/10.1016/j.ejca.2016.09.021

Non-invasive urothelial carcinoma;
Transitional cell carcinoma;
Chemoprevention;
Randomised clinical trial;
Urology

Method: The Selenium and Bladder Cancer Trial (SELEBLAT) was an academic phase III placebo-controlled, double-blind, randomised clinical trial designed to determine the effect of selenium on recurrence of non-invasive urothelial carcinoma conducted in 14 Belgian hospitals. Patients were randomly assigned by a computer program to oral selenium yeast 200 μg once a day or placebo for three years, in addition to standard care. All study personnel and participants were blinded to treatment assignment for the duration of the study. All randomised patients were included in the intention to treat (ITT) and safety analyses. Per protocol analyses (PPAs) included all patients in the study three months after start date.

Results: Between September 18, 2009 and April 18, 2013, 151 and 141 patients were randomised in the selenium and placebo group. Patients were followed until December 31, 2015. The ITT analysis resulted in 43 (28%; 95% CI, 0.21−0.35) and 45 (32%; 95% CI, 0.24−0.40) recurrences in the selenium and placebo group. The hazard ratio (HR) was 0.85 (95% CI, 0.56−1.29; p = 0.44) while the HR for the PPA resulted in 42 and 39 (28%; 95% CI, 0.20−0.35) recurrences in the selenium and placebo group (HR = 0.96 [95% CI, 0.62−1.48]; p = 0.93).

Conclusion: Selenium supplementation does not lower the probability of recurrence in BC patients.

1. Introduction

In 2012, more than 400,000 bladder cancer cases occurred worldwide [1]. Belgium ranks first in the world with the highest age-standardised rate for both sexes before Lebanon and Malta [1]. Indeed, in Belgium, bladder cancer (BC) is the fifth most prevalent cancer for both sexes and for men it is the fourth most common cancer representing 9.6% of all cancers [1]. This number has been stable over the last five years. Incidence and mortality rates increase sharply with age and about two-thirds of patients are 65 years and older [2]. In the United States of America and probably in most Western countries, BC has the greatest per-patient lifetime cost for cancer in terms of health care expenditure compared with all other types of cancer [3]. Any reduction in the number of recurrences would reduce these costs and, more importantly, improve patients' quality of life.

Evidence supporting the use of selenium as a general cancer preventive agent includes proof from geographical, animal, *in vitro*, and epidemiological studies. High selenium intake in Venezuela has been associated with a reduced bladder cancer risk [4,5]. In animal models, antitumourigenic activity has been observed for metabolites of naturally occurring forms of selenium such as selenomethionine, selenocysteine, and methylselenocysteine and inorganic selenium salts, such as selenite and selenate [6]. Recent *in vitro* studies have demonstrated that selenium may be an effective chemopreventive and anti-cancer agent with a broad spectrum against several human cancer cells (prostate, colon, bladder, lung, liver, ovarian, leukaemia) including bladder cancer cells. In total, 28 different selenium compounds have been reported to have anti-cancer, chemopreventive or apoptotic activities [7–9]. As selenium is mainly excreted in the urine, it comes into direct and prolonged contact with the bladder mucosa, making its role as a potential chemoprevention agent biologically plausible. Three case–control studies reported an increased risk of BC, associated with lower serum [10] and toenail [10–12] selenium concentrations. A meta-analysis of BC incidence in five observational studies [13–17] found an inverse association with an overall risk estimate of 0.67 (95% CI, 0.46–0.97) suggesting a strong protective effect of higher selenium levels against BC [18]. The results of subsequent reviews [5,19–22] evaluating selenium for BC risk, available at time of study onset, suggest that selenium may be suitable for chemoprevention as well as for treatment. It is useful to perform a selenium trial in a country such as Belgium with a high non-invasive urothelial carcinoma prevalence where the selenium intake is low due to low soil selenium content [10].

The Selenium and Bladder Cancer Trial (SELEBLAT) trial investigated whether 200 μg/day Selenium-yeast, in addition to standard care, reduced the risk of recurrence for patients with non-invasive urothelial carcinoma.

2. Methods

2.1. Study design

The study was an academic phase III, double-blind, placebo-controlled, multicentre, randomised trial evaluating the effect of 200 μg/day Selenium-yeast supplementation on the recurrence of non-invasive urothelial carcinoma. Patients were recruited in 14 hospitals throughout Belgium (Flanders and Brussels) from

September 18, 2009 to April 18, 2013. Patients were followed-up for another three years until December 31, 2015. The study was approved by the ethical review board of the University Hospital of Leuven acting as the central ethical review board (ML 5220), by the trial steering committee and by the appropriate ethical review boards related to the hospitals in which the study was performed. The study was registered at ClinicalTrials.gov number NCT00729287. The SELEBLAT study protocol has been described in more detail elsewhere [23].

2.2. Participants

Subsequent patients, men and women, at least 18 years of age, were eligible for inclusion in SELEBLAT, if they underwent a transurethral resection (TUR) for a histologically confirmed low-grade or high-grade non-invasive urothelial carcinoma [24] (transitional cell carcinoma of the bladder), stage Ta, T1, or carcinoma *in situ* (Tis). Inclusion and exclusion criteria were described *in extenso* in the design paper [23]. All subjects had to be fluent in the Dutch or French. The most important exclusion criteria were a history of any type of malignancy within the past five years and other serious medical or psychiatric illness that would preclude giving informed consent [23].

Eligible patients received oral and written information from the research nurse and signed an informed consent form. The procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008.

2.3. Randomisation and masking

Patients were randomly allocated to one of the two groups. The pharmacist of the University Hospital of Leuven used a computer programme to generate the sequences stratified by treatment centre in blocks of 50. Once a patient was deemed to be eligible for the trial and had given written consent, a trained research nurse allocated a randomisation number to each patient by using a hospital-based database designed for the SELEBLAT study. All study personnel and participants were blinded to treatment assignment for the duration of the study. Tablets were identical in appearance. To evaluate patient blinding, patients were asked by a questionnaire to indicate which treatment they believed they had received (selenium, placebo, or 'don't know'). If patients answered either selenium or placebo, they were asked to indicate what led to that belief.

2.4. Procedures

Patients in each study arm received an oral tablet (selenium or placebo) to be taken daily, in addition to standard care. The active product was selenium (200 μg/day, in the form of high-selenium yeast, SelenoPrecise from PharmaNord, Vojens, Denmark). Placebos were identical in composition except for the active agents. Standard care was provided as described by Babjuk *et al.* in the 'guidelines from the European Association of Urology (EAU) on non-muscle-invasive urothelial carcinoma of the bladder.' [25,26]. The duration of the treatment in both arms was three years, in absence of concurrent illness that prevented further administration of treatment, an unacceptable adverse event, unacceptable toxicity, or if a patient decided to withdraw from the study. Study participants were supplied with the study drug and followed on a bi-annual basis for a period of up to three years. This follow-up phase entailed questionnaire distribution and monitoring for adverse events including potential selenium-related toxicities. The message of compliance was repeated during each follow-up visit. Patients were encouraged to return unused medication and empty packs. To monitor compliance, the research nurse recorded the number of remaining unused tablets during each follow-up visit.

2.5. Outcome

The primary end-point of SELEBLAT was the recurrence-free interval defined as the time from the date of trial entry (T0) to the date of first recurrence in patients with non-invasive urothelial carcinoma. A recurrence was defined as the new occurrence of BC at the same or at a different site as the index cancer.

The secondary end-point was the progression-free interval defined as the time from the date of trial entry to the date of progression. Progression was defined as a recurrence with an increase in tumour grade from low (G1–G2) to high grade (G3), or an increase in tumour-node-metastasis (TNM) stage, or a new occurrence of carcinoma *in situ* (Tis) in the bladder previously free from such lesions, or a new occurrence of multiple tumours following resection of a solitary tumour, or the need for a cystectomy because of refractory disease.

Safety and adverse events were continuously reported by the individual hospital research nurse and additionally formally assessed bi-annually by questionnaires based on the Common Terminology Criteria for Adverse Events v3.0 [27].

2.6. Data collection

Patients received a self-completion questionnaire after TUR, comprising questions about socio-demographics (age, sex, ethnicity, marital status, education) health-related lifestyle (lifetime smoking, history, passive smoking), medical and drug history, dietary intake (food-type frequency, alcohol, caffeine and total fluid intake, use of vitamins), social support and quality of life. Patients' medical records were examined by the research nurses for information on clinical treatment,

histopathology, and outcome measures, which were reported on case report forms.

Selenium value in serum was measured at baseline and after three years of follow-up. The normal range for serum selenium levels is between 50 and 150 μg/l.

Grading of tumours was performed following the 1973 World Health Organization (WHO) grading system [28] which defines well-differentiated (grade I; orderly arrangement of normal cells lining delicate papillae), moderately differentiated (grade II; focal variation in nuclear appearance) and poorly differentiated transitional cell carcinoma (grade III; the most extreme nuclear abnormalities) and the revised 2004 classification which renamed the disease urothelial cell carcinoma (UCC) and classified tumours into low-grade and high-grade. High-grade disease includes around 1/3 of grade II and all grade III UCC [29]. The current TNM Classification of Malignant Tumours (TNM) [30] of non-invasive urothelial carcinoma describes Ta tumours as non-invasive and papillary lesions confined to the mucosa, Tis, as a flat tumour confined to the mucosa, and T1 invasive tumours that invade the subepithelial connective tissue (lamina propria).

The European Organization for Research and Treatment for Cancer (EORTC) Bladder Cancer Prognosis calculator was used to calculate the risk of recurrence within one year. The calculator was developed to provide tables that allow urologists to easily calculate a non-invasive urothelial carcinoma, patient's short- and long-term risks of recurrence and progression after TUR. The calculator implements the EORTC Scoring System and Risk Tables for Stage Ta T1 Bladder Cancer as published by Sylvester *et al.* [31]. The software is available for Windows, iPhone/iPad and Android phones/tablets.

2.7. Statistical analysis

Sample size calculations were based on the meta-analysis of Malström *et al.* [32] who investigated recurrence after standard treatment (instillations of Mitomycine or BCG [Bacillus Calmette-Guérin]). Their group corresponded best with our placebo-group (standard care). The absolute difference between the two groups was based on the difference in percent of recurrence after three years. A difference of 12.5% in absolute reduction could be expected, considering that the intake of 200 μg of selenium increases serum selenium by ±100 mg/dl [33] and that, according to epidemiological studies, the incidence of BC decreases by 25% if serum selenium increases by 10 mg/dl [10]. To detect an absolute decrease of 12.5% in the recurrence-free rate by selenium versus placebo, 700 patients need to be recruited, taking into account a drop out of 25% with a power of 86% (two-sided test).

All randomised patients were included in the intention to treat (ITT) and safety analyses. Kaplan–Meier estimates of a recurrence-free interval were used to compare treatment groups descriptively, while log-rank tests were used to test the hypothesis of no difference between treatments. Cox proportional hazards regression models were used to estimate the hazard ratio (HR) and 95% confidence intervals (CI) comparing patients randomised to selenium or placebo. The start date of the trial (T0) was the day the informed consent was signed. The end date for each patient was the day of recurrence, cystectomy or death, or the day the patient withdrew from the trial, or three years after T0, or the end date of the trial (December 31, 2015).

Per protocol analysis (PPA) included all patients in the study three months after the starting date (T3). Patients who withdrew as well as patients with cystectomy within the first three months were excluded from PPA. Tumours identified within the first three months were considered as incompletely resected primary tumours in patients who were macroscopically tumour-free after the first resection.

Two sensitivity analyses were performed excluding all patients that ended the trial prematurely, the first based on ITT analysis, the second based on PPA. Furthermore, the estimates were adjusted for age, gender, smoking status, staging, baseline serum selenium level and hospital as well as controlled for interaction between treatment and gender, age, or smoking status.

Furthermore, a Data Safety Monitoring Board (DSMB), comprised individuals with expertise in the areas of medicine and biostatistics, was established to serve as an external review committee to monitor the progress of the study including accrual and adverse events. In May 2012, the DSMB examined the data and the results of the Kaplan–Meier estimates, the log-rank test and the Cox proportional hazards of the first 100 patients and recommended the trial to be continued. In December 2015, the DSMB approved unblinding of the study subjects.

All analyses were performed using STATA (StataCorp. 2009. Stata Statistical Software: Release 14. College Station, TX: StataCorp LP).

3. Results

Six hundred and ninety-four patients were assessed for eligibility between September 18, 2009 and April 18, 2013 of whom 292 were randomised (Fig. 1). Thirty-three patients (15 and 18 in the selenium and placebo group) discontinued intervention before three months: one patient died, three patients underwent cystectomy, 13 patients withdrew consent and 16 patients did not meet inclusion criteria.

3.1. Baseline patient characteristics

Baseline characteristics were similar in both groups and are summarised in Table 1. Median age was 68 years

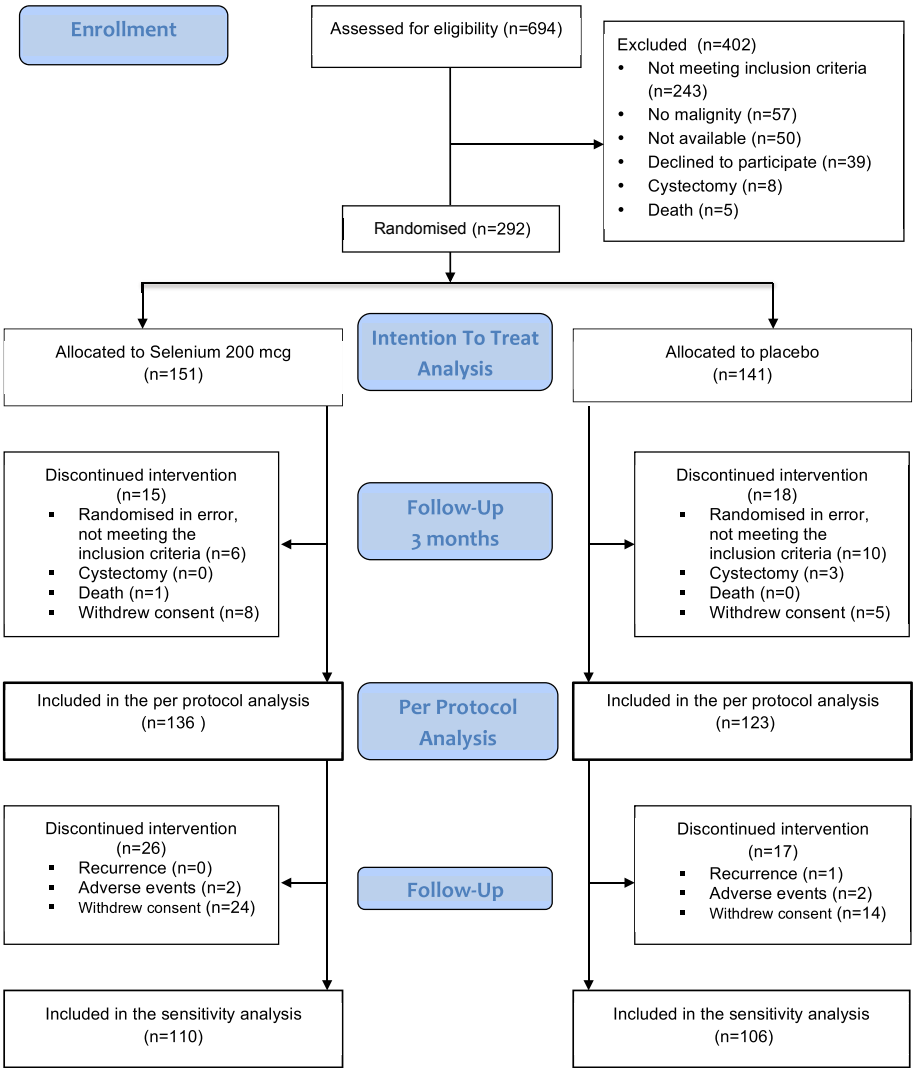

Fig. 1. SELEBLAT trial profile.

ranging from 46 to 90 and 91 years for the selenium and the placebo group. Mean serum selenium values were 83.8 mg/dl and 82.9 mg/dl for the selenium group and placebo group. Most of the patients had a solitary (54% and 57%) primary (67% and 62% for the selenium and placebo group) tumour.

4. Outcome

Median overall follow-up was 17.93 months (range, 0.13–36 months). Detailed characteristics of the patients with recurrence are summarised in Table 2.

4.1. Primary outcome: recurrence

The ITT analysis showed recurrence in 43 (28%; 95% CI, 0.21–0.35) and 45 (32%; 95% CI, 0.24–0.40) patients in the selenium and placebo group. HR was 0.85 (95% CI, 0.56–1.29). The log-rank test was not significant ($p = 0.44$). Adjustment for age, gender, smoking status, staging, baseline serum selenium level and hospital did not alter the results. There was no interaction between treatment and gender, age or smoking status. Excluding those patients who discontinued the intervention did not alter HR of ITT analysis ($p = 0.39$).

PPA, performed for all patients still participating at T3, showed an overall recurrence for 42 and 39 (28%; 95% CI, 0.20–0.35) patients in the selenium and placebo group (HR = 0.96 [95% CI, 0.62–1.48]). The log-rank test was not significant ($p = 0.85$) (Fig. 2). Excluding those patients who discontinued the intervention resulted in an HR of 0.94 (95% CI, 0.61–1.45). The log-rank test was not significant ($p = 0.78$) (Table 3).

4.2. Secondary outcome: progression

Twenty-nine patients showed progression of whom 15 occurred in the selenium group and 14 in the placebo group. Performing the ITT analysis resulted in an HR of 0.97 (95% CI, 0.47–2.00; $p = 0.93$). After three months

Table 1
Baseline demographic and clinical characteristics of the SELEBLAT participants.

Characteristics	Selenium 200 mcg		Placebo	
	N = 151	(%)	N = 141	(%)
Follow-up time (months)				
Median (range)	17.93	(0.13–36)	17.82	(0.36–36)
Sex				
Male	131	(86.8)	111	(78.7)
Female	20	(13.2)	30	(21.3)
Age				
Median (range)	68	(46–90)	68.5	(46–91)
Blood selenium level				
Mean (SD)	83.8	(21.5)	82.9	(14.9)
Smoking status				
Never smoker	26	(17.2)	26	(18.4)
Ever smoker	57	(37.7)	53	(37.6)
Current smoker	27	(17.9)	10	(7.1)
Unknown	41	(27.2)	52	(36.9)
Primary tumour				
Primary	102	(67.3)	88	(62.4)
Recurrent	35	(23.2)	41	(29.1)
Unknown	14	(9.3)	12	(8.5)
Tumour grade				
Low grade	96	(63.5)	94	(66.7)
High grade G3	38	(25.2)	33	(23.4)
Unknown	17	(11.3)	14	(9.9)
Tumour stage (histopathology)				
pTa	93	(61.6)	94	(66.7)
pT1	31	(20.5)	30	(21.3)
Cis	13	(8.6)	3	(2.1)
Unknown	14	(9.3)	14	(9.9)
Tumour diameter				
≤ 3 cm	103	(68.2)	99	(70.2)
> 3 cm	32	(21.2)	23	(16.3)
Unknown	16	(10.6)	19	(13.5)
Number of tumours				
Solitary	81	(53.6)	80	(56.7)
2–7	54	(35.8)	44	(31.2)
≥ 8	2	(1.3)	2	(1.4)
Unknown	14	(9.3)	15	(10.6)
Baseline risk of recurrence within one year				
15%	27	(17.9)	33	(23.4)
24%	41	(27.2)	20	(14.2)
38%	38	(25.2)	40	(28.4)
61%	3	(2.0)	1	(0.7)
Unknown	42	(27.8)	47	(33.3)

SD = standard deviation.

Table 2
Characteristics of the patients with histopathologically confirmed recurrences in the SELEBLAT study.

Characteristics	Selenium 200 mcg		Placebo	
	N = 43	(%)	N = 45	(%)
Sex				
Male	35	(81.4)	36	(80.0)
Female	8	(18.6)	9	(20.0)
Age				
Median (range)	66.5	(46–87)	70.5	(51–90)
Smoking status				
Never smoker	6	(13.9)	10	(22.2)
Ever smoker	16	(37.2)	15	(33.3)
Current smoker	8	(18.6)	3	(6.7)
Unknown	13	(30.2)	17	(37.8)
Baseline risk of recurrence within one year				
15%	8	(18.6)	6	(13.3)
24%	8	(18.6)	10	(22.2)
38%	9	(20.9)	12	(26.7)
61%	2	(4.7)	0	(0.0)
Unknown	16	(37.2)	17	(37.8)
Tumour grade (recurrence)				
Low grade	16	(37.2)	24	(53.4)
High grade G3	12	(27.9)	6	(13.3)
Unknown	15	(34.9)	15	(33.3)
Tumour stage* (recurrence)				
pTa	21	(48.8)	27	(60.0)
pT1	2	(4.7)	5	(11.1)
Cis	7	(16.3)	1	(2.2)
≥T2	2	(4.7)	1	(2.2)
Unknown	11	(25.5)	11	(24.5)
Tumour diameter (recurrence)				
≤3 cm	7	(16.3)	8	(17.8)
>3 cm	2	(4.7)	1	(2.2)
Unknown	34	(79.0)	36	(80.0)
Number of tumours (recurrence)				
Solitary	6	(14.0)	4	(8.9)
2–7	9	(20.9)	6	(13.3)
≥8	0	(0.0)	0	(0.0)
Unknown	28	(65.1)	35	(77.8)

SD = standard deviation.
* Based on histopathology.

(T3), progression was seen in 24 patients. The mean progression time was 22 months for both groups. Patients taking selenium had 48% more chance of progression than those in the placebo group, although not statistically significant (HR = 1.48 [95% CI, 0.65–3.38]; p = 0.35). Excluding those patients from the analysis who discontinued their treatment resulted in a similar HR.

In total, 13 patients underwent cystectomy (6 and 7 in the selenium and placebo group) and 23 patients died (13 and 10 in the selenium and placebo group).

Seven and ten patients reported side-effects in the selenium and placebo group. The side effects were similar in both groups (Table 4) and were grade I with the exception of pain which was grade II. Other side effects reported were: back and neck pain, constipation, disturbed sleep, vertigo and arthralgia. One patient reported nausea, pain and stomach problems, another reported both vertigo and diarrhoea.

Both groups were similar in their blinding assessment of the study drug. When questioned, two thirds of the patients in both groups had no idea whether they were taking selenium or placebo. Almost 20% of the selenium group thought they were taking selenium so did 15% of the placebo group. Seven and four percent of patients were convinced they were taking placebo in the placebo group and the selenium group, respectively. There was no difference in the appreciation of the odour or taste of the tablets between the two groups.

Overall, 82 patients returned their blister bags. For these patients adherence to treatment was excellent (98–100%) with only one patient of the placebo group with a lower adherence of 84%. There was no

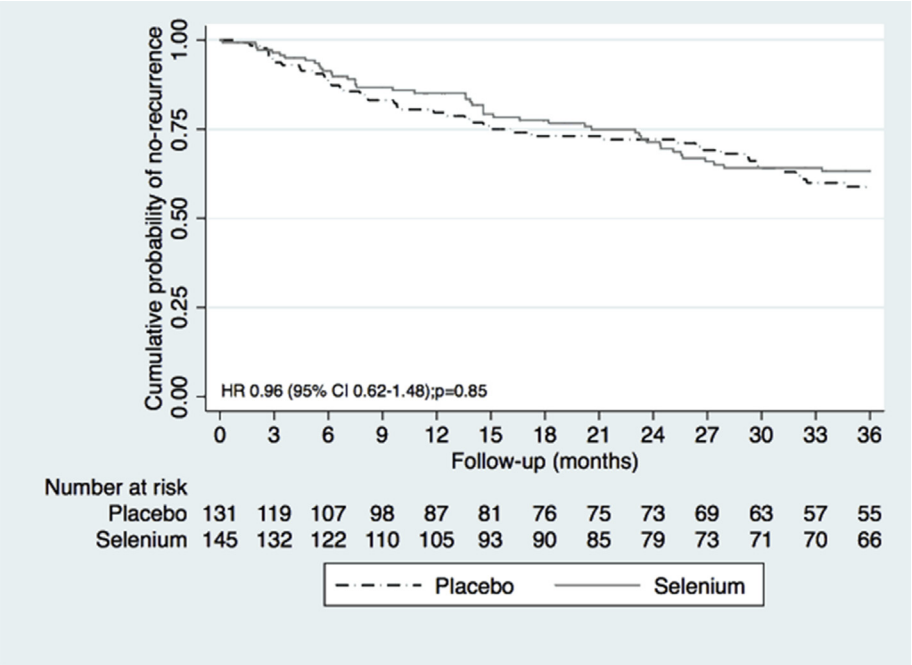

Fig. 2. Cumulative probability of no recurrence in the SELEBLAT study three months after start date.

statistically significant difference in compliance between the two treatment groups.

Serum selenium values at 36 months were available for 67 patients (55%). Three patients in the selenium group did not have elevated serum selenium in their blood (70.1, 75.2 and 80.6 mg/dl), while one patient in the placebo group did have elevated serum selenium (179.3 mg/dl). The mean serum selenium in the selenium group was 187.6 mg/dl ± 57.7 mg/dl versus 88.9 mg/dl ± 22.2 mg/dl in the placebo group. Serum selenium values differed statistically significantly between the two groups ($p = 0.000$).

5. Discussion

Based on the findings from epidemiological studies, we hypothesised that selenium could prevent recurrence in non-invasive urothelial carcinoma. However, our main analysis in this intervention trial showed no difference in recurrence for non-invasive urothelial carcinoma between intervention and control group. ITT analysis and PPA provided similar results.

We hypothesised that selenium could be a suitable chemoprevention drug for recurrence in non-invasive urothelial carcinoma. Our hypothesis was based on the results of geographical [4,5], animal [6], *in vitro* [7–9] and epidemiological studies [10–17]. Those studies reported a decreased risk of BC incidence. At that moment, there were no studies available on the influence of selenium on the recurrence of BC. The SELEBLAT study was the first clinical trial to investigate selenium supplementation to reduce recurrence in BC patients. The Nutritional Prevention of Cancer (NPC) study of Clark in 1996 was the first intervention study with selenium-yeast that showed a decrease in the incidence of prostate, lung, and colorectal cancers in the selenium-supplemented group of older Americans. The effect seemed to be the strongest in the individuals with the lowest selenium status (<123.2 μg/l) [34]. However, a large prevention trial with more than 30,000 participants, the Selenium and Vitamin E Cancer Prevention Trial (SELECT), did not show a benefit of selenium supplementation in reducing the risk of prostate cancer in a population of healthy men [35]. In 2012, a secondary analysis on BC was performed in the SELECT study. No significant difference in the BC incidence was found in this intervention study between 53 men receiving placebo, 56 receiving vitamin E (HR 1.05, IQR 0.64–1.73, $p = 0.79$), 60 receiving selenium (HR 1.13, 0.70–1.84, $p = 0.52$) or 55 receiving vitamin E plus selenium (HR 1.05, 0.63–1.70, $p = 0.86$) [36].

However it could be argued that the lack of positive effect of selenium supplementation on cancer recurrence observed in this study may have been due to the limitations of the study. First of all, the lack of beneficial effect of selenium might be due to a type II error. Indeed we aimed to recruit 700 patients during the three-year lasting recruitment period to answer our research question with sufficient power. Our power analysis was based on the fact that incidence of BC decreases by 25% if serum selenium increases by 10 mg/dl [10]. Intake of 200 μg of selenium increases serum selenium by ±100 mg/dl [33]. We could expect a decrease in BC incidence of at least 50% with an increase of more than 20 mg/dl serum selenium. We hypothesised that a

Table 3
Recurrence and progression in the SELEBLAT study.

Exposure category	Recurrence, N	No recurrence, N	Crude HR (95% CI)		Adjusted[#] HR (95% CI)	
Primary outcome: recurrence						
Intention to treat analysis						
Placebo	45	96	Reference		Reference	
Selenium 200 mcg	43	108	0.85	(0.56–1.29)	0.88	(0.58–1.35)
*Sensitivity analysis**						
Placebo	45	64	Reference		Reference	
Selenium 200 mcg	43	76	0.83	(0.55–1.27)	0.87	(0.57–1.32)
Per protocol analysis*						
Placebo	39	84	Reference		Reference	
Selenium 200 mcg	42	93	0.96	(0.62–1.48)	1.01	(0.65–1.57)
*Sensitivity analysis**						
Placebo	39	66	Reference		Reference	
Selenium 200 mcg	42	75	0.94	(0.61–1.45)	0.99	(0.64–1.54)
	Progression N	No progression N				
Secondary outcome: progression						
Intention to treat analysis						
Placebo	14	127	Reference		Reference	
Selenium 200 mcg	15	136	0.97	(0.47–2.00)	0.89	(0.42–1.86)
*Sensitivity analysis***						
Placebo	14	95	Reference		Reference	
Selenium 200 mcg	15	104	0.95	(0.46–1.98)	0.87	(0.41–1.83)
*Per protocol analysis***						
Placebo	9	112	Reference		Reference	
Selenium 200 mcg	15	120	1.48	(0.65–3.38)	1.45	(0.62–3.35)
*Sensitivity analysis****						
Placebo	9	96	Reference		Reference	
Selenium 200 mcg	15	102	1.45	(0.63–3.32)	1.41	(0.61–3.26)

HR = Hazard Ratio, CI = confidence interval.
* 64 patients stopped prematurely and were excluded from the analysis, 32 in each treatment arm.
** Time starts three months after starting date (T3).
*** 70 patients stopped prematurely and were excluded from the analysis, 34 and 36 in the selenium and placebo group.
[#] Adjusted for age, gender, smoking status, staging, baseline serum selenium level and hospital.

Table 4
Side-effects reported in the SELEBLAT study.

	Selenium 200 mcg	Placebo
Nausea	1 (grade I)	0
Fatigue	1	2 (grade I)
Nail symptoms	0	1 (grade I)
Diarrhoea	1 (grade I)	1 (grade I)
Pain	1 (grade II)	0
Stomach/intestinal	1 (grade I)	4
Vertigo	1	0
Constipation	1	1
Back and neck pain	1	0

reduction of 12.5% in recurrence would be reasonable with a serum selenium increase of 100 mg/dl. Moreover, in the Belgian case control study mean serum selenium level in cases was 78.77 μg/l compared with 92.31 μg/l in controls [10]. This contrasts with the patients enrolled in the NPC and the SELECT trials, who had higher initial serum levels of selenium (113 μg/l and 135 μg/l, respectively) [37]. Hence, Belgian BC patients could benefit more from selenium intake given their low selenium serum level. A second reason for not reaching the target of 700 patients was a high percentage of ineligible patients. We screened 694 patients with BC for eligibility and only 292 patients were suitable for randomisation and met inclusion criteria. Furthermore, the percentage of recurrence was lower than expected. The baseline risk of recurrence for our patient cohort in the first year calculated with the EORTC Bladder Cancer Prognosis calculator [31] was 27%. In our study, however, only 14–15% actually showed recurrence during the first year of follow-up. After nearly three years of follow-up, 30% were diagnosed with recurrence in contrast with 43% in the meta-analysis of Malström [32] on which we had based our power analysis. We do not believe there was a problem of recurrence under-reporting as rigorous controls were performed. We therefore assume that the implementation of the new EAU guidelines concerning standard treatment of non-invasive urothelial carcinoma [25,26] among which the immediate instillation of mitomycin C after TUR might be responsible for the lower recurrence rates. The meta-analysis of Malström was indeed mainly based on records of patients from before the existence of the above-mentioned guidelines (only two of the nine trials recruited patients after 2000), which may explain their higher incidence of recurrence. Other reasons for the lack of beneficial effect of selenium could be the different form of selenium used, selenomethionine, compared to Se-enriched yeast in the NPC study [38]. In our study we used the Se-enriched yeast.

Moreover, selenium levels might not have been different enough between groups to result in a clinically meaningful effect. This explanation seems unlikely since patients had the expected low serum selenium levels at study entry (83.3 μg/l in the control and 82.6 μg/l in the selenium group) which significantly increased to 187.6 μg/l for the experimental group. Furthermore despite efficient randomisation, the two groups were not exactly similar, reflected in differences in baseline characteristics. There were more men, more Tis tumours and more current smokers in the selenium group. This may have negatively influenced the results of this group. However, on the one hand, these differences were not statistically significant, on the other hand, the HRs adjusted for age, gender, smoking status, staging, baseline serum selenium level and hospital were similar to the crude HRs.

Moreover, a similar study was performed in the United Kingdom. This study, the Selenib (ClinicalTRials.gov NCT00553345) [39], has closed with almost the same number of patients and we will be able to pool results after publication. This will enable us to report on a sufficient number of patients.

As Jan Vandenbroucke explained in his Austin Bradford Hill Memorial Lecture in 2007 [40], new discoveries found in epidemiological studies need confirmation. The SELEBLAT study could not confirm the hypothesis that selenium is suitable in chemoprevention of non-invasive urothelial carcinoma. The secondary analysis of the SELECT study evaluating the influence of selenium on the incidence of BC could not confirm a protective effect of selenium on the incidence of BC. These negative results contradict earlier epidemiologic studies and make selenium currently unsuitable as chemopreventive agent in bladder cancer. A very recent meta-analysis reported similar negative results on selenium and bladder cancer [41]. As mentioned earlier the results of the Selenib study [39] will confirm or reject the results of the SELEBLAT study in the near future.

6. Conclusion

Selenium, in addition to standard care, did not diminish recurrence in bladder cancer patients compared to placebo. Based on the result of the SELEBLAT study, we do not recommend prescribing selenium to patients in order to prevent recurrence of early bladder cancer at this moment.

Trial registration

ClinicalTrials.gov identifier: NCT00729287.

Availability of the protocol, data and material

The protocol has been published [23].

Authors' contributions

MG, FB and MZ: study design, conception and design of the article, analysis and interpretation of data, drafting the article. SJ and HvP: study design, revising the article critically for important intellectual content. KA, FA, IB, JB, AB, JD, KD, LG, BT, FV and KV: local investigator, recruitment of patients, revising the article critically for important intellectual content. BV and SvB: recruitment of patients, revising the article critically for important intellectual content. The corresponding author was responsible for the report, had full access to all the data in the study and had final responsibility for the decision to submit for publication. All authors have read and approved the final manuscript.

Conflict of interest statement

None declared.

Acknowledgements

The SELEBLAT study was funded by an unconditional grant of the Agency for Innovation by Science and Technology Belgium (IWT) (project IWT 70699). The funder of the study had no role in study design, data collection, data analysis, data interpretation or writing of the report. Drugs were supplied by PharmaNord, Vojens, Denmark and prepared according to the principles of good manufacturing practice (GMP). The authors acknowledge all participating urologists from the 14 recruiting hospitals for their willingness to participate in the SELEBLAT study. The authors recognize the invaluable contribution made by the research nurses and administrative staff. The authors also acknowledge the members of the data and safety monitoring board and the staff of the Vesalius Research Centrum.

References

[1] Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, et al. GLOBOCAN 2012 v1.0, Cancer incidence and mortality worldwide: IARC CancerBase No. 11 [Internet]. Lyon, France: International Agency for Research on Cancer; 2013. Available from: http://globocan.iarc.fr.

[2] Cancer incidence in Belgium. Belgium cancer registry, Brussels. 2008. 2011;Available from: http://kankerregister.nettools.be/media/docs/StK_publicatie.pdf.

[3] Cooksley CD, Avritscher EB, Grossman HB, Sabichi AL, Dinney CP, Pettaway C, et al. Clinical model of cost of bladder cancer in the elderly. Urology 2008;71(3):519–25.

[4] Bosetti C, La Vecchia C. Cancer mortality in Latin America: implications for prevention. Rev Panam Salud Publica 2005;18(1):1–4.

[5] Brinkman M, Buntinx F, Muls E, Zeegers MP. Use of selenium in chemoprevention of bladder cancer. Lancet Oncol 2006;7(9):766–74.

[6] Whanger PD. Selenocompounds in plants and animals and their biological significance. J Am Coll Nutr 2002;21(3):223–32.

[7] Sanmartin C, Plano D, Palop JA. Selenium compounds and apoptotic modulation: a new perspective in cancer therapy. Mini Rev Med Chem 2008;8(10):1020–31.
[8] Brozmanova J, Manikova D, Vlckova V, Chovanec M. Selenium: a double-edged sword for defense and offence in cancer. Arch Toxicol 2010;84(12):919–38.
[9] Naithani R. Organoselenium compounds in cancer chemoprevention. Mini Rev Med Chem 2008;8(7):657–68.
[10] Kellen E, Zeegers M, Buntinx F. Selenium is inversely associated with bladder cancer risk: a report from the Belgian case-control study on bladder cancer. Int J Urol 2006;13(9):1180–4.
[11] Torun M, Aldemir H, Yardim S. Serum selenium levels in various cancer types. Trace Elem Electrocytes 1995;12(4):186–90.
[12] Yalcin O, Karatas F, Erulas FA, Ozdemir E. The levels of glutathione peroxidase, vitamin A, E, C and lipid peroxidation in patients with transitional cell carcinoma of the bladder. BJU Int 2004;93(6):863–6.
[13] Helzlsouer KJ, Comstock GW, Morris JS. Selenium, lycopene, alpha-tocopherol, beta-carotene, retinol, and subsequent bladder cancer. Cancer Res 1989;49(21):6144–8.
[14] Michaud DS, De Vivo I, Morris JS, Giovannucci E. Toenail selenium concentrations and bladder cancer risk in women and men. Br J Cancer 2005;93(7):804–6.
[15] Michaud DS, Hartman TJ, Taylor PR, Pietinen P, Alfthan G, Virtamo J, et al. No association between toenail selenium levels and bladder cancer risk. Cancer Epidemiol Biomarkers Prev 2002;11(11):1505–6.
[16] Nomura A, Heilbrun LK, Morris JS, Stemmermann GN. Serum selenium and the risk of cancer, by specific sites: case-control analysis of prospective data. J Natl Cancer Inst 1987;79(1):103–8.
[17] Zeegers MP, Goldbohm RA, Bode P, van den Brandt PA. Prediagnostic toenail selenium and risk of bladder cancer. Cancer Epidemiol Biomarkers Prev 2002;11(11):1292–7.
[18] Dennert G, Zwahlen M, Brinkman M, Vinceti M, Zeegers Maurice PA, Horneber M. Selenium for preventing cancer. Cochrane Database Syst Rev [Internet] 2011;5. Available from: http://www.mrw.interscience.wiley.com/cochrane/clsysrev/articles/CD005195/frame.html.
[19] Busby JE, Kamat AM. Chemoprevention for bladder cancer. J Urol 2006;176(5):1914–20.
[20] Altwein JE. Primary prevention of bladder cancer. What's new? Urol A 2007;46(6):616–21.
[21] Grossman HB, Stenzl A, Moyad MA, Droller MJ. Bladder cancer: chemoprevention, complementary approaches and budgetary considerations. Scand J Urology Nephrol 2008;42(Suppl. 218):213–33.
[22] Amaral AF, Cantor KP, Silverman DT, Malats N. Selenium and bladder cancer risk: a meta-analysis. Cancer Epidemiol Biomarkers Prev 2010;19(9):2407–15.
[23] Goossens ME, Buntinx F, Joniau S, Ackaert K, Ameye F, Billiet I, et al. Designing the selenium and bladder cancer trial (SELEBLAT), a phase lll randomized chemoprevention study with selenium on recurrence of bladder cancer in Belgium. BMC Urol 2012;12:8.
[24] Humphrey PA, Moch H, Cubilla AL, Ulbright TM, Reuter VE. The 2016 WHO classification of tumours of the urinary system and male genital organs-part B: prostate and bladder tumours. Eur Urol 2016;70(1):106–19.
[25] Babjuk M, Oosterlinck W, Sylvester R, Kaasinen E, Bohle A, Palou-Redorta J. EAU guidelines on non-muscle-invasive urothelial carcinoma of the bladder. Eur Urol 2008;54(2):303–14.
[26] Babjuk M, Oosterlinck W, Sylvester R, Kaasinen E, Bohle A, Palou-Redorta J, et al. EAU guidelines on non-muscle-invasive urothelial carcinoma of the bladder, the 2011 update. Eur Urol 2011;59(6):997–1008.
[27] Cancer Therapy Evaluation Program. Common Terminology criteria for adverse events, version 3.0, DCTD, NCI, NIH, DHHS March 31. 2003. Publish Date: August 9, 2006, http://ctep.cancer.gov.
[28] Epstein JI, Amin MB, Reuter VR, Mostofi FK. The World Health Organization/International Society of Urological Pathology consensus classification of urothelial (transitional cell) neoplasms of the urinary bladder. Bladder Consensus Conference Committee. Am J Surg Pathol 1998;22(12):1435–48.
[29] Sauter G, Algaba F, Amin MB, Busch C, Cheville J, Gasser T, et al. Tumours of the urinary system: non-invasive urothelial neoplasias. In: Eble JN, Sauter G, Epstein Jl, Sesterhenn I, editors. WHO classification of classification of tumors of the urinary system and male genital organs Lyon. IARCC Press; 2004. p. 29–34.
[30] Sobin LH, Gospodariwicz M, Wittekind C, editors. TNM classification of malignant tumors. UICC International Union against Cancer. 7th ed. Wiley-Blackwell; 2009 Dec. p. 262–5.
[31] Sylvester RJ, van der Meijden AP, Oosterlinck W, Witjes JA, Bouffioux C, Denis L, et al. Predicting recurrence and progression in individual patients with stage Ta T1 bladder cancer using EORTC risk tables: a combined analysis of 2596 patients from seven EORTC trials. Eur Urol 2006;49(3):466–77. discussion 75–7.
[32] Malmstrom PU, Sylvester RJ, Crawford DE, Friedrich M, Krege S, Rintala E, et al. An individual patient data meta-analysis of the long-term outcome of randomised studies comparing intravesical mitomycin C versus bacillus Calmette-Guerin for non-muscle-invasive bladder cancer. Eur Urol 2009;56(2):247–56.
[33] Larsen EH, Hansen M, Paulin H, Moesgaard S, Reid M, Rayman M. Speciation and bioavailability of selenium in yeast-based intervention agents used in cancer chemoprevention studies. J AOAC Int 2004;87(1):225–32.
[34] Rayman MP. The use of high-selenium yeast to raise selenium status: how does it measure up? Br J Nutr 2004;92(4):557–73.
[35] Lippman SM, Klein EA, Goodman PJ, Lucia MS, Thompson IM, Ford LG, et al. Effect of selenium and vitamin E on risk of prostate cancer and other cancers: the selenium and vitamin E cancer prevention trial (SELECT). JAMA 2009;301(1):39–51.
[36] Lotan Y, Goodman PJ, Youssef RF, Svatek RS, Shariat SF, Tangen CM, et al. Evaluation of vitamin E and selenium supplementation for the prevention of bladder cancer in SWOG coordinated SELECT. J Urol 2012;187(6):2005–10.
[37] Hatfield DL, Gladyshev VN. The outcome of selenium and vitamin E cancer prevention trial (SELECT) reveals the need for better understanding of selenium biology. Mol Interv 2009;9(1):18–21.
[38] Goossens ME, Buntinx F, Zeegers MP. Re: selenium and vitamin E: interesting biology and dashed hope. J Natl Cancer Inst 2009;101(19):1363–4. author reply 4.
[39] Zeegers MP, Bryan RT, Langford C, Billingham L, Murray P, Deshmukh NS, et al. The West Midlands Bladder Cancer Prognosis Programme: rationale and design. BJU Int 2009;105(6):784–8.
[40] Vandenbroucke JP. Observational research, randomised trials, and two views of medical science. PLoS Med 2008;5(3):e67.
[41] Cai X, Wang C, Yu W, Fan W, Wang S, Shen N, et al. Selenium exposure and cancer risk: an updated meta-analysis and meta-regression. Sci Rep 2016;6:19213.