Investigational New Drugs (2019) 37:1231–1238
https://doi.org/10.1007/s10637-018-00716-w

PHASE II STUDIES

Phase II trial of continuous treatment with sunitinib in patients with high-risk (BCG-refractory) non-muscle invasive bladder cancer

Haris Zahoor[1] · Maria C. Mir[2] · Pedro C. Barata[3] · Andrew J. Stephenson[4] · Steven C. Campbell[4] · Amr Fergany[4] · Robert Dreicer[5] · Jorge A. Garcia[4,6]

Received: 29 October 2018 / Accepted: 13 December 2018 / Published online: 24 June 2019

Summary

Purpose Sunitinib is a vascular endothelial growth factor receptor (VEGFR) inhibitor with antitumor activity against bladder cancer. We hypothesized that treatment with sunitinib may decrease progression or recurrence in non-muscle invasive bladder cancer (NMIBC) refractory to intra-vesical BCG. *Patients and Methods* This is a single-arm phase II study of sunitinib in patients (pts) with NMIBC who progressed after BCG. Treatment included sunitinib 37.5 g daily for 12 weeks followed by 12± 2-week cystoscopy and surveillance for one year. The primary endpoint was the complete response rate at 12 months. Secondary endpoints included recurrence free survival (RFS), progression free survival (PFS), overall survival (OS), and safety of sunitinib. Correlative studies on effects of sunitinib on myeloid derived suppressor cells (MDSC) and humoral immune responses were also performed. This trial was registered on ClinicalTrials.gov, number NCT01118351. *Results* Between June 2011 and September 2011, 15/19 pts. completed 12 weeks of therapy. The remaining 4 pts. had treatment related adverse events leading to discontinuation of sunitinib with one patient withdrawing consent. On the 12-week cystoscopy, 44% (8/18) of the pts. showed remission, 50% (9/18) progression and 1/18 recurrence. Overall, 22% (4/18) of pts. remained free of progression for >12 months. Grade (G) 4 toxicities were noted in 2 pts. (anemia and thrombocytopenia) while G3 were noted in 58%. Sunitinib resulted in reversal of MDSC mediated immunosuppression. *Conclusions* In NMIBC refractory to BCG, treatment with sunitinib was safe but not associated with improved clinical outcomes. The immune effects of sunitinib deserve further investigation.

Keywords Sunitinib · Non-muscle invasive bladder cancer · BCG-refractory · Angiogenesis · MDSC

Introduction

Bladder cancer is the sixth most common cancer. In 2018, 81,190 new cases of bladder cancer are estimated to be diagnosed and 17,240 of these patients are estimated to die from this disease, making it a leading cause of cancer deaths in United States [1].

Approximately 70% of the newly diagnosed cases are non-muscle invasive cancers including Ta (papillary, 70–75%), T1 (20–25%) and Tis (carcinoma in situ –CIS, 5–10%) tumors [2]. These non-muscle invasive bladder cancers are primarily managed with endoscopic resection [3], however, up to 90% of these patients will have a recurrence within 5 years, if treated with resection alone [2]. Conventional therapy with Transurethral Resection of Bladder Tumor (TURBT) followed by intra-vesical therapy, generally with Bacillus Calmette Guerin (BCG), reduces the recurrence rate by 30%–40%,

✉ Jorge A. Garcia
garciaj4@ccf.org

1 Division of Medical Oncology, USC Norris Comprehensive Cancer Center, Keck School of Medicine, Los Angeles, CA, USA

2 Urology Department, IMED Valencia Hospital, Valencia, Spain

3 Department of Internal Medicine, Section of Hematology and Medical Oncology, Tulane Medical School, New Orleans, Louisiana, USA

4 Cleveland Clinic, Glickman Urological and Kidney Institute, 9500 Euclid Avenue/R35, Cleveland, OH 44195, USA

5 Division Hematology/Oncology, University of Virginia School of Medicine, Charlottesville, Virginia, USA

6 Department of Solid Tumor Oncology and Urology, Cleveland Clinic Taussig Cancer Institute, 9500 Euclid Avenue/R35, Cleveland, OH 44195, USA

improving the chances of bladder preservation and avoiding radical surgery [4–6]. However, 30% to 50% of the patients fail to respond to BCG treatment or will develop a relapse within the first 5 years after treatment [7].

Patients with high risk non-muscle invasive disease, with recurrence or persistent disease after initial treatment with BCG, have limited therapeutic options in terms of bladder preservation [8]. Therefore, radical cystectomy, bilateral extended lymph node dissection and urinary diversion offers the best chance for cure, at the cost of a higher morbidity [9]. This opens up an unmet need for alternative treatment regimens in BCG-refractory non-muscle invasive bladder cancer to decrease recurrence and progression of the disease.

Angiogenesis is well-known to play a key role in the survival, proliferation, and metastatic potential of various cancers including urothelial cancer [10]. The various markers of angiogenesis including Vascular Endothelial Growth Factor (VEGF) are overexpressed in urothelial cancer patients and this overexpression is associated with worse prognosis [11]. These observations have led to the investigation of anti-VEGF agents in bladder cancer. For example, sunitinib has been shown to have clinical activity in preclinical models of urothelial cancer [12]. Clinical studies evaluating the role of sunitinib in metastatic urothelial cancer either alone or as combination have shown modest activity [13, 14]. However, its ability to decrease progression or recurrence in non-muscle invasive bladder cancers has not been evaluated. We hypothesize that treatment with sunitinib in BCG-refractory patients will be efficacious and safe. To this end, we conducted a single center phase II trial to evaluate the clinical activity and safety of sunitinib in patients with BCG-refractory non-muscle invasive transitional cell carcinoma (TCC) of bladder.

Patients and methods

Patient population

Patients were enrolled in the study at the Cleveland Clinic between June 2011 and September 2011. Patients were at least 18 years of age with histologically proven non-muscle invasive TCC of the bladder (TaHG, T1HG, or Tis) which had recurred after treatment with intra-vesical BCG. Prior administration of other intra-vesical therapy within 3 years of registration was allowed. Additional inclusion criteria included European Cooperative Oncology Group (ECOG) performance status of 0–1 and predefined hematological criteria. Patients with history of systemic chemotherapy at any time for bladder cancer or history of radiation therapy or systemic therapy for any malignancy within 3 years of registration were excluded. Patients with prior use of any immunomodulatory or VEGF inhibitors were not eligible. Patients with a major cardiovascular event in the 6 months prior to study enrollments were also excluded.

Study design and treatment

This was a single arm open label phase II clinical study conducted at Cleveland Clinic. A single stage accrual design was employed and sunitinib administered at a dose of 37.5 mg orally once daily for 12 consecutive weeks (total 3 cycles; 1 cycle = 4 weeks). This continuous dosing schedule was selected due to its demonstrated equivalent efficacy and safety compared to an intermittent 4 weeks on, 2 weeks off schedule in renal cell carcinoma [15, 16].

All patients were evaluated at baseline, each cycle during treatment phase, and had a 2-week follow-up cystoscopy after the end of 12 weeks of treatment with sunitinib. Cystoscopic evaluations and urinary cytology were performed every 3 months for one year thereafter. Subsequent transurethral resection of bladder tumor (TURBT) were performed at the discretion of the treating urologist.

The Cleveland Clinic Institutional Review Board reviewed and approved the study, which was conducted per the Declaration of Helsinki in accordance with the World Medicines Association and its amendments. All patients provided written informed consent before enrollment.

Correlative studies

Serum / plasma correlative studies were performed before treatment and at day 28 of each cycle of therapy and included peripheral blood assessment of MDSC, regulatory T cells (Treg) and cytokines to evaluate Th1 humoral response.

Laboratory methods

The underlying biological effect of sunitinib on immunosuppressive MDSCs, type 1 immune response and changes in the proportion of Treg were assessed over time; however, the primary focus was the change after one cycle of therapy. MDSC were identified by two criteria, $CD15^{+}14^{-}$ and $CD33^{+}$ while T cell production of IFN-γ was used to evaluate type 1 response.

Peripheral blood mononuclear cell (PBMC) were isolated from peripheral blood (60 mL) before sunitinib treatment (cycle 1, day 1) and on day 28 after each cycle of treatment, and from age matched normal (AMN) donors using the ficoll-hypaque gradient and then frozen in liquid nitrogen. Samples were thawed in a 37 °C bath for analysis after all time points for a given patient were obtained. Thereafter, cells were washed at room temperature (25 °C) with 20 ml of complete medium (RPMI 1640+ 10% fetal bovine serum), centrifuged at 1500 rpm for 10 min and suspended in the medium. The cells were plated in 6-well tissue culture plates (BD

Falcon™) and incubated at 37 °C in 5% CO2 overnight. For the analysis of type-1 and type-2 response, patients' samples were run for T cell activation and cytokine production with PBMC from AMN donors as a positive control.

Type-1 and Type-2 cytokine response assessment PBMCs were unstimulated (no addition) or stimulated for 72 h at 37 °C, 5% CO2 with 25 μl of Dynabeads™ CD3/CD28 T Cell Expander Beads (Dynal) per 1 × 106 PBMCs and 200 μ/ml rhIL-2. At the end of 72 h, Golgi plugs were added to the cells for 6 h to prevent cytyokine secretion. To detect intracellular levels of IFN γ and IL-4 the unstimulated and stimulated PBMCs were harvested and the wells rinsed with 1xPBS. Thereafter, cells were centrifuged at 1500 rpm for 10 min, resuspended in FACS Buffer (2% Heat Inactivated FBS + 1xPBS) and stained with CD3-APC (BD Pharmingen) and CD4-PerCP (BD Pharmingen) for 30 min, 4 °C. The cells were permeabilized with BD CytoFix/Cytoperm™ Solution (BD Pharmingen) for 30 min at 4 °C. Next cells were washed with BD Perm/Wash™ Solution (BD Pharmingen), resuspended in the same solution, and stained for intracellular IFN-g using IFNg- FITC mAb (BD Pharmingen) and intracellular IL-4 using an IL4-PE mAb (BD Pharmingen) for 30 min, 4 °C. The cells were fixed in 1% paraformaldehyde in 1xPBS and the data acquired on the BD FACSCalibur machine. Data analysis was performed using the FlowJo Software (Tree Star, Inc./FlowJo LLC). Non-stimulated cells from each donor served as a negative control. Additionally, specificity of cytokine staining was confirmed in each sample via subtraction of any non-specific staining occurring in samples pretreated with unlabeled anti-cytokine antibodies.

For the analysis of T regulatory cells in patient PBMCs, samples were thawed, washed and resuspended in complete RPMI and incubated overnight. The following day, surface marker staining of CD3, CD4, and CD25 was done in normal FACS buffer for 30 min. Cells were then permeabilized for 30 min with BD Fix/Perm, and then stained for Foxp3 in BD Perm/Wash solution for 30 min. All staining and permeabilization steps were done at 4 °C. Cells were then resuspended in 1% PFA and subjected to FACS analysis. Data was acquired using Cellquest on a BD FACSCalibur, and analyzed using Flow Jo software (Tree Star Incorporated, Ashland, OR). At least 300,000 live cell events were collected for each tube used in analysis. Results were expressed as percentage of CD25 + hi/Foxp3+ cells out of total CD3+/CD4+ viable cells.

Statistical analyses

The primary endpoint of this trial was to assess the complete response rate at 12 months in patients with BCG-refractory TCC treated with sunitinib for a maximum of 12 weeks. The complete response was defined as the absence of gross tumor on cystoscopy, negative biopsy, and negative urine cytology. Secondary endpoints included safety assessment along with recurrence free probability, progression free probability and overall survival. Survival outcomes were measured from date of registration. A total of 31 patients were required to detect a 45% complete response rate (as compared to historical 20%) with a statistical power of 80% and a two-sided p value of 0.05. A single stage accrual design was employed however efficacy was monitored periodically to allow for early closure of the trial in case of high recurrence or progression rates. It was predefined that consideration to early closure of the trial will be given if 8 or more of the first 10 patients relapse or progress within 12 months.

Baseline characteristics were summarized as numbers and percentages for categorical variables and as medians with interquartile ranges for continuous variables. Survival endpoints were summarized with the Kaplan-Meier method. Toxicity was assessed, graded and tabulated using NCI CTCAE version 3.0.

Exploratory analyses evaluating the effect of sunitinib on the levels of MDSC, Treg and Th1 responses, were also performed. At baseline these levels were compared to AMN donors using Wilcoxon rank-sum test and subsequently evaluated post-treatment using Wilcoxon signed-rank test. All tests of statistical significance were two-sided and there was no adjustment for multiple comparisons. These analyses were exploratory in nature and it is acknowledged that the trial was adequately powered statistically to detect only fairly large effects.

Results

Baseline patient characteristics

Nineteen patients were enrolled in the study. Table 1 summarizes baseline patient and disease characteristics at enrollment. Median age was 72 years (range 54–82), 79% (15/19) male, and 95% having an ECOG performance status of 0. All patients had T1 disease. Six (32%) patients had prior intravesical treatment with BCG only while the remaining 68% (13/19) had exposure to multiple intra-vesical agents in addition to BCG, primarily interferon-α2b and / or mitomycin-C. Two patients had prior radiotherapy, one to the prostate and the other to mediastinum and lymph nodes.

Treatment exposure and efficacy

Fifteen patients (79%) completed the full 12 weeks of therapy with sunitinib, 3 patients discontinued therapy early due to AEs while one patient withdrew from the trial after being on treatment for 15 weeks. This patient was excluded from the endpoint analysis. Overall 44% (8/18) of the patients

Table 1 Baseline clinical characteristics

Characteristic	N (%)
Gender	
Male	15 (79%)
Female	4 (21%)
Age, years; Median (Range)	72 (54–82)
ECOG PS	
0	18 (95%)
1	1 (5%)
Pathological T1 Stage	19 (100%)
Prior Intra-vesical Therapy (BCG ± other therapies)	
BCG only	6 (32%)
BCG and other therapies	13 (68%)[2]
IFN-α2b	9 (47%)
Mitomycin-C	7 (37%)
Thiotepa	1 (5%)
Docetaxel	1 (5%)
Prior Radiation Therapy	
No	17 (89%)
Yes[a]	2 (11%)

[a] RT to prostate - #7; RT to mediastinum and lymph nodes - #17

[b] mitomycin-C and IFN-α2b were the only additional therapy for 3 and 6 patients, respectively; 2 patients were exposed to both agents; one patient had prior mitomycin-C and thiotepa; and one patient had prior mitomycin-C, IFN-α2b, and docetaxel

remained without evidence of disease at the 12-week cystoscopy (95% CI 20–67%), one patient had recurrence (urinary cytology showed atypical cells suggestive of urothelial cancer), while the remaining 9 patients (50%) had progressed.

Accrual to the study was suspended and trial was prematurely closed due to a predefined futility rule that was part of the study's initial statistical design. At 12-month mark (primary endpoint), a total of four patients (4/18) remained without evidence of cancer. Hence, overall 78% (14/18) of the evaluable patients had progressed with an estimated median time to progression of 4.7 months from the 12-week cystoscopy. The median follow-up was 14.9 months (range 3.4–27.9).

Safety and tolerability

The most frequently reported treatment related adverse events are described in Table 2. All the enrolled patients received at least some treatment and therefore all 19 are included in the assessment of AEs. Clinically significant toxicity (G3/4) was noted in 13 patients.

Thrombocytopenia was the most frequent adverse event, noted in nearly 90% of the patients, with one patient having G4 toxicity. Apart from this, the only other G4 AE considered possibly related to treatment was anemia in one patient. Eleven patients (58%) had G3 AEs, primarily hypertension noted in 6 patients (32%). The other G3 AEs included thrombocytopenia, diarrhea, leukopenia (including neutropenia and lymphopenia) and rash. G2 AEs were noted in the remaining 6 patients (32%). Three patients discontinued treatment due to the AEs including a worsening leg ulcer, G2 and G3 thrombocytopenia and G2 generalized malaise with arthralgias.

Immunologic markers

Nine patients (50%) treated on this trial had pre-treatment and cycle 1, 2, and 3 data on immunologic markers. In addition, data from 10 to 22 healthy control subjects were available. Table 3 summarizes baseline immunologic differences between the health control subjects and bladder cancer patients on this trial. The two groups differed in that cancer patients had significantly more MDSC than the control subjects ($p = .001$ for CD15 + CD14-MDSC and $p < .001$ for CD33+ MDSC), but less ability to mount a Th1 response as demonstrated by significantly fewer CD3+ or CD4+ IFN-γ producing T-Cells ($p < .001$ for CD3+ and $p = .002$ for CD4+). The number of cancer patients is small, however there was no suggestion that the two groups differed with respect to the presence of Treg ($p = .40$) prior to treatment with sunitinib.

Table 3 also summarizes changes in these parameters following 1, 2, and 3 cycles of treatment. In aggregate, sunitinib resulted in a sustained increase (compared to pre-treatment) in CD3+ and CD4+ IFN-γ producing T-cells ($p < 0.01$ and $p < 0.07$ respectively). In addition, there was a significant decrease in CD33+ MDSC following cycles 2 and 3 of therapy ($p < 0.008$ and $p < 0.04$). There was also a significant decrease in Treg following 2 cycles of therapy ($p = 0.04$); however, it was not maintained during cycle 3 ($p = 0.36$).

Discussion

This phase II trial is one of the first studies to explore the role of a VEGF inhibitor in BCG refractory non-muscle invasive TCC of the bladder. The treatment with sunitinib failed to show an improvement in progression free survival in this population leading to premature discontinuation of the trial. There was a sunitinib associated reduction in tumor induced immunosuppression as evidenced by improved effector T cell function and decrease in MDSC. An evolving understanding of the critical role of the tumor microenvironment in supporting the growth, invasion and migration of tumors, and immune-escaping has led to widespread evaluation of anti-angiogenic agents in cancer trials [17]. VEGF pathway particularly plays an important role in tumor growth not only by promoting tumor-associated angiogenesis but also through its negative effect on the host antitumor adaptive immunity by promoting suppressive myeloid cells [18]. Sunitinib not only inhibits

Table 2 Adverse Events (AE) considered at least possibly related to treatment

AE	Grade 1	Grade 2	Grade 3	Grade 4	Any Grade
Thrombocytopenia	11	2	3	1	17 (89%)
Taste Alterations	12	4	–	–	16 (84%)
Fatigue	10	4	–	–	14 (74%)
Leukopenia	5	7	1	–	13 (68%)
Diarrhea	10	1	2	–	13 (68%)
Heartburn	9	3	–	–	12 (63%)
Rash/PPE	4	5	1	–	10 (53%)
Mucositis	5	5	–	–	10 (53%)
Hypertension	–	2	6	–	8 (42%)
Anemia	5	2	–	1	8 (42%)
Neutropenia	1	6	1	–	8 (42%)
Lymphopenia	3	2	2	–	7 (36%)
Dermatologic other than Rash/PPE	6	–	–	–	6 (32%)

angiogenesis, but has also been shown to reverse the tumor-mediated immunosuppression by decreasing the MDSC and Treg, and improving effector T cell function [18]. These observations provide the rationale to investigate its use in BCG-refractory non-muscle invasive bladder cancer which may have escaped the host antitumor immune response triggered by BCG treatment.

Although sunitinib has shown clinically activity in multiple solid tumors [19–21] and is a standard first line treatment for metastatic renal cell carcinoma [22], its evaluation in bladder cancer is either lacking or has met with rather disappointing results in the advanced setting. A single institution, open labeled, single arm trial evaluated two different dosing cohorts (intermittent and continuous) of sunitinib in refractory metastatic urothelial cancer (UC). Although the primary end point of the trial, overall response rate (ORR) of 20%, was not met in either cohort, antitumor activity (eight patients with tumor reduction >20%) was observed [13]. The PFS (2.4 and 2.3 months) and OS (7.1 and 6.0 months) were similar, irrespective of the intermittent or continuous dosing schedule of sunitinib, respectively. Although these results were not encouraging and only included a small subset of patients re-staged after 2 cycles of treatment, they

Table 3 Immunological markers

A. Bladder Cancer vs. Healthy Controls

Controls					Bladder Cancer				
Marker	N	Mean ± s.d.	Median	Range	N	Mean ± s.d.	Median	Range	*p*
CD15 + 14MDSC	10	0.19 ± 0.11	0.16	0.05–0.41	9	5.68 ± 10.49	2.37	0.17–32.29	.001
CD33+ MDSC	10	1.14 ± 0.79	1.00	0.24–2.66	9	7.95 ± 10.57	4.43	1.32–35.66	<.001
Treg	22	1.45 ± 0.54	1.45	0.44–2.77	9	2.11 ± 1.57	2.00	0.53–5.06	.40
CD3+ IFN- α2b	21	18.01 ± 6.41	16.22	10.85–31.52	9	9.13 ± 4.35	8.79	2.86–17.87	< .001
CD4+ IFN- α2b	21	16.44 ± 6.14	15.13	7.23–27.92	9	8.18 ± 4.45	7.28	2.78–18.81	.002

B. Relative Changes Following 1, 2 and 3 Cycles of Therapy ($n = 9$ patients in all cases)

	C1 D28 vs Pre-Treatment[a]		C2, D28 vs Pre-Treatment[a]		C3, D28 vs Pre-Treatment[a]	
Marker	Median (Range)	p^2	Median (Range)	p^2	Median (Range)	p^2
CD15 + 14MDSC	−33.2% (−86.5, 562.7)	.73	−55.6% (−96.5–107.9)	.16	5.4% (−91.6–297.4)	.73
CD33+ MDSC	−28.6% (−78.9, 37.5)	.30	−40.9% (−91.1–6.6)	.008	−38.8% (−83.2–31.1)	.04
Treg	−10.7% (−40.9–35.9)	.57	−8.4% (−54.4 7.8)	.04	−20.3% (−61.0–107.1)	.36
CD3+ IFN- α2b	38.9% (−21.3–157.7)	.01	65.4% (8.2–338.5)	.004[3]	50.8% (−13.1–214.5)	.03
CD4+ IFN- α2b	38.1% (−23.2–159.4)	.07	54.9% (8.0–229.9)	.004[3]	24.5% (−24.5–191.9)	.05

[a] Negative values indicate a decrease compared to pre-treatment, positive values an increase

[b] Wilcoxon signed rank test

[c] All 9 patients had an increase compared to baseline

were similar to those observed with other existing second-line treatments. These small improvements in PFS and OS merited the biologic research which proposed VEGF pathway as potential therapeutic target in bladder cancer. Another study in patients with metastatic UC evaluated the role of sunitinib as maintenance therapy to consolidate the response of chemotherapy and delay disease progression [23]. The study was closed prematurely due to slow accrual and maintenance sunitinib did not appear to improve progression free survival. Hence, the role of VEGF directed therapy in the treatment of advanced bladder cancer remains to be defined. Similar studies evaluating the role of VEGF directed therapies in non-muscle invasive bladder cancer, which account for 70% of the bladder cancers, are however lacking. The present study evaluated the effect of sunitinib on clinically relevant endpoints as well as its effect on reversing tumor-induced immunosuppression. Apart from the limitations of a small, single-arm trial design conducted at a single institution, a few other explanations may be offered for these results.

Firstly, the pathogenesis of bladder cancer is believed to progress along two divergent biologic pathways with superficial lesions showing up regulation of βFGF, IL-8 and EGFR while VEGF appears to be significantly up regulated only at later stages in the development of muscle-invasive TCC [24]. The studies which correlated microvessel density (MVD), a quantitative surrogate of angiogenesis, to worse outcomes including progression, metastases and decreased survival were predominantly in patients with advanced bladder cancer [25–27]. Subsequent studies have highlighted a significant step up in angiogenic factors, like VEGF, from non-muscle invasive to muscle invasive disease suggesting an increasingly important role [28]. Even within non-muscle invasive disease, MVD has been shown to have a strong association with clinical stage. [29] Hence it is possible that angiogenesis in non-muscle invasive bladder cancers may not be as critical a driver of tumorigenesis as compared to advanced disease. However, the VEGFR expression was not evaluated in the present study and therefore a definitive conclusion cannot be drawn.

Also, the final angiogenic phenotype of the tumor is determined by the balance of pro-angiogenic and anti-angiogenic factors in the body. As noted above, non-muscle invasive bladder cancers may exhibit lower levels of pro-angiogenic factors like VEGF. Concurrently, studies in non-muscle invasive bladder cancers have shown a significant increase in the amount of thrombospondin-1, a natural anti-angiogenic molecule compared to invasive disease [28]. This imbalance may set the stage for a subdued response to an anti-angiogenic therapy. The expression of these angiogenesis markers were not assessed in this study and therefore this explanation remains hypothesis generating.

Biological rationale to combine VEGF inhibitors with other agents exists, and future studies should focus on investigating the effect of these potential combination therapies on clinical outcomes. The use of anti-angiogenic agents have improved OS in colorectal [30] and non-small cell lung cancer [31] and disease-free survival in breast cancer [32] when combined with chemotherapy. The rationale for this comes from the vascular normalization theory where the pruning of abnormal leaky tumor vasculature by anti-angiogenic agents improves tumor blood flow by host vasculature and synergizes the effects of chemotherapy [33]. An ongoing randomized phase III trial is evaluating the combination of chemotherapy with bevacizumab, a VEGF inhibitor in patients with metastatic or advanced TCC [34].

While systemic chemotherapy has no established role in treatment of non-muscle invasive bladder cancer, its combination with anti-angiogenic therapy may merit further evaluation as a viable alternative to radical cystectomy especially in high risk BCG refractory disease.

Immune checkpoint inhibitors (ICI) are changing the treatment landscape of advanced urothelial cancer, with multiple ICI now approved in metastatic setting [35]. Similarly, several ongoing trials are evaluating the role of ICI in non-muscle invasive bladder cancer patients with BCG refractory disease [36–38]. Although ICI have shown clinical activity in advanced urothelial cancer, only a subset of patients responds to the treatment [35]. Understanding the biology and treatment options for these ICI refractory patients is an area of active research. An evolving concept in cancer immunotherapy is that of tumor induced immature myeloid precursors, MDSC, which can inhibit T cell function directly as well as indirectly via induction of Treg [39, 40]. . Hence the combination of VEGF inhibitors to enhance the clinical efficacy of ICI has been suggested and is the rationale for several ongoing trials in solid tumors including urothelial cancer [41, 42]. In the present study, sunitinib has shown the ability to decrease the number of MDSC and increase Treg, thereby reversing the tumor induced immunosuppression, in line with previous studies [18]. These data provide the rationale to investigate the combination of sunitinib with ICI in future.

Recent studies also showed enhancement of the BCG-mediated cytotoxicity by sunitinib through the apoptosis pathway and may offer another combination approach to improve outcomes [43]. These findings led to the clinical trial which evaluated the combination of BCG and sunitinib in high risk s non-muscle invasive bladder cancer patients [44]. Patients received induction BCG followed by 28 days of sunitinib. This study showed that the combination of BCG with sunitinib is active in non-muscle invasive bladder cancer with two thirds of patients achieving complete responses, and lower risk of recurrence and progression. Despite the lack of a comparator arm, these provocative results warrant further investigation of this combination.

In summary, sunitinib monotherapy does not appear to have meaningful clinical efficacy in the management of patients with BCG-refractory non-muscle invasive TCC of the bladder. The correlative studies from this trial offer

preliminary evidence that sunitinib can reverse MDSC derived immunosuppression, providing a rationale to investigate combinations of inhibitors of VEGF with novel immunotherapeutics in refractory non-muscle invasive disease.

Authors' contributions HZ contributed to the data acquisition, drafting and final revision of the manuscript. MCM participated in data acquisition and final revision of the manuscript. PCB participated in data acquisition and final revision of the manuscript. AJS participated in data acquisition and final revision of the manuscript. SCC participated in data acquisition and final revision of the manuscript. RD participated in data acquisition and final revision of the manuscript. JAG contributed to the conception and coordination of the study, data acquisition, drafting and final revision of the manuscript. All authors read and approved the manuscript.

Funding The work was supported by the Pfizer Inc.

Compliance with ethical standards

Conflicts of interest HZ declares that he has no conflict of interest. MCM declares that he has no conflict of interest. PCB declares that he has no conflict of interest. AJS declares that he has no conflict of interest. SCC declares that he has no conflict of interest. AF declares that he has no conflict of interest. RD has served as a consultant for Astra Zeneca, Janssen, Pfizer, Lilly and Astellas. The other authors declare no competing interests directly related to this manuscript. JAG has honoraria from Bayer, Sanofi, Pfizer, Genentech, Janssen, Eisai and Exelixis.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

References

1. Siegel RL, Miller KD, Jemal A (2018) Cancer statistics, 2018. CA Cancer J Clin 68:7–30
2. Pasin E, Josephson DY, Mitra AP, Cote RJ, Stein JP (2008) Superficial bladder cancer: an update on etiology, molecular development, classification, and natural history. Rev Urol 10:31–43
3. Spiess PE, Agarwal N, Bangs R, Boorjian SA, Buyyounouski MK, Clark PE, Downs TM, Efstathiou JA, Flaig TW, Friedlander T, Greenberg RE, Guru KA, Hahn N, Herr HW, Hoimes C, Inman BA, Jimbo M, Kader AK, Lele SM, Meeks JJ, Michalski J, Montgomery JS, Pagliaro LC, Pal SK, Patterson A, Plimack ER, Pohar KS, Porter MP, Preston MA, Sexton WJ, Siefker-Radtke AO, Sonpavde G, Tward J, Wile G, Dwyer MA, Gurski LA (2017) Bladder Cancer, version 5.2017, NCCN clinical practice guidelines in oncology. J Natl Compr Cancer Netw 15:1240–1267
4. Smith JA Jr, Labasky RF, Cockett AT et al (1999) Bladder cancer clinical guidelines panel summary report on the management of nonmuscle invasive bladder cancer (stages ta, T1 and TIS). The American Urological Association. J Urol 162:1697–1701
5. Soloway MS, Sofer M, Vaidya A (2002) Contemporary management of stage T1 transitional cell carcinoma of the bladder. J Urol 167:1573–1583
6. Thalmann GN, Markwalder R, Shahin O et al (2004) Primary T1G3 bladder cancer: organ preserving approach or immediate cystectomy? J Urol 172:70–75
7. Lamm DL, Griffith JG (1992) Intravesical therapy: does it affect the natural history of superficial bladder cancer? Semin Urol 10:39–44
8. Herr HW, Sogani PC (2001) Does early cystectomy improve the survival of patients with high risk superficial bladder tumors? J Urol 166:1296–1299
9. Shabsigh A, Korets R, Vora KC, Brooks CM, Cronin AM, Savage C, Raj G, Bochner BH, Dalbagni G, Herr HW, Donat SM (2009) Defining early morbidity of radical cystectomy for patients with bladder cancer using a standardized reporting methodology. Eur Urol 55:164–174
10. Bradley DA, Dunn R, Nanus D, Stadler W, Dreicer R, Rosenberg J, Smith DC, Hussain M (2007) Randomized, double-blind, placebo-controlled phase II trial of maintenance sunitinib versus placebo after chemotherapy for patients with advanced urothelial carcinoma: scientific rationale and study design. Clin Genitourin Cancer 5:460–463
11. Brown LF, Berse B, Jackman RW, Tognazzi K, Manseau EJ, Dvorak HF, Senger DR (1993) Increased expression of vascular permeability factor (vascular endothelial growth factor) and its receptors in kidney and bladder carcinomas. Am J Pathol 143:1255–1262
12. Sonpavde G, Jian W, Liu H, Wu MF, Shen SS, Lerner SP (2009) Sunitinib malate is active against human urothelial carcinoma and enhances the activity of cisplatin in a preclinical model. Urol Oncol 27:391–399
13. Gallagher DJ, Milowsky MI, Gerst SR, Ishill N, Riches J, Regazzi A, Boyle MG, Trout A, Flaherty AM, Bajorin DF (2010) Phase II study of sunitinib in patients with metastatic urothelial cancer. J Clin Oncol 28:1373–1379
14. Bellmunt J, Gonzalez-Larriba JL, Prior C, Maroto P, Carles J, Castellano D, Mellado B, Gallardo E, Perez-Gracia JL, Aguilar G, Villanueva X, Albanell J, Calvo A (2011) Phase II study of sunitinib as first-line treatment of urothelial cancer patients ineligible to receive cisplatin-based chemotherapy: baseline interleukin-8 and tumor contrast enhancement as potential predictive factors of activity. Ann Oncol 22:2646–2653
15. Harshman L, Srinivas S (2008) Continuous daily dosing of sunitinib in patients with metastatic renal cell cancer. Onkologie 31:432–433
16. Escudier B, Roigas J, Gillessen S, Harmenberg U, Srinivas S, Mulder SF, Fountzilas G, Peschel C, Flodgren P, Maneval EC, Chen I, Vogelzang NJ (2009) Phase II study of sunitinib administered in a continuous once-daily dosing regimen in patients with cytokine-refractory metastatic renal cell carcinoma. J Clin Oncol 27:4068–4075
17. Chen F, Zhuang X, Lin L, Yu P, Wang Y, Shi Y, Hu G, Sun Y (2015) New horizons in tumor microenvironment biology: challenges and opportunities. BMC Med 13:45
18. Ko JS, Zea AH, Rini BI, Ireland JL, Elson P, Cohen P, Golshayan A, Rayman PA, Wood L, Garcia J, Dreicer R, Bukowski R, Finke JH (2009) Sunitinib mediates reversal of myeloid-derived suppressor cell accumulation in renal cell carcinoma patients. Clin Cancer Res 15:2148–2157
19. Demetri GD, van Oosterom AT, Garrett CR, Blackstein ME, Shah MH, Verweij J, McArthur G, Judson IR, Heinrich MC, Morgan JA, Desai J, Fletcher CD, George S, Bello CL, Huang X, Baum CM, Casali PG (2006) Efficacy and safety of sunitinib in patients with advanced gastrointestinal stromal tumour after failure of imatinib: a randomised controlled trial. Lancet 368:1329–1338
20. Motzer RJ, Hutson TE, Tomczak P, Michaelson MD, Bukowski RM, Rixe O, Oudard S, Negrier S, Szczylik C, Kim ST, Chen I, Bycott PW, Baum CM, Figlin RA (2007) Sunitinib versus interferon alfa in metastatic renal-cell carcinoma. N Engl J Med 356:115–124
21. Raymond E, Dahan L, Raoul JL, Bang YJ, Borbath I, Lombard-Bohas C, Valle J, Metrakos P, Smith D, Vinik A, Chen JS, Hörsch D, Hammel P, Wiedenmann B, van Cutsem E, Patyna S, Lu DR,

Blanckmeister C, Chao R, Ruszniewski P (2011) Sunitinib malate for the treatment of pancreatic neuroendocrine tumors. N Engl J Med 364:501–513
22. Motzer RJ, Jonasch E, Agarwal N, Bhayani S, Bro WP, Chang SS, Choueiri TK, Costello BA, Derweesh IH, Fishman M, Gallagher TH, Gore JL, Hancock SL, Harrison MR, Kim W, Kyriakopoulos C, LaGrange C, Lam ET, Lau C, Michaelson MD, Olencki T, Pierorazio PM, Plimack ER, Redman BG, Shuch B, Somer B, Sonpavde G, Sosman J, Dwyer M, Kumar R (2016) Clinical practice guidelines in oncology: kidney cancer. J Natl Compr Canc Netw 15(6):804-834. https://doi.org/10.6004/jnccn.2017.0100
23. Grivas PD, Daignault S, Tagawa ST, Nanus DM, Stadler WM, Dreicer R, Kohli M, Petrylak DP, Vaughn DJ, Bylow KA, Wong SG, Sottnik JL, Keller ET, al-Hawary M, Smith DC, Hussain M (2014) Double-blind, randomized, phase 2 trial of maintenance sunitinib versus placebo after response to chemotherapy in patients with advanced urothelial carcinoma. Cancer 120:692–701
24. Izawa JI, Slaton JW, Kedar D, Karashima T, Perrotte P, Czerniak B, Grossman HB, Dinney CP (2001) Differential expression of progression-related genes in the evolution of superficial to invasive transitional cell carcinoma of the bladder. Oncol Rep 8:9–15
25. Bochner BH, Cote RJ, Weidner N, Groshen S, Chen SC, Skinner DG, Nichols PW (1995) Angiogenesis in bladder cancer: relationship between microvessel density and tumor prognosis. J Natl Cancer Inst 87:1603–1612
26. Canoglu A, Gogus C, Beduk Y et al (2004) Microvessel density as a prognostic marker in bladder carcinoma: correlation with tumor grade, stage and prognosis. Int Urol Nephrol 36:401–405
27. Dickinson AJ, Fox SB, Persad RA et al (1994) Quantification of angiogenesis as an independent predictor of prognosis in invasive bladder carcinomas. Br J Urol 74:762–766
28. Donmez G, Sullu Y, Baris S, Yildiz L, Aydin O, Karagoz F, Kandemir B (2009) Vascular endothelial growth factor (VEGF), matrix metalloproteinase-9 (MMP-9), and thrombospondin-1 (TSP-1) expression in urothelial carcinomas. Pathol Res Pract 205:854–857
29. Goddard JC, Sutton CD, Furness PN, O'Byrne KJ, Kockelbergh RC (2003) Microvessel density at presentation predicts subsequent muscle invasion in superficial bladder cancer. Clin Cancer Res 9:2583–2586
30. Hurwitz HI, Fehrenbacher L, Hainsworth JD, Heim W, Berlin J, Holmgren E, Hambleton J, Novotny WF, Kabbinavar F (2005) Bevacizumab in combination with fluorouracil and leucovorin: an active regimen for first-line metastatic colorectal cancer. J Clin Oncol 23:3502–3508
31. Sandler A, Gray R, Perry MC, Brahmer J, Schiller JH, Dowlati A, Lilenbaum R, Johnson DH (2006) Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer. N Engl J Med 355:2542–2550
32. Miller K, Wang M, Gralow J, Dickler M, Cobleigh M, Perez EA, Shenkier T, Cella D, Davidson NE (2007) Paclitaxel plus bevacizumab versus paclitaxel alone for metastatic breast cancer. N Engl J Med 357:2666–2676
33. Goel S, Duda DG, Xu L, Munn LL, Boucher Y, Fukumura D, Jain RK (2011) Normalization of the vasculature for treatment of cancer and other diseases. Physiol Rev 91:1071–1121
34. Gemcitabine Hydrochloride and Cisplatin With or Without Bevacizumab in Treating Patients With Advanced Urinary Tract Cancer. Available at: https://clinicaltrials.gov/ct2/show/NCT00942331. [Last accessed August 25, 2018]
35. Gopalakrishnan D, Koshkin VS, Ornstein MC, Papatsoris A, Grivas P (2018) Immune checkpoint inhibitors in urothelial cancer: recent updates and future outlook. Ther Clin Risk Manag 14:1019–1040
36. Motzer RJ, Bander NH, Nanus DM (1996) Renal-cell carcinoma. N Engl J Med 335:865–875
37. Lamiell JM, Salazar FG, von Hsia YE (1989) Hippel-Lindau disease affecting 43 members of a single kindred. Medicine (Baltimore) 68:1–29
38. Latif F, Tory K, Gnarra J, Yao M, Duh F, Orcutt M, Stackhouse T, Kuzmin I, Modi W, Geil L et al (1993) Identification of the von Hippel-Lindau disease tumor suppressor gene. Science 260:1317–1320
39. Ohki S, Shibata M, Gonda K et al (2012) Circulating myeloid-derived suppressor cells are increased and correlate to immune suppression, inflammation and hypoproteinemia in patients with cancer. Oncol Rep 28:453–458
40. Kusmartsev S, Gabrilovich DI (2006) Role of immature myeloid cells in mechanisms of immune evasion in cancer. Cancer Immunol Immunother 55:237–245
41. George DJ, Kaelin WG Jr (2003) The von Hippel-Lindau protein, vascular endothelial growth factor, and kidney cancer. N Engl J Med 349:419–421
42. Rini BI, Campbell SC, Escudier B (2009) Renal cell carcinoma. Lancet 373:1119–1132
43. Ping SY, Wu CL, Yu DS (2010) Sunitinib can enhance BCG mediated cytotoxicity to transitional cell carcinoma through apoptosis pathway. Urol Oncol
44. Alexander M, Helfand CTL, Hafez K, Hussain M, Liebert M, Daignault S, Montgomery JS, Miller DC, Drnek L, Hollenbeck BK, Weizer AZ (2015) Phase II clinical trial of intravesical bacillus Calmette-Guerin (BCG) followed by sunitinib for the treatment of high-risk nonmuscle-invasive bladder cancer (NMIBC). J Clin Oncol 33(suppl 7; abstr 293):2015