Bladder Cancer

An Oncofetal Glycosaminoglycan Modification Provides Therapeutic Access to Cisplatin-resistant Bladder Cancer

Roland Seiler a,b,c,†, Htoo Zarni Oo a,b,†, Davide Tortora a,b, Thomas M. Clausen a,b,d,e, Chris K. Wang a,b,†, Gunjan Kumar a,b,f, Marina Ayres Pereira d,e, Maj S. Ørum-Madsen a,b, Mette Ø. Agerbæk a,b,d,e, Tobias Gustavsson d,e, Mie A. Nordmaj d,e, Jamie R. Rich g, Nada Lallous a,b, Ladan Fazli a,b, Sherry S. Lee b, James Douglas h, Tilman Todenhöfer a,b, Shaghayegh Esfrandia a,b, Dulgun Battsogt b, John S. Babcock g, Nader Al-Nakouzi a,b, Simon J. Crabb j, Igor Moskalov b, Bernhard Kiss c, Elai Davicioni j, George N. Thalmann c, Paul S. Rennie a,b, Peter C. Black a,b, Ali Salanti d,e, Mads Daugaard a,b,g,*

*Department of Urologic Sciences, University of British Columbia, Vancouver, BC, Canada; †Vancouver Prostate Centre, Vancouver, BC, Canada; ‡Department of Urology, University of Bern, Bern, Switzerland; §Department of Immunology and Microbiology, Centre for Medical Parasitology, University of Copenhagen, Copenhagen, Denmark; ¶Department of Infectious Diseases, Copenhagen University Hospital, Copenhagen, Denmark; ‰Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada; †Zymeworks Inc., Vancouver, BC, Canada; ‡Department of Urology, University Hospital of Southampton, Hampshire, UK; †Department of Medical Oncology, University Hospital of Southampton, Hampshire, UK; ‡GenomeDx Biosciences, Inc., Vancouver, BC, Canada

Abstract

**Background:** Although cisplatin-based neoadjuvant chemotherapy (NAC) improves survival of unselected patients with muscle-invasive bladder cancer (MIBC), only a minority responds to therapy and chemoresistance remains a major challenge in this disease setting.

**Objective:** To investigate the clinical significance of oncofetal chondroitin sulfate (ofCS) glycosaminoglycan chains in cisplatin-resistant MIBC and to evaluate these as targets for second-line therapy.

**Design, setting, and participants:** An ofCS-binding recombinant VAR2CSA protein derived from the malaria parasite *Plasmodium falciparum* (rVAR2) was used as an in situ, in vitro, and in vivo ofCS-targeting reagent in cisplatin-resistant MIBC. The ofCS expression landscape was analyzed in two independent cohorts of matched pre- and post-NAC-treated MIBC patients.

**Intervention:** An rVAR2 protein armed with cytotoxic hemiasterlin compounds (rVAR2 drug conjugate [VDC] 886) was evaluated as a novel therapeutic strategy in a xenograft model of cisplatin-resistant MIBC.

**Outcome measurements and statistical analysis:** Antineoplastic effects of targeting ofCS.

**Results and limitations:** In situ, ofCS was significantly overexpressed in residual tumors after NAC in two independent patient cohorts (p < 0.02). Global gene-expression profiling and biochemical analysis of primary tumors and cell lines revealed syndican-1 and chondroitin sulfate proteoglycan 4 as ofCS-modified proteoglycans in MIBC. In vitro, ofCS was expressed on all MIBC cell lines tested, and VDC886 eliminated these cells in the low-nanomolar IC50 concentration range. In vivo, VDC886 effectively retarded...
growth of chemoresistant orthotopic bladder cancer xenografts and prolonged survival ($p = 0.005$). The use of cisplatin only for the generation of chemoresistant xenografts are limitations of our animal model design. 

**Conclusions:** Targeting oFCS provides a promising second-line treatment strategy in cisplatin-resistant MIBC.

**Patient summary:** Cisplatin-resistant bladder cancer overexpresses particular sugar chains compared with chemotherapy-naïve bladder cancer. Using a recombinant protein from the malaria parasite *Plasmodium falciparum*, we can target these sugar chains, and our results showed a significant antitumor effect in cisplatin-resistant bladder cancer. This novel treatment paradigm provides therapeutic access to bladder cancers not responding to cisplatin.

© 2017 European Association of Urology. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. **Introduction**

Bladder cancer is the fifth most common cancer in the world and the most costly cancer to treat on a per-person basis due to required clinical surveillance and multiple therapeutic interventions [1]. Muscle-invasive bladder cancer (MIBC) is a highly aggressive flavor of the disease with a 5–yr survival probability of $\sim$50% [2]. Despite efforts in refining surgical techniques and optimizing systemic therapy, the prognosis has remained unchanged for more than 20 yrs [3]. Clinical improvement can be ascribed cisplatin-based neoadjuvant chemotherapy (NAC), which offers an overall survival benefit of 5–6% [4] and is currently standard of care in MIBC [5]. However, only $\sim$40% of patients demonstrate a major response to NAC and adverse treatment effects and delay in surgery, potentially harm 60%. Therefore, second-line treatment options for MIBC are currently in great demand [6]. Although 60% of patients with metastatic MIBC demonstrate an objective response to cisplatin-based chemotherapy, this response is rarely durable, and most of these patients succumb to disease [7]. Atezolizumab has recently become the first Food and Drug Administration-approved agent for second-line treatment of metastatic bladder cancer [8], but there remains a great need for additional agents in all states of MIBC.

Glycosaminoglycans (GAGs) are carbohydrate modifications attached to proteins in the cell membrane. Changes in expression and composition of GAGs have been reported in bladder cancer over the past 3 decades [9–11]. Chondroitin sulfate (CS) is a major cancer-associated GAG that also has a key role in malaria pathogenesis [12]. The malaria parasite *Plasmodium falciparum* has evolved a protein, called VAR2CSA that mediates attachment of infected erythrocytes to a distinct type of CS in the placental syncytium [13]. CS chains comprise repeated disaccharide units, made up of glucuronic acid and N-acetylgalactosamine (GalNAc) residues. CS chains are highly heterogeneous showing variations in both chain length and disaccharide modifications. Placental-type CS is mainly sulfated on carbon-4 of the GalNAc hexose, and this particular modification is required for exclusive sequestration of VAR2CSA-positive erythrocytes in the placenta [14]. Placenta and cancer share obvious phenotypic features such as highly proliferating cells, the ability to invade adjacent tissue, and promoting angiogenesis [15]. In the placenta, these features are partly facilitated by CS, and many tumors re-express placental-type CS as an oncofetal GAG [16]. Therefore, recombinant malarial VAR2CSA (rVAR2) proteins can be conveniently utilized to detect oncofetal chondroitin sulfate (oFCS) in human cancer [16,17]. Finally, rVAR2 conjugated with the hemiasterlin toxin analog KT886 derived from the marine sponge *Hemiasterella minor* (rVAR2 drug conjugate [VDC] 886), showed antitumor activity in non-Hodgkin’s lymphoma, prostate cancer, and metastatic breast cancer with no adverse treatment effects in non-pregnant mice [16].

We noticed that several cancer-associated proteins can be modified with an oFCS chain, including CD44 [16], syndican-1 (SDC1) [18], and chondroitin sulfate proteoglycan 4 (CSPG4) [19], and that CD44 and SDC1 have previously been described as candidate biomarkers in bladder cancer [20–25]. Accordingly, we hypothesized that human MIBC might display oFCS chains that could be exploited for rVAR2-based targeted therapy. In particular, we aimed to interrogate the oFCS expression landscape in cisplatin-resistant MIBC with the intention of developing an additional line treatment option for this disease state.

2. **Patients and methods**

2.1. **Bladder cancer patient cohorts**

Bladder tumor tissue was collected at three institutions (University of Bern, Switzerland; Vancouver General Hospital, Canada; and University Hospital of Southampton, UK) from patients receiving at least three cycles of cisplatin-based NAC prior to radical cystectomy with pelvic lymph node dissection (Supplementary Table 1). Tissue was harvested from prechemotherapy transurethral resection of bladder tumor (TURBT) specimens in all patients and from postchemotherapy radical cystectomy specimens in patients with residual MIBC (ypT). The Bern cohort was used as the discovery cohort, and the Vancouver and Southampton cohorts were amalgamated as the validation cohort.

A complete and detailed description of the patients and methods used in this study can be found in the Supplementary Information.

3. **Results**

To analyze the oFCS expression in bladder cancer pre- and post-treatment with cisplatin-based chemotherapy, we performed immunohistochemical (IHC) analysis of two
independent cohorts of matched primary chemotherapy-naïve and cisplatin-resistant bladder tumors using rVAR2 as the ofCS detection reagent (Fig. 1A and B, and Supplementary Fig. 1A–E). The ofCS expression was absent in urothelium of the adjacent normal bladder (Fig. 1A, left) and was highly restricted to the bladder tumors (Fig. 1A, right). To differentiate ofCS expression between tumor environment and cancer cell compartments, we performed IHC analysis of an epithelial marker, E-cadherin, and a mesenchymal marker, vimentin (Fig. 1A). The pre-chemotherapy bladder tumors were sampled by transurethral resection (TURBT) and the matched cisplatin-resistant tumors after NAC were sampled from subsequent radical cystectomy specimens (Fig. 1B). The discovery cohort comprised more advanced tumors from a single institution as compared with the validation cohort combined from two institutions (Supplementary Table 1). Overall, high ofCS expression was seen in approximately 92% (n = 110/120) of chemotherapy-naïve bladder tumors in both cohorts. Among these, 17% (n = 8/47) of the tumors in the discovery cohort (Fig. 1C, left) and 33% (n = 24/73) of the validation cohort (Fig. 1C, right) showed high membranous expression of ofCS (ofCSHigh) in the cancer cell compartment. In chemotherapy-naïve tumors, ofCS expression failed to predict response to chemotherapy (p = 0.4, odds ratio = 2.28, 95% confidence interval (CI): 0.35–23.87) and inform on survival in univariable and multivariable analyses. In cisplatin-resistant disease (ypT), ofCS expression increased; 57% (n = 16/28, p = 0.001; vs 29%, n = 8/28 in TURBT) of tumors had ofCSHigh cancer cells in the discovery cohort (Fig. 1D, left) and 70% (n = 23/33, p = 0.01; vs 33%, n = 11/33 in TURBT) in the validation cohort (Fig. 1D, right). In cisplatin-resistant MIBC, ofCSHigh was associated with extravesical extension in the discovery cohort (p = 0.005) (Fig. 1E, left, and Fig. 1F) but this was not significant in the validation cohort (p = 0.081; Fig. 1E, right). The shift in ofCS expression was associated with poor overall survival in the discovery cohort (p = 0.045; Fig. 1G, upper part) but not in the validation cohort (p = 0.5), which had a shorter clinical follow-up of 3.3 yr (95% CI: 2.52–3.3; Fig. 1G, lower part). In multivariable analysis, only pathological tumor stage added independent prognostic information (Supplementary Table 2). Aggregated, these data indicate that ofCS is upregulated in cisplatin-resistant bladder cancer, and this event may be associated with a poor outcome.

Several proteoglycans can carry ofCS GAG modifications [16]. To search for ofCS-modified proteoglycans in bladder cancer, we analyzed gene expression data of known CS-modified proteoglycans [16,28] in two independent bladder cancer cohorts. In both datasets, luminal-type tumors were enriched for SDCC1, SDCC4, and APPL2, while more basal tumors were enriched for a number of genes including CD44 and CSPG4 (Fig. 2A, and Supplementary Fig. 2A–E). SDCC1, CSPG4, and CD44 have been widely associated with multiple types of cancer [16,18,19], and CD44 and SDCC1 have previously been suggested as candidate biomarkers in bladder cancer [20–25]. Indeed, protein expression of CD44 (Fig. 2B), SDCC1 (Fig. 2C), and CSPG4 (Fig. 2D) showed a strong correlation with mRNA expression (Fig. 2E–G) that correlated with ofCSHigh cases (Fig. 2H–J). Together, these data suggest that CD44, SDCC1, and CSPG4 are expressed in human bladder cancer and associated with ofCS modifications.

We next analyzed a panel of seven bladder cancer cell lines derived from MIBC patients for ofCS expression by flow cytometry using rVAR2 as the ofCS detection reagent. All MIBC cell lines analyzed expressed ofCS as detected by rVAR2 in a concentration- and CS-dependent manner (Fig. 3A). This was supported by sensitivity to the ofCS-targeting VDC886 drug conjugate [16] in the low-nanomolar IC50 concentration range (Fig. 3B). Interestingly, four out of seven cell lines expressed CSPG4, seven out of seven expressed splice variants of CD44, and five out of seven expressed SDCC1 (Fig. 3C), but this expression pattern was not obviously related to VDC886 sensitivity. This might indicate that not all of the selected proteoglycans (CD44, SDCC1, and CSPG4) are modified with ofCS in the individual cell lines and/or that additional proteoglycans contribute to the ofCS presentation. To investigate this further, we analyzed whether or not CD44, SDCC1, and CSPG4 were modified with ofCS in UM-UC13 cells. The ofCS-modified proteins were purified from cell lysates using immobilized rVAR2. The resulting fractions were treated with chondroitinase ABC to reduce the CSPGs down to their core protein component. In this analysis, a shift in gel mobility toward the predicted protein size on SDS-PAGE upon chondroitinase ABC treatment would imply CS substitution. Surprisingly, while CD44, SDCC1, and CSPG4 were expressed in UM-UC13 cells, only SDCC1 and CSPG4 produced a shift in molecular weight after chondroitin treatment (Fig. 3D). Chondroitinase ABC treatment did not reduce the size of SDCC1 to its predicted core protein size, but merely produced an intermediary sized protein. This is in line with the heparan sulfate substitution status of SDCC1, a GAG that will not be digested with chondroitinase ABC. These data indicate that SDCC1 and CSPG4, but not CD44, are ofCS modified in UM-UC13 cells. To validate this result, we treated UM-UC13 cells with validated siRNAs targeting CD44, SDCC1, and CSPG4, and assayed for binding to rVAR2 as an indication of ofCS expression. While the CD44 siRNA had no effect on rVAR2 binding, SDCC1 and CSPG4 siRNAs reduced rVAR2 binding to UM-UC13 cells by 50% and 20%, respectively (Fig. 3E). This corroborates the biochemical analysis showing that SDCC1 and CSPG4, but not CD44, are ofCS modified in UM-UC13 cells, reflecting that ofCS modification of eligible proteins is not mandatory and likely a dynamic event.

To assess the expression of ofCS-modified proteoglycans in cells treated with cisplatin in vivo we inoculated UM-UC13 cells into the bladder wall of nude mice with ultrasound guidance (Supplementary Fig. 3A and B) [26,27]. As the UM-UC13 tumors developed, the mice were subjected to cisplatin treatment and resistant tumors were allowed to recover. To analyze the ofCS modification status of proteoglycans in UM-UC13 tumors and to potentially identify novel proteoglycans, we purified ofCS-associated proteoglycans from ex vivo cells and analyzed these by mass spectrometry. This analysis identified eight ofCS-modified
Fig. 1 – ofCS expression in chemotherapy-naïve (TURBT) and cisplatin-resistant (ypT) bladder cancer. (A) Representative H&E and IHC images in matched adjacent normal bladder (left panel) and bladder cancer cases (right panel) with an epithelial marker, E-cadherin, and a mesenchymal marker, Vimentin, expression in parallel with ofCS expression. Scale bar represents 200 µm. (B) Representative IHC images of ofCS expression in matched bladder cancer cases showing cellular ofCS expression in paired TURBT (left panel) and cisplatin-resistant tumors at cystectomy (ypT) (right panel). Scale bar represents 50 µm. (C) ofCS expression was examined in chemotherapy-naïve bladder cancers (TURBT) in two independent cohorts (discovery: left; validation: right). “Tumor” represents ofCS expression in overall bladder tumor including the surrounding microenvironment, and “cancer cells” represent membranous ofCS expression only in cancer cells. (D) Plots indicating paired analysis of ofCS expression in cancer cells in chemotherapy-naïve (TURBT) and cisplatin-resistant (ypT) tumors in discovery (left) and validation (right) cohorts. Each box indicates the tumor of a given patient, and the lines indicate the pairs between TURBT to ypT. (E) Barplots indicating the relation of cellular ofCS expression in ypT compared with tumor stage in discovery (left) and validation (right) cohorts. (F) Representative IHC images of ofCS expression according to ypT stages of MIBC in eight different patients from a validation cohort. Scale bar represents 50 µm. (G) Kaplan–Meier plots for overall survival stratified according to high and low cellular ofCS expression in ypT (discovery: upper part; validation: lower part). H&E = hematoxylin and eosin; IHC = immunohistochemical; MIBC = muscle-invasive bladder cancer; ofCS = oncofetal chondroitin sulfate; TURBT = transurethral resection of bladder tumor.
Fig. 2 – ofCS-carrying proteoglycans in bladder cancer. (A) Heatmap of gene expression of proteoglycans that have been shown to be ofCS modified. The TURBT samples from both cohorts have been selected. Virtually all samples show high expression of at least one validated proteoglycan. (B) Representative IHC images showing high and low CD44 protein expression of bladder cancer. For statistical analysis in the subsequent panel (E), low expressing tumors (first–third quartile) were compared with high expression tumors (fourth quartile). Scale bar represents 100 μm. (C) Representative IHC images showing high and low SDC1 protein expression of bladder cancer. For statistical analysis in the subsequent panel (F), low expressing tumors (first–third quartile) were compared with high expression tumors (fourth quartile), respectively. Scale bar represents 100 μm. (D) Representative IHC images showing high and low CSPG4 protein expression of bladder cancer. For statistical analysis in the subsequent panel (G), low expressing tumors (first–third quartile) were compared to high expression tumors (fourth quartile), respectively. Scale bar represents 100 μm. (E) Boxplot indicates the CD44 mRNA expression (y-axis) of samples with low and high CD44 protein expression. (F) Boxplot indicates the SDC1 mRNA expression (y-axis) of samples with low and high SDC1 protein expression. (G) Boxplot indicates the CSPG4 mRNA expression (y-axis) of samples with low and high CSPG4 protein expression. (H) Boxplot indicating CD44 protein expression levels in ofCSHigh and ofCSLow bladder tumors. (I) Boxplot indicating SDC1 protein expression levels in ofCSHigh and ofCSLow bladder tumors. (J) Barplot indicating CSPG4 protein expression levels in ofCSHigh and ofCSLow bladder tumors. IHC = immunohistochemical; ofCS = oncofetal chondroitin sulfate; ofCSHigh = high membranous expression of ofCS; ofCSLow = low membranous expression of ofCS; TURBT = transurethral resection of bladder tumor.
proteoglycans including SDC1 and CSPG4 (Supplementary Table 3). Similar to the in vitro cell line study, this analysis showed that CD44 was not modified by ofCS in UM-UC13 cells, and that ofCS modification of SDC1 and CSPG4 was retained in vivo. Proximity-ligation assay analysis of ofCS chains and CD44, SDC1, and CSPG4 confirmed the mass spectrometry result (Supplementary Fig. 3C), but surprisingly, CD44 was also in proximity to ofCS. This suggests that while CD44 is not itself modified with ofCS in UM-UC13 cells, it is indeed expressed in close proximity to ofCS-modified proteoglycans.

Capitalizing on our findings that human cisplatin-resistant MIBC upregulates ofCS (Fig. 1A–F) and that MIBC cells are sensitive to VDC886 in vitro (Fig. 3B), we next tested whether VDC886 had efficacy against cisplatin-resistant MIBC in vivo. Again, UM-UC13 cells were inoculated into the bladder wall of nude mice with ultrasound guidance (Supplementary Fig. 3A and B) and tumor growth was subsequently monitored using ultrasound over a 40–60 days period (Supplementary Fig. 4A). As the UM-UC13 tumors developed, the mice were subjected to repeating cycles of cisplatin treatment and the resultant tumors were passaged through six generations of mice (G1–G6) creating highly stable cisplatin-resistant xenografts (Fig. 4A). Importantly, the ex vivo tumor cells retained their rVAR2 binding in a concentration- and CS-dependent manner (Fig. 4B). They also retained the same rVAR2 internalization capacity as the parental line, G0 (Fig. 4C). Notably, the completely cisplatin-resistant G6 and cisplatin-sensitive G0 cells showed equal sensitivity to VDC886 ex vivo (Fig. 4D and Supplementary Fig. 4B).

Next, mice with established G6-initiated cisplatin-resistant tumors were randomized into four groups and treated bi-weekly (four treatments in total) with vehicle, rVAR2, KT886, or VDC886 (Fig. 4E) in combination with cisplatin. The G6 cisplatin-resistant tumors expressed SDC1,
CSPG4, and ofCS (Supplementary Fig. 4C). Remarkably, VDC886 treatment strongly retarded growth of cisplatin-resistant tumors (Fig. 4F and G) and significantly prolonged survival of the mice (Fig. 4H). Importantly, a clinicopathological examination of VDC886-treated mice demonstrated no organ toxicity (Supplementary Fig. 4D) and the weight of the mice was stable in all groups (Supplementary Fig. 4E). In the VDC886-treated group, one mouse had complete response, one mouse presented with significant tumor regression (Supplementary Fig. 4F), and four mice had stable tumor growth inhibition without progression during the experiment, with viable tumor cells in histology. In summary, our work demonstrates that ofCS-modified proteoglycans can provide therapeutic access to cisplatin-resistant MIBC.

4. Discussion

We have tested an unconventional approach for treating cisplatin-resistant MIBC based on the evolutionarily refined parasite–host anchor protein VAR2CSA derived from the malaria parasite *P. falciparum*. We found that ofCS was
present at high levels in cisplatin-resistant MIBC, promoting ofCS as a potential access point for targeted therapy. Supporting this notion, VDC886 was able to suppress the growth of cisplatin-resistant MIBC. High ofCS expression was associated with advanced tumor stage, cisplatin resistance, and poor overall survival of MIBC patients. These associations were more robust in the discovery cohort, which contained more advanced disease and a longer follow-up. In the validation cohort, presentation of ofCS was not prognostic. While this suggests that high ofCS may only relate to survival in more advanced MIBC, this association requires further investigation.

Several different proteoglycans can be modified with ofCS chains [16]. As a result, intratumoral heterogeneity in proteoglycan expression does not necessarily translate into varying levels of ofCS modifications. CD44 has previously been shown to carry ofCS chains in melanoma [16,29,30]. We found that CD44 mRNA and protein expression correlated with high ofCS presentation in MIBC, but biochemical interrogation showed that CD44 was not modified with ofCS, at least not in UM-UC13 cells. Contrary to CD44, SDC1 and CSPG4 were modified in UM-UC13 cells and correlated with ofCS expression in primary tumors. Mass spectrometry of rVAR2-purified proteoglycans identified another six ofCS-modified candidates. It is likely that additional proteoglycans contribute to ofCS presentation in MIBC.

The most important finding of our study was the increased cellular ofCS expression in cisplatin-resistant MIBC when compared with paired chemotherapy-naïve MIBC. These findings suggest that targeting ofCS with VDC886 provides an attractive approach, particularly in cisplatin-resistant MIBC. The first-generation VDC compound VDC886 comprises the DBL1X-Id2a domains of the malarial VAR2CSA protein loaded with an average of three KT886 hemiasterlin toxin analogs derived from the marine sponge H. minor [16]. VDC886 eliminated all MIBC cell lines in the low-nanomolar IC50 concentration range. As all the MIBC cell lines investigated express high levels of ofCS, the small differences in IC50 are most likely due to differences in internalization kinetics. In an animal model of cisplatin-resistant MIBC, VDC886 was able to efficiently target established cisplatin-resistant tumors and rescue the mice from tumor-associated morbidity and death. The completely cisplatin-resistant G6 and cisplatin-sensitive G0 cells isolated from mice showed equal sensitivity to VDC886 ex vivo. This is likely due to high baseline levels of ofCS in MIBC cell lines and suggests that the ofCS modification is a passenger, rather than a driver, of cisplatin resistance. Nevertheless, ofCS GAG modifications constitute a therapeutic access point in cisplatin-resistant MIBC.

5. Conclusions

We have found an oncofetal GAG antigen in cisplatin-resistant MIBC that can be targeted by a malarial host–cell anchor protein armed with hemiasterlin compounds. This discovery potentially offers a new treatment paradigm for human MIBC patients not responding to cisplatin.

Author contributions: Mads Dauggaard had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Dauggaard, Black.


Drafting of the manuscript: Seiler, Oo, Black, Salanti, Dauggaard.

Critical revision of the manuscript for important intellectual content: Tortora, Ørum-Madsen, Agerbæk, Clausen, Gustavsson.

Statistical analysis: Seiler.

Obtaining funding: Seiler, Dauggaard, Salanti.

Administrative, technical, or material support: Seiler, Oo, Clausen, Agerbæk, Rich, Fazli, Douglas, Todenhöfer, Battsgott, Al-Nakouzi, Babcook, Crabb, Moskalev, Kiss, Davicioni, Thalmann, Rennie, Black, Salanti, Dauggaard.

Supervision: Dauggaard, Black, Salanti.

Other: None.

Financial disclosures: Mads Dauggaard certifies that all conflicts of interest, including specific financial interests and relationships and affiliations relevant to the subject matter or materials discussed in the manuscript (e.g. employment/affiliation, grants or funding, consultancies, honoraria, stock ownership or options, expert testimony, royalties, or patents filed, received, or pending), are the following: M. Dauggaard, A. Salanti, M.B. Agerbæk, and T.M. Clausen are shareholders in VAR2 Pharmaceuticals Aps. VAR2 Pharmaceuticals is a biotechnology company that specializes in clinical development of VAR2CSA technology. J.S. Babcook and J.R. Rich are employees of Zymeworks Inc. Zymeworks is a biotechnology company that specializes in protein-based cancer therapeutics.

Funding/Sponsor and role of the sponsor: This work was awarded by Prostate Cancer Canada and is proudly funded by a VCH Research Institute Innovation and Translational Research Award funded by the VGH & UBC Hospital Foundation; the Vancouver Prostate Centre, the Danish Cancer Society; the European Research Councils (ERC) through the MalOnco program, the Danish Innovation Foundation, the Svend Andersen Foundation, the Danish Research Councils, Gangsted Fonden and Lundbeck foundation, Kirsten and Freddy Johansen Foundation, and the Swiss National Foundation.

Acknowledgments: The authors would like to acknowledge Alireza Moeen, Estelle Li, and Janet Liew for their technical assistance in pathology work; Matthew Sommerlad for providing patient materials; and Mafalda Resende for assisting with protein production.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.eururo.2017.03.021.

References


