Original Article

Identification of somatic mutations and their effects in a Thai population with both non-muscle invasive and muscle invasive bladder cancer using whole exome sequencing analysis

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Abstract

Objective: Whole exome sequencing is a new technology which enables the detection of genetic mutation in cancer. Genetic alterations in urothelial carcinoma have been identified and studies are being carried out with regard to clinical applications. Proposals have been made pertinent to molecular classifications for the prediction of treatment response and prognosis. To date, there is a paucity of data regarding somatic mutation of bladder cancer in Thailand, therefore, the aim of this study is to identify specific somatic mutations associated with different types of bladder cancer in Thailand.

Materials and Methods: Fourteen patients were enrolled onto this study, 7 with non-muscle invasive (NMIBC) and 7 with muscle invasive bladder cancer (MIBC). DNA was isolated from peripheral blood mononuclear cells and tumor tissue for whole exome sequencing to identify any tumor somatic mutations and the mutation burden in each patient. The results were analyzed and correlated with the clinical status of the patients after treatment.

Results: In the NMIBC group, the most common mutated genes were found to be HLA-F, KDM6A, and TTN. In the MIBC group, the most common mutated genes were TP53, TTN, and KMT2D. Patients with urothelial carcinoma with small cell variant show TP53 and RB1 mutation. This is the same as the current consensus on molecular classification. The disease has usually metastasized after 1 year. This supports the evidence that Neuroendocrine-like groups have poorer prognosis.

Conclusion: The somatic mutations of bladder cancer in this Thai population showed greater diversity of genetic alteration in comparison with the worldwide database. The mutations in the muscle invasive bladder cancer were the same as previous findings. We also found a similar association in neuroendocrine-like genomic mutations. Despite the number of patients in this study being small, there is evidence of genetic diversity and tumor origins of mutation in our patients.

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Introduction

Bladder cancer, the tenth most-common malignancy worldwide, is a major cause of morbidity and mortality. Currently, approximately 573,000 cases of bladder cancer have been diagnosed globally, and 212,000 fatalities recorded.[1] Due to the differences in prognosis and management, urothelial carcinoma (UC), the most common type of bladder cancer, is divided into non-muscle-invasive bladder cancer (NMIBC) and muscle-invasive bladder cancer (MIBC).[2,3]

The rapid advancement of next-generation sequencing (NGS) technology has facilitated the investigation of molecular characterization of bladder cancer. In 2014, a group of researchers in The Cancer Genome Atlas (TCGA) project published an integrated genomic analysis of 131 MIBC samples, finding statistically significant recurrent mutations in 32 genes, including several chromatin regulators.[4] In a more recent study of 412 MIBC patients the expression of five subtypes was demonstrated which may help in treatment response stratification.[5] In 2020, a consensus molecular classification had been proposed for MIBC, the sub-types being divided into Luminal Papillary, Luminal non specified, Luminal unstable, stroma-rich, Basal/Squamous and Neuroendocrine-like.[6] This study also provided insight into the specific mutation and clinical characteristics of each subtype. The molecular characteristics of NMIBC were also studied. In 2016, Hedegaard et al.[7] published the UROMOL 2016 subtyping system that classified NMIBC into three categories (Class 1-3). Although the global trend of precision medicine has expanded by the use of modern genomic sequencing technologies, in Thailand the application of genomic knowledge for medical purposes is still relatively rare. Furthermore, there is no dataset pertinent to the association between somatic mutation and bladder cancer in a Thai population.

This is a descriptive pilot study aiming to identify the somatic mutated genes in UC of the bladder, including both NMIBC and MIBC in a Thai population. The focus was on comparing the common genetic alterations of UC of the bladder between Thai patients and large public databases to identify differences that may impact our treatment and clinical practice with regard to bladder cancer patients. We believe that our study will be the first small step toward long-term development in genomic and molecular research in our country.

Materials and Methods

Patient selection

All patients newly diagnosed with a bladder tumor that was suspected bladder cancer who underwent transurethral resection of bladder tumor (TUR-BT) in the King Chulalongkorn Memorial Hospital (KCMH) between August 2019 to March 2020 were enrolled onto this study. Patients were excluded if they had any other histological type besides UC, or if tissue specimens were limited or of too low quality for DNA extraction.

Specimen Collection

Blood was collected to isolate peripheral blood mononuclear cells (PBMCs) from the participants before the operation. Germline DNA samples were isolated from PBMCs. All tumor tissues were gathered immediately after transurethral resection to maintain the viability of tissues and to lessen DNA breakdown. Tumor tissue sampling was performed randomly from multiple areas of the tumor to ensure samples were diverse and then rapidly preserved in liquid nitrogen for transport to the laboratory. Some specimens were preserved in formalin and sent to uropathologists for subtype classification.

DNA isolation

DNA was isolated from peripheral blood mononuclear cells (germline DNA) and tumor tissue (somatic DNA) using the genUp dDNA kit (Promega, Madison, WI, USA), in accordance with the manufacturer's instructions. The quantity of extracted genomic DNA was assessed by a fluorimetric method with a Qubit device.

Whole exome sequencing of PBMC and tumor tissues

Libraries from the PBMC and the fresh tissue samples from all patients were prepared starting from 200 ng of extracted DNA by using the SureSelect All Exon v6 (Agilent Technologies, Santa Clara, USA) in accordance with the protocol described by the manufacturer. All libraries were sequenced on the MGC sequencer and 2x150 bp paired end reads were generated.

Data analysis

The short reads were aligned with the human reference genome (GRCh38) by using Burrows Wheeler Aligner (BWA). After the alignment, PCR duplicates were removed using Picard MarkDuplicates. The alignments were then recalibrated and filtered using the Genome Analysis Toolkit (GATK). Varscan was then applied to identify somatic mutations by comparing tumor against normal tissues. The oncoprint diagram was created using the Maftools software.

Results

Patient demographics, clinical and pathological characteristics of patients

During the study period, 14 patients underwent TUR-BT at our institution and were included in this study. Baseline characteristics and pathologic features of patients with NMIBC and MIBC are summarized in table 1 and 2, respectively. The mean age of the population was 72.5 years, with a male-to-female ratio of 1.3:1. The variant histology of UC may result in a negative impact on patient outcome.8 In the NMIBC group all patients had high grade UC and two patients showed mixed histologic features between UC and variant histology including micropapillary features and squamous differentiation. In the MIBC group all the patients had high grade UC, with two patients having small cell differentiation and glandular/nested variant. Among the 14 patients, there was only one patient with the carcinoma in situ (CIS) and two with lymphovascular invasion (LVI).

Summary of somatic mutations

Figure 1 shows the distribution of the mutations in our study population. The six most common genes harboring somatic mutations were TTN, NRXN1, MUC19, HYDIN, CTNNA2 and TP53. The CA-B3 sample shows the highest mutation burden followed by CA-B21 and CA-B15 respectively. In the NMIBC group the most common mutated genes are TTN, HYDIN, DMD, and ADGRV1 (Fig. 2). In comparison, in the MIBC group the most common genes are TP53, KMT2D, TTN and MUC4 (Fig. 3). Missense mutation and single nucleotide polymorphism (SNP) were the main variant class and type respectively in both NMIBC and MIBC groups. The dominant single nucleotide variation (SNV) classes were C>T in both NMIBC and MIBC.

From the TCGA database 5, from this Thai population we identified TP53 and KMT2D as among the top mutations, findings the same as published in the TCGA database. However, in the NMIBC group the top 10 mutated genes did not match the top 10 mutated genes listed in the UROMOL study.

Consideration of variant histology

Many studies have shown that the variant histology may affect the outcome of the patient.[8,9] These studies have been working to identify the genomic mutation of each subtype. It has been shown that the small cell/neuroendocrine variant is strongly associated with the TP53 and RB1 mutation.[10] One of the MIBC samples had UC with small cell variant. This sample harbored both the TP53 and RB1 mutations, the same as in the neuroendocrine-like group in the consensus molecular classification of MIBC.[6]

Nested variant histology is rare, one study has shown that this variant is associated with the TP53 and JAK3 mutations which were not found in our specimens.11 Another study shows that the most common genomic alterations in UC with squamous cell variants are TP53 (67.7%), KMT2D (48.4%) and ARIDIA (32.3%).[12] The patients with the squamous cell variant in our study did not harbor any of the aforementioned genetic mutations.

Summary of tumor mutation burden (TMB)

TMB is the total number of mutations per megabase detected in the DNA of cancer cells. The higher TMB may correlate with the response of immune checkpoint inhibitors in solid organ tumors, especially in bladder cancer.[13,14] In our study CA B3 had the highest TMB which may correlate with the high stage of the tumor in that patient.

Discussion

Genomic sequencing is now used to identify the mutations of the molecular signature in cancer. Studies are looking for potential actionable genomic alterations which could lead to clinical implications, especially with regard to new treatment. In bladder cancer, NMIBC and MIBC are two almost distinct clinicopathological conditions due to their behavior as well as their

Table 1. Characteristics of patients with non-muscle invasive bladder cancer

Sample	Age (yrs)	Sex	Presence of Histologic Subtype	Tumor Grading	TNM Stage	Presence of CIS	Presence of LVI	Multi-focality	Size (> 3 cm)	Smoking History	BCG	Ref.
CA-B4	70	M	Focal micropapillary features	High grade	pTaN0M0	No	No	Solitary	No	Yes	No	No
CA-B8	75	F	No	High grade	cTaN0M0	Yes	No	Multifocal	No	No	No	Yes
CA-B9	82	M	No	High grade	cTaN0M0	No	No	Multifocal	No	Yes	Yes	Yes
CA-B10	74	M	No	High grade	cTaN0M0	No	No	Multifocal	No	No	No	Yes
CA-B19	70	F	No	High grade	cTaN0M0	No	No	Multifocal	Yes	No	Yes	No
CA-B20	88	F	No	High grade	cT1N0M0	No	No	Multifocal	No	No	No	Yes
CA-B21	67	F	Focal squamous differentiation	High grade	cT1N0M0	No	No	Solitary	Yes	No	Yes	No

CIS = carcinoma in situ, LVI = lymphovascular invasion, BCG = Bacillus Calmette-Guérin, F = female, M = male, Ref. = references, yrs = years

Table 2. Characteristics of patients with muscle invasive bladder cancer

Sample	Age (yrs)	Sex	Presence of Histologic Subtype	Tumor Grading	TNM Stage	Presence of CIS	Presence of LVI	Multi-focality	Size (> 3 cm)	Smoking History	Neoadjuvant Chemo-therapy	Adjuvant Chemo-therapy
CA-B2	65	M	Small cell differentiation	High grade	ypT2aN0M0	No	No	Solitary	Yes	No	Yes	No
CA-B3	72	F	No	High grade	ypT3bpN1 M1a	No	Yes	Solitary	Yes	No	Yes	Yes
CA-B5	70	F	No	High grade	pT3a pN1cM0	No	Yes	Multifocal	Yes	No	No	No
CA-B14	80	M	No	High grade	cT2N0M1	No	No	Solitary	No	No	No	No
CA-B15	69	M	No	High grade	cT2N0M0, (pT1N0M0)	No	No	Multifocal	Yes	No	No	No
CA-B17	70	M	No	High grade	pT3aN0M0	No	No	Solitary	Yes	Yes	No	No
CA-B18	62	M	Glandular variant & Nested variant	High grade	ypT4aN0M0	No	No	Solitary	Yes	Yes	Yes	Yes

CIS = carcinoma in situ, LVI = lymphovascular invasion, BCG = Bacillus Calmette-Guérin, F = female, M = male, yrs = years

somatic mutations. By identifying the mutation, clinicians may be able to evaluate the risk of disease progression, response to chemotherapy, or even tailor treatment for each specific mutation.

In this study, we explored the common somatic mutations of NMIBC and MIBC in a Thai population. In the NMIBC group TTN, HYDIN, DMD, and ADGRV1 are the most common mutations in our study. However, according to the UROMOL study FGFR3, KIAA1109 and SYNE2 are the genes which most frequently mutate.[7] In the MIBC group in our study the most common mutations were in the TP53, KMT2D, TTN and MUC4 genes. In the TCGA database the genes which show the most frequent mutations are TP53, KMT2D and KDM6A.[5] Despite the study population in our study being small, the results still show that the somatic mutations may prove to be the same as in the global population.

Being able to identify somatic mutations in specimens by performing TUR-BT may benefit the prediction of the response to neoadjuvant

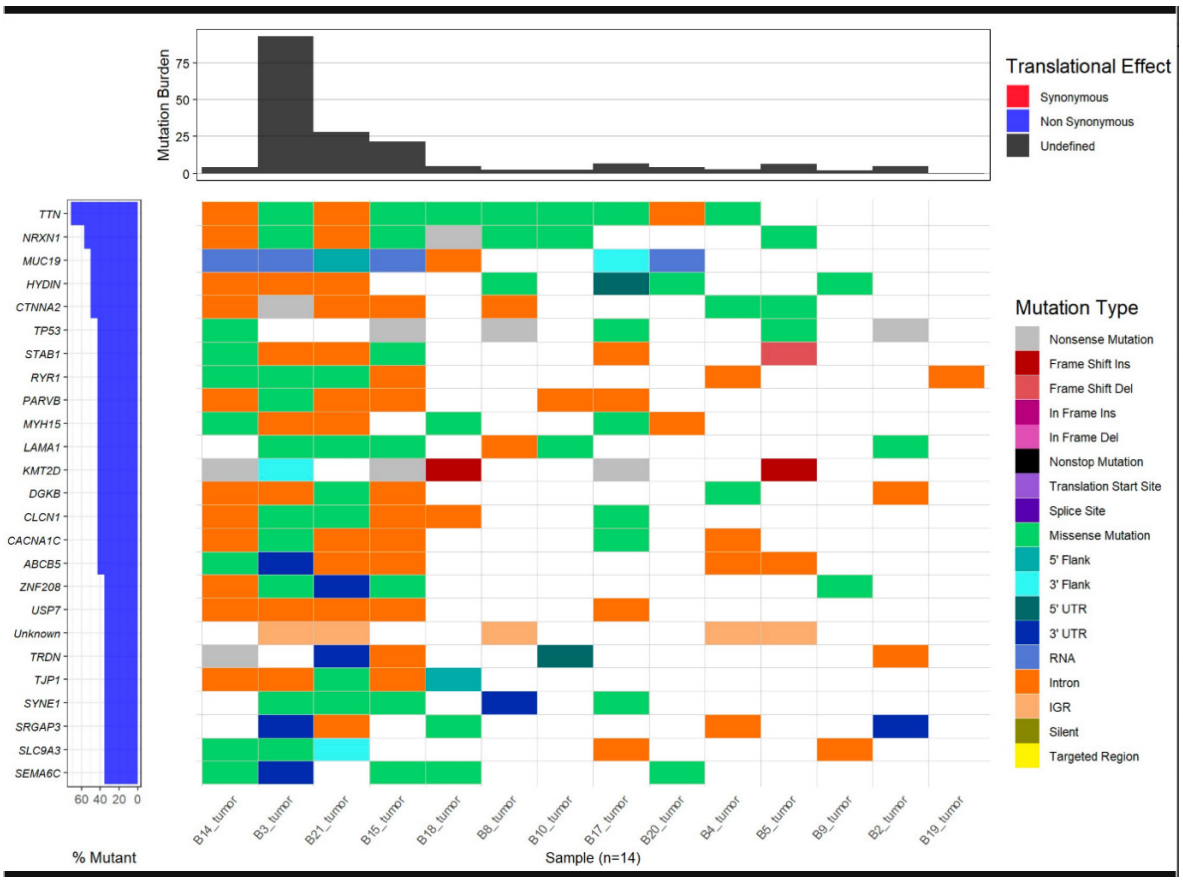

Figure 1. Somatic mutations of all study participants

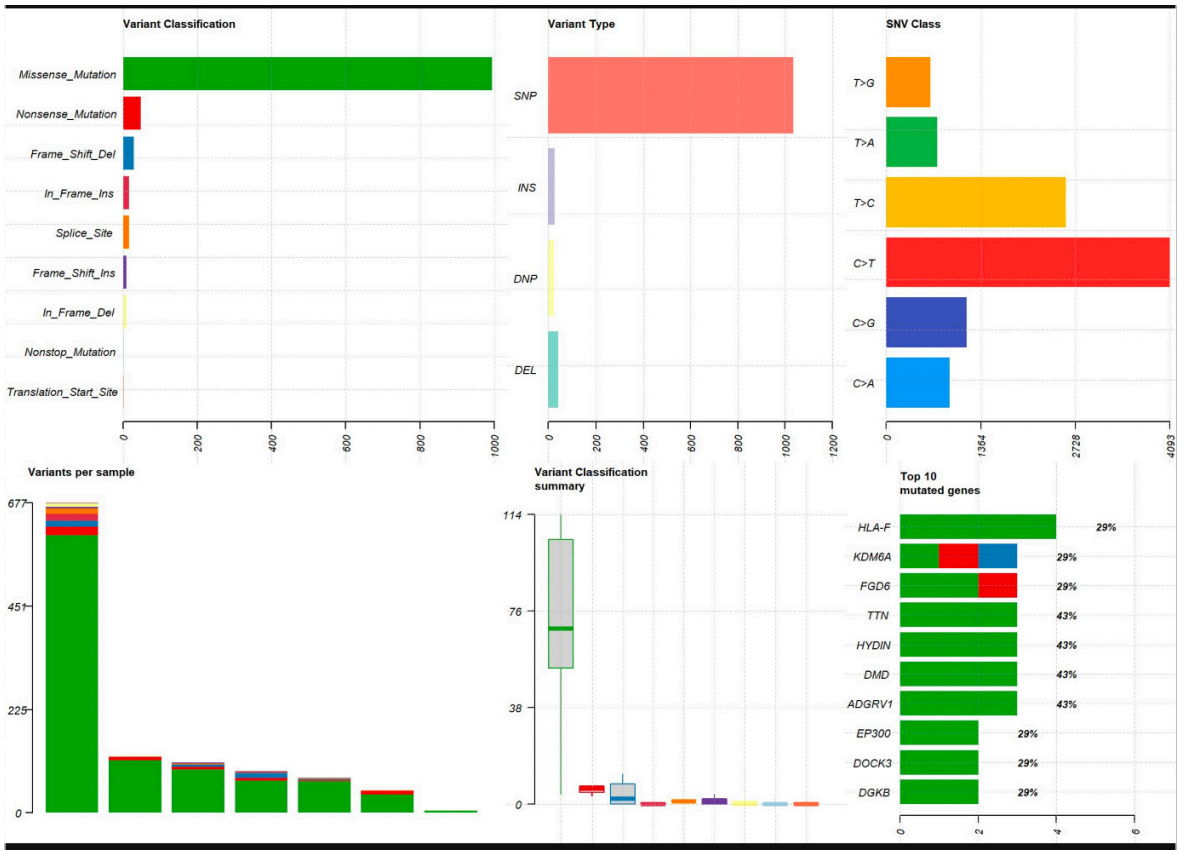

Figure 2. Somatic mutations of all study participants

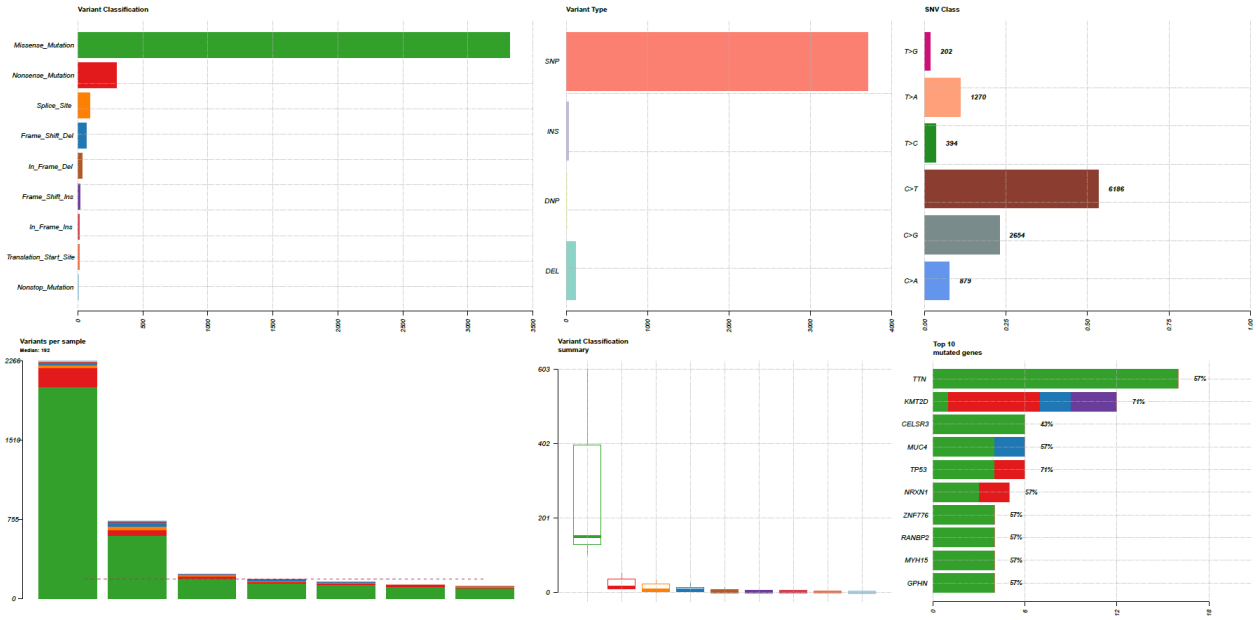

Figure 3. Bar chart of somatic mutations in the MIBC group

Cisplatin-based chemotherapy. One study has shown that luminal tumors have a lower rate of pathological up staging to non-organ-confined-disease in comparison to non-luminal tumors.[15] The TMB has now also been found to be associated with immune checkpoint inhibitor response.[16] Fibroblast growth factor receptors (FGFRs) have been a potential target for the development of cancer treatment. The FGFR3 mutation is known to facilitate the development of cancer by promoting cell proliferation, survival, migration, invasion, and angiogenesis in bladder cancer.[17] FGFR inhibitors have been recognized as a promising targeted therapy of bladder cancer with FGFR3 mutation. Studies are ongoing to verify the clinical benefits for bladder cancer patients with an FGFR mutation.[18] Erdafitinib, a tyrosine kinase inhibitor of FGFR1-4, is currently the only available treatment for locally advanced or metastatic UC with an FGFR3 mutation. It showed a 40.0% response rate and 13.8 months of median overall survival.[19] These findings highlight the potential treatment which may be developed to target a specific genetic mutation for other genes to inform the selection of candidates for chemotherapy, immunotherapy or up-front radical surgery.

Special consideration for neuroendocrine-like group

We also investigated the incidence of mutations in UC with variant histology. We found that in the small cell variant in this study there was a consistent occurrence of TP 53 and RB1 mutation as in other study. This can be classified as a neuroendocrine-like variant.[10] The patient with small cell variant in this study received neoadjuvant chemotherapy followed by radical cystectomy. Within the 1 year follow up period the patient lung metastasis developed. One study has demonstrated that small cell carcinoma of the bladder with an ERCC2 mutation can have a complete pathologic response after neoadjuvant chemotherapy of 50.0%.[10] Our patient did not harbor the ERCC2 mutation and did not respond to neoadjuvant chemotherapy. Pembrolizumab, a PD-1 inhibitor, was initiated and the disease was stable for 1.5 years. Immunotherapy is an established option for the treatment of advance UC as a second line treatment.[20, 21] but is now emerging as a first line treatment in stage IV bladder cancer.[22,23] There are few studies into histologic variants and the response of immunotherapy, but some case reports show a good response.[24] A study combining chemotherapy with immunotherapy compared to chemotherapy alone has also shown improved median overall survival in locally advanced or metastatic small cell genitourinary cancer.[25] Hopefully in the future with more clinical data, the establishment of a specific treatment for a specific mutation will emerge.

Limitations

Our study is limited by a small population, and the lack of clinical outcomes and statistics. However, the study is of value because it is the first to provide clinical data for NMIBC and MIBC genomic variants in a Thai population. In a future study data should be collected for other types of variant histology such as sarcomatoid. We believe that the description of some of the genomic mutations found in association with the variant histology could play a role in facilitating the selection of treatment in the near future.

Conclusion

Whole exome sequencing has provided greater insight into the genomic alterations associated with UC. This will surely play a role in future treatment of UC. NMIBC, MIBC, and their variant histology have distinct mutations and oncogenic pathways which lead to the development of UC. We have identified common somatic mutations in a Thai population which have similarities with the global database for example TP53 and KMT2D. We have also shown that some common mutations are found in the cancers in this population including FGFR3, TP53, and RB1. Even though our study is limited, this is an exciting first step into the application of whole exome sequencing for the treatment of bladder cancer in a Thai population.

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