Clinical sequencing identifies potential actionable alterations in a high rate of urachal and primary bladder adenocarcinomas

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Abstract

Administration of targeted therapies provides a promising treatment strategy for rare cancers such as urachal adenocarcinoma (UrC) or primary bladder adenocarcinoma (PBAC), however, the selection of appropriate drugs remains difficult. Therefore, in the present study, we aimed to establish a routine compatible methodological pipeline for the identification of the most important therapeutic targets and potentially effective drugs for UrC and PBAC. Next-generation sequencing using a 161 cancer driver gene panel was performed on 41 UrC and 13 PBAC samples. Clinically relevant alterations were filtered by four publicly available databases. Therapeutic interpretation has been performed by *in silico* evaluation of drug-gene interactions using an evidence-based decision support tool. After data processing, 45/54 samples (33 UrC and 12 PBAC) passed the quality control. The sequencing analyses revealed a total of 191 pathogenic SNVs in 68 genes. The most frequent gain-of-function mutations in UrC were found in *KRAS* (33%), *MYC* (15%), *EGFR* (9%) and *ERBB2* (9%), while in PBAC *KRAS* (25%), *MYC* (25%), *FLT3* (17%) and *TERT* (17%) were recurrently affected. The most frequently affected pathways in both tumour types were related to cell cycle regulation, DNA damage control and the MAPK/RAS pathway. Actionable mutations with at least one available, regulatory agency-approved drug could be identified for 31/33 (94%) of UrC and 8/12 (67%) of PBAC patients. In this study, we used a commercially available assay and developed a data processing pipeline for the detection and therapeutic interpretation of genetic alterations in two rare cancers. Our analyses revealed actionable mutations in a high rate of cases, including *EGFR, BRCA, CCND1/2/3, ERBB2, METex14* suggesting a potentially feasible strategy for both UrC and PBAC treatment.

1. Introduction

According to various definitions, cancers with annual incidence rates of 2–15 cases per 100 000 persons are considered rare (1). Together, rare tumours account for more than 20% of all reported cancers, which is higher than the most commonly occurring single tumour types (2, 3). Rare cancers face specific challenges such as late and often incorrect diagnosis, and lack of clinical expertise, research interest and standard treatments (3). Consequently, the average survival time for rare cancer patients is inferior to those having more common malignancies (2).

While urothelial carcinoma of the bladder is a common malignancy, other histological types like primary adenocarcinoma of the bladder (PBAC) account for < 1% of newly diagnosed bladder cancers. PBAC is derived from the urothelium but exhibits a pure glandular phenotype (4). Although a urachal remnant is not a direct anatomical component of the urinary bladder, urachal adenocarcinoma (UrC) is usually described together with bladder adenocarcinoma as it is detected in most cases after its invasion into the bladder (4). UrC is an aggressive malignancy deriving from the embryological remnant of the urogenital sinus and allantois. It represents an extremely low proportion of bladder cancers with an incidence of ~ 0.3/100 000 000 (2, 5, 6). UrC shares histological and molecular features with colorectal adenocarcinoma (CRC), potentially reflecting their similar embryological origin from the cloaca (7). As UrC is commonly diagnosed at an advanced stage, up to 50% of cases require systemic treatment (8).

Currently, there are no standard, evidence-based guidelines for the management of PBAC and UrC. According to small retrospective studies and case reports on UrC, 5-FU-based treatments seem to provide superior response rates compared to platinum-based therapies, while 5-FU/platinum combinations showed the best oncological results, although with highest toxicity (8, 9). With the lack of clinically proven systemic therapies, targeted treatments based on genomic profiling and a biological rationale represent a promising personalized strategy for rare cancers. However, for UrC and PBAC, only a few published series are available relating to targeted therapies (10–14).

In the perspective of treatment personalization, it is essential to understand the molecular mechanisms as well as the genetic alterations driving tumour development and conferring response to specific therapies. In the past few years, the mutational pattern of UrC has been intensely investigated, although the number of examined cases and genes remains limited. Most studies focused on genes in the RAS/PI3K signaling pathways and found frequently recurring mutations in the *KRAS* gene. In addition, *NF1, GNAS, NRAS* and *PIK3CA* mutations were also recurrently detected (10, 11, 15–19). Considering these overlapping alterations, the genomic background of UrC seems to be similar to that of CRC. On the other hand, we formerly also found some characteristic differences as the *APC* gene was much less frequently affected in UrC (10%) compared to CRC (80%). In addition, microsatellite instability can be detected in 15% of CRCs, but was rarely found in UrC (20). Based on these findings, UrC represents a similar but clearly distinct molecular pattern when compared with CRC.

Much less data is available on the genetic background of PBAC, showing alterations mainly in MAPK or Wnt pathway genes (16, 21). Therefore, further investigation is needed to gain a more detailed insight into the molecular backgrounds of both UrC and PBAC. An additional missing step towards the clinical implementation of genomic profiling is the lack of a systematic approach to interpretation its potential to guide therapeutic intervention. Thus, in this multicentre study, we performed a genomic analysis of UrC and PBAC samples using a large, commercially available next-generation sequencing panel with 161 cancer-related genes. In addition, to identify potentially effective drugs, a clinical interpretation was performed by applying an evidence-based decision support tool.

2. Materials And Methods

2.1. Clinical samples and data collection

Formalin-fixed paraffin embedded (FFPE) tumour tissues from 41 UrC and 13 PBAC patients were retrospectively collected from 9 academic centres. Clinicopathological characteristics including age, sex, tumour localization, Sheldon/Mayo-stage, grade, lymph node status, presence of distant metastasis and survival outcomes were retrieved from medical records using a standardized datasheet. Histopathology slides of all cases were reviewed and verified in accordance with World Health Organization (WHO) criteria by a genitourinary pathologist (H.R.). The study conformed to the declaration of Helsinki and the institutional ethics committee also approved the study protocol (SE TUKEB 74/2016).

2.2. Nucleic acid extraction and next-generation sequencing (NGS)

Samples from either radical/partial cystectomy or transurethral resection of bladder (TURB) were processed for next-generation sequencing. Tumour DNA and RNA was extracted from 4µm-thick FFPE tissue slides. In order to minimize contamination with non-malignant tissue, macrodissection was performed. For this, a board-certified genitourinary pathologist (H.R.) marked tumour areas on haematoxylin and eosin (H&E) stained tissue slides. Corresponding areas were scraped carefully, and DNA extraction was performed using High Pure PCR Template Preparation Kit (Roche, Mannheim, Germany) and RNA was isolated using MagMAX™ FFPE DNA/RNA Ultra Kit (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's recommendations. Extracted nucleic acid concentration was quantified with Qubit™ dsDNA and RNA High-Sensitive Assay kit (Thermo Fisher Scientific, Waltham, MA, USA) on the Qubit fluorometer (Thermo Fisher Scientific, Waltham, MA, USA).

We used an amplicon-based, targeted, next-generation sequencing (NGS) assay (Oncomine Comprehensive Assay v3, OCAv3, Thermo Fisher Scientific, Waltham, MA, USA) to identify relevant single nucleotide variants (SNVs), copy number variants (CNVs), gene fusions, small insertions and deletions (indels) from 161 unique genes. NGS library preparation was performed with the Ion AmpliSeq Library Preparation on the Ion Chef System (Thermo Fisher Scientific, Waltham, MA, USA). PCR amplification was conducted using an input nucleic acid concentration of 10 ng. Sequencing was performed on the IonTorrent™S5 XL platform, following the manufacturer's protocol. The average coverage of all runs was around 2500x. We used the Ion Reporter™ Software (Thermo Fisher Scientific, Waltham, MA, USA) for initial automated analysis. Sequencing reads were aligned with the human genome assembly 19 (hg19), embedded as the standard reference genome in the software. Coverage analysis reports from the Ion Reporter™ Software providing measurements of mapped reads, mean depth, uniformity, and alignment over a target region were used as quality assessment of the sequencing reactions. Sample quality control (QC) was carried out according to the following criteria: total number of reads > 3M, mean read length > 75 bp, mean depth > 800x and uniformity of base coverage > 80%. Exonic variants detected at an allele frequency of > 15% were filtered and annotated using the database provided in Ion Reporter™ software, in addition variants were checked in four freely available online databases (Ensembl Variant Effect Predictor (VEP)/ClinVar / VarSome / COSMIC) to identify pathogenic variants. A variant was considered as a pathogenic alteration when the Ion Reporter™ software and/or two external databases described it as a pathogenic/likely pathogenic mutation.

2.3. Clinical interpretation of sequencing data

Pathogenic variants were evaluated for their therapeutic relevance using an evidence-based decision support software, the QIAGEN® Clinical Insight Interpret (QCI®, Qiagen, Hilden, Germany) which assesses genomic variants from a therapeutic perspective in the context of published biomedical literature, professional association guidelines, publicly available databases, drug labels, and clinical trials. As the software works with curated information from several relevant sources, the list of drugs is constantly

expanding. Our database search was performed on 16.12.2021. The somatic cancer workflow was used in order to match each patient's molecular profile to relevant therapeutic information. The software automatically computes actionability classifications (Tier 1–3) for each alteration according to the AMP/ASCO/CAP guidelines (Supplementary Table S1). In addition, it provides recommendations and contraindications by categorizing drug-mutation interactions as "sensitive", "resistant", "not recommended" or "predictive". For this study, we considered sensitive, resistant and not recommended drug-mutation interactions but excluded the category called "predictive" and focused only on FDA/EMA approved drugs. Since for UrC and PBAC no Tier 1 recommendations exist and we excluded recommendations with low evidence level (Tier 3), only variants with Tier 2 recommendations were included (Fig. 1).

3. Results

3.1. Cohort characteristics

Forty-one patients with UrC and 13 patients with PBAC were included in this study. A full description of patients' characteristics is shown in Table 1.

3.2. Genomic alterations detected by NGS

Eighty percent (33/41) of UrC and 92% (12/13) of PBAC samples passed sequencing quality control. Mutational patterns of UrC and PBAC are shown in Figs. 2 and 3. Our sequencing analyses revealed a total of 191 pathogenic SNVs in 68 genes (176 in the UrC and 18 in the PBAC cohort), including 89 missense mutations, 87 nonsense mutations, 9 frameshift deletions and 6 frameshift insertions. The Ion Reporter™ software and the manual annotation were concordant in 63% (121/191) of the variants. The majority of SNVs were loss-of-function alterations (74% for UrC and 83% for PBAC, respectively). The most frequently affected genes in both UrC and PBAC were *TP53* (UrC: 79%; PBAC: 42%) and *KRAS* (UrC: 33% PBAC: 25%).

Amplifications were present in 16 genes in the UrC cohort and in 10 genes of PBAC cohort. *MYC* amplification was the most frequently identified CNV in both groups (UrC: 15%, PBAC: 25%).

For the UrC cohort 37 genes were recurrently (at least in two patients) affected. However, only 7 recurrently affected genes were detected in PBAC.

RNA was analysed for 24 UrC and 10 PBAC cases. Due to low quality of input RNA the sequencing results of two UrC samples proved to be invalid. The only alteration detected on the RNA level was the *MET* exon 14 skipping alteration, which was present in two UrC and one PBAC samples.

Considering alterations at the pathway level provides a more functional insight. Therefore, we assigned the examined genes to different pathways according to the Vogelstein's classification (22) (Table 2). Since *TP53*, the most frequently altered gene, was assigned to both the cell cycle and the DNA damage control pathways, these proved to be the most affected pathways, followed by the RAS and PIK3 (MAPK)

pathways. More than 90% (30/33) of UrC patients and 67% (8/12) of PBAC patients carried mutations in one or more genes assigned to the cell cycle pathway.

3.3. Therapeutic interpretation / In silico therapy prediction

We used the QCI® software to search for targeted and chemotherapy recommendations and contraindications for FDA/EMA approved drugs with at least Tier 2 evidence level. Below, we only describe the sensitive (recommended) mutation-drug combinations, while resistant and not recommended combinations are given in the supplementary Table S2. FDA and/or EMA approved therapies were found for 31 (94%) UrC and 8 (67%) PBAC patients. Notably, none of these drugs have been approved for the treatment of patients with UrC or PBAC. In the UrC cohort, 53 targeted therapeutic and 12 chemotherapeutic agents were listed as recommended drugs based on SNVs. In the PBAC cohort, 18 potentially effective targeted therapeutic and 6 chemotherapeutic compounds were reported based on alterations of *TP53* and *KRAS* genes (Fig. 4). Therapeutic recommendations for individual patients are given in supplementary Table S2.

When investigating the involvement of different pathways, we found that the majority of the recommended agents for both UrC and PBAC were those targeting members of the RAS/PIK3 pathways. Regorafenib, a multi-kinase inhibitor was recommended for mutations of four different RAS pathway genes (*KRAS, NRAS, BRAF* and *FGFR2*) (Fig. 4A).

Therapy recommendations based on gene amplifications were also considered. Ten of 19 amplified genes (*CCND1, CDK4, EGFR, ERBB2, FGFR2, FGFR3, FLT3, KRAS, MET* and *MYC*) proved to be relevant for therapy prediction. Drugs were recommended for 11 (33%) UrC and four (33%) PBAC patients based on their CNVs. Forty-six targeted therapeutic agents and 5 chemotherapeutic compounds were assigned based on sensitive alteration-drug interactions. As with SNVs, the majority of recommended drugs based on copy number gains were RAS/PIK3 pathway inhibitors, mainly multi-kinase inhibitors (e.g., nintedabin, ponatinib) (Fig. 4B). Therapeutic recommendations for individual patients are given in supplementary Table S2.

4. Discussion

PBAC and UrC are rare and aggressive malignancies with a median survival for locally advanced or metastatic disease ranging between 12–24 months (9). This poor prognosis is the result of many different factors, such as 1) delayed symptoms resulting in diagnosis at advanced tumour stages, 2) no standard-of-care therapeutic recommendations, and 3) poorly known molecular pathogenesis and genomic landscape of the tumour (23). As randomized trials for the evaluation of clinical benefit of various drugs in UrC and PBAC are not feasible, precision medicine is of prominent therapeutic interest in these rare cancers (24). Comprehensive genome profiling could therefore be fundamental to drive therapeutic decisions in these patients. In the present study, we performed mutational analyses of tumour tissues from UrC and PBAC patients and sought to identify targetable alterations and corresponding drugs that could potentially be effective for these patients.

Our genomic analysis, in line with previous reports, found *TP53* (UrC: 79%, PBAC: 42%) to be the most commonly affected gene in both UrC and PBAC (10, 15, 16, 18, 19, 21). Since *TP53* mutated tumours progress faster and have a poor response to anticancer therapy, targeting p53 for cancer therapy seems to be an attractive strategy (25). Although *TP53* has previously been considered as undruggable due to its essential role in cell survival, recently many drugs targeting *TP53* mutant tumours are being tested in early-phase (Phase I/II) clinical trials (26).

KRAS mutations in UrC have been extensively investigated, as it is a commonly affected oncogene in CRC, a tumour type sharing large histological and molecular similarity with UrC. After summarizing published literature on the prevalence of *KRAS* mutations in both UrC and PBAC, it proved the most frequently tested gene with alterations in ~ 30% of UrC and 25% of PBAC cases, which is in agreement with our present results showing 33% mutational frequency in UrC and 25% in PBAC (11, 15, 16, 18, 19). These data underscore the importance of the RAS pathway in both UrC and PBAC (16, 18, 21, 27). The vast majority (11/14) of *KRAS* alterations were missense mutations in codon 12 (G12V, G12D and G12A) which is a similar pattern to that found in CRC (28). In recent years, several breakthrough structural and mechanistic studies have led to the clinical development of selective *KRAS* inhibitors. Last year, the FDA granted accelerated approval to sotorasib, the first *KRAS*-blocking drug for non-small cell lung cancer (NSCLC) patients. Phase I/II studies (e.g. NCT03600883, NCT04699188) are currently investigating the efficacy of sotorasib and other G12C-inhibitors in other tumour types as well.

In our UrC cohort, four patients carried loss-of-function alterations in their *BRCA2* gene. When considering the therapeutic significance of these alterations, only one patient proved to bear a potentially significant (Tier 2) SNV, the rest were categorized into Tier 3. Recently, PARP inhibitors (olaparib, rucaparib, niraparib) have becomeavailable for patients with alterations in their *BRCA1/2* or other homologue recombinant repair genes. Accordingly, our drug prediction recommended PARP inhibitors for the UrC patient with Tier 2 mutation. Although little is known about the efficacy of PARP inhibitors in UrC. The only report on the use of a PARP inhibitor for the treatment of an UrC patient came from a Japanese phase I dose escalation study of niraparib and described progression during therapy. As *BRCA* positivity was not an inclusion criterion in the study, the *BRCA* status of the UrC patient is unknown(13).

When considering copy number alterations, *MYC* amplifications were detected at the highest frequency (5/33, 15%) in our UrC cohort, which is lower compared to a previously published study (6/17, 35%). In addition, we found *EGFR* amplification in 6% (2/33) of our UrC patients, which was also lower, compared to the frequency of 20% found by Lee *et al*. (19). Furthermore, we found recurrent copy number gains in members of the FGF/FGFR signalling pathway (in four UrC and six PBAC patients). Although, previous genomic analyses of PBAC did not reveal amplifications of the *MYC* gene, here we found *MYC* as the most frequently amplified gene (25%).

MYC is a global transcription factor and a driver of many human malignancies, that has proven to be difficult to inhibit directly (29). In this context, it is interesting that *CDK12* was found to be a synthetic lethal gene with *MYC*. These findings were corroborated in an independent study demonstrating that CDK

inhibition triggered massive downregulation of *MYC* expression and its related genes (30). The overlap between *MYC* and the known cellular functions of *CDK12*, as well as the requirement of *CDK12* for optimal processing of *MYC*, collectively indicate *CDK12* as a potential therapeutic target for *MYC*-dependent cancers (31).

EGFR is a widely used therapeutic target. To date, numerous anti-EGFR compounds, both tyrosine kinase inhibitors (TKIs) and monoclonal antibodies, have been developed and approved for different cancers (32). This is also reflected by our results identifying the second highest number of recommended drugs for patients with tumours harboring an *EGFR* amplification. In the literature, two UrC patients have been reported to receive an EGFR inhibitor. One patient with an *EGFR* amplification experienced a persistent partial response to cetuximab in the third line setting (10), while the other UrC patient with immunohistochemically proven EGFR overexpression experienced a transient 55% decrease in tumour size with gefitinib treatment (14). These results suggest *EGFR* as a potent therapeutic target in UrC.

In addition, we found for the first time D-type cyclin (*CCND1/2/3*) genes to be affected by activating mutations in both UrC and PBAC samples with frequencies of 15% and 25%, respectively. CCNDs promote cell cycle progression from G1 to S phase by binding to and activating the cyclin dependent kinases CDK4/CDK6, thereby imparting oncogenic properties. The cyclin D-CDK4/6 complex is often hyperactivated in various tumours (e.g. NSCLC, head and neck, renal cell, breast, pancreatic and colorectal cancer) partly by gene amplification, and is therefore an attractive therapeutic target (33, 34). Recently, multiple CDK4/6 inhibitors have been approved for the treatment of breast cancer (33). In addition, several clinical trials are ongoing to assess the efficacy of palbociclib, abemaciclib, or ribociclib in other cancers (e.g. NCT03446157, NCT02022982 and NCT03356223). Although, molecular alterations of cell cycle pathway genes are not mandatory for the prescription of these drugs, their presence suggest a favourable effect. Accordingly, this mutation-drug association is being tested in an ongoing phase II pan-cancer trial (NCT04439201) assessing the efficacy of palbociclib in patients with various malignancies harboring *CCND1/2/3* amplifications. Due to our present results, UrC and PBAC patients carrying activating amplification in their *CCND* genes may be good candidates for future studies.

Our analyses identified one UrC and one PBAC patient with MET amplification. MET alterations besides their primary cancer driver role can mediate resistance to other targeted therapies such as EGFR inhibitors. They do this through activation of downstream signal transduction that leads to escape from therapy-induced cell death (35). This effect was reflected in our QCI® drug prediction as it suggested resistant association between MET amplification and anti-EGFR drugs.

According to recent data, not only the above mentioned amplification of MET but also its exon 14 skipping alteration (METex14) is associated with acquired resistance to EGFR-targeting compounds (36). METex14 has been identified in about 3% of lung NSCLCs and other solid tumours like breast cancer and glioblastoma. In 2020 and 2021, two MET-inhibitors; capmatinib and tepotinib, were approved for use as monotherapies in NSCLC patients carrying METex14 (37). Here we are the first to report the METex14 alteration in treatment-naïve UrC and PBAC with an overall incidence of 3/38 (7–8%). Considering the

durable response observed in NSCLC patients with METex14 alteration, UrC and PBAC patients with this alteration may also benefit from tepotinib or capmatinib therapy. Accordingly, in the only published UrC patient treated with tepotinib a durable disease stabilization could be observed (12).

We identified copy number gain of ERBB2 (HER2) in one UrC and one PBAC sample. In breast cancer, ERBB2 amplification is a well-known prognostic biomarker for worse survival in the absence of anti-HER2 therapy (38). There is an abundance of approved HER2-targeted agents not just for breast cancer but also for other cancer entities such as metastatic gastric or gastroesophageal junction cancers (39). Currently, several ongoing clinical trials are evaluating the potential benefit of targeting HER2 in various tumour types (e.g. NCT02465060, NCT02675829). Little is known about the prevalence of ERBB2 amplification in UrC and PBAC. A study investigating the prevalence of ERBB2 amplification in different tumours identified ERBB2 amplification in 2 of 7 (28%) UrC samples (39). This study also showed clinical benefit of HER2-targeted therapy in tumours for which HER2-inhibitors are not yet approved (39). In the present study, the QCI® drug prediction algorithm recommended the highest number of drugs for ERBB2 amplified tumours, suggesting this alteration as an attractive therapeutic target.

FLT3 amplification might also be a potentially actionable molecular alteration, although the majority of the kinase inhibitors approved so far are relatively nonspecific for FLT3 (e.g. nintedanib, ponatinib, sorafenib and sunitinib). Off-target activities of these multi-kinase inhibitors can contribute to higher toxicity causing severe adverse events (40). Accordingly, in the case report of Loh et al. an UrC patient with FLT3 amplification received sorafenib therapy, but it had to be discontinued shortly after drug initiation due to a serious adverse event. Sunitinib was subsequently administered without toxicity, but an additional treatment change was required due to disease progression (11). Next-generation inhibitors, such as quizartinib or gilteritinib, are more specific and potent FLT3 inhibitors, with more favourable toxicity profiles, however, to date these drugs are approved only for acute myeloid leukaemia and thus no data on their activity in solid tumours is available (41).

This study has several key strengths. This is one of the largest studies of UrC and PBAC with respect to case number and number of the assessed genes. It is the first study in UrC and PBAC that systematically applies a clinical decision support tool to match driver aberrations with clinically approved drugs. Finally, we report here for the first-time recurrent alterations in *CCND1-2, NOTCH3, RNF43, CDK12, FGFR4, CREBBP* and *SMARCA4* genes in UrC and mutations in *MYC, CDKN2A and FLT3* genes in PBAC.

On the other hand, this study has several limitations. Due to the rarity of UrC and PBAC, a retrospective approach was needed to collect samples from multiple institutions over a long time period. Associated differences in sample handling and specimen age may result in heterogeneous quality of FFPE tissues. Consequently, a relatively high rate (~ 9%) of samples did not pass quality control. In addition, as the clinical interpretation could be carried out in a retrospective manner, we were not able to assess whether the recommended drugs would have been effective in the assessed UrC and PBAC patients. A further limitation is the heterogeneity of databases regarding both judgment of pathogenicity and druggability of certain variants. In addition, as the CNVs were predicted by bioinformatics tools, orthogonal validation for

example by *fluorescence in-situ* hybridization (FISH) would be needed to validate the presence of the alteration. Some of these limitations could be addressed with the addition of *in vitro* (e.g., organoid) and *in vivo* (patient-derived tumour xenografts) models to improve our ability to predict drug response, which could then improve treatment selection for patients with rare cancers.

In conclusion, our results suggest significant overlaps in the genomic landscape of UrC and PBAC. The cell cycle pathway is the most affected pathway, followed by the DNA damage control, RAS and PI3K pathways. However, large individual heterogeneity was observed in the mutation patterns. In the majority of cases at least one potentially druggable alteration was identified, highlighting the promise of genetic profiling to guide treatment of these rare malignancies.

Declarations

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Tables

Table 1. Clinicopathological characteristics of UrC and PBAC patients

		UrC cohort		PBAC cohort	
		n=41	*%*	n=13	*%*
Age (year)	Median (Range)	49.5 (22-77)	*-*	59 (38-75)	*-*
Sex	Male	22	*53.7*	8	*61.5*
	Female	19	*46.3*	5	*38.5*
Haematuria	Yes	28	*84.8*	5	*83.3*
	No	5	*15.2*	1	*16.7*
	NA	8	*-*	7	*-*
Abdominal pain	Yes	2	*6.5*	2	*28.6*
	No	29	*93.5*	5	*71.4*
	NA	10	*-*	6	
Palpable tumor	Yes	1	*3.2*	1	*14.3*
	No	30	*96.8*	6	*85.7*
	NA	10	*-*	6	*-*
UrC type	Intestinal	18	*46.2*	17	*53,1*
	Mucinous	12	*30.8*	9	*28,1*
	NOS	5	*12.8*	4	*12,5*
	SRC	2	*5.1*	2	*6,3*
	Mixed	2	*5.1*	-	*-*
	NA	2	*-*	9	*-*
Calcification	Yes	2	*5.4*	-	*-*
	No	35	*94.6*	-	*-*
	NA	4	*-*	-	*-*
Signet ring cell component	Yes	-	*-*	3	*25.0*
	No	-	*-*	9	*75.0*
	NA	-	*-*	1	*-*
Sheldon staging	I	0	*0.0*	-	*-*
	II	0	*0.0*	-	*-*
	IIIA	28	*70.0*	-	*-*

	IIIB	1	*2.5*	-	*-*
	IIIC	1	*2.5*	-	*-*
	IIID	0	*0.0*	-	*-*
	IVA	3	*7.5*	-	*-*
	IVB	7	*17.5*	-	*-*
	NA	1	*-*	-	*-*
Mayo staging	I	10	*25.6*	1	*9.1*
	II	19	*48.7*	6	*54.5*
	III	3	*7.7*	4	*36.4*
	IV	7	*18.0*	-	*-*
	NA	2	*-*	2	*-*
Pathological stage	T1	-	*-*	0	*0.0*
	T2	-	*-*	3	*23.1*
	T3	-	*-*	5	*38.5*
	T4	-	*-*	5	*38.5*
Grade	I	-	*-*	1	*9.1*
	II	-	*-*	6	*54.5*
	III	-	*-*	4	*36.4*
	NA	-	*-*	2	*-*
LN status	LN-/LNx	19	*76.0*	8	*61.5*
	LN+	6	*24.0*	5	*38.5*
	NA	16	*-*	-	*-*
M status	M-	28	*77.8*	-	*-*
	M+	8	*22.2*	-	*-*
	NA	5	*-*	-	*-*
LN/M status	LN/M+	11	*26.8*	7	*53.8*
	LN/M- / LN/Mx	30	*73.2*	6	*46.2*
Surgery	TURB	1	*2.7*	0	*0.0*
	Partial CE	28	*75.7*	1	*7.7*

	Radical CE	8	***21.6***	12	***92.3***
	NA	4	**-**	-	**-**
Umbilectomy	Yes	21	***63.6***	-	**-**
	No	12	***36.4***	-	**-**
	NA	8	**-**	-	**-**

Abbreviations: *NOS* not otherwise specified, *SRC* signet ring cell carcinoma, *LN* lymph node, *LN+* positive lymph node status, *LN-* negative lymph node status, *LNx* unknown lymph node status, *M+* positive distant metastatic status, *M-* negative distant metastatic status, *Mx* unknown distant metastatic status, *CE* cystectomy, *TURB* transurethral resection of bladder, *NA.* not available

Table 2. Affected pathways in UrC and PBAC according to Vogelstein's classification

Vogelstein's pathways	**Genes**	**Patients with mutation n (%)**	
		UrC	**PBAC**
Cell Cycle	*CCND1; CCND2; CCND3; CDKN2A; CDK4; CHEK2; MDM2; MYC; MYCL; MYD88; PPP2R1A; TP53**	30 (91%)	8 (67%)
DNA Damage Control	*ATM; BRCA1; BRCA2; FANCA; FANCD2; FANCI; MLH1; MSH2; MSH6; PALB2; TP53**	26 (79%)	5 (42%)
RAS	*BRAF; EGFR*; ERBB2*; FGFR2*; FGFR3*; FGFR4*; FLT3*; GNAQ*; GNAS*; HRAS; KRAS; MAP2K1; MET*; NF1; NRAS; RET**	20 (61%)	6 (50%)
PI3K	*AKT1; EGFR*; ERBB2*; FGFR2*; FGFR3*; FGFR4*; FLT3*; GNAQ*; GNAS*; MET*; PIK3CA; PIK3R1; PTEN; RET*; TSC2*	14 (42%)	4 (33%)
NOTCH	*FBXW7; NOTCH1; NOTCH2; NOTCH3*	6 (18%)	3 (25%)
TGF-β	*GNAS*; SMAD4*	4 (12%)	1 (8%)
*** Gene assigned to more than one pathway**			

Figures

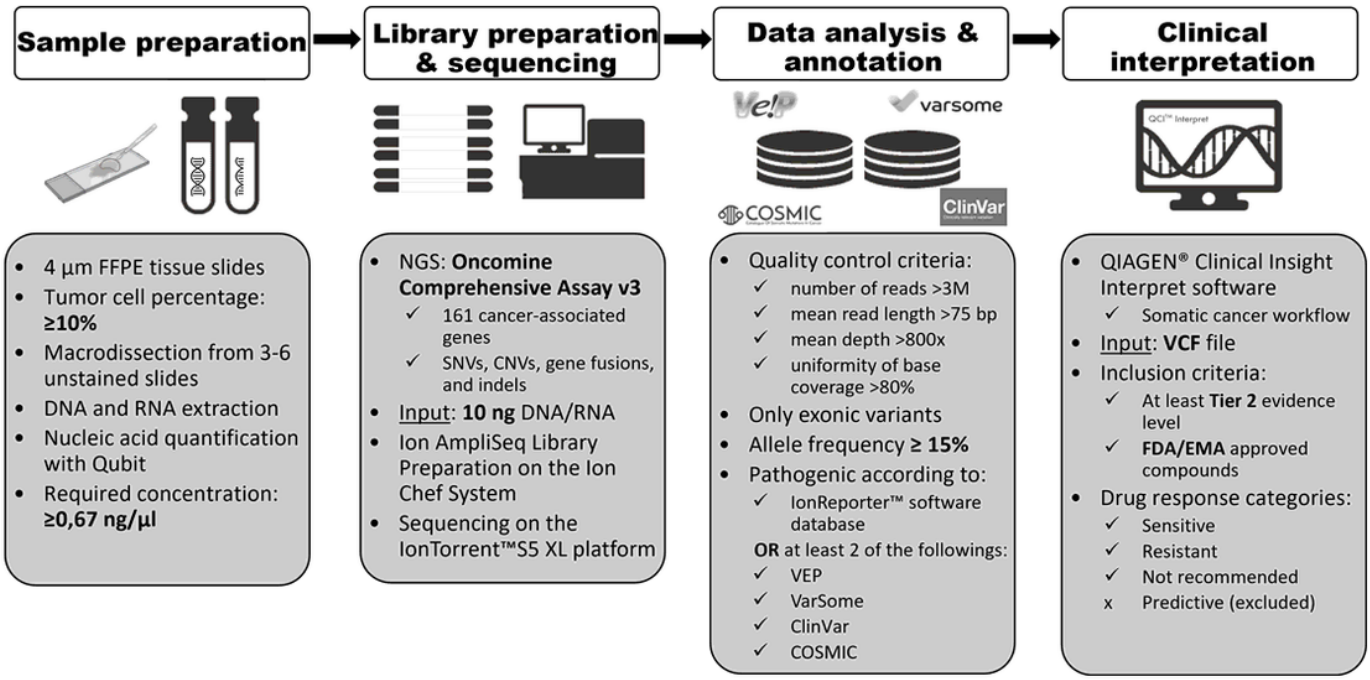

Figure 1

Methodological pipeline for identification of therapeutic targets and drugs for UrC and PBAC

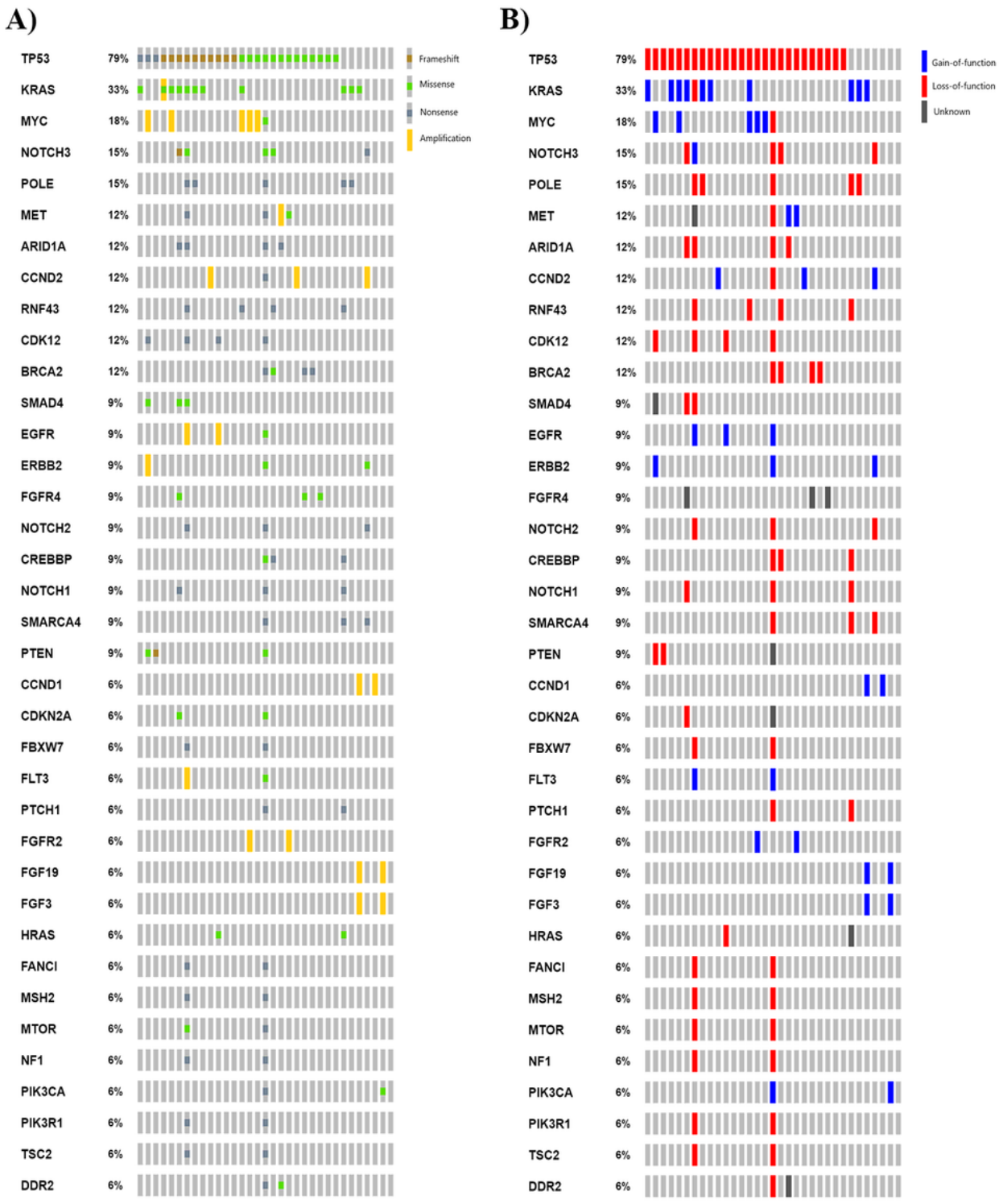

Figure 2

Oncoprint presentation of recurrently mutated genes in UrC by the type (A) and functional effect (B) of alterations

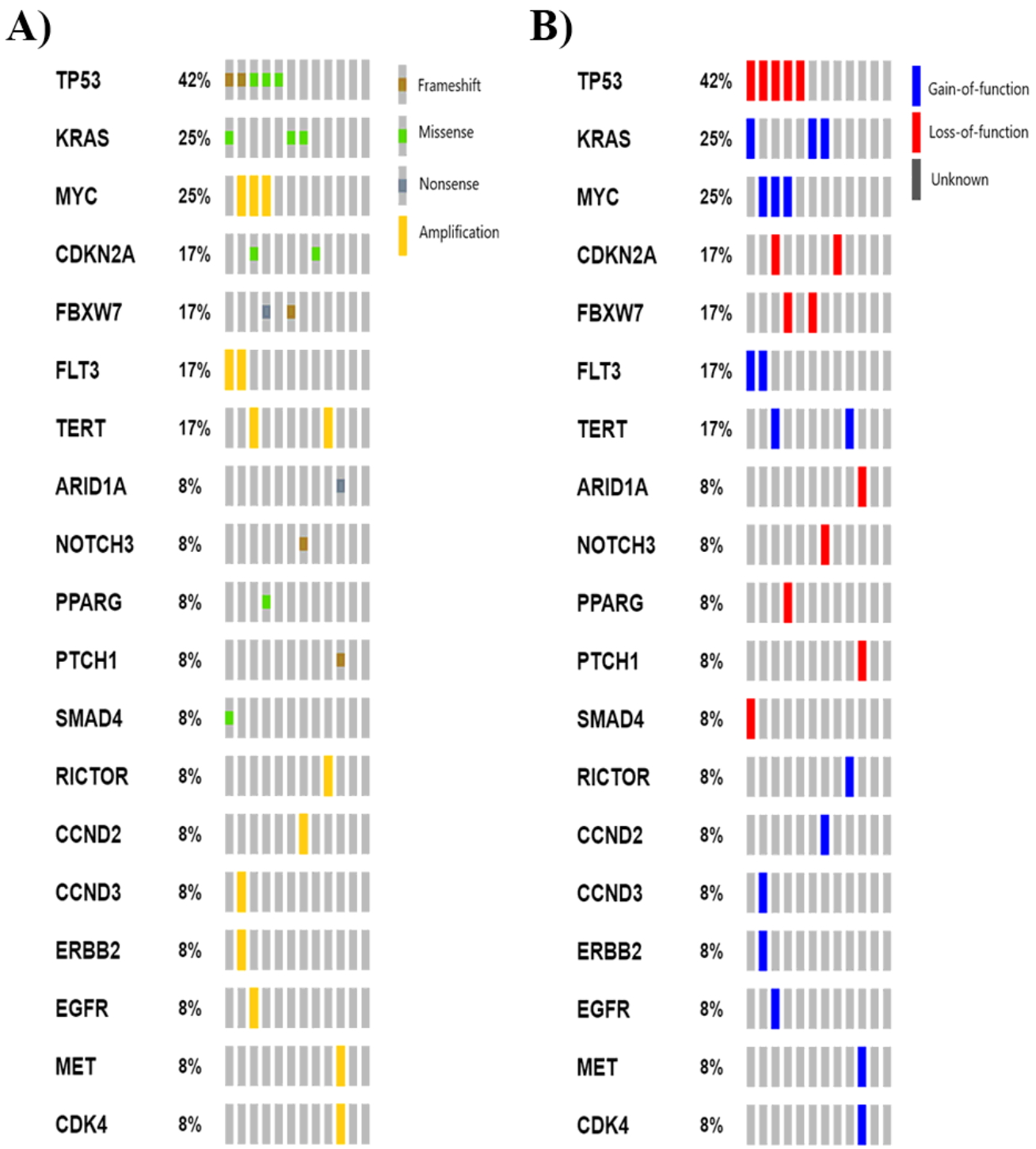

Figure 3

Oncoprint presentation of recurrently mutated genes in PBAC by the type (A) and functional effect (B) of alterations

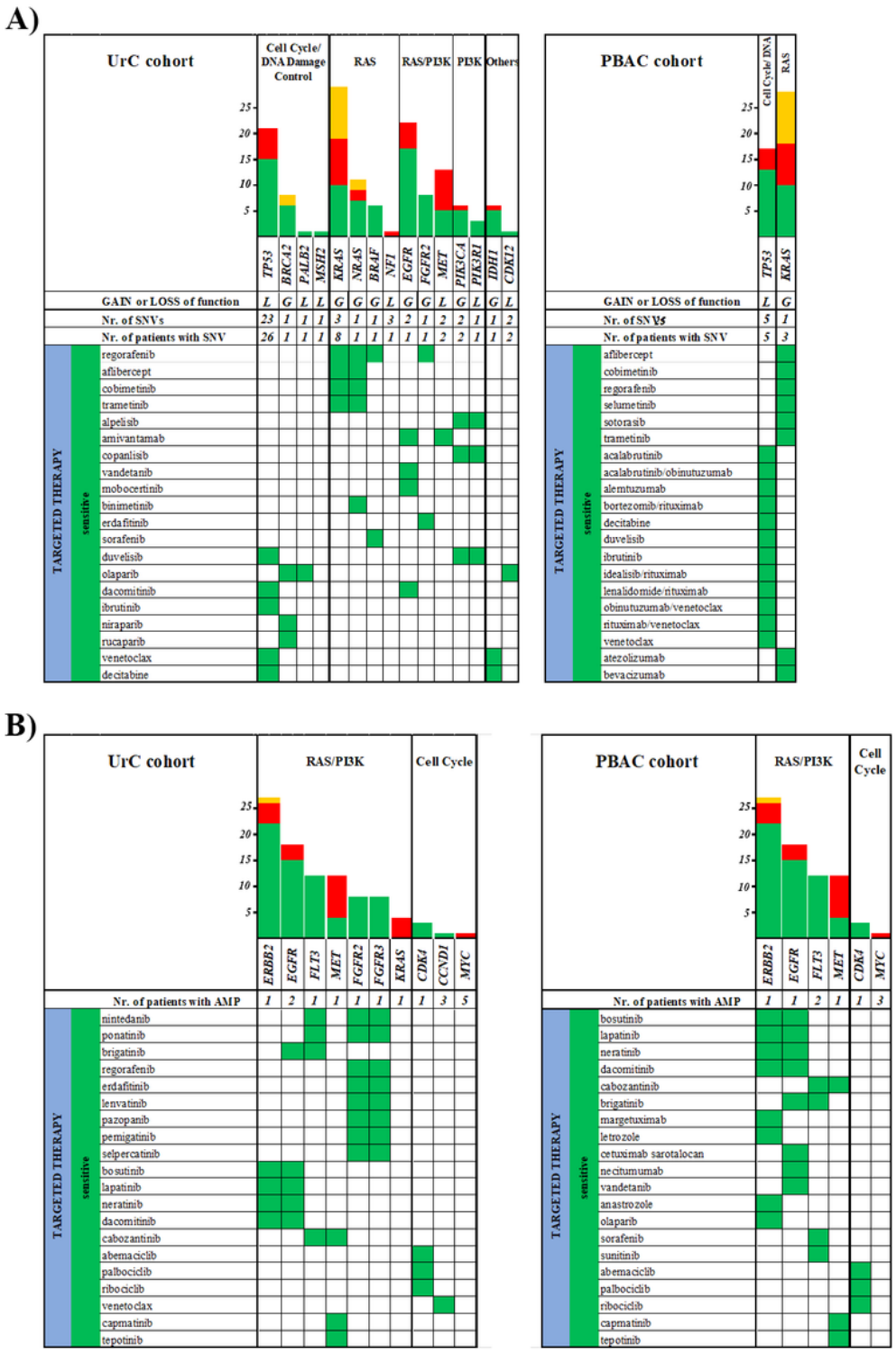

Figure 4

Most recommended targeted therapeutic agents for A) SNVs and B) CNVs

Supplementary Files

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- SupplemetaryTableS1.docx
- SupplementaryTableS2.xlsx