*Article*

# Identification of Novel Rare *ABCC1* Transporter Mutations in Tumor Biopsies of Cancer Patients

**Onat Kadioglu [1], Mohamed Saeed [1], Markus Munder [2], Andreas Spuller [3], Henry Johannes Greten [4,5] and Thomas Efferth [1,*]**

[1] Department of Pharmaceutical Biology, Institute of Pharmacy and Biochemistry, Johannes Gutenberg University, 55128 Mainz, Germany; kadioglu@uni-mainz.de (O.K.); saeedm@uni-mainz.de (M.S.)

[2] Third Department of Medicine (Hematology, Oncology, and Pneumology), University Medical Center of the Johannes Gutenberg University Mainz, 55131 Mainz, Germany; markus.munder@unimedizin-mainz.de

[3] Clinic for Gynecology and Obstetrics, 76131 Karlsruhe, Germany; andreas-spuller@web.de

[4] Abel Salazar Biomedical Sciences Institute, University of Porto, 4099-030 Porto, Portugal; heidelbergschool@aol.com

[5] Heidelberg School of Chinese Medicine, 69126 Heidelberg, Germany

[*] Correspondence: efferth@uni-mainz.de; Tel.: +49-6131-392-5751; Fax: 49-6131-392-3752

check for updates

**Abstract:** The efficiency of chemotherapy drugs can be affected by ATP-binding cassette (ABC) transporter expression or by their mutation status. Multidrug resistance is linked with ABC transporter overexpression. In the present study, we performed rare mutation analyses for 12 *ABC* transporters related to drug resistance (ABCA2, -A3, -B1, -B2, -B5, -C1, -C2, -C3, -C4, -C5, -C6, -G2) in a dataset of 18 cancer patients. We focused on rare mutations resembling tumor heterogeneity of ABC transporters in small tumor subpopulations. Novel rare mutations were found in *ABCC1*, but not in the other ABC transporters investigated. Diverse *ABCC1* mutations were found, including nonsense mutations causing premature stop codons, and compared with the wild-type protein in terms of their protein structure. Nonsense mutations lead to truncated protein structures. Molecular docking and heat map analyses of *ABCC1*/MRP1 pointed out that Lys498* appeared in a separate cluster branch due to the large deletion, leading to a massive disruption in the protein conformation. The resulting proteins, which are nonfunctional due to nonsense mutations in tumors, offer a promising chemotherapy strategy since tumors with nonsense mutations may be more sensitive to anticancer drugs than wild-type *ABCC1*-expressing tumors. This could provide a novel tumor-specific toxicity strategy and a way to overcome drug resistance.

**Keywords:** ABC transporters; cancer; multidrug resistance

## 1. Introduction

ATP-binding cassette (ABC) transporters are drug efflux pumps hampering the effectiveness of cytotoxic anticancer drugs. ABC transporter overexpression is associated with multidrug resistance (MDR) in cancer cells, possibly leading to chemotherapy failure [1]. The overexpression of ABC transporters is also associated with shorter survival rates of cancer patients [2–4]. RNA-sequencing and other genome- and transcriptome-wide techniques paved the way to address the MDR phenomenon and the corresponding influence of ABC transporters in a more comprehensive fashion.

The advances in next-generation sequencing methodology have spurred researchers to study a large amount of genomic data to identify patients' tumor-specific mutations. Indeed, this knowledge will assist clinicians in making personalized treatment decisions to improve treatment strategies [5,6]. In recent years, medical oncologists have focused on targeted sequencing to identify genetic aberrations

that may be predictive of response to anticancer therapies [7]. While most analyses focused on mutation profiling of the main cell populations of a tumor, the mutations in small subpopulations may be especially decisive for the refractoriness of tumors. Tumor cells with mutations in drug resistance genes may survive and grow if the predominant tumor population has been eradicated by chemotherapy. Tumor heterogeneity within a patient has been recognized as a major factor of drug resistance [8,9]. It is worth mentioning that the advancement of genome-wide DNA mutation analyses have enabled the identification of hundreds of low-frequency mutated genes in tumors and triggered scientists to unravel their impact on the prognosis and pathogenesis of cancer [10]. Structural comparisons of truncated forms of *ABC* transporters with wild-type proteins have shed light on the effect of these nonsense mutations. Nonsense mutations were clearly associated with truncated protein structures. Tumors carrying nonsense mutations in ABC genes possess nonfunctional proteins for the corresponding ABC transporter. The determination of truncated nonfunctional ABC transporters may imply a promising chemotherapy strategy. Tumors with nonsense mutations in ABC transporters may be sensitive to chemotherapy with ABC transporter substrates. While tumors carry a nonsense-mutated ABC transporter, this transporter is not mutated in normal tissues and is still intact. Hence, chemotherapy would preferentially affect tumor tissues with nonsense-mutated and nonfunctional ABC transporters rather than normal tissues. This strategy may trigger a novel tumor-specific chemotherapy strategy to overcome drug resistance. We analyzed low-frequency mutations in 12 ABC transporters associated with drug resistance (ABCA2, -A3, -B1, -B2, -B5, -C1, -C2, -C3, -C4, -C5, -C6, -G2) [11–15]. Novel *ABCC1* transporter mutations, including nonsense mutations causing premature stop codons, were identified that have not been reported before.

In the present study, we performed RNA-sequencing in tumors from 16 patients with different tumor types at a late stage who had not responded to conventional chemotherapy and two leukemia patients' biopsies were collected during the initial diagnosis (n = 18 in total). We specifically focused on low-frequency mutations. Additionally, we identified novel nonsense and missense mutations in the *ABCC1* gene and speculate that substrates of MDR-associated protein 1 (MRP1, encoded by the *ABCC1* gene), such as doxorubicin, docetaxel, etoposide, and teniposide could be administered to patients with nonsense *ABCC1* mutations. Furthermore, we selected three missense and one nonsense mutations, in order to evaluate the binding mode of MRP1 substrates and inhibitors. By applying heat map analyses, we compared the binding patterns with those of wild-type MRP1.

## 2. Material and Methods

### 2.1. RNA Sequencing and Mutation Analysis

The ABC transporter mutations in our dataset of 18 patients with various cancer types were identified by RNA sequencing. Informed consent was collected from all patients. The procedure of RNA sequencing has been described previously [16]. The clinical data of the patients is described in Table 1. Considering frequent mutations, none of the patients possess nonsense mutations. In order to identify the low frequent mutations, Strand NGS 3.4 software (Strand Life Sciences Pvt. Ltd., Bangalore, India) was used. Twelve ABC transporters together with their chromosomal position were selected and imported as a gene list. As a first step, the patients' ".vcf" files and a ".bam" file as a reference human genome alignment were imported. Then, by using the "filter by region list" option, read lists (aligned reads) and region lists (patient data) were selected to generate a further read list. The next round of "filter by region list" was performed by selecting the read list from the previous step and the imported ABC transporter gene list as the region list. This final read list was used to perform low-frequency SNP detection by clicking on "SNP detection" and "perform low frequency SNP detection" with default options. Default lower threshold of the base quality range for the binomial test iteration is 20 and default upper threshold of the base quality range for the binomial test iteration is 30 for low-frequency SNP detection. Detailed explanation for low-frequency SNP detection is listed at the user manual

Section 11.5.4 of Strand NGS software. We took the same threshold for low-frequency mutations. Afterwards, SNP effect analysis was performed, and the gene lists and the mutations were exported.

**Table 1.** Patient Information.

| Patient ID | Tumor Type | Age | Gender |
|---|---|---|---|
| P01 | vulva cancer, colon cancer | 82 | Female |
| P02 | breast | 64 | Female |
| P03 | breast | 75 | Female |
| P04 | lung | 75 | Female |
| P05 | breast | 76 | Female |
| P06 | non-Hodgkin lymphoma | 56 | Male |
| P07 | non-Hodgkin lymphoma | 68 | Male |
| P08 | lymphoma | 43 | Female |
| P09 | breast | 50 | Female |
| P10 | cholangiocellular cancer of bile duct | 54 | Male |
| P11 | liver | 80 | Male |
| P12 | cervix | 54 | Female |
| P13 | invasive lobular breast cancer | 50 | Female |
| P14 | pancreas cancer | 62 | Male |
| P15 | squamous epithelium cancer of the base of the tongue | 57 | Male |
| P16 | acute myeloid leukemia | 64 | Female |
| P17 | serous adeno cancer of the tube | 57 | Female |
| P18 | acute myeloid leukemia | 82 | Male |

### 2.2. Homology Modeling, Protein Structure Preparation

A human MRP1 homology model was created by using bovine Mrp1 (PDB ID: 5UJ9) as template. The Modeller 9.23 algorithm embedded in UCSF Chimera software was used as previously described [17]. Structural comparisons were performed for human MRP1 focusing on nonsense mutations which cause premature stop codons. Corresponding protein structure files were prepared by using the human MRP1 homology model.

### 2.3. Molecular Docking and Clustering

The modeled wild-type MRP1, three missense mutations (T550A, T556A, and V1101F) and one nonsense mutation (Lys498*) were subjected to molecular docking analyses against a panel of MRP1 substrates (zoledronic acid, irinotecan, etoposide, epirubicin, doxorubicin, docetaxel, dactinomycin, 7-ethyl-10-hydroxycamptothecin, and camptothecin) and MRP1 inhibitors (reversan, mk-571, glibenclamide, pak104p, sulfinpyrazone, indomethacin, probenecid, quercetin, genistein, and diltiazem) [18–28]. The two missense mutations, T550A and T556A, were hotspot mutations in the transmembrane domain. Thr550 and Thr556 were located in the inner leaflet region of transmembrane domain 10 (TM10), playing a vital role in determining the drug resistance profile [29]. The V1101F mutation was also observed in COSMIC database, present in the HCT15 colon cancer cell line. Lys498* was the nonsense mutation observed in our patients leading to the largest deletion in MRP1. A total of four human MRP1 homology models, 9 classical anticancer drugs that are MRP1 substrates, and 10 MRP1 inhibitors have been subjected to an automated and comprehensive virtual molecular docking campaign. Each molecular docking was based on at least three independent dockings, each consisting of 2,500,000 calculations. The AutoDock 4 algorithm was used for the defined molecular docking calculations on the drug-binding pocket of MRP1 as previously described [30].

Hierarchical clustering was performed with the CIM miner software (https://discover.nci.nih.gov/cimminer/home.do) (Bethesda, MD, USA) as previously described by applying ward cluster algorithm [31,32]. The heat maps were visualized in order to classify the compounds and the mutant models in terms of their binding energy distributions.

## 3. Results

### 3.1. ABC Transporters Mutation Analysis

　　Low-frequency missense and nonsense mutations were only observed for *ABCC1*, but not for the other 11 ABC transporters investigated. These low-frequency nonsense mutations in *ABCC1* are listed in Table 2. Low-frequency missense and deletion/insertion mutations in *ABCC1* are listed in Table 3. All identified nonsense mutations in our patient dataset are new and were not listed in the COSMIC database (https://cancer.sanger.ac.uk/cosmic/gene/analysis?ln=ABCC1#variants). Therefore, they can be considered as novel mutations.

**Table 2.** Low frequent nonsense mutations in *ABCC1*.

| Mutation | Patient |
|---|---|
| Ala519_Glu521delinsAlaGlnTer | P16, P03, P04, P17, P08 |
| Cys555Ter | P16, P03, P13, P08, P15 |
| Glu1065Ter | P16, P01, P02, P03, P05, P06, P13, P08, P11, P09, P10 |
| Glu507Ter | P03, P04, P17, P08 |
| Glu521Ter | P16, P03, P04, P17, P13, P08 |
| Leu562Ter | P16, P18, P02, P03, P05, P06, P17, P13, P08, P11, P12, P15, P14, P09 |
| Lys498Ter | P03, P04, P17, P08 |
| Lys537Ter | P16, P03, P17, P13, P08 |
| Lys540Ter | P16, P03, P17, P13, P08 |
| Ser546Ter | P16, P03, P13, P08 |
| Thr1082Ter | P16, P01, P02, P03, P05, P06, P07, P13, P08, P11, P09, P10 |
| Trp520Ter | P16, P03, P04, P17, P08 |
| Tyr518Ter | P16, P03, P04, P17, P08 |
| Tyr544Ter | P16, P03, P13, P08 |

Table 3. Low frequent missense and del/ins mutations in *ABCC1*.

| Mutation | Patient | Mutation | Patient | Mutation | Patient |
|---|---|---|---|---|---|
| Ala1104Asp | P16, P01, P03, P04, P07, P17, P13, P08, P11, P12 | His494Leu | P03, P04, P17, P08 | Phe1064Cys | P16, P01, P02, P03, P05, P06, P13, P08, P11, P09, P10 |
| Ala493Ser | P03, P04, P17, P08 | His494Phe | P03, P04, P17, P08 | Phe1064Val | P16, P01, P02, P03, P05, P06, P13, P08, P11, P09, P10 |
| Ala519Thr | P16, P03, P04, P17, P08 | His494Tyr | P03, P04, P17, P08 | Phe1076Leu | P16, P01, P02, P03, P05, P06, P07, P13, P08, P11, P09, P10 |
| Ala523_Asp526delinsValGluLeuSer | P16, P03, P04, P17, P13, P08 | His701Pro | P01, P01, P03, P03, P07, P07, P13, P13, P08, P08, P15, P15 | Phe1076Ser | P16, P01, P02, P03, P05, P06, P07, P13, P08, P11, P09, P10 |
| Ala523Val | P16, P03, P04, P17, P13, P08 | Ile1087Leu | P16, P01, P02, P03, P05, P06, P07, P13, P08, P11, P09, P10 | Phe1094_Gly1096delinsTrpSerGln | P16, P01, P02, P03, P05, P06, P07, P13, P08, P11, P09, P10 |
| Ala530_Ile531delinsGlyHis | P16, P03, P17, P13, P08 | Ile1087Thr | P16, P01, P02, P03, P05, P06, P07, P13, P08, P11, P09, P10 | Phe1094Cys | P16, P01, P02, P03, P05, P06, P07, P13, P08, P11, P09, P10 |
| Ala530Gly | P16, P03, P17, P13, P08 | Ile1091_Lys1092delinsMetGlu | P12 | Phe1094Leu | P16, P01, P02, P03, P05, P06, P07, P13, P08, P11, P12, P09, P10 |
| Ala543Val | P16, P03, P13, P08 | Ile1091Asn | P16, P01, P02, P03, P05, P06, P07, P13, P08, P11, P09, P10 | Phe1099Leu | P16, P01, P03, P07, P13, P08, P11, P12 |
| Ala547Asp | P16, P03, P13, P08 | Ile1091Met | P16, P01, P02, P03, P05, P06, P07, P13, P08, P11, P12, P09, P10 | Phe1099Ser | P16, P01, P03, P07, P13, P08, P11, P12 |
| Ala547Ser | P16, P03, P13, P08 | Ile1091Val | P16, P01, P02, P03, P05, P06, P07, P13, P08, P11, P09, P10 | Phe314Ala | P07, P13, P10 |
| Ala693_Glu694delinsMetLeu | P01, P03, P07, P13, P08, P15 | Ile1102Leu | P16, P01, P03, P04, P07, P17, P13, P08, P11, P12 | Phe314Ser | P07, P13, P10 |

**Table 3.** *Cont.*

| Mutation | Patient | Mutation | Patient | Mutation | Patient |
|---|---|---|---|---|---|
| Ala693Thr | P01, P03, P07, P13, P08, P15 | Ile1102Pro | P16, P01, P03, P04, P07, P17, P13, P08, P11, P12 | Phe314Val | P07, P13, P10 |
| Ala693Val | P01, P03, P07, P13, P08, P15 | Ile1102Thr | P16, P01, P03, P04, P07, P17, P13, P08, P11, P12 | Phe321Val | P07, P13, P10 |
| Ala703Pro | P01, P03, P07, P13, P08, P15 | Ile345Leu | P04, P07, P13, P10 | Phe325Leu | P07, P13, P10 |
| Ala703Val | P01, P03, P07, P13, P08, P15 | Ile508Asn | P03, P04, P17, P08 | Phe524Leu | P16, P03, P04, P17, P13, P08 |
| Arg1066Pro | P16, P01, P02, P03, P05, P06, P13, P08, P11, P09, P10 | Ile508Leu | P03, P04, P17, P08 | Phe524Tyr | P16, P03, P04, P17, P13, P08 |
| Arg1075_Ser1077delinsLysProGly | P16, P01, P02, P03, P05, P06, P07, P13, P08, P11, P09, P10 | Ile512_Lys513delinsArgGlu | P16, P03, P04, P17, P08 | Phe524Val | P16, P03, P04, P17, P13, P08 |
| Arg1075His | P16, P01, P02, P03, P05, P06, P07, P13, P08, P11, P09, P10 | Ile512_Lys513delinsSerGlu | P03, P08 | Phe551Ser | P16, P03, P13, P08, P15 |
| Arg1075Ser | P16, P01, P02, P03, P05, P06, P07, P13, P08, P11, P09, P10 | Ile512Ser | P16, P03, P04, P17, P08 | Phe558Ile | P16, P03, P13, P08, P15 |
| Arg501Leu | P03, P04, P17, P08 | Ile531Asn | P16, P03, P17, P13, P08 | Phe558Ser | P16, P03, P13, P08, P15 |
| Arg532Met | P16, P03, P17, P13, P08 | Ile531Leu | P16, P03, P04, P17, P13, P08 | Phe565Leu | P16, P18, P02, P03, P05, P06, P17, P13, P08, P11, P12, P15, P14, P09 |
| Arg532Phe | P16, P03, P17, P13, P08 | Ile704Phe | P01, P03, P07, P13, P08, P15 | Phe565Ser | P16, P18, P02, P03, P05, P06, P17, P13, P08, P11, P12, P15, P14, P09 |
| Arg532Ser | P16, P03, P17, P13, P08 | Ile704Thr | P01, P03, P07, P13, P08, P15 | Phe565Val | P16, P18, P02, P03, P05, P06, P17, P13, P08, P11, P12, P15, P14, P09 |

**Table 3.** *Cont.*

| Mutation | Patient | Mutation | Patient | Mutation | Patient |
|---|---|---|---|---|---|
| Arg532Trp | P16, P03, P17, P13, P08 | Leu1098_Val1101delins ProProValSer | P16, P01, P03, P07, P13, P08, P11, P12 | Pro1068Ala | P16, P01, P02, P03, P05, P06, P13, P08, P11, P09, P10 |
| Asn1071His | P16, P01, P02, P03, P05, P06, P07, P07, P13, P08, P11, P09, P10 | Leu1098Pro | P16, P01, P02, P02, P03, P05, P05, P06, P06, P07, P13, P08, P11, P12, P09, P09, P10, P10 | Pro1068Leu | P16, P01, P02, P03, P05, P06, P13, P08, P11, P09, P10 |
| Asn1071Lys | P16, P01, P02, P03, P05, P06, P07, P13, P08, P11, P09, P10 | Leu313Val | P07, P13, P10 | Pro1088Gln | P16, P01, P02, P03, P05, P06, P07, P13, P08, P11, P09, P10 |
| Asn1071Thr | P16, P01, P02, P03, P05, P06, P13, P08, P11, P09, P10 | Leu317Val | P07, P13, P10 | Pro1088Lys | P16, P01, P02, P03, P05, P06, P07, P13, P08, P11, P09, P10 |
| Asn1074Ile | P16, P01, P02, P03, P05, P06, P07, P13, P08, P11, P09, P10 | Leu504_Met505delinsHisLeu | P03, P04, P17, P08 | Pro1088Thr | P16, P01, P02, P03, P05, P06, P07, P13, P08, P11, P09, P10 |
| Asn1074Phe | P16, P01, P02, P03, P05, P06, P07, P13, P08, P11, P09, P10 | Leu504Gln | P03, P04, P17, P08 | Pro557_Phe558delinsArgThr | P16, P03, P13, P08, P15 |
| Asn1074Tyr | P16, P01, P02, P03, P05, P06, P07, P13, P08, P11, P09, P10 | Leu509Phe | P03, P04, P17, P08 | Pro557_Phe558delinsLeuThr | P16, P03, P13, P08, P15 |
| Asn1100Asp | P16, P01, P02, P03, P04, P06, P07, P17, P13, P08, P11, P12 | Leu515Ile | P16, P03, P04, P17, P08 | Pro557Leu | P16, P03, P13, P08, P15 |
| Asn1100Ile | P16, P01, P02, P03, P06, P07, P13, P08, P11, P12 | Leu515Pro | P16, P03, P04, P17, P08 | Ser1069Arg | P16, P01, P02, P03, P05, P06, P13, P08, P11, P09, P10 |
| Asn1100Lys | P16, P01, P03, P07, P13, P08, P11, P12 | Leu517Val | P16, P03, P04, P17, P08 | Ser1069Thr | P16, P01, P02, P03, P05, P06, P13, P08, P11, P09, P10 |

**Table 3.** *Cont.*

| Mutation | Patient | Mutation | Patient | Mutation | Patient |
|---|---|---|---|---|---|
| Asn1100Val | P02, P06 | Leu522Arg | P16, P16, P03, P03, P04, P04, P17, P17, P13, P13, P08, P08 | Ser1077Ala | P16, P01, P02, P03, P05, P06, P07, P13, P08, P11, P09, P10 |
| Asn310Asp | P07, P13, P10 | Leu529Met | P16, P03, P04, P17, P13, P08 | Ser1077Cys | P16, P01, P02, P03, P05, P06, P07, P13, P08, P11, P09, P10 |
| Asn500Asp | P03, P04, P17, P08 | Leu529Pro | P16, P03, P04, P17, P13, P08 | Ser1085Phe | P16, P01, P02, P03, P05, P06, P07, P13, P08, P11, P09, P10 |
| Asn500Ile | P03, P04, P17, P08 | Leu536_Val538delinsHisCysArg | P16, P03, P17, P13, P08 | Ser1097Cys | P16, P01, P02, P03, P05, P06, P07, P13, P08, P11, P12, P09, P10 |
| Asn500Lys | P03, P04, P17, P08 | Leu536Gln | P16, P03, P17, P13, P08 | Ser1097Trp | P16, P01, P02, P03, P05, P06, P07, P13, P08, P11, P12, P09, P10 |
| Asn506His | P03, P04, P17, P08 | Leu559Pro | P16, P03, P13, P08, P15 | Ser497_Arg501delins ArgCysSerValIle | P03, P04, P17, P08 |
| Asn506Ile | P03, P04, P17, P08 | Leu562_Phe565 delinsTerLysAspAla | P16, P18, P02, P03, P05, P06, P17, P13, P08, P11, P12, P15, P14, P09 | Ser497Arg | P03, P04, P17, P08 |
| Asn510His | P03, P04, P17, P08 | Leu691His | P01, P03, P07, P13, P08, P15 | Ser497Cys | P03, P04, P17, P08 |
| Asn510Pro | P03, P04, P17, P08 | Leu691Phe | P01, P03, P07, P13, P08, P15 | Ser542Leu | P16, P03, P17, P13, P08 |
| Asn510Thr | P03, P04, P17, P08 | Leu691Tyr | P01, P03, P07, P13, P08, P15 | Ser542Phe | P16, P03, P17, P13, P08 |
| Asp1081Phe | P16, P01, P02, P03, P05, P06, P07, P13, P08, P11, P09, P10 | Leu692Trp | P01, P03, P07, P13, P08, P15 | Ser542Pro | P16, P03, P17, P13, P08 |
| Asp1081Tyr | P16, P01, P02, P03, P05, P06, P07, P13, P08, P11, P09, P10 | Lys1078_Glu1079delinsAsnLys | P16, P01, P02, P03, P05, P06, P07, P13, P08, P11, P09, P10 | Ser546_Ala547delinsGlyTyr | P16, P03, P13, P08 |

**Table 3.** *Cont.*

| Mutation | Patient | Mutation | Patient | Mutation | Patient |
|---|---|---|---|---|---|
| Asp1081Val | P16, P01, P02, P03, P05, P06, P07, P13, P08, P11, P09, P10 | Lys1078Asn | P16, P01, P02, P03, P05, P06, P07, P13, P08, P11, P09, P10, P12 | Ser546Ala | P16, P03, P13, P08 |
| Asp1084Val | P16, P01, P02, P03, P05, P06, P07, P13, P08, P11, P09, P10 | Lys1092Asn | P16, P01, P02, P03, P05, P06, P07, P13, P08, P11, P12, P09, P10 | Thr1067_Ser1069delinsThrValPro | P16, P01, P02, P03, P05, P06, P13, P08, P11, P09, P10 |
| Asp292His | P07, P13, P10 | Lys1092Glu | P16, P01, P02, P03, P05, P06, P07, P13, P08, P11, P09, P10 | Thr1067Ala | P16, P01, P02, P03, P05, P06, P13, P08, P11, P09, P10 |
| Asp499Ala | P03, P04, P17, P08 | Lys1092Met | P16, P01, P02, P03, P05, P06, P07, P13, P08, P11, P09, P10 | Thr1082Arg | P16, P01, P02, P03, P05, P06, P07, P13, P08, P11, P09, P10 |
| Asp499Tyr | P03, P04, P17, P08 | Lys315Thr | P07, P13, P10 | Thr1082Ser | P16, P01, P02, P03, P05, P06, P07, P13, P08, P11, P09, P10 |
| Asp526Ala | P16, P03, P04, P17, P13, P08 | Lys319_Phe321delinsAsnLeuVal | P07, P13, P10 | Thr1242Leu | P01, P02, P03, P13, P11, P14 |
| Asp526Glu | P16, P03, P04, P17, P13, P08 | Lys319Asn | P07, P13, P10 | Thr1242Met | P16, P01, P02, P03, P07, P17, P13, P08, P11, P14, P10 |
| Asp526Tyr | P16, P03, P04, P17, P13, P08, P01, P03, P07, P13, P08, P15 | Lys319Glu | P07, P13, P10 | Thr1242Pro | P01, P02, P03, P13, P11, P14 |
| Asp696Tyr | P01, P03, P07, P13, P08, P15 | Lys496_Ser497delinsLeuCys | P03, P04, P17, P08 | Thr320Ile | P07, P13, P10 |
| Asp696Val | P01, P03, P07, P13, P08, P15 | Lys496Asn | P03, P04, P17, P08 | Thr320Pro | P07, P13, P10 |
| Cys555Arg | P16, P03, P13, P08, P15 | Lys496Gln | P03, P04, P17, P08 | Thr550Ala | P16, P03, P13, P08 |
| Cys555Ser | P16, P03, P13, P08, P15 | Lys496Met | P03, P04, P17, P08 | Thr550Gly | P16, P03, P13, P08 |

| Mutation | Patient | Mutation | Patient | Mutation | Patient |
|---|---|---|---|---|---|
| Cys563Ser | P16, P18, P02, P03, P05, P06, P17, P13, P08, P11, P12, P15, P14, P09 | Lys498Arg | P03, P04, P17, P08 | Thr550Ser | P16, P03, P13, P08, P15 |
| Cys563Trp | P16, P18, P02, P03, P05, P06, P17, P13, P08, P11, P12, P15, P14, P09 | Lys498Asn | P03, P04, P17, P08 | Thr552_Thr556delins TrpLeuArgProGly | P16, P03, P13, P08, P15 |
| Cys563Tyr | P16, P18, P02, P03, P05, P06, P17, P13, P08, P11, P12, P15, P14, P09 | Lys503Arg | P03, P04, P17, P08 | Thr552Ser | P16, P16, P03, P03, P13, P13, P08, P08, P15, P15 |
| Gln344_Ile345delinsHisLeu | P04, P07, P13, P10 | Lys503Asn | P03, P04, P17, P08 | Thr556Ala | P16, P03, P13, P08, P15 |
| Gln344His | P04, P07, P13, P10 | Lys503Glu | P03, P04, P17, P08 | Thr556Arg | P16, P03, P13, P08, P15 |
| Gln533_Glu535delinsHisLeuGln | P16, P03, P17, P13, P08 | Lys503Gly | P03, P04, P17, P08 | Thr564Ala | P16, P18, P02, P03, P05, P06, P17, P13, P08, P11, P12, P15, P14, P09 |
| Gln533His | P16, P03, P17, P13, P08 | Lys513_Leu515delins LysGlnThr | P16, P03, P04, P17, P08 | Thr564Lys | P16, P18, P02, P03, P05, P06, P17, P13, P08, P11, P12, P15, P14, P09 |
| Glu1065Asp | P16, P01, P02, P03, P05, P06, P13, P08, P11, P09, P10 | Lys513Glu | P16, P03, P04, P17, P08 | Trp309_Asn310delinsCysAsp | P07, P13, P10 |
| Glu1065Gly | P16, P01, P02, P03, P05, P06, P13, P08, P11, P09, P10 | Lys516Gln | P16, P03, P04, P17, P08 | Trp309Arg | P07, P13, P10 |
| Glu1079Lys | P16, P01, P02, P03, P05, P06, P07, P13, P08, P11, P09, P10 | Lys525Gln | P16, P03, P04, P17, P13, P08 | Trp309Cys | P07, P13, P10 |
| Glu1089_Met1093delins ArgProGluValLeu | P16, P01, P02, P03, P05, P06, P07, P13, P08, P11, P09, P10 | Lys525Met | P16, P03, P04, P17, P13, P08 | Trp520Arg | P16, P03, P04, P17, P08 |

**Table 3.** *Cont.*

| Mutation | Patient | Mutation | Patient | Mutation | Patient |
|---|---|---|---|---|---|
| Glu1089Gln | P16, P01, P02, P03, P05, P06, P07, P13, P08, P11, P09, P10 | Lys527Met | P16, P03, P04, P17, P13, P08 | Trp553Arg | P16, P03, P13, P08, P15 |
| Glu1089Gly | P16, P01, P02, P03, P05, P06, P07, P13, P08, P11, P09, P10 | Lys537Arg | P16, P03, P17, P13, P08 | Trp553Cys | P16, P03, P13, P08, P15 |
| Glu308_Trp309delinsAspArg | P07, P13, P10 | Lys537Asn | P16, P03, P17, P13, P08 | Trp553Leu | P16, P03, P13, P08, P15 |
| Glu308Asp | P07, P13, P10 | Lys540_Lys541delinsCysGly | P16, P03, P17, P13, P08 | Tyr1243Arg | P16, P01, P02, P03, P07, P17, P13, P08, P11, P14, P10 |
| Glu507_Ile508delinsCysHis | P03, P04, P17, P08 | Lys540Arg | P16, P03, P17, P13, P08 | Tyr1243Cys | P16, P01, P02, P03, P07, P17, P13, P08, P11, P14, P10 |
| Glu507Asp | P03, P04, P17, P08 | Lys540Asn | P16, P03, P17, P13, P08 | Tyr1243His | P16, P01, P02, P03, P07, P17, P13, P08, P11, P14, P10 |
| Glu507Gly | P03, P04, P17, P08 | Lys541Arg | P16, P03, P17, P13, P08 | Tyr518_Ala519delinsTerThr | P16, P03, P04, P17, P08 |
| Glu521Asp | P16, P03, P04, P17, P13, P08 | Lys541Glu | P16, P03, P17, P13, P08 | Tyr544His | P16, P03, P13, P08 |
| Glu534Asp | P16, P03, P17, P13, P08 | Lys697Asn | P01, P03, P07, P13, P08, P15 | Tyr568Ser | P02, P03, P05, P13, P08, P09 |
| Glu534Gln | P16, P03, P17, P13, P08 | Lys697Gln | P01, P03, P07, P13, P08, P15 | Val1073Leu | P16, P01, P02, P03, P05, P06, P07, P13, P08, P11, P09, P10 |
| Glu534Val | P16, P03, P17, P13, P08 | Lys697Thr | P01, P03, P07, P13, P08, P15 | Val1083Gly | P16, P01, P02, P03, P05, P06, P07, P13, P08, P11, P09, P10 |
| Glu535Gln | P16, P03, P17, P13, P08 | Lys705Arg | P01, P03, P07, P13, P08, P15 | Val1090Ala | P16, P01, P02, P03, P05, P06, P07, P13, P08, P11, P09, P10 |

**Table 3.** *Cont.*

| Mutation | Patient | Mutation | Patient | Mutation | Patient |
|---|---|---|---|---|---|
| Glu694Gln | P01, P03, P07, P13, P08, P15 | Lys705Glu | P01, P03, P07, P13, P08, P15 | Val1090Leu | P16, P01, P02, P03, P05, P06, P07, P13, P08, P11, P09, P10 |
| Glu694Val | P01, P03, P07, P13, P08, P15 | Met1086_Ile1087delinsSerPro | P16, P01, P02, P03, P05, P06, P07, P13, P08, P11, P09, P10 | Val1101Ala | P16, P01, P03, P04, P07, P17, P13, P08, P11, P12 |
| Glu699_Gly700delinsGlyHis | P01, P03, P07, P13, P08, P15 | Met1086Ile | P16, P01, P02, P03, P05, P06, P07, P13, P08, P11, P09, P10 | Val1101Phe | P16, P01, P03, P07, P13, P08, P11, P12 |
| Glu699Asp | P01, P03, P07, P13, P08, P15 | Met1086Leu | P16, P01, P02, P03, P05, P06, P07, P13, P08, P11, P09, P10 | Val1240Gly | P01, P02, P03, P13, P11, P14 |
| Glu699Gly | P01, P03, P07, P13, P08, P15 | Met1086Thr | P16, P01, P02, P03, P05, P06, P07, P13, P08, P11, P09, P10 | Val316Ala | P07, P13, P10 |
| Gly1070_Asn1071delinsHisPro | P16, P01, P02, P03, P05, P06, P13, P08, P11, P09, P10 | Met1093Ile | P16, P01, P02, P03, P05, P06, P07, P13, P08, P11, P09, P10 | Val492Gly | P03, P04, P17, P08 |
| Gly1070Arg | P16, P01, P02, P03, P05, P06, P13, P08, P11, P09, P10 | Met1093Leu | P16, P01, P02, P03, P05, P06, P07, P13, P08, P11, P09, P10 | Val492Met | P03, P04, P17, P08 |
| Gly1070Glu | P16, P01, P02, P03, P05, P06, P13, P08, P11, P09, P10 | Met1095Ile | P16, P01, P02, P03, P05, P06, P07, P13, P08, P11, P09, P10 | Val514Glu | P16, P03, P04, P17, P08 |
| Gly1096Arg | P16, P01, P02, P03, P05, P06, P07, P13, P08, P11, P12, P09, P10 | Met1095Leu | P16, P01, P02, P03, P05, P06, P07, P13, P08, P11, P09, P10 | Val514Leu | P16, P03, P04, P17, P08 |
| Gly1096Asp | P16, P01, P02, P03, P05, P06, P07, P13, P08, P11, P12, P09, P10 | Met1095Thr | P16, P01, P02, P03, P05, P06, P07, P13, P08, P11, P12, P09, P10 | Val528_Leu529delinsThrThr | P16, P03, P04, P17, P13, P08 |

**Table 3.** *Cont.*

| Mutation | Patient | Mutation | Patient | Mutation | Patient |
|---|---|---|---|---|---|
| Gly1096Gln | P12 | Met495Arg | P03, P03, P04, P04, P17, P17, P08, P08 | Val528Ala | P16, P03, P04, P17, P13, P08 |
| Gly1103Ala | P16, P01, P03, P04, P07, P17, P13, P08, P11, P12 | Met495Leu | P03, P04, P17, P08 | Val528Met | P16, P03, P04, P17, P13, P08 |
| Gly1103Arg | P16, P01, P03, P04, P07, P17, P13, P08, P11, P12 | Met505_Asn506delinsIleLeu | P03, P04, P17, P08 | Val538Gly | P16, P03, P17, P13, P08 |
| Gly1103Pro | P16, P01, P03, P04, P07, P17, P13, P08, P11, P12 | Met505Ile | P03, P04, P17, P08 | Val538Met | P16, P03, P17, P13, P08 |
| Gly511Ala | P03, P04, P17, P08 | Met505Leu | P03, P04, P17, P08 | Val548_Gly549delinsGlyCys | P16, P03, P13, P08 |
| Gly511Arg | P03, P04, P17, P08 | Met695_Lys697delinsSerPhePro | P01, P03, P07, P13, P08, P15 | Val548Gly | P16, P03, P13, P08 |
| Gly511Pro | P03, P03, P04, P17, P08, P08 | Met695Ile | P01, P03, P07, P13, P08, P15 | Val554Gly | P16, P03, P13, P08, P15 |
| Gly549Cys | P16, P03, P13, P08 | Met695Leu | P01, P03, P07, P13, P08, P15 | Val554Ile | P16, P03, P13, P08, P15 |
| Gly700Arg | P01, P03, P07, P13, P08, P15 | Met695Thr | P01, P03, P07, P13, P08, P15 | Val698Ala | P01, P03, P07, P13, P08, P15 |
| Gly700Glu | P01, P03, P07, P13, P08, P15 | Phe1064_Thr1067delins GlyCysThrAla | P16, P01, P02, P03, P05, P06, P13, P08, P11, P09, P10 | Val702_Lys705delinsAlaLeuSerGly | P01, P03, P07, P13, P08, P15 |
| | | | | Val702Ala | P01, P03, P07, P13, P08, P15 |

14 of 20

For visualization of these low-frequency nonsense mutations, their read values and the corresponding alignments are depicted in Figure 1.
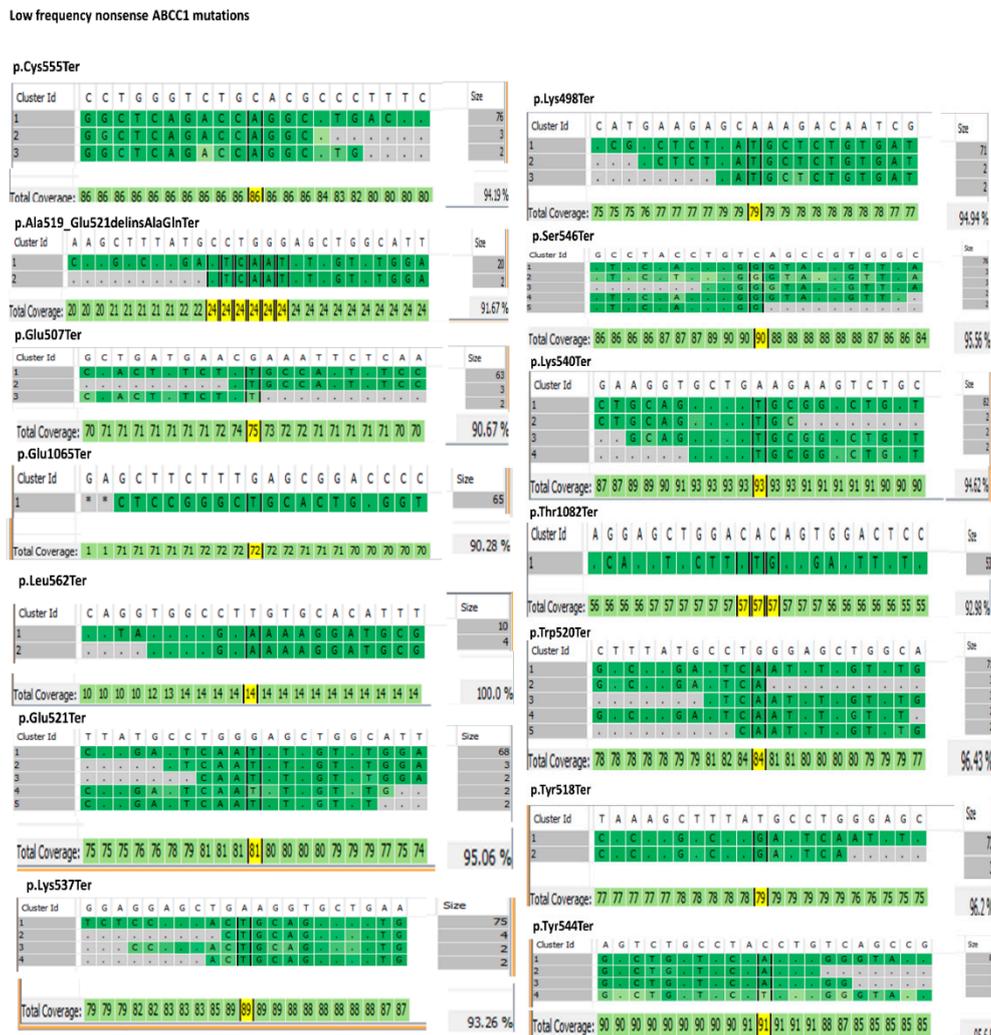


**Figure 1.** Low-frequency nonsense *ABCC1* mutation sequence reads and the corresponding alignments. Reference genome sequence (gray) is on top of each screenshot. Sequence reads from the tumor biopsy with different clusters are labeled in green. Read numbers of each cluster sequence for that region are shown on the right side of each screenshot. On the bottom right side, the coverage percentage for the shown cluster reads is depicted for that region. One patient for each mutation was selected for visualization.

## 3.2. Structural Comparison of the Nonsense MRP1 Mutant Models

Low-frequency *ABCC1* nonsense mutation caused truncated MRP1 protein structures. A structural comparison of each low-frequency nonsense mutant with the wild-type MRP1 structure is visualized in Figure 2.
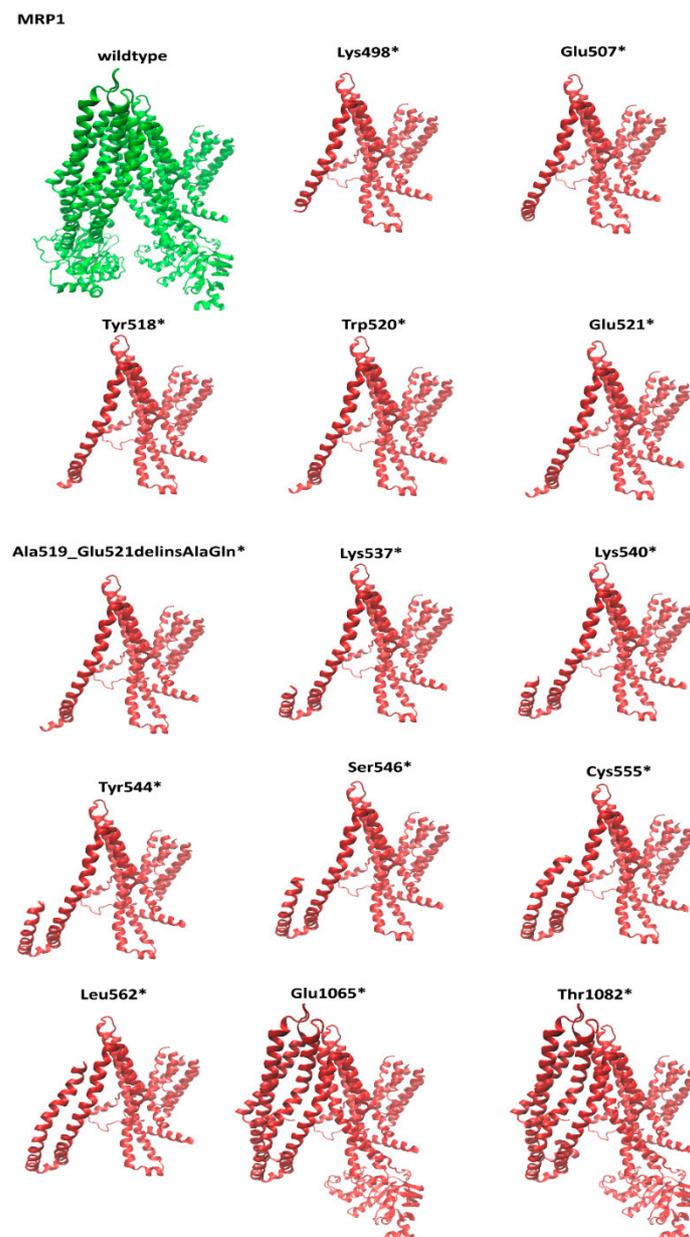
**Figure 2.** Influence of nonsense mutations on the *ABCC1* gene and structural comparison with wild-type MRP1 protein structure. Wild-type MRP1 protein (green) and mutant MRP1 homology models (red).

### 3.3. Molecular Docking Clustering

Hierarchical clustering and heat maps are visualized for MRP1 inhibitors in Figure 3A and substrates in Figure 3B. For both inhibitors and substrates, Lys498* appeared in a separate cluster branch as expected due to the large deletion, leading to a massive disruption in the conformation. T550A and T556A mutants clustered together on the heat map for both inhibitors and substrates, indicating that both mutants have the same pattern of conformation. Interestingly, the V1101F mutant clustered with the wild type for inhibitors and clustered in close distance for substrates, suggesting this mutant has little effect on the MRP1 transport function. The inhibitor Mk-571 showed higher affinity to the mutants than the wild type. Glibenclamide and pak104p revealed lower binding energies for the T550A and T556A mutants than for the wild type and V1101F mutant. Intriguingly, epirubicin showed higher affinity to the nonsense-mutated and truncated MRP1 than the wild type and the other mutants.

This could be attributed to a unique binding site that might be reachable for epirubicin in the deleted form of MRP1.
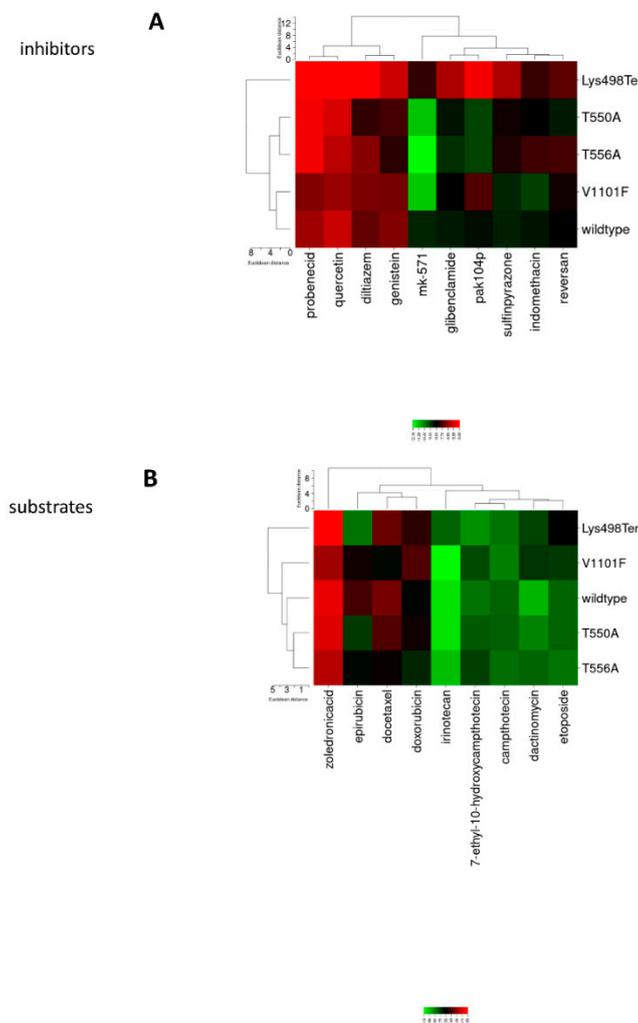


**Figure 3.** Heat maps for the docking analyses of (**A**) MRP1 inhibitors, (**B**) MRP1 substrates (green color: strong interaction; red color: weak interaction) on wild-type and mutant MRP1.

## 4. Discussion

The development of MDR phenotypes is a major obstacle in the treatment of malignancies as it leads to therapeutic failure. Overexpression of *ABCC1* is considered as one of the mechanisms that elicits MDR development in cancer cells due to its capacity to expel the chemotherapeutic drugs from the cells [33]. MRP1 localizes primarily at the plasma membrane of the cells and in the membranes of sub-cellular organelles such as mitochondria, endoplasmic reticulum and endocytic vesicles [34]. *ABCC1* mRNA overexpression has been reported in small-cell lung carcinoma [35], prostate [36], neuroblastoma [37], acute lymphoblastic leukemia [38], breast [39], and glioblastoma [25].

In the present study, we performed rare mutation analyses for 12 *ABC* transporters related to drug resistance (ABCA2, -A3, -B1, -B2, -B5, -C1, -C2, -C3, -C4, -C5, -C6, -G2) in a dataset consisting of RNA sequencing data from 18 cancer patients. Novel rare mutations were found only in *ABCC1*. Low-frequency *ABCC1* mutations have been identified, which reflect tumor heterogeneity and the presence of various missense and nonsense mutations at the *ABCC1* coding region for each patient. Cases with a large deletion as result of a nonsense mutation were characterized by dramatic structural changes. These kind of nonsense mutations presumably led to aberrant structures and thus nonfunctional or only partially functional MRP1 transporters. As a result, MRP1 substrates could not be recognized by

the transporter anymore and could remain within the tumor cells without being expelled. This implies a novel personalized chemotherapy approach in those cases, where nonsense mutations in *ABCC1* are determined. Tumors with nonfunctional/partially functional MRP1 due to the presence of premature stop codon formations may be more sensitive towards anticancer substrates of MRP1, but normal tissues expressing wild-type *ABCC1*/MRP1 are not sensitized at the same time, because these nonsense mutations occur as somatic mutations only in tumor cells, but not as germline mutations in normal tissues. The identification of *ABCC1*/MRP1 nonsense mutations, therefore, could be critical in this regard and could take place on an individual basis by sequencing each tumor transcriptome.

All identified nonsense mutations in our patient dataset are novel. These results considerably expand upon the findings of Yin et al. [40] in analyzing the role of MRP1 polymorphisms in drug resistance, toxicity, and prognosis prediction. They identified only 14 non-synonymous polymorphisms with very low frequencies, but no nonsense polymorphism has been found [40]. Single nucleotide polymorphisms occur in healthy tissues at variable high percentages and may therefore also occur in tumors derived from these normal tissues. The results we obtained in our analysis are somatic mutations in tumors. Saito et al. reported 95 genetic variations in *ABCC1* out of the identified 779 genetic variations in eight ABC genes in the Japanese population; only one of them is a missense mutation (R723Q) and none of them are nonsense mutations [41]. Fukushima-Ueseka et al. identified 86 genetic variations in *ABCC1*, including 31 novel variations in the same population, of which 11 were missense and none were nonsense mutations [42]. Leschziner et al. screened five ABC transporter genes in a Caucasian population and observed 61 out of 221 variations are in *ABCC1*, among which 22 are novel [43]. In our study, we focused on rare missense, del/ins, and nonsense mutations. We identified 14 novel, rare *ABCC1* nonsense mutations (Table 2) and 301 novel, rare *ABCC1* missense and del/ins mutations (Table 3).

As shown by our molecular docking results, the truncated *ABCC1*/MRP1 forms revealed different patterns of drug binding than the wild type and other mutants for specific MRP1 inhibitors and substrates, because they clearly appeared as a separate cluster. Importantly, the known MRP1 inhibitor, Mk-571, showed higher affinity to missense mutations than wild-type MRP1. Combining MRP1 chemotherapeutic substrates with Mk-571 might be a favorable therapeutic option for patients whose *ABCC1* gene bears these missense mutations.

The two polar residues, Thr550 and Thr556, located in the inner leaflet region of TM10, play a vital role in determining the drug resistance profile [29]. Both residues were mutated and led to an amino acid exchange and placement of alanine. Increased resistance to vincristine and decreased resistance to doxorubicin and etoposide were observed in the T550A and T556A mutations which was attributed to the smaller size of the Ala side chain that favors transport of the larger drug [29]. Moreover, our heat map analyses based on the molecular docking results clearly showed that these two missense mutations were clustered together and had the same pattern of substrate/inhibitor bindings. To the best of our knowledge, this is the first time to report those two missense mutations in clinical samples. Since both mutations are determinants for drug resistance, this warrants further care in the selection of chemotherapeutic agents for patients bearing these mutations.

In conclusion, a common assumption is that the low-frequency-mutation analyses may have more prognostic significant values for driver mutations [44]. Hence, mutations in driver genes have been mainly in the spotlight. In the present investigation, we intentionally did not focus on driver mutations that are important for carcinogenesis and tumor progression. Instead, we concentrated on ABC transporters as important mediators of chemotherapeutic drug resistance. In fact, we found that pointing to nonsense mutations in ABC transporters may offer a unique opportunity for precision medicine. Nonsense mutations in ABC transporters lead to truncated and nonfunctional proteins offering unique chances to sensitize tumor patients to chemotherapy. RNA-sequencing of tumor transcriptomes on an individual patient-to-patient basis may, therefore, unravel opportunities to successfully treat otherwise refractory cancer patients with drugs that are substrates of specific ABC transporters.

Furthermore, rare mutations contribute to intratumoral heterogeneity and sub-clonal diversity for primary and metastatic tumors. Therefore, identification of rare mutations in ABC transporters, coupled with knowledge of protein structure and functional insight, may allow the design of novel and appropriate strategies for individualized cancer treatment.

## Abbreviations

| | |
|---|---|
| ABC | ATP-binding cassette transporter |
| MRP | multidrug resistance-associated protein |

## References

1. Efferth, T.; Zeino, M.; Volm, M. Modulation of P-Glycoprotein-Mediated Multidrug Resistance by Synthetic and Phytochemical Small Molecules, Monoclonal Antibodies, and Therapeutic Nucleic Acids. In *Resistance to Targeted ABC Transporters in Cancer*; Efferth, T., Ed.; Springer: Cham, Switzerland, 2015; pp. 153–181. [CrossRef]

2. Volm, M.; Efferth, T. Role of P-Glycoprotein for Resistance of Tumors to Anticancer Drugs: From Bench to Bedside. In *Resistance to Targeted ABC Transporters in Cancer*; Efferth, T., Ed.; Springer: Cham, Switzerland, 2015; pp. 1–26. [CrossRef]

3. Roundhill, E.A.; Fletcher, J.I.; Haber, M.; Norris, M.D. Clinical Relevance of Multidrug-Resistance-Proteins (MRPs) for Anticancer Drug Resistance and Prognosis. In *Resistance to Targeted ABC Transporters in Cancer*; Efferth, T., Ed.; Springer: Cham, Switzerland, 2015; pp. 27–52. [CrossRef]

4. Natarajan, K.; Baer, M.R.; Ross, D.D. Role of Breast Cancer Resistance Protein (BCRP, ABCG2) in Cancer Outcomes and Drug Resistance. In *Resistance to Targeted ABC Transporters in Cancer*; Efferth, T., Ed.; Springer: Cham, Switzerland, 2015; pp. 53–88. [CrossRef]

5. Frampton, G.M.; Fichtenholtz, A.; Otto, G.A.; Wang, K.; Downing, S.R.; He, J.; Schnall-Levin, M.; White, J.; Sanford, E.M.; An, P.; et al. Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing. *Nat. Biotechnol.* **2013**, *31*, 1023–1031. [CrossRef] [PubMed]

6. Conley, B.A.; Doroshow, J.H. Molecular analysis for therapy choice: NCI MATCH. *Semin. Oncol.* **2014**, *41*, 297–299. [CrossRef] [PubMed]

7. Zhao, X.; Little, P.; Hoyle, A.P.; Pegna, G.J.; Hayward, M.C.; Ivanova, A.; Parker, J.S.; Marron, D.L.; Soloway, M.G.; Jo, H.; et al. The Prognostic Significance of Low-Frequency Somatic Mutations in Metastatic Cutaneous Melanoma. *Front. Oncol.* **2018**, *8*, 584. [CrossRef] [PubMed]

8. Liu, J.; Dang, H.; Wang, X.W. The significance of intertumor and intratumor heterogeneity in liver cancer. *Exp. Mol. Med.* **2018**, *50*, e416. [CrossRef] [PubMed]

9. Saunders, N.A.; Simpson, F.; Thompson, E.W.; Hill, M.M.; Endo-Munoz, L.; Leggatt, G.; Minchin, R.F.; Guminski, A. Role of intratumoral heterogeneity in cancer drug resistance: Molecular and clinical perspectives. *EMBO Mol. Med.* **2012**, *4*, 675–684. [CrossRef] [PubMed]

10. Lusito, E.; Felice, B.; D'Ario, G.; Ogier, A.; Montani, F.; Di Fiore, P.P.; Bianchi, F. Unraveling the role of low-frequency mutated genes in breast cancer. *Bioinformatics* **2019**, *35*, 36–46. [CrossRef]

11. Leitner, H.M.; Kachadourian, R.; Day, B.J. Harnessing drug resistance: Using ABC transporter proteins to target cancer cells. *Biochem. Pharm.* **2007**, *74*, 1677–1685. [CrossRef]

12. Dharmapuri, G.; Doneti, R.; Philip, G.H.; Kalle, A.M. Celecoxib sensitizes imatinib-resistant K562 cells to imatinib by inhibiting MRP1-5, ABCA2 and ABCG2 transporters via Wnt and Ras signaling pathways. *Leuk. Res.* **2015**, *39*, 696–701. [CrossRef]

13. Steinbach, D.; Gillet, J.P.; Sauerbrey, A.; Gruhn, B.; Dawczynski, K.; Bertholet, V.; de Longueville, F.; Zintl, F.; Remacle, J.; Efferth, T. ABCA3 as a possible cause of drug resistance in childhood acute myeloid leukemia. *Clin. Cancer Res.* **2006**, *12*, 4357–4363. [CrossRef]

14. Xu, M.; Li, L.; Liu, Z.Q.; Jiao, Z.J.; Xu, P.; Kong, X.Y.; Huang, H.J.; Zhang, Y.L. ABCB2 (TAP1) as the downstream target of SHH signaling enhances pancreatic ductal adenocarcinoma drug resistance. *Cancer Lett.* **2013**, *333*, 152–158. [CrossRef]

15. Kawanobe, T.; Kogure, S.; Nakamura, S.; Sato, M.; Katayama, K.; Mitsuhashi, J.; Noguchi, K.; Sugimoto, Y. Expression of human ABCB5 confers resistance to taxanes and anthracyclines. *Biochem. Biophys. Res. Commun.* **2012**, *418*, 736–741. [CrossRef] [PubMed]

16. Kadioglu, O.; Cao, J.; Kosyakova, N.; Mrasek, K.; Liehr, T.; Efferth, T. Genomic and transcriptomic profiling of resistant CEM/ADR-5000 and sensitive CCRF-CEM leukemia cells for unravelling the full complexity of multi-factorial multidrug resistance. *Sci. Rep.* **2016**, *6*, 36754. [CrossRef] [PubMed]

17. Tajima, Y.; Nakagawa, H.; Tamura, A.; Kadioglu, O.; Satake, K.; Mitani, Y.; Murase, H.; Regasini, L.O.; Bolzani, V.D.; Ishikawa, T.; et al. Nitensidine A, a guanidine alkaloid from Pterogyne nitens, is a novel substrate for human ABC transporter ABCB1. *Phytomed. Int. J. Phytother. Phytopharm.* **2014**, *21*, 323–332. [CrossRef] [PubMed]

18. de Groot, D.J.; van der Deen, M.; Le, T.K.; Regeling, A.; de Jong, S.; de Vries, E.G. Indomethacin induces apoptosis via a MRP1-dependent mechanism in doxorubicin-resistant small-cell lung cancer cells overexpressing MRP1. *Br. J. Cancer* **2007**, *97*, 1077–1083. [CrossRef] [PubMed]

19. Hill, C.R.; Jamieson, D.; Thomas, H.D.; Brown, C.D.; Boddy, A.V.; Veal, G.J. Characterization of the roles of ABCB1, ABCC1, ABCC2 and ABCG2 in the transport and pharmacokinetics of actinomycin D in vitro and in vivo. *Biochem. Pharmacol.* **2013**, *85*, 29–37. [CrossRef]

20. Burkhart, C.A.; Watt, F.; Murray, J.; Pajic, M.; Prokvolit, A.; Xue, C.; Flemming, C.; Smith, J.; Purmal, A.; Isachenko, N.; et al. Small-molecule multidrug resistance-associated protein 1 inhibitor reversan increases the therapeutic index of chemotherapy in mouse models of neuroblastoma. *Cancer Res.* **2009**, *69*, 6573–6580. [CrossRef]

21. Ebert, R.; Meissner-Weigl, J.; Zeck, S.; Maatta, J.; Auriola, S.; Coimbra de Sousa, S.; Mentrup, B.; Graser, S.; Rachner, T.D.; Hofbauer, L.C.; et al. Probenecid as a sensitizer of bisphosphonate-mediated effects in breast cancer cells. *Mol. Cancer* **2014**, *13*, 265. [CrossRef]

22. Deeley, R.G.; Cole, S.P. Substrate recognition and transport by multidrug resistance protein 1 (ABCC1). *FEBS Lett.* **2006**, *580*, 1103–1111. [CrossRef]

23. Van Zanden, J.J.; van der Woude, H.; Vaessen, J.; Usta, M.; Wortelboer, H.M.; Cnubben, N.H.; Rietjens, I.M. The effect of quercetin phase II metabolism on its MRP1 and MRP2 inhibiting potential. *Biochem. Pharmacol.* **2007**, *74*, 345–351. [CrossRef]

24. Vulsteke, C.; Lambrechts, D.; Dieudonne, A.; Hatse, S.; Brouwers, B.; van Brussel, T.; Neven, P.; Belmans, A.; Schoffski, P.; Paridaens, R.; et al. Genetic variability in the multidrug resistance associated protein-1 (ABCC1/MRP1) predicts hematological toxicity in breast cancer patients receiving (neo-)adjuvant chemotherapy with 5-fluorouracil, epirubicin and cyclophosphamide (FEC). *Ann. Oncol. Off. J. Eur. Soc. Med. Oncol.* **2013**, *24*, 1513–1525. [CrossRef]

25. Tivnan, A.; Zakaria, Z.; O'Leary, C.; Kogel, D.; Pokorny, J.L.; Sarkaria, J.N.; Prehn, J.H. Inhibition of multidrug resistance protein 1 (MRP1) improves chemotherapy drug response in primary and recurrent glioblastoma multiforme. *Front. Neurosci.* **2015**, *9*, 218. [CrossRef] [PubMed]

26. Brangi, M.; Litman, T.; Ciotti, M.; Nishiyama, K.; Kohlhagen, G.; Takimoto, C.; Robey, R.; Pommier, Y.; Fojo, T.; Bates, S.E. Camptothecin resistance: Role of the ATP-binding cassette (ABC), mitoxantrone-resistance half-transporter (MXR), and potential for glucuronidation in MXR-expressing cells. *Cancer Res.* **1999**, *59*, 5938–5946. [PubMed]

27. Castro, A.F.; Altenberg, G.A. Inhibition of drug transport by genistein in multidrug-resistant cells expressing P-glycoprotein. *Biochem. Pharmacol.* **1997**, *53*, 89–93. [CrossRef]

28. Marbeuf-Gueye, C.; Salerno, M.; Quidu, P.; Garnier-Suillerot, A. Inhibition of the P-glycoprotein- and multidrug resistance protein-mediated efflux of anthracyclines and calceinacetoxymethyl ester by PAK-104P. *Eur. J. Pharmacol.* **2000**, *391*, 207–216. [CrossRef]

29. Zhang, D.W.; Nunoya, K.; Vasa, M.; Gu, H.M.; Cole, S.P.C.; Deeley, R.G. Mutational analysis of polar amino acid residues within predicted transmembrane helices 10 and 16 of multidrug resistance protein 1 (ABCC1): Effect on substrate specificity. *Drug Metab. Dispos.* **2006**, *34*, 539–546. [CrossRef]

30. Kadioglu, O.; Saeed, M.E.M.; Valoti, M.; Frosini, M.; Sgaragli, G.; Efferth, T. Interactions of human P-glycoprotein transport substrates and inhibitors at the drug binding domain: Functional and molecular docking analyses. *Biochem. Pharm.* **2016**, *104*, 42–51. [CrossRef]

31. Kadioglu, O.; Saeed, M.; Kuete, V.; Greten, H.J.; Efferth, T. Oridonin Targets Multiple Drug-Resistant Tumor Cells as Determined by in Silico and in Vitro Analyses. *Front. Pharmacol.* **2018**, *9*. [CrossRef]

32. Ooko, E.; Kadioglu, O.; Greten, H.J.; Efferth, T. Pharmacogenomic Characterization and Isobologram Analysis of the Combination of Ascorbic Acid and Curcumin-Two Main Metabolites of Curcuma longa-in cancer Cells. *Front. Pharmacol.* **2017**, *8*. [CrossRef]

33. Robey, R.W.; Pluchino, K.M.; Hall, M.D.; Fojo, A.T.; Bates, S.E.; Gottesman, M.M. Revisiting the role of ABC transporters in multidrug-resistant cancer. *Nat. Rev. Cancer* **2018**, *18*, 452–464. [CrossRef]

34. Roundhill, E.A.; Burchill, S.A. Detection and characterisation of multi-drug resistance protein 1 (MRP-1) in human mitochondria. *Br. J. Cancer* **2012**, *106*, 1224–1233. [CrossRef]

35. Ji, L.L.; Li, H.; Gao, P.; Shang, G.G.; Zhang, D.N.D.; Zhang, N.; Jiang, T. Nrf2 Pathway Regulates Multidrug-Resistance-Associated Protein 1 in Small Cell Lung Cancer. *PLoS ONE* **2013**, *8*. [CrossRef] [PubMed]

36. Liu, C.; Li, Z.H.; Bi, L.K.; Li, K.Q.; Zhou, B.F.; Xu, C.; Huang, J.; Xu, K.W. NOTCH1 signaling promotes chemoresistance via regulating ABCC1 expression in prostate cancer stem cells. *Mol. Cell. Biochem.* **2014**, *393*, 265–270. [CrossRef] [PubMed]

37. Yu, D.M.T.; Huynh, T.; Truong, A.M.; Haber, M.; Norris, M.D. ABC Transporters and Neuroblastoma. *Adv. Cancer Res.* **2015**, *125*, 139–170. [CrossRef] [PubMed]

38. Bakos, E.; Homolya, L. Portrait of multifaceted transporter, the multidrug resistance-associated protein 1 (MRP1/ABCC1). *Pflug. Arch. Eur. J. Physiol.* **2007**, *453*, 621–641. [CrossRef] [PubMed]

39. Abaan, O.D.; Mutlu, P.K.; Baran, Y.; Atalay, C.; Gunduz, U. Multidrug resistance mediated by MRP1 gene overexpression in breast cancer patients. *Cancer Investig.* **2009**, *27*, 201–205. [CrossRef] [PubMed]

40. Yin, J.; Zhang, J. Multidrug resistance-associated protein 1 (MRP1/ABCC1) polymorphism: From discovery to clinical application. *Zhong Nan Da Xue Xue Bao Yi Xue Ban* **2011**, *36*, 927–938. [CrossRef] [PubMed]

41. Saito, S.; Iida, A.; Sekine, A.; Miura, Y.; Ogawa, C.; Kawauchi, S.; Higuchi, S.; Nakamura, Y. Identification of 779 genetic variations in eight genes encoding members of the ATP-binding cassette, subfamily C (ABCC/MRP/CFTR. *J. Hum. Genet.* **2002**, *47*, 147–171. [CrossRef]

42. Fukushima-Uesaka, H.; Saito, Y.; Tohkin, M.; Maekawa, K.; Hasegawa, R.; Kawamoto, M.; Kamatani, N.; Suzuki, K.; Yanagawa, T.; Kajio, H.; et al. Genetic variations and haplotype structures of the ABC transporter gene ABCC1 in a Japanese population. *Drug Metab. Pharm.* **2007**, *22*, 48–60. [CrossRef]

43. Leschziner, G.; Zabaneh, D.; Pirmohamed, M.; Owen, A.; Rogers, J.; Coffey, A.J.; Balding, D.J.; Bentley, D.B.; Johnson, M.R. Exon sequencing and high resolution haplotype analysis of ABC transporter genes implicated in drug resistance. *Pharm. Genom.* **2006**, *16*, 439–450. [CrossRef]

44. Nussinov, R.; Tsai, C.J.; Jang, H. Why Are Some Driver Mutations Rare? *Trends Pharmacol. Sci.* **2019**, *40*, 919–929. [CrossRef]