SUMMARY REPORT

NKFIH – grant 124431

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Short summary

In the present translational research, we comparatively assessed on the protein (LC-MS/MS) and DNA (whole exome sequencing) level prostate cancer cell lines that are resistant vs. sensitive to the three most commonly used first-line treatments (docetaxel, abiraterone and enzalutamide) in metastatic castration-resistant prostate cancer. From the large number of differentially expressed proteins we selected >20 proteins for quantitative determination in pre- and on-treatment serum samples of patients who underwent respective therapies. Using this approach, we identified proteins independently associated with response and survival under docetaxel- (n=5), abiraterone- (n=3) and enzalutamide-treated (n=3) mCRPC patients. Our *in vitro* analyses found CD44 and ALCAM to be functionally involved in docetaxel and enzalutamide resistance, respectively.

Additionally, our comparative exome sequencing identified a large number of genetic alterations (SNVs, CNVs) potentially involved in resistance to the above three therapies. If their functional involvement could successfully be validated in subsequent analyses, these alterations may ideally supplement sequencing panels that we just developed for routine use for metastatic prostate cancer patients.

Overall, our results will potentially contribute to the improvement of therapeutic decision-making regarding the type and timing of systemic treatment of metastatic castration-resistant prostate cancer.

Rövid összefoglaló

Jelen transzlációs kutatásunk során a metasztatikus prosztatarákban leggyakrabban használt szisztémás kezelésekre (docetaxel, abirateron, enzalutamid) érzékeny és rezisztens prosztatatrák sejtek összehasonlító protein (LC-MS/MS) és DNS (exom szekvenálás) szintű vizsgálatát végeztük el. Az így azonosított nagy számú fehérje közül több, mint 20 fehérje mennyiségi vizsgálatát végeztük el az adott terápiának alávetett betegek kezelés előtt és alatt vett szérum mintáiban. Ìgy a docetaxel esetében 5, az abirateron és az enzalutamid esetében pedig 3-3 olyan fehérjét találtunk, melyek az adott kezelésre adott terápiás válasz és túlélés független prognosztikai tényezőinek bizonyultak. In vitro vizsgálataink rámutattak, hogy a docetaxel esetében a CD44, míg az enzalutamid esetében az ALCAM funkcionális szerepet tölt be a rezisztencia kialakításában.

Összehasonlító exom szekvenálási adataink nagy számú genetiakai eltérést (SNV/CNV) azonosítottak, melyek szerepet játszhatnak a fenti terápiákkal szembeni rezisztencia kialakításában. Amennyiben az itt azonosított eltérések a rezisztenciában történő funkcionális érintettsége későbbi vizsglatokban igazolódik, akkor az érintett gének jól kiegészíthetik azt a génpanelt, melyet a közelmúltban terveztünk a metasztatikus prosztatarák genetikai eltéréseinek rutinszerű vizsgálatára.

Összeségében, eredményeink hozzájárulhatnak a metasztatikus prosztatarák kezelésének helyes megválasztásához, ezáltal javítva a kezelés hatékonyságát.

Detailed summary

1. Literature review

1.1 Molecular patterns of local and progressed prostate cancer

In the last few years, the emergence of new high throghput molecular technologies allowed a never-before-seen insight into the genetic, epigenetic, transcriptomic and proteomic background of prostate cancer (PC). As a result of this development the most frequent molecular alterations and affected pathways responsible for the formation and progression of PC have been identified. Therefore, in order to place our research in an up-to-date context, we prepared a comprehensive review on current progress in primary and metastatic prostate cancer (PC) research focusing on the molecular subtype classification and the most frequently dysregulated pathways, such as androgen signaling, PI3K pathway, cell cycle and DNA repair regulation. We highlighted therapies already approved or going through clinical investigation for PC.

Accepted publication Nr. 1: This review has been published in Orvosi Hetilap (Q3, IF=0.322).

1.2 Molecular patterns of local and progressed prostate cancer

Due to current development in the therapeutic landscape of metastatic castration-resistant prostate cancer (mCRPC) therapeutic decision-making has become increasingly complex. This development makes it important to understanding of resistance mechanisms to each therapy. In order to provide an overview on this rapidly improving field, we prepared a less detailed review with focus on clinical aspects (in Hungarian) and a more comprehensive molecular review (in English). In these papers, we provided an overview on the resistance mechanisms against the most frequently applied systemic treatments of mCRPC such as docetaxel (DOC), abiraterone (ABI) and enzalutamide (ENZA). We summarized i.a. mechanisms by MDR (multidrug resistance) protein expression, alterations of androgen receptor-, Wnt-, p53 and DNA repair-pathways (BRCA/ATM) as well as resistance through therapy induced neuroendocrine differentiation of PC.

Accepted publication: This review has been published in Orvosi Hetilap (Q3, IF=0.322).

Accepted publication: This review has been published in Urologic Oncology (Q1, IF=3.397).

2. Clinical sample collection

The serum collection from CRPC patients treated with DOC, ABI or ENZA is continuously ongoing. The collection reached >1000 samples from >400 CRPC patients. The retrospective collection of detailed clinical (including PSA, Gleason score, metastasis, ECOG performance status, primary therapy) and follow-up data (including PSA response, radiographic progression and patients' survival) from DOC- as well as ABI- and ENZA-treated CRPC cohorts has been finished.

3. Culturing PC cells

Culturing of DOC sensitive (PC3, DU145) and resistant (PC3-DR, DU145-DR), ENZA sensitive (LNCaPabl, DUCaP and LAPC4) and resistant (LNCaPabl-ER, DUCaP-ER and LAPC4-ER) as well as ABI resistant (LNCaPabl-AR, DUCaP-AR and LAPC4-AR) cells with 9 parallel for each 16 cell lines (2 million cells / culture) has been finished. Each cell line was cultured in 9 parallel 75 cm^2 plastic tissue culture flasks until subconfluent density was reached; six cell culture dishes for proteome analysis and three for genomic analyses. Each culture dish contained approximately two million cells.

For exome analysis, genomic DNA and total RNA were isolated using the AllPrep DNA/RNA kit (Qiagen) according to the manufacturer's protocol. The purified DNA was eluted in 50 µl RNase/DNase-free water, while total RNA was eluted in 50 µl RNase-free water. DNA/RNA concentration and purity was determined using a NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific) and Qubit 2.0 Fluorometer (Thermo

Fisher Scientific). Total RNA quality was also determined by agarose gel electrophoresis. The genomic DNA is stored at -20 °C, while the total RNA is stored at -80 °C.

4. Comparative proteome analysis

Proteome LC-MS/MS analyses of 16 cell lines (8 therapy sensitive vs. resistant pairs) with 6 biological parallels (sum of 96 mass-spec. measurements) have been completed. In the resistant sublines at least two-fold significantly higher abundant proteins detected at least by two peptides were considered. By this method we found 177, 278 and 68 significantly upregulated proteins in DOC-, ENZA- and ABI-resistant cell lines (Fig. 1).

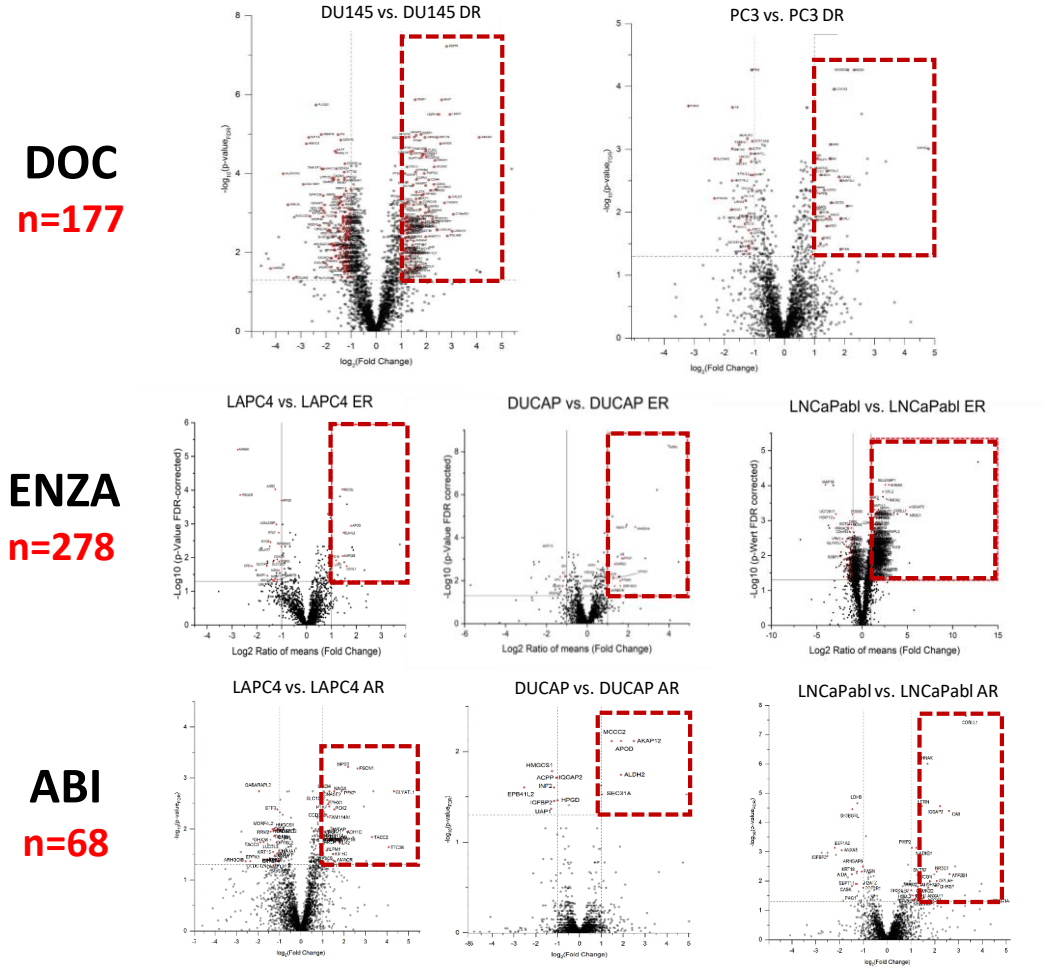

Fig. 1 *Volcano plot presentation of differentially expressed proteins between DOC/ABI/ENZA sensitive and resistant PC cell lines detected by LC-MS/MS analysis.*

5. Bioinformatics evaluation of proteome results in DOC, ABI and ENZA sensitive vs. resistant cell lines

We used three different bioinformatics methods in order to select the most promising protein candidates for further analyses.

5.1 Cross-reference analysis with published comparative proteome or transcriptome datasets

For this approach, we identified publicly available datasets for DOC/ABI/ENZA sensitive vs. resistant cell lines or patients' samples. This method identified 4 proteins (NAMPT, FLNC, ANXA3, KCRB) for DOC and 9 proteins (GALK2, PTGR1, SCIN, IDH1, AHCY, NEDD4L, THOP1, SF3B5, HEBP2) for ENZA resistant cells, while for ABI no published proteomics or transcriptome datasets were available for comparison (Fig. 2).

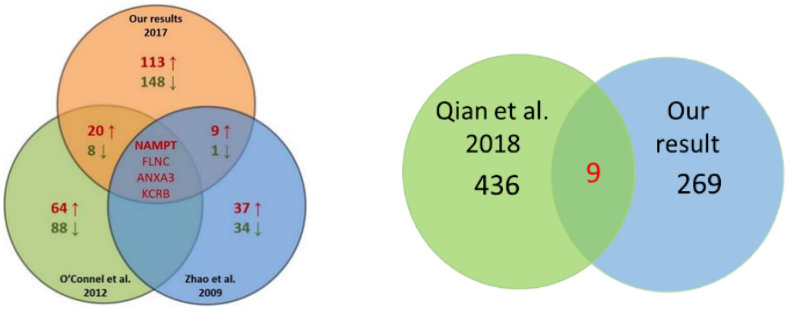

Fig. 2 *Cross-reference analyses were performed with two published datasets (Zhao et al. and O'Connel et al.) from comparative proteome of DOC (left) sensitive and resistant PC cell lines, while one transcriptome dataset (Qian et al.) was available for ENZA. Red numbers represent in resistant cells upregulated, while green numbers the downregulated proteins.*

5.2 Prediction of potentially secreted proteins in DOC, ABI or ENZA sensitive and resistant cell lines

For the selection of potentially secreted proteins, we used a combinatory algorithm developed by our cooperation partners (Ilona Tornyi and Prof. László Takács, Department of Human Genetics, University of Debrecen). This algorithm uses various protein databases (Uniprot, NVBI, ExoCarta, Human Protein Atlas) and prediction programmes (SignalP 4.1, SecretomeP 2.0, TargetP 1.1, TMHMM 2.0). This method identified 11 proteins (IL13RA2, COL6A1, MET, AUP1, ERAP1, LNPEP, CD44, GSN, CALU, COASY, HBS1L) for DOC treatment, 7 proteins (AGR2, PFKP, APOD, ENPP5, ALCAM, MINPP1, DYNLT3) for ABI and 13 proteins (PRCP, CTX, LIPA, DNASE2, UTRN, NAGA, SEC31A, EPHX2, DHRS7, APOD, PTK7, PFKP, KLK2) for ENZA therapy. These proteins are expected to be detectable in patients' serum samples.

5.3 Literature- and pathway-based selection method

The third method focused on the known oncological role (according to literature) and signal pathway context of proteins (using the String database) as well as on the availability of specific inhibitors against the given proteins. Based on this, we identified further 5 proteins (GAP43, MET, ANXA1, ANXA3, ROCK1) for DOC, 12 proteins (AMACR, FSCN1, NR3C1, ALDH2, KLK2, NQO1, HSF1, CTAG1A, AKAP12, PFKP, AHNAK, PTK7) for ABI and 7 proteins (NR3C1, AGR2, RHOA, SQSTM1, NDRG1, RRM2, ALCAM) for ENZA therapy.

6. Quantitative analysis of selected proteins in serum samples of mCRPC patients who underwent DOC, ABI or ENZA treatment

6.1 Proteins selected based on former promising results of our research group

6.1.1 MMP-7, sFas and sFasL serum levels in DOC, ABI and ENZA treated patients

We formerly found high preoperative serum MMP-7 level as an independent predictor of poor disease-specific survival in clinically localized PC *(Szarvas et al. IJC 2011)*. In addition, high MMP-7 levels were associated with the presence of bone metastasis and in accordance, MMP-7 was shown to be causally involved in the formation of PC bone metastases *(Lynch et al. Cancer Cell 2005)*. A growing body of evidence support the hypothesis that MMP-7 is involved in therapy resistance by allowing tumour cells to escape from chemotherapy-induced apoptosis. The degrading and downregulating effects of MMP-7 on receptor Fas and FasL have been identified as a key mechanism responsible for MMP-7-related chemotherapy resistance. Therefore, we determined the serum MMP-7 levels in 987 serum samples of 96 mCRPC patients and found that pre-treatment serum MMP-7 levels are independently associated with poor survival in DOC-treated patients *(Szarvas et al. BJU 2018)*. These results could be validated in an independent patient cohort *(Szarvas et al. Urol Oncol 2020) (Fig. 3).* We could show that increase of MMP-7 levels during DOC therapy (measured at the 3rd treatment cycle) were associated with poor patients' prognosis (Fig. 4). In addition,

our results suggested MMP-7 to be associated with shorter survival also in the ABI/ENZA treatment groups, however these associations were weaker compared to that of found in the DOC treated group (Fig. 3).

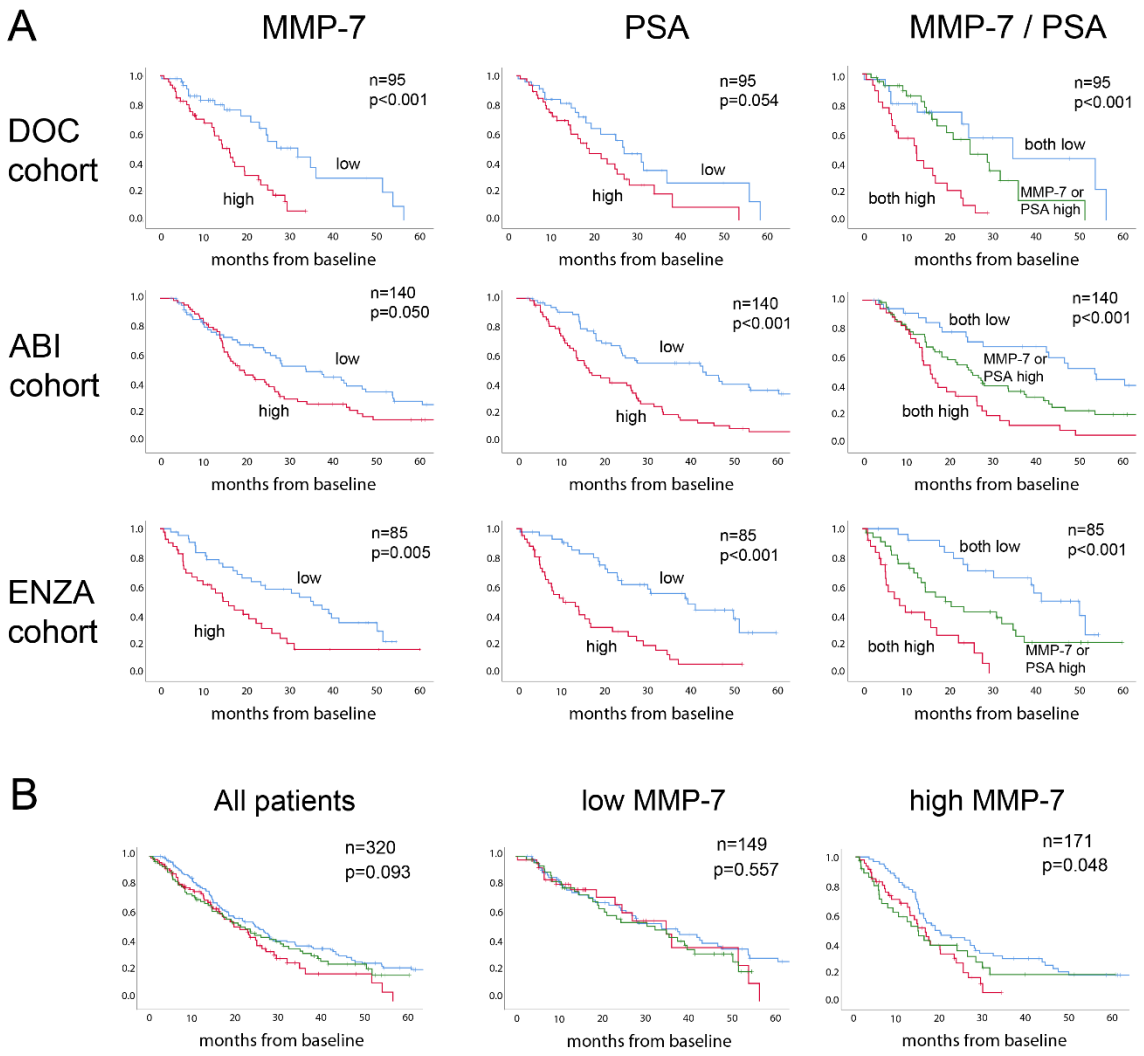

Fig. 3 *Kaplan–Meier curves of overall survival according to pre-treatment serum MMP-7 (left) and PSA levels (middle) and their combinations (right) in the DOC, ABI and ENZA treatment groups (A). Risk-stratification by treatment (DOC – red line, Abi – green line, ENZA – blue line) in the whole cohort (left) in MMP-7 low and MMP-7 high groups (B).*

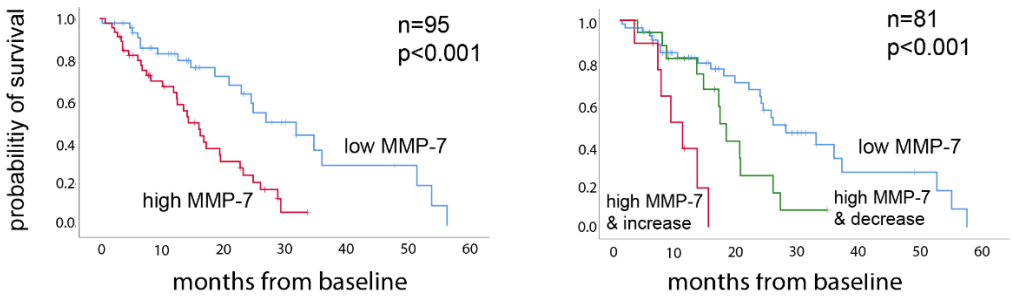

Fig. 4 *Kaplan-Meier overall survival curves in DOC-treated patients stratified by baseline serum MMP-7 levels (left) and (B) by combining baseline MMP-7 level with its changes (increase vs. decrease) at the 3rd DOC cycle.*

Accepted publication: These results have been published in Urologic Oncology (Q1, IF=3.397).

6.1.2 Neuroendocrine serum marker levels (CGA and NSE) in DOC, ABI and ENZA treated mCRPC patients

CGA is a glycoprotein commonly expressed in neuroendocrine cells. When a tumor develops in a neuroendocrine tissue, it becomes the main source of circulating/serum CGA. Neuroendocrine differentiation in PC has received attention in the last years because of its potential implication as a prognostic and/or diagnostic factor. Both *in vitro* and *in vivo* studies showed that treatment of PC cell lines with various androgen-targeted therapies lead to transdifferentiation to neuroendocrine PC (NEPC). This is probably triggered by the selective pressure of androgen targeting therapies leading to rapidly progressing disease. NEPC detected in biopsies of patients who were initially diagnosed with PCA and underwent antiandrogen therapy is referred to as treatment-related NEPC. Accordingly, treatment-related NEPC is increasingly detected following androgen deprivation therapy and is associated with poor prognosis. We therefore, in cooperation with the BRAHMS GmbH (part of ThermoFisher) conducted a large retrospective study for the determination of the predictive value of CGA and NSE in DOC, ABI and ENZA treated mCRPC patients by using the KRYPTOR method. We found an independent prognostic value for CGA/NSE combined score in ABI/ENZA- but not in DOC-treated mCRPC patients *(Szarvas et al. BJU 2020) (Fig. 5)*. Our analysis showed that patients with low CGA/NSE levels may rather benefit from an ABI or ENZA treatment, while for patients with high CGA/NSE level DOC chemotherapy could be the better choice *(Fig. 6)*.

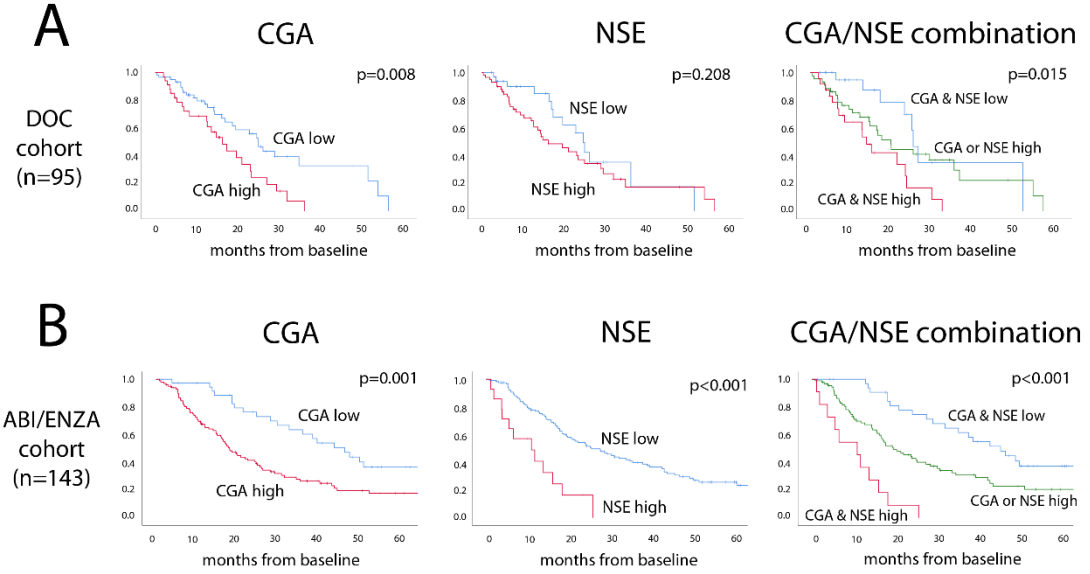

Fig. 5 *Kaplan–Meier overall survival curves in the (A) docetaxel (DOC) and (B) abiraterone (ABI)/enzalutamide (ENZA) cohorts stratified by chromogranin A (CGA), neuron-specific enolase (NSE) and their combination. In the DOC cohort receiver-operating characteristic-based thresholds (CGA: 168.0 ng/mL, NSE: 10.8 ng/mL) were used, while in the ABI/ENZA cohort the formerly published 'BJU score' thresholds (CGA: 85 ng/mL, NSE: 19 ng/mL) were applied for dichotomization.*

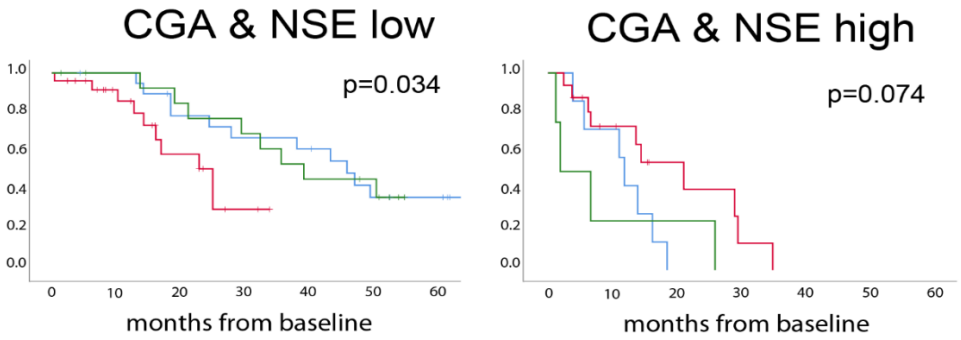

Fig. 6 *CGA/NSE high (left) and low (right) patient groups stratified by received treatment (DOC – red line, Abi – green line, ENZA – blue line).*

Accepted publication: These results have been published in British Journal of Urology (D1, IF=4.806).

6.1.3 Serum YKL-40 levels in DOC-treated mCRPC patients

Based on its promising literature, we assessed the serum levels of YKL-40 in 109 DOC-treated mCRPC patients. YKL-40 levels were significantly higher in patients with baseline resistance to DOC. Higher YKL-40 serum levels were detected in patients with bone metastasis and in those who were not pre-treated with radical prostatectomy. High YKL-40 levels were associated with shorter survival in patients who received DOC in the first-line setting. In multivariable analysis, ECOG performance status, presence of any metastases and high PSA levels remained independent predictors for survival *(Darr and Szarvas et al. Urol Int 2018)*.

Accepted publication: These results have been published in Urologia Internationalis (Q2, IF=1.698).

6.1.4 Serum SDC1 levels in DOC-treated mCRPC patients

We have formerly found that circulating serum syndecan-1 (SDC1) levels are elevated in PC patients with higher Gleason scores as well as with shorter disease-specific survival in patients with clinically localized PC. In addition, a more recent study showed that high pre-treatment soluble SDC1 (sSDC1) serum levels were associated with decreased response to chemotherapy in colorectal cancer. The authors demonstrated that SDC1 shedding was induced by MMP-7 and resulted in reduced chemotherapeutic sensitivity of colorectal cancer cells. Our above presented (6.1.1) analyses identified serum MMP-7 as a predicting factor for DOC therapy in mCRPC. Based on these findings, we hypothesized that circulating SDC1 levels may predict response to DOC chemotherapy in CRPC. Therefore, we determined the pre-treatment sSDC1 serum concentrations in 75 mCRPC patients who received with DOC therapy. Our results demonstrated that pre-treatment SDC1 serum levels correlate with MMP-7 concentrations and independently associated with poor survival *(Szarvas et al. Urol Oncol 2018)*.

Accepted publication: These results have been published in Urologic Oncology (Q1, IF=3.397).

6.2 Serum levels of proteins identified by the proteomics analyses

6.2.1 Serum protein analysis in DOC-treated patients

Based on our LC-MS/MS analysis and subsequent bioinformatics evaluation we selected 7 proteins for evaluation in serum samples of mCRPC patients who underwent DOC therapy. We measured serum NAMPT, CD44, MET, IL13RA2, LNPEP, GSN and GAP43 levels in samples of 66 DOC-treated mCRPC patients and found high pre-treatment NAMPT and CD44 serum levels to be independently associated with shorter survival (Table 1, Fig. 7). In addition to the 66 pre-treatment serum samples also samples obtained at 3rd (n=48), 5th (n=36), 7th (n=30), 9th (n=16) and 10th (n=6) therapy cycles were assessed. Our analyses found no correlation between a CD44, MET, IL13RA2, GSN, NAMPT, GAP43 and LNPEP serum levels and patients clinicopathological parameters (age, primary local therapy, ECOG performance status, PSA response or the presence of lymph node, visceral or bone metastases). Higher pre-treatment NAMPT and CD44 levels were independently associated with shorter patients' survival (Table 1, Fig. 7).

Variables		Overall survival		
		HR	95% CI	p
Age	< 71	ref.		
	≥ 71	1.456	0.851 - 2.490	0.170
ECOG	0	ref.		
	1-2	1.774	1.029 - 3.059	**0.039**
Visceral mets.	no	ref.		
	yes	1.351	0.720 - 2.537	0.349
Lymph node mets.	no	ref.		
	yes	1.024	0.596 - 1.759	0.933
Bone mets.	no	ref.		
	yes	0.678	0.164 - 2.804	0.592
Primary RPE	no	ref.		
	yes	1.181	0.606 - 2.301	0.624
Primary RAD	no	ref.		
	yes	1.331	0.566 - 3.132	0.512
PSA baseline (median)	< 88 ng/ml	ref.		
	> 88 ng/ml	1.506	0.866 - 2.619	0.146
PSA response	no response	ref.		
	response	0.291	0.141 - 0.602	**0.001**
PSA response	< 30%	ref.		
	> 30%	0.455	0.250 - 0.828	**0.001**
PSA response	< 50%	ref.		
	> 50%	0.45	0.249 - 0.814	**0.008**
PSA response	< 90%	ref.		
	> 90%	0.634	0.327 - 1.227	0.176
CD44 (median)	< 872.7 pg/ml	ref.		
	> 872.7 pg/ml	1.976	1.150 - 3.395	**0.014**
CD44 (upper 25%)	< 1237 pg/ml	ref.		
	> 1237 pg/ml	1.313	1.069 - 1.612	**0.009**
MET (median)	< 345.9 ng/ml	ref.		
	> 345.9 ng/ml	1.119	0.655 - 1.913	0.680
LNPEP	negative	ref.		
	positive	0.837	0.429 - 1.634	0.602

Table 1. *Univariable Cox-analysis. Patients' clinical parameters were correlated with OS. ECOG PS (1-2), PSA response and the median and upper 25% of pre-treatment CD44 values were found to be significantly correlated with poor OS. Significant p-values are indicated in bold.*

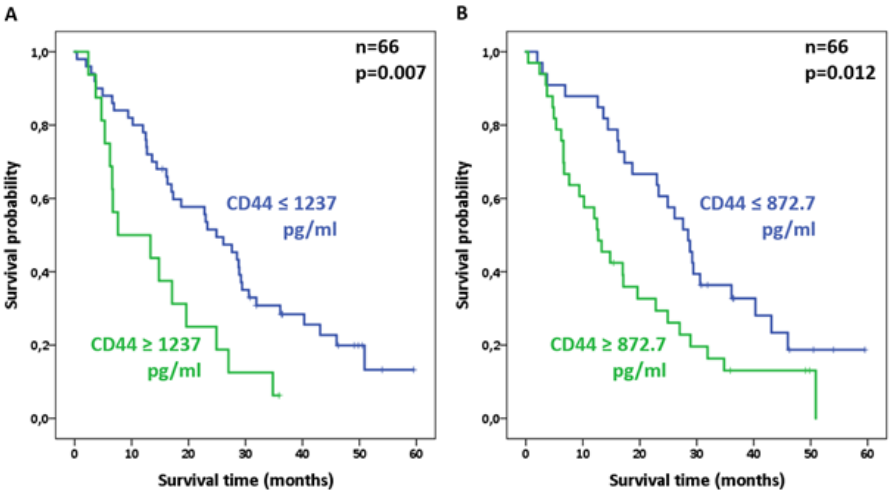

Fig. 7 *Kaplan-Meier plots. Survival analyses revealed that elevated baseline serum NAMPT (left) and CD44 (right) levels were associated with shorter survival of mCRPC patients who received DOC therapy.*

Poster: These results were presented at the 2019 Annual Congress of the European Association of Urology

Manuscript under review: These results have been submitted to The Prostate

6.2.2 Serum protein analysis in ENZA-treated patients

Based on our proteome analyses in ENZA sensitive and resistant cell lines, we selected 6 proteins (AGR2, ALCAM, NR3C1, IDH1, RRM2 and NDRG1) for ELISA analysis. These proteins were measured in baseline samples of 72 ENZA-treated patients and in 64 cases also in on-treatment serum samples taken 3 months after starting therapy. None of the marker levels correlated with patients' age, primary local therapy, ECOG performance status, PSA response or the presence of lymph node metastases. In contrast, IDH1 levels were decreased in bone metastatic cases, while AGR2 levels were lower in visceral metastatic patients. Of the 5 protein markers only ALCAM showed a correlation with patients' overall survival (Table 2, Fig. 8). High ALCAM level proved to be an independent risk-factor for survival in mCRPC patients who underwent ENZA treatment (Fig. 8).

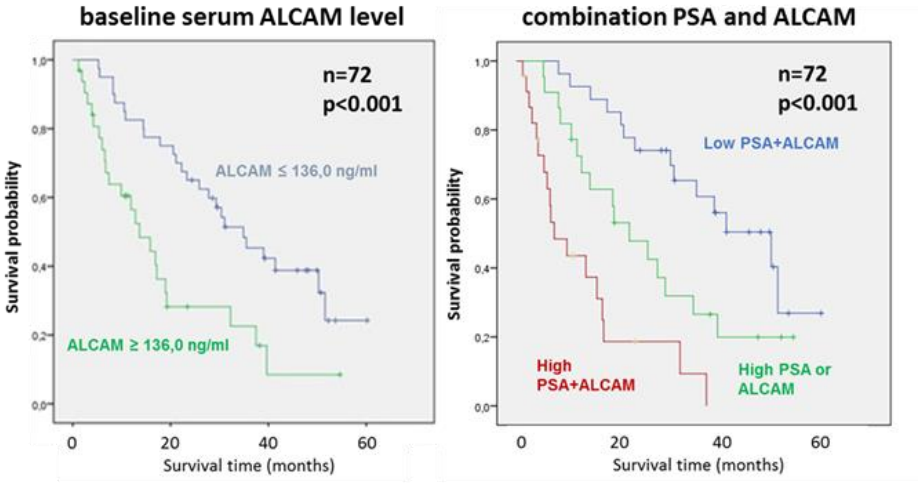

Fig. 8 *Kaplan-Meier plots. Survival analyses revealed that elevated baseline serum ALCAM levels (left) alone or in combination with PSA (right) significantly improving risk-stratification of ENZA-treated mCRPC patients.*

Variables		Overall survival		
		HR	95% CI	p
Age	≤ 72	ref.		
	> 72	1 038	0.589 - 1.837	0.898
ECOG	0	ref.		
	1-2	1 357	0.638 - 2.886	0.428
Any mets.	no	ref.		
	yes	4 488	1.078 - 18.678	**0.039**
Visceral mets.	no	ref.		
	yes	3 511	1.017 - 12.122	**0.047**
LN mets	no	ref.		
	yes	0.622	0.290 - 1.330	0.221
Bone mets.	no	ref.		
	yes	5.796	1.773 - 18.945	**0.004**
Primary RPE	no	ref.		
	yes	0.779	0.512 - 1.651	0.919
Primary RAD	no	ref.		
	yes	0.661	0.329 - 1.328	0.245
ENZA therapy	1st line	ref.		
	2nd or later line	2.769	1.169 - 6.559	**0.021**
PSA baseline (median)	< 90.5 ng/ml	ref.		
	> 90.5 ng/ml	3.844	1.968 - 7.507	**<0.001**
PSA response	no response	ref.		
	response	0.608	0.291 - 1.269	0.185
PSA response	< 30%	ref.		
	> 30%	0.740	0.385 - 1.419	0.364
PSA response	< 50%	ref.		
	> 50%	0.735	0.394 - 1.373	0.335
PSA response	< 90%	ref.		
	> 90%	0.471	0.255 - 0.870	0.016
AGR2 (median)	< 439.15 pg/ml	ref.		
	> 439.15 pg/ml	0.818	0.463 - 1.444	0.488
ALCAM (median)	< 127.8 ng/ml	ref.		
	> 127.8 ng/ml	2 002	1.124 - 3.564	**0.018**
ALCAM (cut off)	< 136.0 ng/ml	ref.		
	< 136.0 ng/ml	2 570	1.434 - 4.607	**0.002**
GR (median)	< 52.26 pg/ml	ref.		
	> 52.26 pg/ml	0.922	0,523 - 1.626	0.780
IDH1 (median)	> 2694.0 ng/ml	ref.		
	< 2694.0 ng/ml	0.816	0.416 - 1.442	0.484
NDRG1 (median)	> 25800.0 ng/ml	ref.		
	< 25800.0 ng/ml	1.083	0.609 - 1.926	0.786

Table 2. *Univariable Cox-analysis. Patients' clinical parameters were correlated with OS. ECOG PS (1-2), PSA response and the median and upper 25% of pre-treatment CD44 values were found to be significantly correlated with poor OS. Significant p-values are indicated in bold.*

Submitted abstract: These results were submitted to the 2021 Annual Congress of the EAU

6.2.3 Serum protein analysis in ABI-treated patients

Based on our LC-MS/MS-based proteome data in ABI sensitive and resistant cell lines, we selected 4 target proteins (CTAG1A, KLK2, AMARC and FSCN1) for ELISA analyses. These proteins were measured in pre-treatment serum samples of 100 ABI-treated patients and in 40 cases also in samples taken at the 3rd months of therapy. None of the marker levels correlated with patients' age, primary local therapy, ECOG performance status or the presence of lymph node or visceral metastases. CTAG1 serum levels were higher in patients who experienced PSA response to ABI therapy. High KLK2 and FSCN1 pre-treatment serum levels were associated with patients' overall survival (Table 3, Fig. 9). Both proteins (FSCN1 and KLK2) remained significant risk factors in the multivariable analysis.

Variables		Overall survival		
		HR	95%CI	p
Age	≤ 72	ref.		
	> 72	1.577	0.967 - 2.571	0.068
ECOG	0	ref.		
	1-2	5.386	2.566 - 11.351	**<0.001**
Any mets.	no	ref.		
	yes	2.517	0.612 - 10.360	0.201
Visceral mets.	no	ref.		
	yes	1.031	0.443 - 2.399	0.944
LN mets	no	ref.		
	yes	1.344	0.719 - 2.514	0.354
Bone mets.	no	ref.		
	yes	2.426	0.971 - 6.058	0.058
Primary local treatment	no	ref.		
	yes	0.600	0.369 - 0.974	**0.039**
Primary RPE	no	ref.		
	yes	0.804	0.499 - 1.296	0.370
Primary RAD	no	ref.		
	yes	0.729	0.390 - 1.360	0.321
ABI therapy	1st line	ref.		
	2nd or later line	0.792	0.490 - 1.279	0.340
PSA baseline (median)	< 66.45 ng/ml	ref.		
	> 66.45 ng/ml	2.529	1.535 - 4.168	**<0.001**
PSA response	no response	ref.		
	response	1.351	0.584 - 3.126	0.483
PSA response	< 30%	ref.		
	> 30%	0.600	0.341 - 1.056	0.076
PSA response	< 50%	ref.		
	> 50%	0.628	0.379 - 1.042	0.072
PSA response	< 90%	ref.		
	> 90%	0.597	0.349 - 1.020	0.059
CTAG1A (median)	< 2.240 ng/ml	ref.		
	> 2.240 ng/ml	1.084	0.674 - 1.743	0.740
CTAG1A (median)	< 2.285 ng/ml	ref.		
	> 2.285 ng/ml	0.977	0.912 - 1.047	0.509
KLK2 (median)	< 4.088 pg/ml	ref.		
	> 4.088 pg/ml	1.549	0.963 - 2.493	0.071
KLK2 (upper_40prz)	< 9.595 pg/ml	ref.		
	> 9.595 pg/ml	1 154	1.017 - 1.309	**0.026**
FSCN1 (median)	> 9.385 ng/ml	ref.		
	< 9.385 ng/ml	1.764	1.086 - 2.866	**0.022**

Table 3. *Univariable Cox-analysis. Patients' clinical parameters were correlated with OS. ECOG PS (1-2), PSA baseline level and high pre-treatment KLK2 and FSCN1 levels were significantly correlated with poor OS. Significant p-values are indicated in bold.*

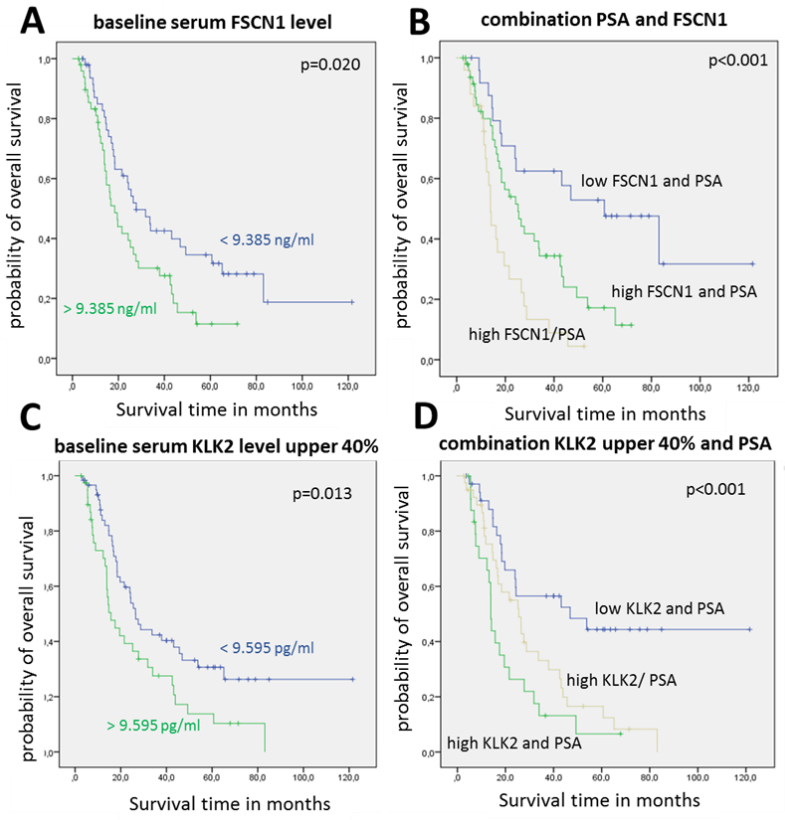

Fig. 9 *Kaplan-Meier survival analysis revealed that elevated baseline serum FSCN1 (A) and KLK2 (C) alone and in combination with serum PSA (B and D) are able to significantly improve risk-stratification in ABI-treated mCRPC patients.*

7 Functional analyses of target molecules in therapy-resistant vs. parental PC cells

7.1 Functional analysis in DOC-resistant cells

We selected three target molecules (CD44, NAMPT and GAP43) to assess their functional involvement in DOC resistance. CD44 and NAMPT were selected based on the association of their serum concentrations with treatment response and survival of DOC treated mCRPC patients. GAP43 is a neuronal growth factor, which showed a strong, 28-fold upregulation in PC3-DR compared to the DOC-sensitive PC3 cells. First, expression levels of CD44, NAMPT and GAP43 have been determined in DOC sensitive and resistant PC cell lines on the protein and gene expression levels (Fig. 10). CD44, NAMPT and GAP43 expressions were knocked-down by using the siRNA technique. Western blot analysis confirmed that silencing of these proteins' expression was effective (Fig. 9). Cell viability assay showed that silencing of CD44, NAMPT and GAP43 did not resensitized PC3-DR and DU145-DR cells to DOC (Fig. 11). However, apoptosis analysis by flow cytometry revealed that apoptotic cell rate was higher in CD44 knocked-down DOC resistant DU145-DR cells compared to control (non-transfected) DU145-DR cells (Fig. 12).

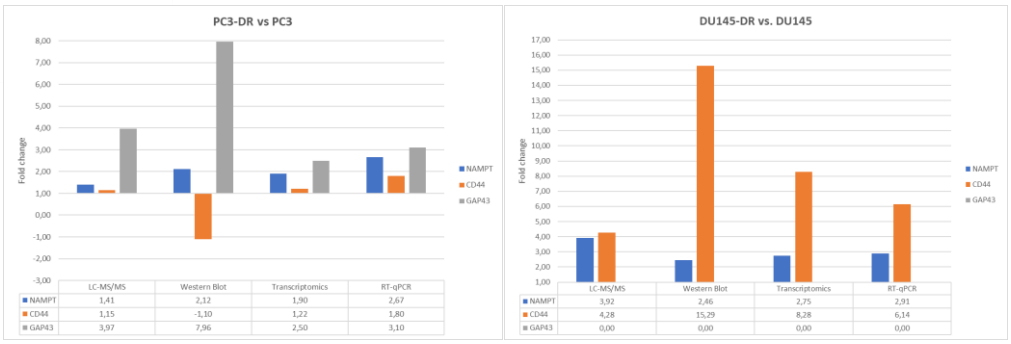

Fig. 10 *Protein and gene expression levels of selected molecules in DOC sensitive (PC3 and DU145) and DOC resistant (PC3-DR and DU145-DR) PC cells. NAMPT protein and gene expression levels were significantly higher abundant compared to DOC sensitive PC cells. CD44 protein and gene expression levels were elevated in DU145-DR cells, while GAP43 gene and protein levels were elevated in PC3-DR. *GAP43 expression values were divided by 7.*

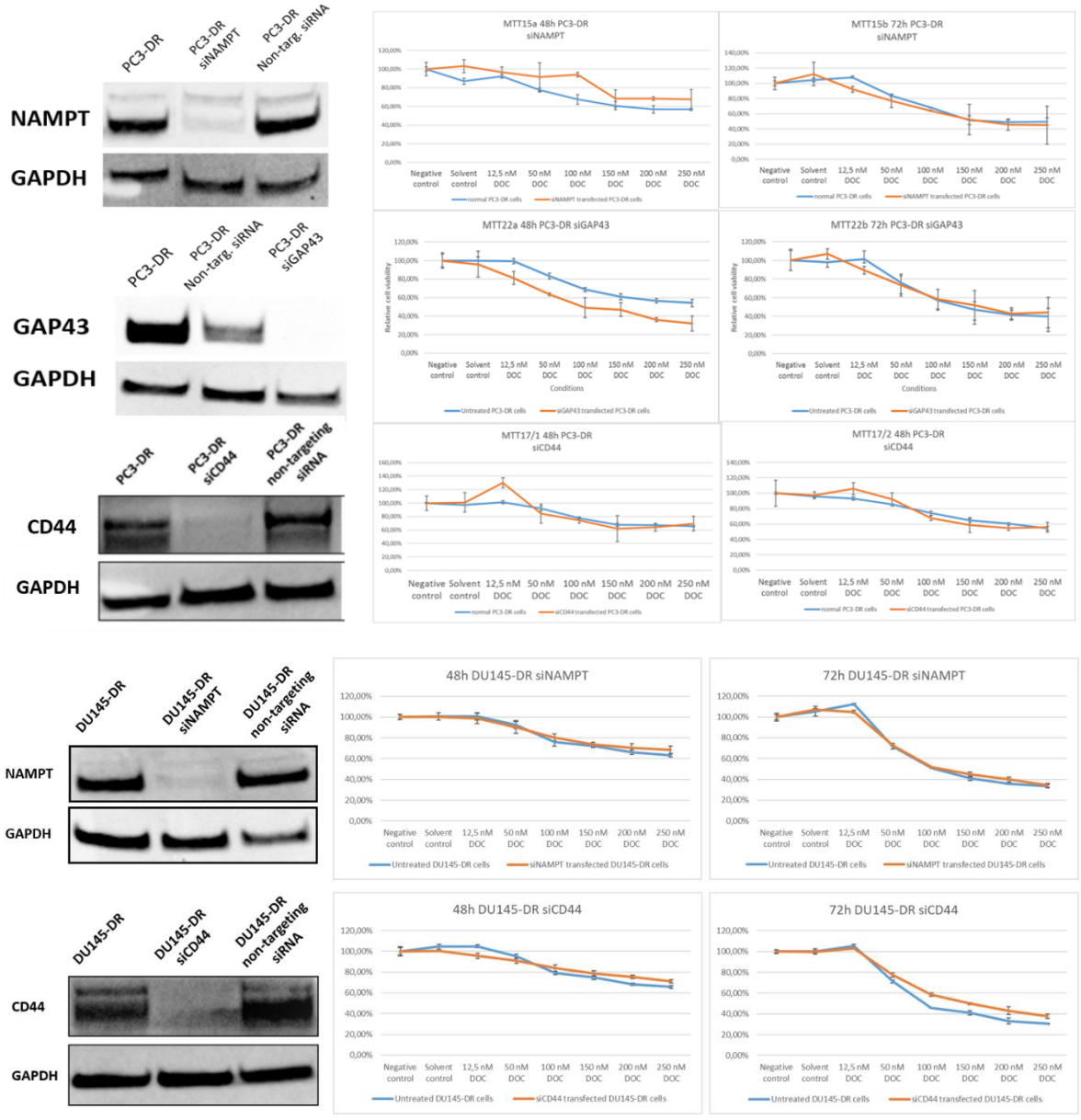

Fig. 11 *Western blot and cell viability analyses in PC3-DR cells with and without siRNA knockdown of NAMPT, GAP43 and CD44. 20 µg protein sample were loaded into each well. The anti-NAMPT, GAP43 and CD44 antibodies and as loading control anti-GAPDH antibody were blocked on the blot by NFDM/TBST (5%). Super Signal West Pico Plus Chemiluminescent Substrate (Thermo Scientific) was used for the detection of target proteins.*

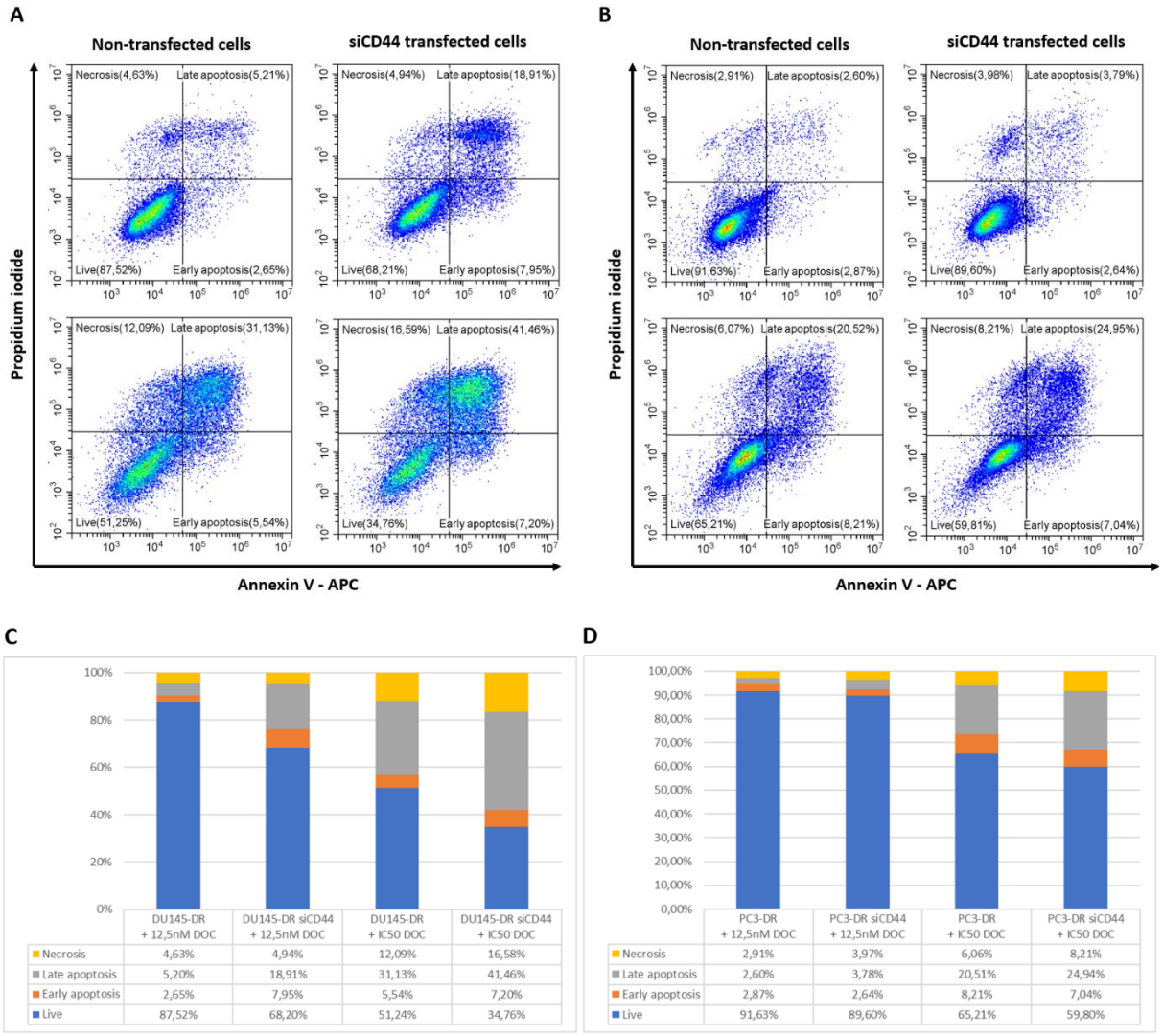

Fig. 12 *Apoptosis analysis by flow cytometry was performed on both DOC resistant cell lines. Regarding the experiments done on DU145-DR cells (A and C) we could detect significant changes. The rate of the living DU145-DR cells due to siCD44 transfection changed from 87.52% to 68.20 % (under 12.5 nM DOC treatment), and 51.24% to 34.76% (IC50 DOC). Meanwhile the rate of late apoptotic cells in the population raised from 5.20% to 18.91% (12.5 nM DOC), and from 31.13% to 41.46% (IC50 DOC). The same experiments with PC3-DR cells did not show similar changes (B and D).*

Manuscript under review: These results have been submitted to The Prostate

7.2 Functional analysis in ENZA and ABI-resistant cells

Based on our proteome analyses in ENZA sensitive and resistant cell lines, we selected 3 target molecules (ALCAM, AGR2 and IDH1) to examine their potential mechanistic involvement in ENZA resistance (Fig.13). For ABI-treatment, we selected FSCN1 and KLK2 proteins for functional cell culture analyses (Fig. 14). Western blot analysis for selected ABI and ENZA resistance molecules confirmed the results of proteome analyses. ALCAM protein and gene expression levels were elevated in LNCaPabl-ER and LAPC4-ER cells, while IDH1 gene and protein levels were elevated in DUCAP-ER. FSCN1 was higher abundant in the DUCAP-AR cells both in at the RNA and protein level and its protein but RNA expression was higher in the ABI resistant LAPC4-AR compared to ABI sensitive LAPC4 (Fig. 14).

Viability (WST-assay) analysis confirmed that LAPC4 and DUCAP cells show significantly higher sensitivity against ENZA, while LNCaP cells exhibit higher resistance to ENZA than LAPC4 and DUCAP cells. ALCAM knockdown was successful in LAPC4-ER cell lines as demonstrated by Western blot analysis. ALCAM knockdown significantly increased ENZA sensitivity of LAPC4-ER cells. In addition, IDH1 knockdown was successful in DUCAP-ER cell lines at the protein level as shown by Western blot analysis; however, IDH1 knockdown did not significantly change ENZA sensitivity of LAPC4-ER cells (Fig. 15).

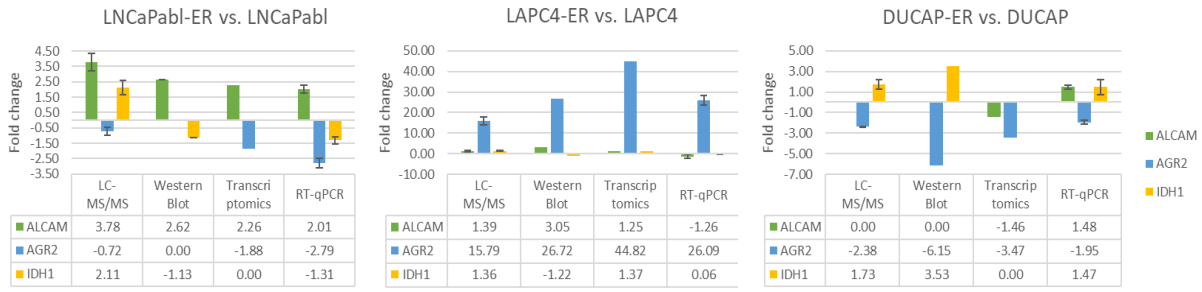

Fig. 13 *Protein and gene expression levels of selected molecules in ENZA sensitive (LNCaPabl, LAPC4, DUCAP) and ENZA resistant (LNCaPabl-ER, LAPC4-ER, DUCAP-ER) PC cells. ALCAM protein and gene expression levels were significantly higher abundant in LNCaPabl-ER and LPAC4-ER cells compared to ENZA sensitive parental cell line. AGR2 and IDH1 protein and gene expression levels were higher in LAPC4-ER and DUCAP-ER cells compared to ENZA sensitive parental cell lines, respectively.*

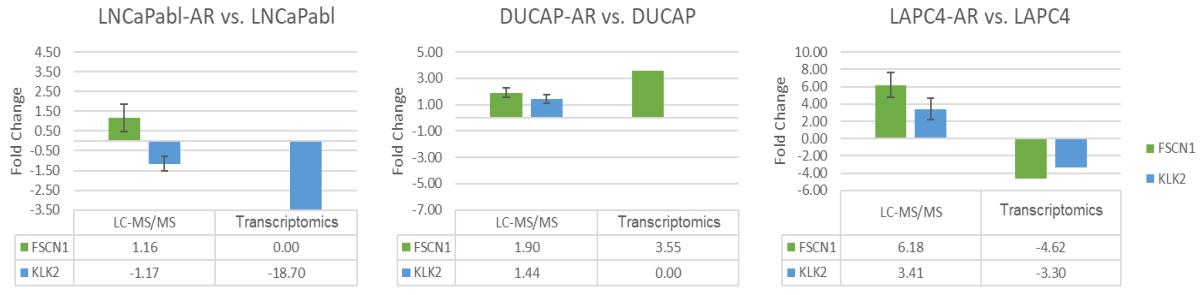

Fig. 14 *Protein and gene expression levels of selected molecules in ABI sensitive (LNCaPabl, LAPC4, DUCAP) and ABI resistant (LNCaPabl-AR, LAPC4-AR, DUCAP-AR) PC cells. FSCN1 and KLK2 protein levels were significantly higher abundant in LAPC4-AR compared to ABI sensitive parental cell line.*

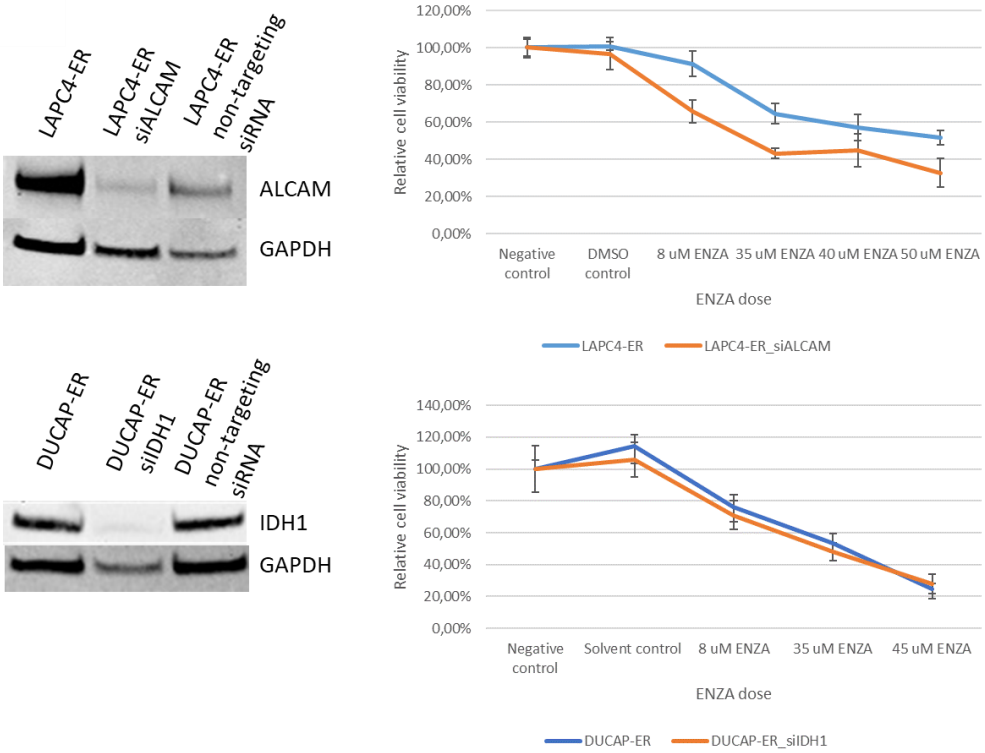

Fig. 15 *Western blot and cell viability analyses in (LAPC4-ER and DUCAP-ER) cells with and without siRNA knockdown of ALCAM and IDH1. 20 ug protein sample was loaded into each well. The anti-ALCAM and IDH1 antibodies were blocked on the blot by using milk (5%), while anti-GAPDH antibody was blocked by BSA (5%). Super Signal West Pico Plus Chemiluminescent Substrate (Thermo Scientific) was used for the detection of the target proteins.*

Submitted abstract: These results were submitted to the 2021 Annual Congr. of the EU Association of Urol.

8 DNA-sequencing and construction of a predictive sequencing panel

8.1 Introduction of BRCA1/2 sequencing in the clinical routine

Current development in systemic therapy of progressed PC and our increasing insight into the molecular background of this disease pave the way for molecularly informed decision-making in PC. This rapid development has already been partly translated into the clinical routine as current NCCN (National Comprehensive Cancer Network) guidelines recommend the routine analysis of some alterations such as microsatellite instability and BRCA1/2 mutations in progressed cases of PC. BRCA1/2 positive PCs represent a distinct subtype of PCs with resistance to androgen targeting agents and DOC, while show a higher sensitivity to platinum chemotherapy and PARP inhibitor treatments. Accordingly, in cooperation with the Institute of Pathology, Semmelweis University, we introduced a BRCA1/2 analysis in our clinical routine. Our first mCRPC patient with BRCA2 mutation positivity had a symptomatic disease, which rapidly progressed on DOC treatment. Based on the BRCA2 finding the patient received platinum chemotherapy, which resulted in a biochemical (PSA decrease from >500 ng/ml to 6 ng/ml), radiographic (partial remission) and symptomatic (analgesia could be omitted) improvement of the disease.

Accepted manuscript: The above described case has been published in Orvosi Hetilap

8.2 Construction of a sequencing panel for therapy prediction of PC

In addition, several ongoing clinical studies include companion molecular analysis or molecular alteration-based selection criteria, which will result in an increase of molecular analyses in clinical decision-making for PC. We provided a comprehensive overview on this field and defined the most promising candidate molecules for further clinical decision-making. Accordingly, we designed a sequencing panel with relevant genes for further routine analysis of PC. This panel includes genes involved in DNA-damage repair (BRCA1/2, ATM, FANCD2, FANCA, CHEK2) with relevance to PARP inhibitor and platinum therapy, mismatch repair genes (MLH1, MSH2, MSH6, PMS2) and CDK12 with relevance for immune checkpoint inhibitor therapy (pembrolizumab), androgen receptor for the prediction of androgen signaling targeting agents, PTEN for the prediction of PIK3 pathway inhibitors (e.g. ipartasterib) RB1, p53 and FOXA1 for the prediction of aggressive variant PC and platinum therapy. We are currently working on the pilot introduction of these analyses in the clinical routine.

Accepted manuscript: The above mentioned preclinical molecular insights have been summarized in Orvostovábbképző Szemle.

8.3 Single nucleotide alterations

Whole exome sequencing an overall number of 540 single nucleotide alterations with functional impact on the protein product. Of these 276 were found in ENZA, 236 in ABI and 28 in DOC resistant cell lines. *MSTL1* and *CEP63* was found as repetitively occurring mutations for the same treatments. In addition, 161 mutations occurred recurrently between ABI and ENZA resistant cell lines, suggesting an overlap in their potential resistance mechanisms. Further 6 mutations were identified as pathogenic *BRCA2, NEB, CASR, POGZ, FTCD, ATM* (Fig. 16). The objective of this work package to define a list of differentially mutated genes between therapy sensitive and resistant cell lines has been reached. Identified alterations are planned to be validated in clinical samples as cell free DNA and functionally assessed in future studies.

Enzalutamide			**Abiraterone**			**Docetaxel**	
LnCaPabl-ER	*LAPC4-ER*	*DUCAP-ER*	*LnCaPabl-AR*	*LAPC4-AR*	*DUCAP-AR*	*PC3-DR*	*DU145 -DR*
1	2	3	4	5	6	7	8
MST1L	HSPA4	MST1L	POGZ	CEP63	CEP63	ZSWIM2	ATM
BRCA2	LIX1L	LOC284009	BRCA2	FTCD	FCGR2C	CAMK2B	SUPT5H
NEB	FCGR2A	SLC9C2	NEB	TOR1AIP1	SCGB2A1	CCT6P1	MST1L
CASR	RP11-25K21.6	NLRP3	CASR	HBZ	ANKLE1		LOC102724562
UBC	NARG2	ANKRD26	VWA8	KRTAP4-1	CYP2A13		FCGR2A
NGEF	ICE2	SAMD8	ZNF225	ARHGAP15	OTOP1		RP11-25K21.6
USF3	FAM57A	MS4A14	NPHP3	FAT2	DRD5		CR1
TMEM88B	RAB40B	CD3G	KIF1B	HLA-DRB5	GYPA		BRF1
ENO1	PRAM1	CAND1	SPEN	HLA-DRB3$030	XRCC4		UNC45A
KIF1B	LILRA6	NEMF	HP1BP3	HLA-DRB3	HLA-DRB1		TRPV3
C1orf189	LILRB3	WDHD1	PTPRU				NFKBIB
COL9A2	CYP2D7P	CMTR2	PHC2				LILRA6
HOOK1	CYP2D7	METTL16	HOOK1				LILRB3
DOCK7	SLC45A2	AC006435.1	FUBP1				CD8B
TYW3	ROS1	CWC25	BRDT				OTOP1
FUBP1	ZNF680	MYOM1	PALMD				HYAL4
COL24A1	CCT6P1	COL5A3	PRPF3				SGK223
DCST1	PCOLCE	KIRREL2	CGN				PRAG1
C1orf85	AC243547.1	SLC3A1	FLG				PRSS55
NES	AC012314.2	DGKD	C1orf189				RP1L1
PYHIN1		AC019221.4	C1orf85				R3HCC1
RASAL2		TFF1	NES				ZCCHC7

Fig. 16 *Single nucleotide alterations with functional impact found in enzalutamide, abiraterone and docetaxel resistant cell lines (a maximal number of 25 alterations are shown). Recurrent mutations in the same treatment group are highlighted, while alterations with clinical significance (pathogenicity) according to the VEP database are marked by red letters.*

8.4 Copy number alterations in DOC/ABI/ENZA resistant and parental cell lines

Our whole exome sequencing analyses identified a large number of copy number variations (CNVs) between resistant and sensitive cell lines in terms of deletion/loss and amplification/gain. Obtained results were compared to RNA sequencing data (generated from the same cell lines in another research project) in order to filter those alterations with potential biological impact. We considered only those amplifications/deletions that resulted an at least 2-fold significant increase/decrease at the gene expression level (Fig. 17). We found 4, 8 and 110 amplifications in ABI, ENZA and DOC resistant cell lines that could be confirmed at the RNA levels. In addition 27, 3 and 0 deletions were found (in ABI-, ENZA- and DOC-resistant cell sublines) with significant down-regulating consequence on the mRNA expression of the affected gene. Interestingly, no deletions were detected in DOC resistant cells but on the other hand 110 amplifications could be observed at the mRNA levels. In addition, only amplifications in the DOC resistant cells could be confirmed at the proteome level.

Abiraterone					Enzalutamide					Docetaxel				
CNV	**RNA-Seq**		**Proteomics**		**CNV**	**RNA-Seq**		**Proteomics**		**CNV**	**RNA-Seq**		**Proteomics**	
Gene name	*Fold change*	*p-adj*	*Fold change*	*FDR p-value*	*Gene name*	*Fold change*	*p-adj*	*Fold change*	*FDR p-value*	*Gene name*	*Fold change*	*p-adj*	*Fold change*	*FDR p-value*
FGFR1	2.47	0.0000			UCA1	24.08	0.009289			ANXA1	**13.82**	0.000000	**5.06**	0.000046
ANKRD17	2.21	0.0000	-1.11	0.930320	ADAMTS3	7.80	0.000000			OGFR	**2.76**	0.000000	**4.80**	0.002092
HSD17B7P2	2.42	0.0000			ORM1	5.83	0.041237			NUDCD1	**1.73**	0.000000	**4.75**	0.001414
ANKRD30A	2.82	0.0039			AEBP2	3.55	0.000000			MRGBP	**3.13**	0.000000	**4.45**	0.002795
KLHDC7B	-78.05	0.0000			SELENOP	2.64	0.000000			TPD52L2	**4.07**	0.000000	**3.64**	0.000032
MOV10L1	-44.33	0.0016			OR2A1-AS1	2.53	0.009604			ATP6V1C1	**1.56**	0.000003	**3.25**	0.010679
IL17REL	-14.41	0.0046			ANKRD17	2.28	0.000000	-1.04	0.797885	SIGMAR1	**2.17**	0.000000	**2.93**	0.021803
ODF3B	-11.95	0.0000			SYNGAP1	2.11	0.000000			ARFGEF1	**1.54**	0.000000	**2.76**	0.002890
TYMP	-9.92	0.0000			HIST3H2A	-4.30	0.000000			EEF1A2	**1.56**	0.000858	**2.73**	0.006471
ADM2	-6.13	0.0000			OLA1	-2.40	0.000000	1.16	0.763038	STK3	1.41	0.000005	**2.49**	0.041068
MIOX	-4.98	0.0075			MTHFD2L	-2.35	0.000000			TLN1	**1.47**	0.000000	**2.29**	0.009032
PANX2	-4.98	0.0000			CHN1	-2.04	0.000000			PLEC	**2.09**	0.037384	**2.25**	0.004180
SHANK3	-4.85	0.0000								VCPIP1	**1.26**	0.014945	**2.20**	0.000100
HOPX	-3.52	0.0000								PTK2	**1.18**	0.005957	**2.05**	0.000100
HIST3H2A	-3.49	0.0000								OSTF1	1.53	0.000000	2.05	0.125853
ADTRP	-3.27	0.0000								TJP2	**3.11**	0.000000	**2.04**	0.001527
PLEKHG7	-3.10	0.0000								UBAP2	1.63	0.000000	1.96	0.045336
DENND6B	-2.88	0.0000								VAPB	**2.69**	0.000000	**1.91**	0.025905
CRADD	-2.78	0.0000								YWHAZ	1.47	0.000000	1.77	0.054358
NMU	-2.60	0.0015								GNAS	**2.10**	0.000000	**1.74**	0.052194
MTHFD2L	-2.34	0.0000								VCP	1.27	0.000004	**1.64**	0.002097
TPMT	-2.32	0.0000	-1.20	0.935791						SPAG1	**2.57**	0.000000	1.61	0.156727
TRABD	-2.29	0.0010								APCDD1L-AS	**388.35**	0.000000		
MAPK11	-2.23	0.0000								SCRT1	**90.36**	0.000000		
MAPK8IP2	-2.20	0.0000								STMN3	**51.15**	0.000000		
MRPL42	-2.17	0.0001								BIRC7	**49.97**	0.000089		
SBF1	-2.13	0.0026								PRSS3	**46.31**	0.000000		
PCID2	-2.11	0.0000	1.09	0.924664						ATP6V0D2	**41.94**	0.000618		
UBE2N	-2.07	0.0000	-1.13	0.810155						APCDD1L	**41.88**	0.000374		
CHEK2P2	-2.06	0.0027								COL20A1	**33.07**	0.000040		
PIM3	-2.04	0.0089								FNDC11	**26.26**	0.000000		

Fig. 17 *Copy number variations (CNVs) in abiraterone, enzalutamide and docetaxel resistant prostate cancer cell lines. Only those CNVs (amplifications –red letters or deletions – blue letters) with a significant effect on the gene expression of the given gene were listed. Additionally, proteome data were also listed for those genes that were detected by the LC-MS/MS analyses.*

As in another research project we performed methylation, and transcriptome sequencing analyses in the same cell lines, as a next step, we will perform an integrated evaluation of exome, proteome, methylation and mRNA expression data. This may help to identify driver alterations of therapy resistance for each cell lines.

9 Final conclusions

1) We analyzed 2 DOC, 3 ABI and 3 ENZA sensitive PC cell lines and their resistant sublines by LC-MS/MS. This identified 177, 278 and 68 significantly at least two-fold upregulated proteins in DOC, ENZA and ABI resistant cells.

2) We quantitatively assessed 12 proteins (CGA, NSE, MMP-7, SDC1, YKL-40, NAMPT, CD44, MET, IL13RA2, LNPEP, GSN and GAP43) in serum samples of DOC-treated mCRPC patients and identified high pre-treatment MMP-7, SDC1, NAMPT and CD44 as significant and independent predictor of poor treatment response and survival.

3) For serum analyses of ENZA-treated patients, 9 proteins (CGA, NSE, MMP-7, AGR2, ALCAM, NR3C1, IDH1, RRM2 and NDRG1) were selected. High CGA/NSE and ALCAM serum levels were independently associated with poor treatment response and survival in ENZA-treated mCRPC patients.

4) For serum analyses of ABI-treated patients, 7 proteins (CGA, NSE, MMP-7, CTAG1A, KLK2, AMARC and FSCN1) were selected. Of these, high CGA/NSE and FSCN1 proved to be independently associated with poor survival in ABI-treated mCRPC patients.

5) Functional siRNA knock-down analyses were performed for NAMPT, CD44 and GAP43. These analyses revealed that knock-down of CD44 in DOC-resistant cells resulted in increased rate of apoptotic cells suggesting the possible involvement of CD44 in DOC resistance. Based on this CD44 may serve as a potential therapy target.

6) In ENZA resistant cells, siRNA knock-down was performed for IDH1 and ALCAM. These analyses found that downregulation of ALCAM is able to re-sensitizes resistant cells to ENZA, suggesting a mechanistic involvement of this molecule in ENZA resistance.

7) Our analyses identified eight potentially predictive serum proteins for DOC, ABI or ENZA treatments and two of these proteins (CD44 and ALCAM) were found to be functionally involved in resistant mechanisms and may therefore serve as target for future therapies.

8) As available data on the therapeutic consequences of specific molecular alterations in PC is rapidly growing and current PC guidelines increasingly recommend molecularly informed therapeutic decision making, we performed a thorough review of relevant literature. Based on this work, we designed a gene PC sequencing gene panel for introduction into the clinical practice.

9) We identified a large number of SNVs and CNVs with impact on gene and protein expression and with potential involvement in resistance mechanisms against DOC, ABI, ENZA-treatments. The found alterations clinical relevance as prognostic or predictive factors need to be tested and validated in additional analyses on clinical samples. Furthermore, presented data together with other already generated mRNA and DNA methylation results will be integratively assessed in order to descript the molecular background of the therapy resistance in prostate cancer.

10 Published and submitted manuscripts on the topic of this research grant

The grant support by NKFIH/OTKA has been acknowledged in all the below listed papers and manuscripts. A copy of the published manuscripts has been placed at the MTMT repository.

1. ***Szarvas T***, *Sevcenco S, Módos O, Keresztes D, Nyirády P, Csizmarik A, Ristl R, Puhr M, Hoffmann MJ, Niedworok C, Hadaschik B, Maj-Hes A, Shariat SF, Kramer G. Matrix metalloproteinase 7, soluble Fas and Fas ligand serum levels for predicting docetaxel resistance and survival in castration-resistant prostate cancer. BJU INT 122(4):695-704. (2018)* *IF: 4.688 / D1*
2. *Darr C, Krafft U, Hadaschik B, Tschirdewahn S, Sevcenco S, Csizmarik A, Nyirady P, Küronya Z, Reis H, Maj-Hes A, Shariat SF, Kramer G,* ***Szarvas T****. The Role of YKL-40 in Predicting Resistance to Docetaxel Chemotherapy in Prostate Cancer. UROL INT 101(1):65-73. (2018)* *IF: 1.508 / Q2*
3. ***Szarvas T***, *Csizmarik A, Szücs M, Nyirády P [Molecular subtypes and perspectives of targeted therapies in prostate cancer ORVOSI HETILAP 160(7):252-263. (2019)* *Markusovszky díj 2019* *IF: 0.322 / Q3*
4. ***Szarvas T***, *Sevcenco S, Módos O, Nyirády P, Kubik A, Romics M, Kovalszky I, Reis H, Hadaschik B, Shariat SF, Kramer G. Circulating syndecan-1 is associated with chemotherapy-resistance in castration-resistant prostate cancer. UROL ONCOL 36:312.e9-312.e15. (2018)* *IF: 3.397 / Q1*
5. *Reis H,* ***Szarvas T***, *Grünwald V. Predictive biomarkers in oncologic uropathology. Pathologe 40:264-275 (2019).* *IF: 0.547 / Q3*
6. *Maj-Hes A, Sevcenco S,* ***Szarvas T***, *Kramer G. Claros System: A Rapid Microfluidics-Based Point-of-Care System for Quantitative Prostate Specific Antigen Analysis from Finger-Stick Blood. ADVANCES IN THERAPY 36(4):916-922 (2019)* *IF: 3.260 / Q1*
7. *Küronya Z, Sükösd F, Varga L, Bíró K, Gyergyay F, Géczi L, Nagyiványi K, Jorgo K,* ***Szarvas T***, *Kovács A, Varga Z, Kahán Z, Maráz A. ERG Expression and the Efficacy of Docetaxel Combined with Androgen Deprivation Therapy in Metastatic Hormone-Sensitive Prostate Cancer. UROL ONCOL. 37(4):289.e1-289.e9. (2019)* *IF:3.397 / Q1*
8. ***Szarvas T***, *Csizmarik A, Fazekas T, Hüttl A, Nyirády P, Hadaschik B, Grünwald V, Püllen L, Jurányi Z, Kocsis Z, Shariat SF, Sevcenco S, Maj-Hes A, Kramer G. Comprehensive analysis of serum chromogranin A and neuron-specific enolase levels in localized and castration-resistant prostate cancer. BJU INT. 127(1):44-55. (2021)* *IF: 4.688 / D1*
9. ***Szarvas T***, *Csizmarik A, Nagy N, Keresztes D, Váradi M, Küronya Z, Riesz P, Nyirády P. Molecular underpinnings of systemic treatment resistance in metastatic castration-resistant prostate cancer. ORVOSI HETILAP 161(20):813-820. (2020)* *IF: 0.322 / Q3*
10. ***Szarvas T***, *Csizmarik A, Váradi M, Fazekas T, Hüttl A, Nyirády P, Hadaschik B, Grünwald V, Tschirdewahn S, Shariat SF, Sevcenco S, Maj-Hes A, Kramer G. The prognostic value of serum MMP-7 levels in prostate cancer patients who received docetaxel, abiraterone, or enzalutamide therapy. UROL. ONCOL. S1078-1439(20)30436-1. (2020)* *IF:3.397 / Q1*
11. *Csizmarik A, Hadaschik B, Kramer G, Nyirády P,* ***Szarvas T.*** *Mechanisms and markers of resistance to androgen signaling inhibitors in patients with metastatic castration-resistant prostate cancer. UROL. ONCOL. (accepted for publication) (2021)* *IF:3.397 / Q1*
12. *Nagy D, Fazekas T, Baghy K, Papp G, Csizmarik A, Szucs M, Nyirady P,* ***Szarvas T.*** *Efficacy of carboplatin chemotherapy in a BRCA2 mutation positive metastatic castration-resistant prostate cancer patient. ORVOSI HETILAP (accepted for publication) (2021)* *IF: 0.322 / Q3*
13. ***Szarvas T***, *Csizmarik A, Váradi M, Olah C, Nyirady P. Therapeutically relevant molecular alterations in prostate and bladder cancer. ORVOSTOVABBKEPZO SZEMLE (invited manuscript) (2021)*

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14. *Maj-Hes A,* ***Szarvas T***, *Sevcenco S, Kramer G. Multiple docetaxel retreatments without prednisone for metastatic castration-resistant prostate cancer in the docetaxel-only era: effects on PSA kinetics and survival. ADVANCES IN THERAPY 36(4):916-922 (2019)* *(revisions submitted)* *IF: 3.871 / Q1*
15. *Keresztes D, Nagy N, Csizmarik A, Módos O, Fazekas T, Bracht T, Sitek B, Witzke K, Puhr M, Sevcenko S Kramer G, Shariat SF, Takács T, Tornyi I, Lázár J, Hadaschik B, Szűcs M, Nyirády P,* ***Szarvas T.*** *Comparative proteome analysis identified CD44 as possible serum marker for docetaxel resistance in castration-resistant prostate cancer THE PROSTATE* *(under review)* *IF: 3.297 / D1*

11 Presentations on the topic of current research grant

11.1 Presentations in Hungarian between

1) 31. Füvészkerti Urológus Napok, 2018. Február 16-17. Budapest
   Szerzők: Csizmarik A, Módos O, Keresztes D, Nagy N, Kubik A, Romics M, Sevcenco S, Reis H, Schmid KW, Niedworok C, Hadaschik B, Kovalszky I, Shariat SF, Kramer G, Nyirády P, **Szarvas T**
   A Syndecan-1 prognosztikai és terápia prediktív értéke korai és előrehaladott stádiumú prosztatarákban
2) Semmelweis Egyetem, PhD napok, 2018. Április 20 11. Budapest
   Keresztes D, Bracht T, Sitek B, Puhr M, Módos O, Csizmarik A, Nagy N, Kramer G, Shariat SF, Nyirády P, **Szarvas T**
   Identification of predictive serum biomarkers for chemotherapy resistance in prostate cancer using mass spectrometric proteome analysis
3) Magyar Onkológusok Gyógyszerterápiás Tudományos Társasága IX. Kongresszusa, 2018. Május 17-19. Budapest, Csizmarik A, Módos O, Keresztes D, Nagy N, Kubik A , Romics M, Sabina S, Reis H, Schmid KW, Niedworok C, Hadaschik B, Kovalszky I, Shariat SF, Kramer G, Nyirády P, **Szarvas T**
   A Syndecan-1, a korai és előrehaladott prosztatarák prediktív és prognosztikus biomarkere
4) Magyar Uroonkológus Társaság 10. Kongresszus, Budapest, 2018. Május 25-26.
   Keresztes D, Bracht T, Sitek B, Witzke K, Puhr M, Orsolya M, Csizmarik A, Nagy N, Kramer G, Shariat SF, Nyirády P, **Szarvas T**
   A prosztatarák kemoterápia rezisztenciáját előrejelző fehérjemarkerek azonosítása tömegspektroszkópiai módszerrel
5) Magyar Uroonkológus Társaság 10. Kongresszus, Budapest, 2018. Május 25-26.
   Csizmarik A, Módos O, Keresztes D, Nagy N, Kubik A, Romics M, Sevcenco S, Reis H, Schmid KW, Niedworok C, Hadaschik B, Kovalszky I, Shariat SF, Kramer G, Nyirády P, **Szarvas T**
   Szérum biomarkerek a prosztatarák várható viselkedésének előrejelzésében
6) Magyar Uroonkológus Társaság 10. Kongresszus, Budapest, 2018. Május 25-26.
   Szarvas T Az előrehaladott prosztatarák terápia érzékenységének előjelzése
7) Magyar Urológusok Társasága - XXIII. Kongresszus, Budapest, 2018. November
   Keresztes D, Bracht T, Sitek B, Witzke K, Puhr M, Módos O, Csizmarik A, Nagy N, Kramer G, Shariat SF, Szűcs M, Nyirády P, **Szarvas T**
   Összehasonlító proteomikai vizsgálat alapján a NAMPT fehérje szérumkoncentrációja segíthet a prosztatarák kemoterápia
8) Minimál Invazív Eljárások az Urológiában, Budapest, 2019. Január 19.
   Szarvas T Az előrehaladott prosztatarák molekuláris háttere és annak terápiás vonatkozásai
9) Magyar Sugárterápiás Társaság 14. Kongresszusa, 2019. Május 16-18. Lillafüred
   Szarvas T Urológiai daganatok molekuláris altípusai és terápiás vonatkozásaik
10) Magyar Uroonkológus Társaság 10. Kongresszus, 2019. Május 24-25. Budapest
   Csizmarik A, Nagy N, Kubik A, Szendrői A, Módos O, Oláh C, Keresztes D, Kenessey I, Szász AM, Oroszi M, Nagy Z, Francz M, Korodi-Gál B, Jamool N, Hadaschik B, Nyirády P, **Szarvas T**
   Az enzalutamid rezisztenciában szerepet játszó fehérje markerek azonosítása prosztatarákban
11) Semmelweis Egyetem, PhD napok, 2019. Április 25-26. Budapest
   Csizmarik A, Bracht T, Sitek B, Puhr M, Tornyi I, Lázár J, Takács L, Keresztes D, Nagy N, Hadaschik B, Nyirády P, **Szarvas T**
   Identification of proteins with potential involvement in enzalutamide resistance of prostate cancer
12) Magyar Urológusok Társasága - XXIV. Kongresszus, 2019. Október 10-13. Budapest
   Keresztes D, Módos O, Szűcs M, Hüttl A, Csizmarik A, Nagy N, Bracht T, Sitek B, Witzke K, Takács L, Tornyi I, Puhr M, Sevcenko S, Kramer G, Shariat SF, Nyirády P, **Szarvas T**
   NAMPT és CD44, mint potenciális szérummarkerek a prosztatarák docetaxel-rezisztenciájában
13) Magyar Urológusok Társasága - XXIV. Kongresszus, 2019. Október 10-13. Budapest
   Nagy D, Fazekas T, Csizmarik A, Nyirády P, **Szarvas T**, Szűcs M
   Platina-alapú kemoterápia hatékonysága egy BRCA2 mutáció pozitív prosztatarákos betegnél
14) 33. Füvészkerti Urológus Napok, 2020. Február 14-15. Budapest

Csizmarik A, Bracht T, Sitek B, Puhr M, Tornyi I, Lázár J, Takács L, Keresztes D, Nagy N, Váradi M, Hadaschik B, Nyirády P, **Szarvas T** A prognosztarák enzalutamid elleni rezisztenciájában szerepet játszó fehérjék azonosítása.

15) Semmelweis Egyetem, PhD napok, 2020. Augusztus 31. – Szeptember 1. Budapest
Keresztes D, Módos O, Szűcs M, Hüttl A, Csizmarik A, Nagy N, Váradi M, Bracht T, Sitek B, Witzke K, Puhr M, Sevcenko S, Kramer G, Shariat SF, Takács L, Tornyi I, Lázár J, Nyirády P, **Szarvas T** NAMPT and CD44 as possible serum biomarkers in docetaxel-resistance of castration resistant prostate cancer.
Best poster prize

16) Semmelweis Egyetem, PhD napok, 2020. Augusztus 31. – Szeptember 1. Budapest
Csizmarik A, Bracht T, Sitek B, Witzke K, Puhr M, Takács L, Tornyi I, Lázár J, Keresztes D, Nagy N, Váradi M, Sevcenko S, Kramer G, Shariat SF, Maj-Hes A, Hadaschik B, Nyirády P, **Szarvas T** Comparative proteome analysis identified ALCAM as a potential serum biomarker for enzalutamide resistance in castration-resistant prostate cancer.
Best poster prize

17) Magyar Urológusok Társasága - XXV. Kongresszus, 2020. Október 08-10. On-line
Csizmarik A, Bracht T, Sitek B, Puhr M, Tornyi I, Lázár J, Takács L, Keresztes D, Nagy N, Váradi M, Hadaschik B, Nyirády P, **Szarvas T** Az abirateron rezisztenciában szerepet játszó fehérje markerek azonosítása prosztatarákban.

11.2 Presentations at international congresses

18) 18th Central European Meeting (CEM) of the Europeran Association of Urology
2018. October 12. Cluj Napoca, Romania
Keresztes D, Bracht T, Sitek B, Puhr M, Módos O, Csizmarik A, Nagy N, Kramer G, Shariat SF, Szűcs M, Nyirády P, **Szarvas T** Identification of predictive serum biomarkers for chemotherapy resistance in prostate cancer using mass spectrometric proteome analysis

19) 34th Annual Meeting of the Europeran Ass. of Urology, 2019. March 15-19. Barcelona, Spain
Keresztes D, Bracht T, Sitek B, Witzke K, Puhr M, Szücs M, Módos O, Csizmarik A, Nagy N, Hüttl A, Kramer G, Shariat S, Nyirády P, **Szarvas T** Comparative proteome analysis identified NAMPT as a potential serum marker for the prediction of docetaxel-resistance in prostate cancer

20) International Workshop on Prostate Cancer Biomarkers / Biomarkers improving management in NEPC (Neuroendocrine prostate cancer) Webinar
Szarvas T Molecular mechanisms of therapy resistance in CRPC – the role of neuroendocrine transdifferentiation
2020. December 9. Internet

21) 36th Annual Meeting of the Europeran Ass. of Urology, 2021. July 9-12. Milan, Italy
Csizmarik A, Bracht T, Sitek B, Witzke K, Puhr M, Tornyi I, Lázár J, Takács L, Keresztes D, Nagy N, Váradi M, Kramer G, Sevcenco S, Maj-Hes A, Shariat SF, Hadaschik B, Nyirády P, **Szarvas T** Activated leukocyte cell adhesion molecule (ALCAM) as a possible serum biomarker for enzalutamide resistance in castration-resistant prostate cancer
Abstract submitted

11.3 Further achievements reached by the NKFIH/OTKA grant support

11.3.1 Support of young researchers

- The NKFIH/OTKA grant support provided the basis for two PhD doctoral works (Anita Csizmarik and Dávid Keresztes) with proposed thesis submission dates of 08/2022 and 08/2021.
- A further ongoing PhD research work by Dr. Borbála Kovács (Department of Molecular Biology, Semmelweis University, Supervisor: Prof. Dr. Péter Csermely), based on the proteome data generated by the support of this grant support.
- The NKFIH/OTKA grant support provided the basis for a diploma work (Réka Bújdosó) closed: 04/2021.

11.3.2 Prizes

- Our review paper on the molecular background of prostate cancer published in Orvosi Hetilap has received the Markusovszky-prize in 2019.
- Anita Csizmarik received best poster prize for their research/presentation at the PhD Scientific Days 2020 of the Semmelweis University
- David Keresztes received best poster prize for their research/presentation at the PhD Scientific Days 2020 of the Semmelweis University

11.3.2 Further grant support

- Dávid Keresztes based on her achievement in the present project and deduced research plan received a Scholarship for the Young Talents of the Nation (NTP-NFTÖ-20-B-0314) provided by the Hungarian Ministry of Human Capabilities.
- Anita Csizmarik based on her achievement in the present project and deduced research plan received a New National Excellence Program of the Ministry for Innovation and Technology from the source of the National Research, Development and Innovation Fund.
- Achieved results provide a solid foundation of data for further research and grant applications for the future.