D6.5

Generate cell line drug sensitivity/resistance validation assays

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Abstract:	This report describes the validation of computational predictions from gene expression data and analysis of phosphoproteomic perturbation data using optimized cell line drug sensitivity/resistance validation assays. PI3K and MAPK pathways and the Hsp90 involvement have been confirmed by differential sensitivity in the used prostate cancer cell lines. Based on our data specific inhibitors could be of clinical significance.
Keywords:	drug sensitivity and resistance validation, cell viability, in vitro validation

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Executive Summary

The main task of D6.5 was to generate drug sensitivity cellular assays in order to validate computational models developed in work package 5 (WP5). We have generated cell line drug sensitivity/resistance validation assays based on several prostate cell lines. In our protocol we have optimized an end-point cell viability assay as well as a cell perturbation analysis technique that is capable of measuring androgen dependent and independent drug-induced changes in cell viability, proliferation and adhesion in a real-time fashion.

Phosphoproteomic data generated in D5.2 showed significant global differences in differential expression phosphorylation patterns between LNCaP and LNCaP-abl cell lines, but harder to detect differences in PI3K and MAPK pathway related proteins upon perturbation with different inhibitors and ligands. Using real-time cellular analysis system we could confirm that cell perturbation induced by PI3K/AKT and MEK kinase inhibitors was cell line specific, but was androgen independent. Furthermore, the androgen independent cell line, LNCaP_abl was more sensitive to PI3K/AKT inhibitors pictilisib and PI-103. Thus, PI3K/AKT inhibitors could be possible effective drugs that could target castration resistant prostate cancers, and several candidate PI3K inhibitors are currently in clinical trials.

Another protein target, Hsp90 was also found in WP5 using bioinformatics analysis of diverse prostate cancer gene expression data. We tested two different Hsp90 inhibitors on both cell lines using end-point and real-time cellular assays. Like in the case of PI3K/AKT inhibition, similar differential sensitivity could be recorded: the androgen independent cell line LNCaP_abl was more sensitive than normal LNCaP cells. Additionally, Hsp90 inhibitors are very promising drugs in anticancer therapy. According to our results these drugs could be also used in castration resistant prostate cancer patients.

Other targets suggested by Boolean model were MAX, Foxo1, HTERT and MEK 1/2 kinase. We also evaluated them in our assays using specific inhibitors with and without androgen treatment. No significant differences in cytotoxic effects could be detected in case of Foxo1 and telomerase inhibitors between the different prostate cell lines used in the assays. However, slight difference in cytotoxicity could be detected in case of all the three applied MEK inhibitors (trametinib, PD184352 and selumetinib) in LNCaP cell line. Besides cytotoxic effects, we could detect differences in cell morphology and cell attachment only in the LNCaP, but not in the LNCaP_abl cells. Interestingly, in case of all the three applied MEK inhibitors induced changes in cell morphology or cell attachment only in the LNCaP, but not in the LNCaP_abl cells. This could underline the possible therapeutic potential of MEK inhibitors in androgen sensitive prostate cancer patients, but not in castration resistant cases.

Additionally, this deliverable describes methods, alternative to Logical models of WP5, that propose new drug targets. These two methods have been proposed by TUDA and IBM and are based on methods developed within WP3 and WP4, however, their validation is beyond the scope of the PrECISE project.

Contents

Chapter 1 Introduction

Predicting cellular phenotypes (e.g.: disease states, cancer drug sensitivity etc.) from different high-coverage molecular ('omics') data is a key question of current systems biology research. Due to increasing affordability of large-scale acquisition and improving methodologies for analysis, genomics, transcriptomics and proteomic have become indispensable source for these studies [1]. Relating the molecular data to the patient therapy is a multi-step process. Complexity of the data requires development of *in silico* models. These models also help to understand the mechanism of anti-cancer drugs.

Bioinformatic models highlight proteins and pathways, essential for prostatic cancer cell growth, proliferation and metastasis. Logic model inference from cell line and patient (phospho-)proteomic data or chemical compound analysis correlation with transcriptomic data can be used to identify the pathways, inhibition of which is likely to hinder cancer cell growth or to trigger cell death. To determine whether inhibition of these pathways indeed affects cell viability – initiate cell death or prevent proliferation, cell viability assays were used.

The aim of this deliverable is to develop and execute cell line drug sensitivity assays, or cell viability assays. There are numerous conventional cell viability methods (MTT assay, Luminescent Cell Viability Assay, Trypan blue assay, etc.) but most of them provide only one aspect of cellular state at a given time point (endpoint assays). For the validation of computationally predicted cell cytotoxicity and/or phosphoproteomic evaluations it is more relevant to validate the data with label-free and real-time monitoring of cells to complement results of the end-point measurements [3], which provides information on cellular behavior in a continuous manner. Moreover, cell proliferation, attachment and migration potential of prostate cancer cells are highly depending on androgens present or absent in cell culture medium [4], which could be also followed in these assays.

For validation, different inhibitors need to be screened. These inhibitors have been selected to inhibit intervention points, proposed by upstream package (WP5). Below we give a short overview of these methods and the resulting list of chemical compounds, that are similar to proposed drugs or are expected to inhibit proteins (intervention points) proposed by the models.

Chapter 2 From computational models to intervention points

2.1 Overview of computational models used for drug sensitivity prediction

In PrECISE several approaches have been used to propose the list of intervention points and drug targets. These approaches are described in detail in D5.4. Briefly, the following methods were used:

- **Use of prostate cancer logical models to identify points of intervention**

CI has built a logical model of prostate cancer, involving the main signaling pathways altered in prostate cancer cells that was delivered in D5.1. This model was tailored to a set of prostate TCGA patients and cell lines used by the consortium as described in D5.3 and D5.4. Stochastic simulations allow the partners to obtain quantitative output probabilities summarizing complex asynchronous dynamics. The model predicted a set of cell-line-specific phenotypic behaviors for a prostate cell given its micro-environmental and genetic condition. The effects of different perturbations of the model on these phenotypes show how genomic alterations initiate and contribute to the different hallmarks composing cancer progression. These models were used to uncover points of intervention to hamper cells' proliferation and/or enhance apoptosis in LNCaP-specific models as described in D5.4.

- **Use of proteomic data sets in cancer cell lines to identify points of intervention**

ETH provided the two SWATH-MS proteomic data sets that were described in D5.2. LNCaP and LNCaP_abl cell lines were chosen as they are closely related, but differ in major component – castration sensitivity, major component of prostate cancer aggressiveness. For these cell lines major kinases - PI3K and MEK have been inhibited. This setup allows to address specifically the changes that are related to the androgen insensitive growth of tumors and is associated with the switch to metastasis. This experiment shows that the general logical model developed in T5.1 and D5.1 is sufficiently covered by phosphopeptides measures. Moreover, additional cancer-related pathways are well represented in the data matrix acquired. The use of the Boolean model approach of PHONEMeS in combination with this data suggested that the perturbation of MAPK proteins (related to MEK signaling) and CDK proteins (related to cell cycle) could have different effects in these two cell lines.

- **Use of public transcriptomic and drug sensitivity data to identify points of intervention**

UKAACHEN analyzed the recently released LINCS-L1000 dataset together with public drug sensitivity (CTRP) and gene essentiality (Achilles) data to create models predicting cell viability from perturbation gene expression data. LINCS-L1000 dataset contains perturbation gene expression profiles, i.e. transcriptomics changes after different (compounds, gene overexpression or knockout / knockdown) perturbations. UKAACHEN used these models to

predict cell viability for the prostate cancer cell lines (VCaP and PC3) in the LINCS-L1000 data. As in this study the cell lines were perturbed with more than 20,000 drugs (most of them are not traditional anti-cancer compounds) this opened the possibility to identify new, prostate cancer specific toxic compounds.

2.2 Selected chemical compounds, protein targets and cell lines

Based on the list of candidates suggested by the consortium partners (CI, ETH, UKAACHEN) in D5.4 Table 2 “List of proposed drug and targets to be tested in validation experiments”, we selected small molecule inhibitors of putative targets against prostate cancer cells (Table 1) based on feasibility and commercial availability. The drugs, their targets and the corresponding models are summarized in Table 1. These targets represent some of the hallmark signalling pathways that are related to cell proliferation. Based on chemical database (Selleckchem) and scientific references, inhibitors were purchased and tested at 0, 3.3, 10 and 30 µM concentrations. The viability test of the treated cells was carried out with a dilution series of each inhibitor. The cell lines were treated and investigated in parallel.

Table 1 Selected inhibitors used in the validation assays

TARGET	TYPE	NOTES / PROPOSED BY	COMPOUND / INHIBITOR NAME	Clinical stage
HSP90AA1	gene	Highlighted by the Boolean model and found in other datasets used within PrECISE	**17-DMAG**	Phase 2
			NMS-E973	preclinical
PI3K/AKT	gene	Relevant in cancer progression	**PI-103**	preclinical
			Pictilisib	Phase 2
MEK	gene	Highlighted by the use of PHONEMeS in the phosphoproteomic data because of the MAPK proteins, some of which also highlighted by the Boolean model. Also relevant in cancer progression.	**PD184352 (CI-1040)**	Phase 2
			Trametinib	approved
			Selumetinib (AZD6244)	Phase 3
MAX	gene	Highlighted by the Boolean model and found in other datasets used within PrECISE	**10058-F4**	preclinical
TERT	gene	Highlighted by the Boolean model and found in other datasets used within PrECISE	**BIBR 1532**	preclinical
AR	gene	Relevant in cancer progression and a target of some of the drugs proposed by the statistical model of perturbation data	**Enzalutamide**	approved
			Abiraterone	approved
CAY-10585	drug	HIF-1 inhibitor	**CAY-10585**	preclinical
Androstanol	drug	Agonist of the androgen receptor (AR)	**5α-Androstan-3β-ol**	preclinical
testosterone-propionate	drug	Agonist of the androgen receptor (AR)	**Testosterone propionate**	approved
formestane	drug	Aromatase inhibitor, also related to AR/steroids	**Formestane**	approved
ornidazole	drug	Anti-protozoa antibiotic	**Ornidazole**	approved
meclocycline	drug	Tetracycline antibiotic	**Meclocycline**	approved

Chapter 3 Experimental Protocol

3.1 Experimental design

During the optimization we had to take into consideration that the tasks under work package 5 (WP5) used highly different datasets: cell line (phospho-)proteomic data, patient proteomic and public genomic and transcriptomic data, drug sensitivity databases and massive public cell line transcriptomic data. As part of validation plan, we address incompleteness of information. For example, to acquire phosphoproteomic data, cells were measured under short treatment period with different inhibitors (a few hours), moreover the computational models lack information about drug concentrations or incubation times. That is why ABT tested different inhibitors of key protein targets of selected pathways in two independent viability assays (real time and end-point) (Figure 1).

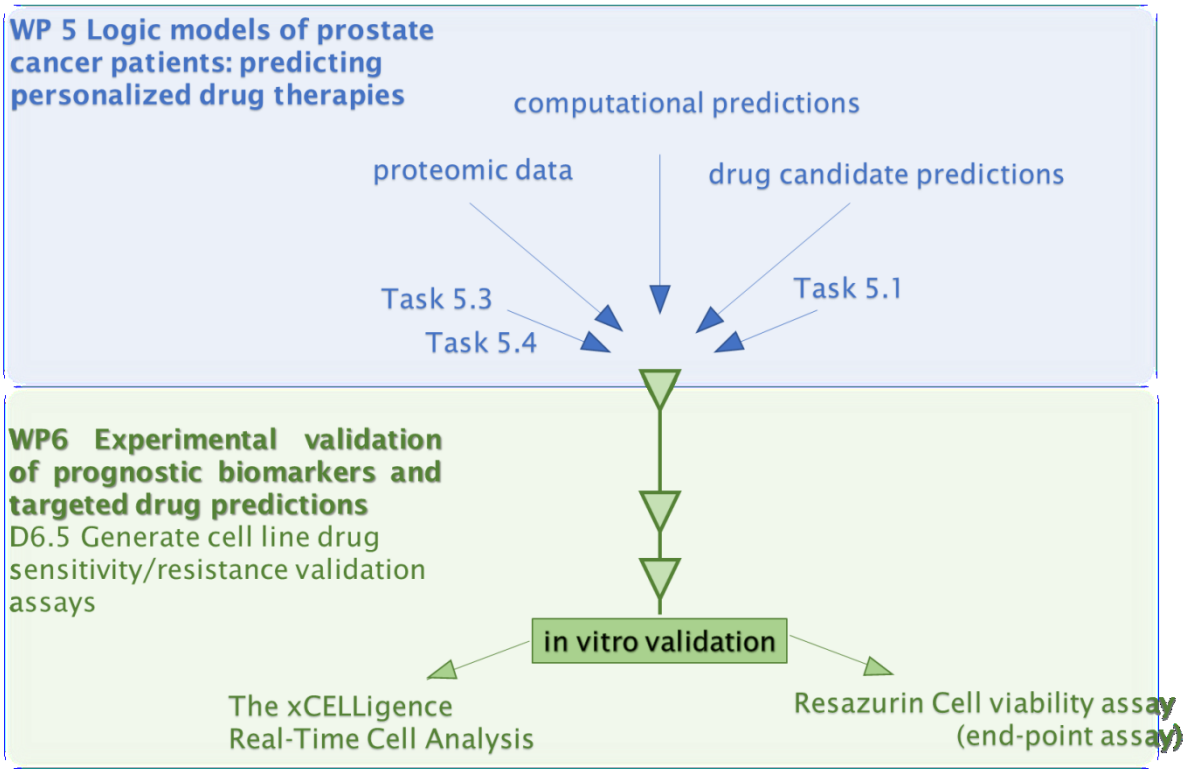


Figure 1. Schematic representation of the encompassed datasets

3.2 Cell lines and culture methods

For *in vitro* drug perturbation validations we used prostate adenocarcinoma cell lines, the androgen sensitive LNCaP and its androgen independent derivative LNCaP-abl cell line [5] and androgen insensitive PC3 and androgen sensitive VCaP cell lines for LINCS-L1000 prediction validation. Cells were maintained in culture media by manufacturer recommendation supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin with or without 1 nM dihydrotestosterone (DHT). Once 70% confluency was reached, the cells were seeded into 96-well microtitre plates and the media were replaced with media containing the tested compound after 16 hours.

Cells were tested either in an end-point assay (3.2) after 72 h incubation period, or followed in real-time for 48-72-96 h.

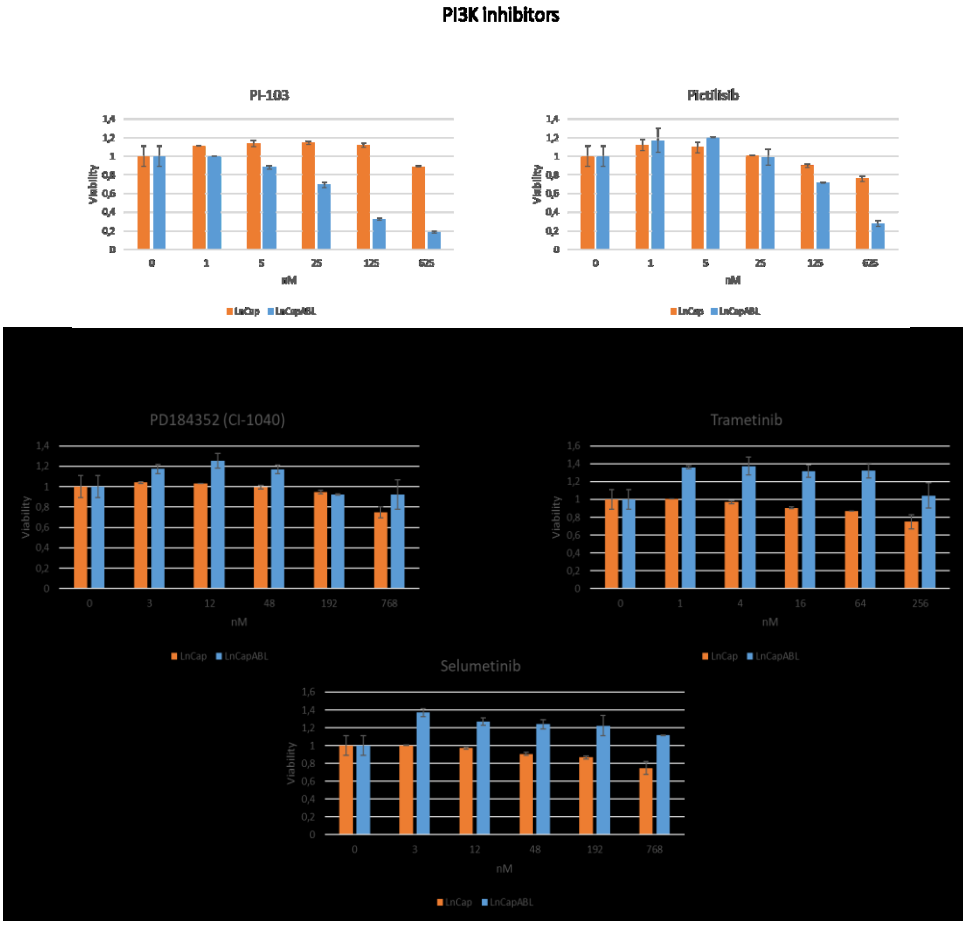


Figure 2. Cell viability assay show dose dependent response to PI3K inhibitors

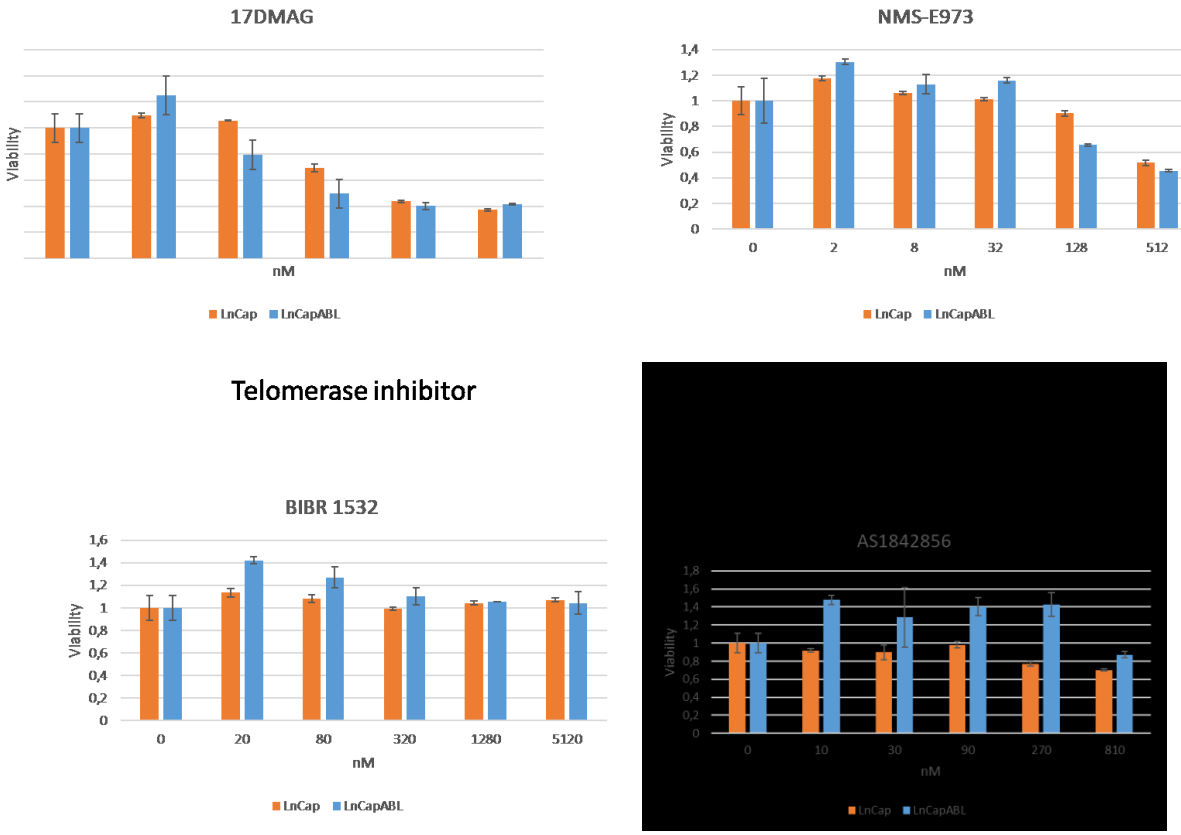
3.3 End-point cell viability measurements

In vitro toxicity of the selected inhibitors was determined on cell lines using the fluorescent Resazurin assay. The cells were seeded into 96-well plates (Corning Life Sciences, Tewksbury,MA, USA) in media and incubated overnight. Test compounds were dissolved in dimethyl sulfoxide (DMSO). Cells were treated with an increasing concentration of test compounds. The highest applied DMSO content of the treated cells was 0.4%. Cell viability was determined after 72 hours incubation. Resazurin reagent (Sigma–Aldrich, Budapest, Hungary) was added at a final concentration of 25µg/mL. After a 2-h at 37°C 5%, CO2 fluorescence (530 nm excitation/580 nm emission) was recorded on a multimode microplate reader (Cytofluor4000, PerSeptive Biosytems, Framingham, MA, USA). Viability was calculated with relation to untreated control cells and blank wells containing media without cells. IC 50 values (50% inhibiting concentration) were calculated by GraphPad Prism® 5 (La Jolla, CA, USA).

Compounds targeting Hsp90 (NMS-E973,17-DMAG) and PI3K/AKT pathway (PI-103, Pictilisib) showed dose dependent activity in the end-point cell viability assay after 72-hour in both cell line (Figure 2 and 3). Moreover, the PI-103 (PI3K/AKT) inhibitor showed 100x selectivity to LNCaP_abl cell line with estimated 0,1 µM IC50 value, comparing to LNCaP cell's with ~10 µM IC50 value. The 17-DMAG (Hsp90 inhibitor) showed similarity with more than 20-times selectivity to LNCaP cell line (~0,4 µM vs. ~10 µM IC50).

We found that the MEK 1/2 inhibition did not affect cell viability significantly, moreover no cytotoxic effects could be detected in case of Foxo1 and telomerase inhibitors (AS1842856 and BIBR 1532, respectively) between the different prostate cell lines used in the assays.

Figure 3. From predicted three inhibitors only Hsp90 inhibition showed dose dependent response in the two tested LNCaP cell lines



The calculated IC50 values are shown in Table 2. When cell lines responded differently to the same drug treatment we marked the difference with green highlight.

Table 2 Experimentally calculated IC50 values

INHIBITOR NAME	TARGET	IC 50 LNCaP	IC 50 LNCaP_abl	SENSITIVE TO DRUG	DIFFERENCE BETWEEN USED CELL LINES	CONDITION
PD184352 (CI-1040)	MEK inh.	~20μM	~20μM	No	No	DHT -/+
PI-103	PI3K/AKT inh.	~10μM	~0,1μM	Yes	Yes	DHT -/+
Pictilisib	PI3K/AKT inh.	~3μM	~0,4μM	Yes	Yes	DHT -/+
Trametinib	MEK inh.	>30 μM	~30 μM	No	No	DHT -/+
Selumetinib (AZD6244)	MEK inh.	>30 μM	>30 μM	No	No	DHT -/+
10058-F4	MyC-MAX inh.	>30 μM	>30 μM	No	No	DHT -/+
BIBR 1532	HTERT inh.	>30 μM	>30 μM	No	No	DHT -
AS1842856	Foxo1 inh.	~4 μM	~2 μM	Yes	No	DHT -
NMS-E973	Hsp90 inh.	~2 μM	~0,5 μM	Yes	Yes	DHT -
17-DMAG	Hsp90 inh.	~10 μM	~0,4 μM	Yes	Yes	DHT -
		IC50 VCaP	IC50 PC3			
CAY-10585	HIF1-alpha inh.	7,38 μM	16,72 μM	Yes	No	DHT -
5α-Androstan-3β-ol	AR agonist	4,44 μM	24,2 μM	Yes	Yes	DHT -
Testosterone propionate	AR agonist	3,26 μM	>30 μM	Yes/VCaP	Yes	DHT -
Formestane	Aromatase inh.	7,17 μM	>30 μM	Yes/VCaP	Yes	DHT -
Ornidazole	Anti-protozoa antibiotic	2,29 μM	23,75 μM	Slightly	Yes	DHT -
Meclocycline	Tetracycline antibioti	11,2 μM	4,24 μM	Yes	Yes	DHT -

3.4 xCELLigence Real Time Cell Analysis System

The xCELLigence System analyzes label-free and noninvasively the electronic impedance through microelectrodes placed on the bottom of the specially designed microtiter plates in real-time manner. These impedance events give quantitative information about the biological status of cells, such as cell number, viability and morphology. [6] The real-time impedance data cannot be obtained by traditional endpoint assays.

3.4.1 Cellular impedance

The functional unit of a cellular impedance assay is a set of gold microelectrodes fused to the bottom surface of a microtiter plate well (Figure 4). When submersed in an electrically conductive solution (culture medium), the application of an electric potential across these electrodes causes electrons to exit the negative terminal, pass through bulk solution, and then deposit onto the positive terminal to complete the circuit. Because this phenomenon is dependent upon the electrodes interacting with bulk solution, the presence of adherent cells at the electrode-solution interface impedes electron flow. The magnitude of this impedance is dependent on the number of cells, the size and shape of the cells, and the cell-substrate attachment quality.

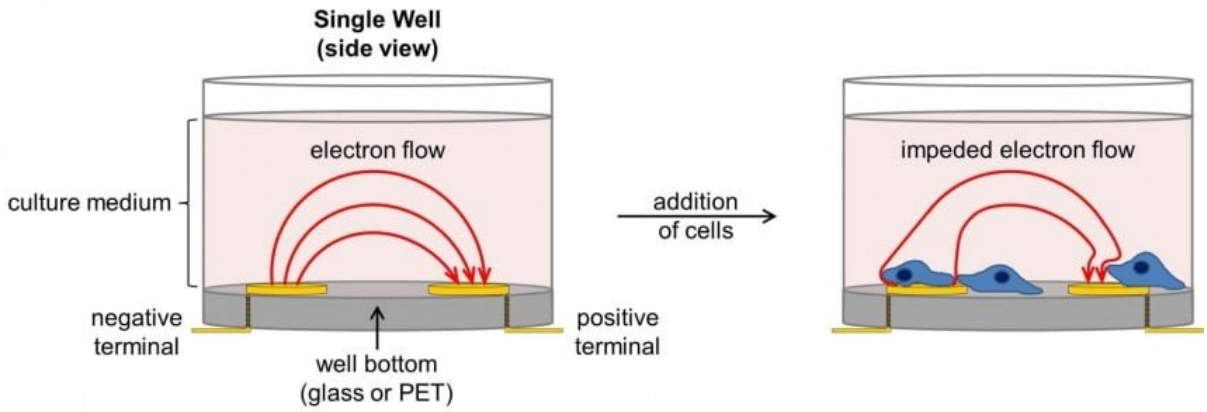


Figure 4. Overview of cellular impedance apparatus. A side view of a single well is shown before and after cells have been added.
(source: https://www.aceabio.com/products/icelligence/)

3.4.2 The Cell Index (CI)

The impedance of electron flow caused by adherent cells is reported using a unitless parameter called Cell Index (CI), where CI = (impedance at time point n – impedance in the absence of cells)/nominal impedance value.

On Figure 5 we show a generic example of a real-time impedance trace throughout the course of setting up and running an apoptosis experiment. For the first few hours after cells have been added to a well there is a rapid increase in impedance. This is caused by cells falling out of suspension, depositing onto the electrodes, and forming focal adhesions. When cells reach confluence the CI value plateaus, reflecting the fact that the electrode surface area that is accessible to bulk media is no longer changing. The addition of a cytotoxic inducer at this point causes a decrease in CI back down to zero. This is the result of cells rounding and then detaching from the well bottom. While this generic example involves drug addition when cells are confluent, impedance-based assays are extremely flexible and can also evaluate the rate and extent of initial cell adhesion to the electrodes, or the rate and extent of cell proliferation.

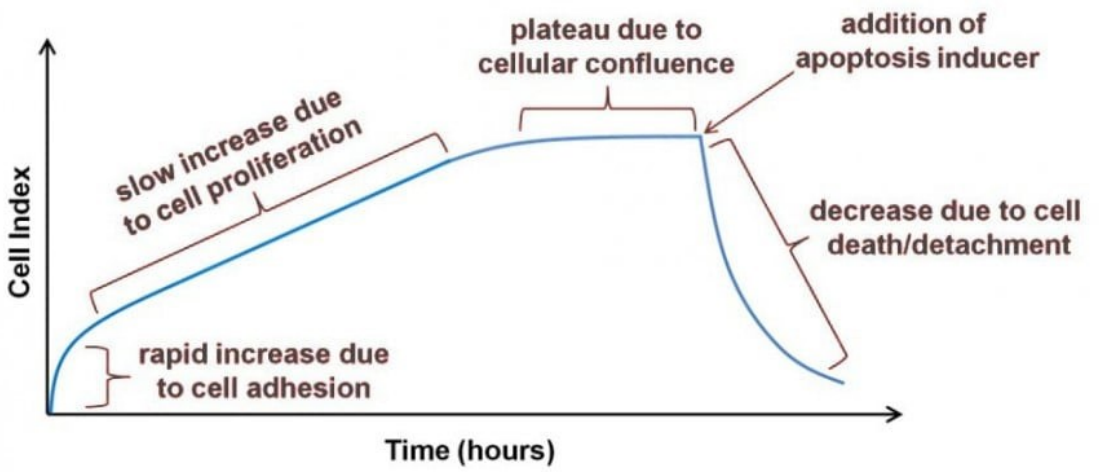


Figure 5. Real-time impedance trace for setting up and running a cytotoxicity assay.
(source: https://www.aceabio.com/products/icelligence/)

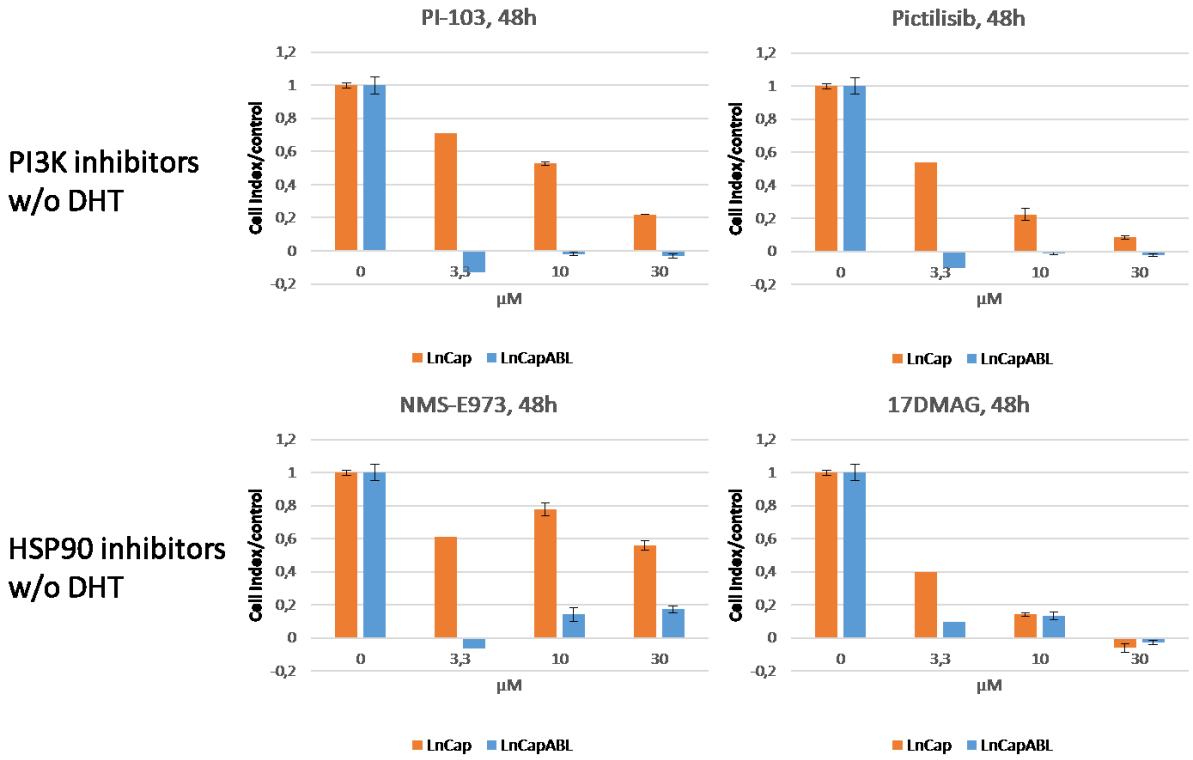


Figure 6. PI3K and Hsp90 inhibitors resulted dose dependent changes in both cell lines

The real-time cytotoxicity tests were carried out with all the inhibitors on both cell lines to follow in real time the changes in viability of the treated cells with higher concentrations than in the end-point assay, but the same conclusions could be drawn, that targeting Hsp90 or PI3K resulted in differential cytotoxic effects when comparing the two cell lines. Selective cytotoxicity could be recorded for LNCaP_abl cell line (Figure 6), which showed higher sensitivity to the drugs used.

Chapter 4 Validation of proposed targets and related pathways

4.1 PI3K/AKT Signalling Pathway:

The PI3K/AKT signaling pathway controls many different cellular processes like cell growth, motility, proliferation, and apoptosis. This pathway is frequently uncontrolled in different cancer cells [7]. Many inhibitors are in different stages of clinical development and some of them are approved for clinical use. The realization that the LNCaP (androgen sensitive) and LNCaP_abl (androgen independent) are sensitive to PI3K inhibitors (Figure 7), moreover LNCaP_abl is 100x sensitive than the initial LNCaP cell line, gives valuable treatment option to clinicians in treatment of castration resistant prostate cancers.

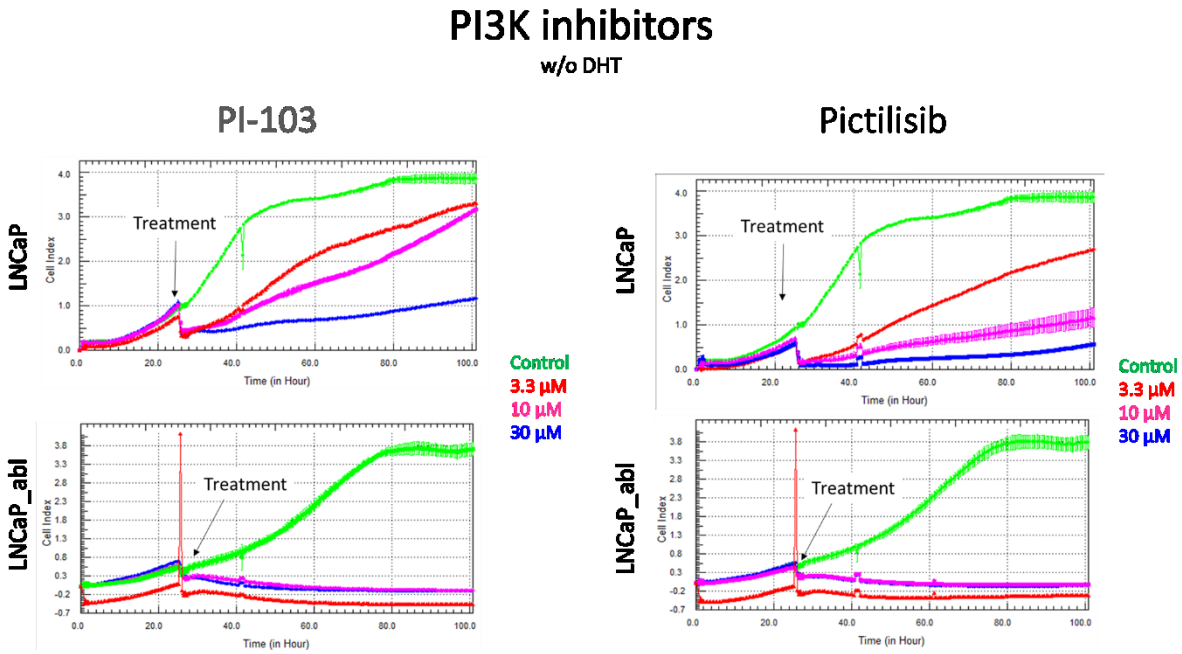


Figure 7 PI3K/AKT pathway inhibition with different PI3K/AKT inhibitors shows dose dependent response in LNCaP and LNCaP_abl cell lines

ETH's (D5.2) pharmacological inhibition of PI3K/AKT pathway resulted in no differences in the level of pAKT (Ser473) protein level with or without DHT treatment. Phosphorylation level of p-AKT upon enzalutamide treatment concentrations ranging from 0 to 107 µM after a 1h exposure time had no effect in protein expression related to the PI3K signaling pathway.

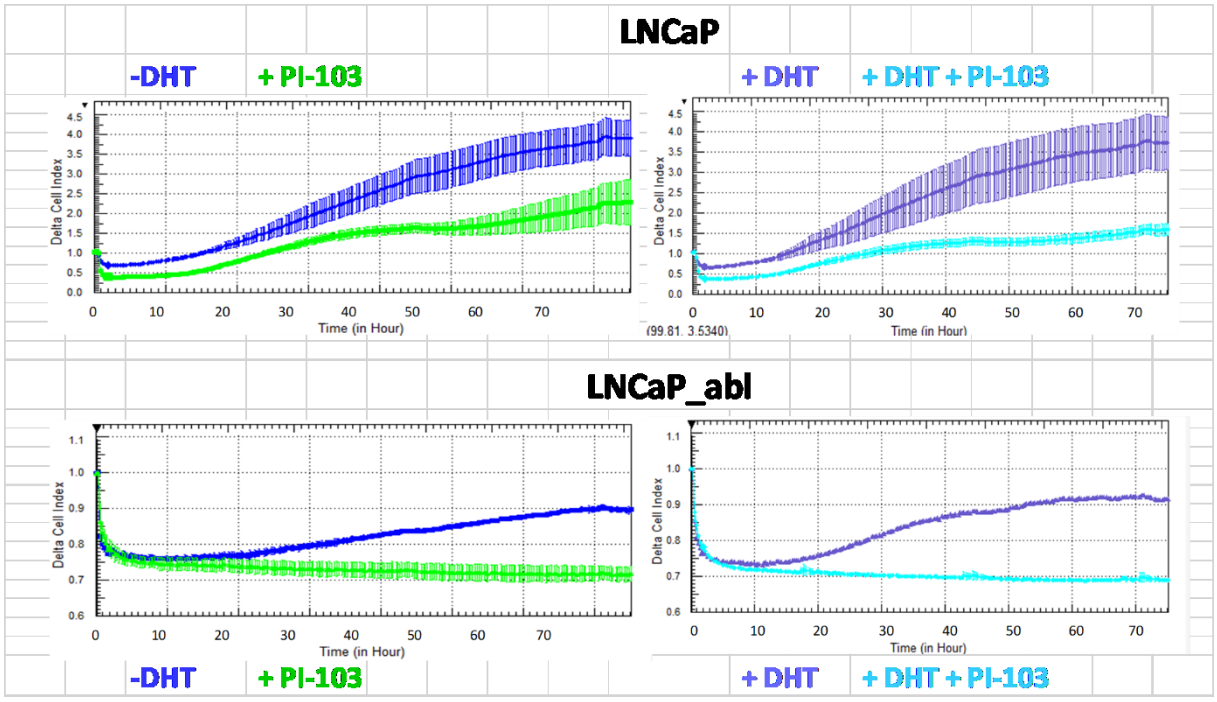


Figure 8 DHT added at 1 nM concentration did not change the sensitivity of cell lines to PI-103 PI3K inhibitor at 3 μM

ABT's *in vitro* data resulted in similar observation: i.e. sensitivity of prostate cancer cells to PI3K inhibitors (PI-103, and pictilisib) were androgen independent (Figure 8). Iinterestingly it was also observed that LNCaP_abl cell line was highly selective to the two PI3K/AKT pathway inhibitors PI-103 and Pictilislib. Even at the lowest 3,33 μM inhibitor concentration cells responded dramatically, and after few hours they lost their adhesion from the bottom of the plates (Figure 7. bottom charts), which correspond to high cytotoxic effects. The LNCaP cell line responded quickly to inhibitor treatment as well, but these cells started to proliferate and the adhesion increased again after 8-10 hours. In case of PI-103 inhibition only at 30 μM moderate inhibition in LNCaP cells could be seen. Pictilisib had slightly stronger effects, it inhibited more than 50 percent of cells viability at 10 μM concentration.

4.2 MAPK Signalling Pathway:

Mitogen-activated protein kinase (MAPK) pathways are very conserved kinases which forward extracellular signals to the intracellular machinery that controls fundamental cellular processes such as gene expression, growth, differentiation, proliferation, migration and survival. In different cancer types the abnormal MAPK signalling may lead to increased or uncontrolled cell proliferation and resistance to apoptosis [8].

The phospho-proteomic results showed that inhibition of androgen receptor did not have any effects on MAPK signaling pathway related proteins (ERK and p-ERK). According to these data ABT selected the upstream partner of the MAPK pathway, the MEK1/2 protein. After careful investigation of the literature three different inhibitors were selected to inhibit the MEK1/2 kinase: trametinib, PD184352 and selumetinib. Selective effects on cell proliferation

of the analyzed two cell lines were investigated using the real-time cellular analysis assay. We found that the MEK 1/2 inhibition did not affect cell viability significantly with and without androgen (1 nM DHT) during culturing in LNCaP_abl cell line, but it decreased cell growth at 3 µM in LNCaP cell line (Figure 9 and 10).

This slight difference in cytotoxicity could be detected in case of all the three applied MEK inhibitors in LNCaP cell line at lower concentrations. Besides cytotoxic effects, we could detect differences in cell morphology and cell attachment again only in the LNCaP, but not in the LNCaP_abl cells. However, we could detect these changes by recording higher cell index values, additional cell biology methods would be needed for further confirmation. This result would be very interesting to assess the effects of these drugs on metastasis and/or cell detachment and migration. This study is out of scope of the present project.

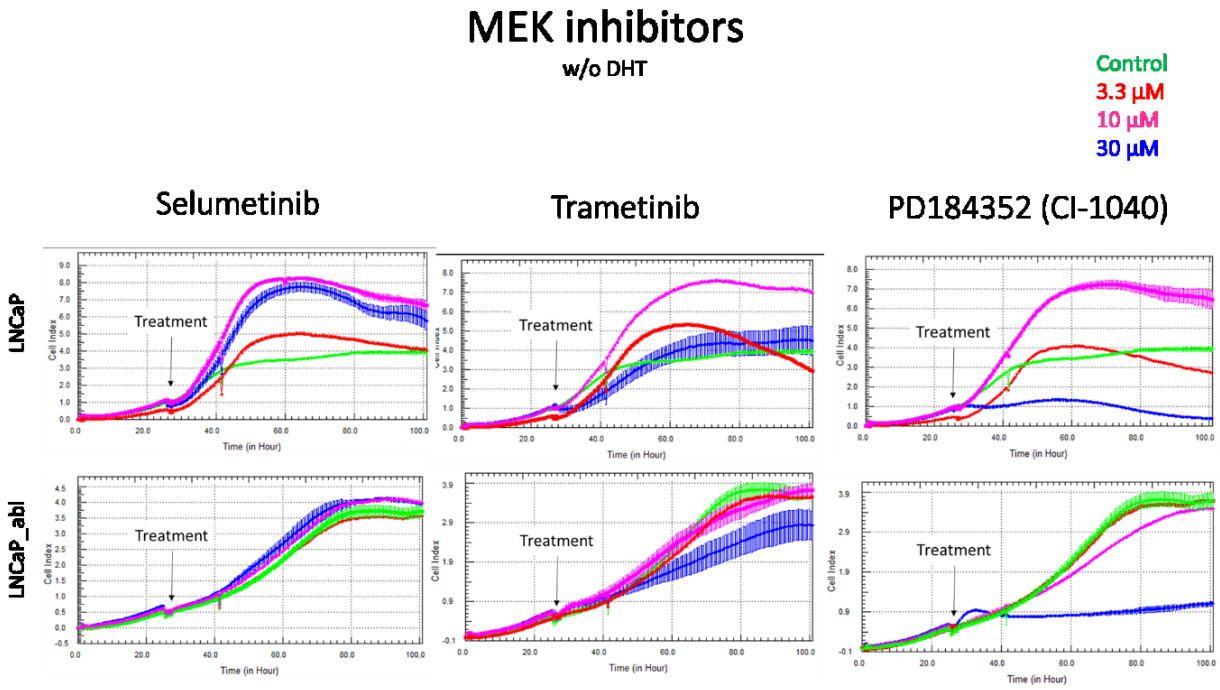


Figure 9. Neither of the cell lines are responsive for MEK 1/2 inhibitions at tested concentrations after 72 hours

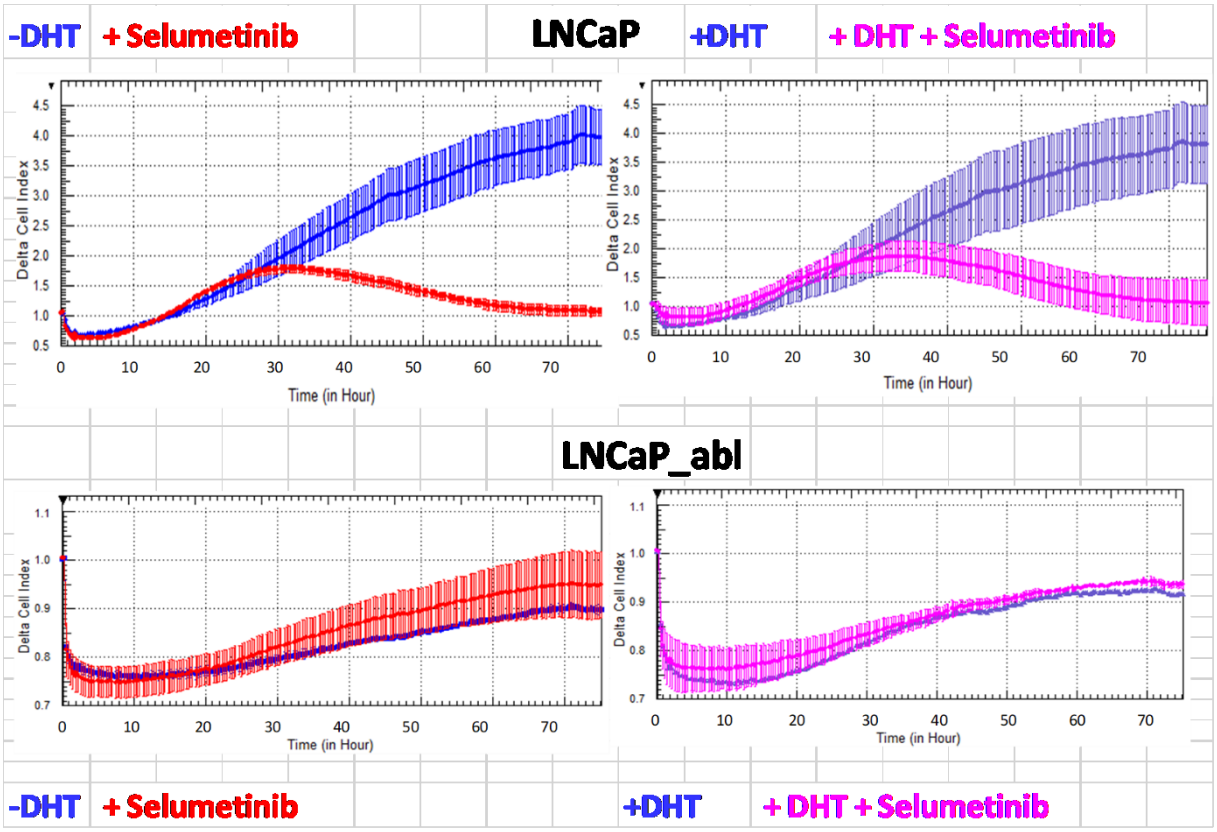


Figure 10 In the presence of DHT at 1 nM MEK inhibition had no effect (Selumetinib (AZD6244) was used at 6 μM)

4.3 Hsp90 molecular chaperone:

The Hsp90 chaperone is expressed abundantly and plays a crucial role in the correct folding of wide variety of proteins such as protein kinases and steroid hormone receptor [9]. Hsp90 is essential for the correct folding and function of many signaling protein kinases that are crucial for cell proliferation, so the Hsp90 can act as a protector of less stable proteins produced by DNA mutations in cancer cells [10]. Currently, Hsp90 inhibitors are in clinical trials for multiple indications in cancer.

Logical models, developed by CI in Task 5.4 provided ABT a list of compounds which were predicted to be promising candidates for application in clinics for the treatment of different prostate cancer types. It was predicted, that the inhibition of Hsp90 in prostate cancer could potentially decrease the growth of cancer cells. ABT *in vitro* tested two Hsp90 inhibitors and validated that the inhibition of Hsp90 indeed decreased the viability of the two cell lines in a concentration dependent manner. We found that the androgen independent LNCaP_abl cell line was more than 20 times sensitive to the 17-DMAG inhibitor than the LNCaP androgen sensitive cell line. (Figure 11) The LNCaP cells responded to both inhibitors, and the higher concentrations of 17-DMAG increased the cellular impedance for 8-10 hours, and after dose dependently killed the cells. In case of the LNCaP_abl cell line we detected cell death after 24-48 hour with all inhibitor concentrations applied. NMS-E973 inhibitor was also very effective in both cell lines, with experimentally calculated IC50 values of 2 μM for LNCaP and 0,4 μM for LNCaP_abl cell line, respectively. Selectivity to this drug in LNCaP_abl cell could be also observed, but to a smaller extent.

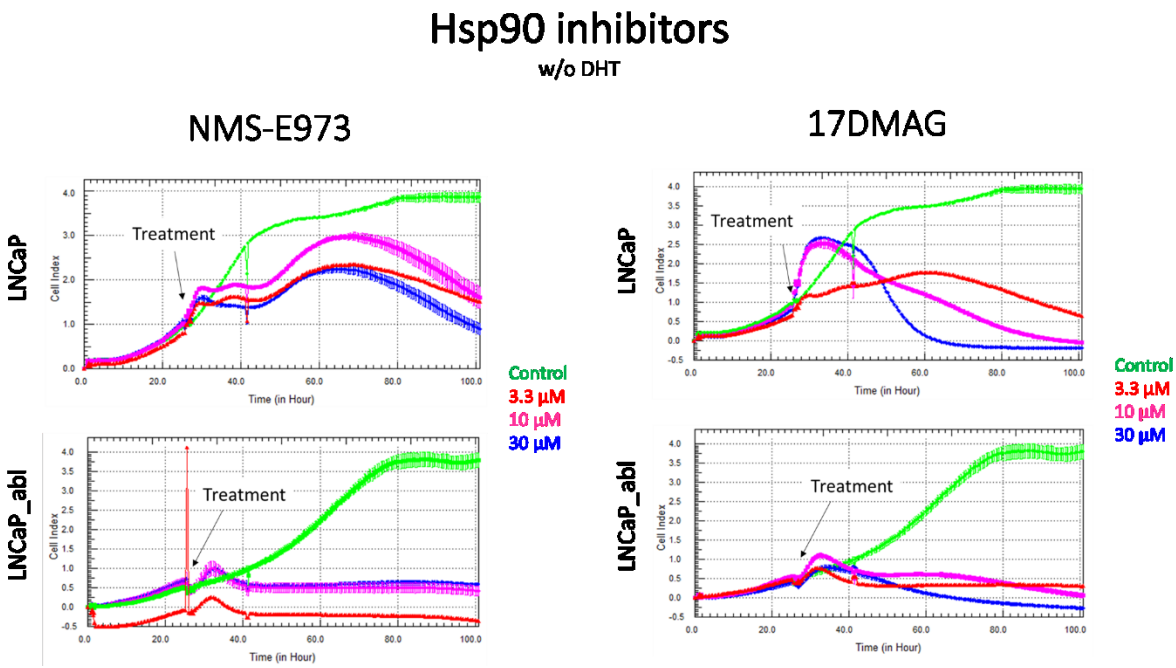


Figure 11The LNCaP and LNCaP_abl cell lines were sensitive to HSP90 inhibition *in vitro*

4.4 Androgen receptor (AR) inhibition

The effects of androgens on prostate cancer growth are mediated through the AR signalling.[11] The androgen receptor plays a critical role in the development of castration-resistant prostate cancer (CRPC) as well as in the resistance to the second-generation AR antagonist enzalutamide and the selective inhibitor of cytochrome P450 17A1 [12]. The human AR gene is located on chromosome Xq11-12 and is a steroid hormone receptor member of the larger nuclear receptor family that includes the estrogen, progesterone, and glucocorticoid receptors [13]. The AR is found in benign epithelial cells as well as in all grades and stages of prostate cancer.

Enzalutamide and Abiraterone are very effective antiandrogens which are used in the treatment of prostate cancer. They are specifically indicated for use in conjunction with castration for the treatment of metastatic castration-resistant prostate cancers.

The real-time cellular measurements demonstrated well that the LNCaP_abl, androgen independent cell line is less responsive to AR and CYP17A1 inhibition than the original LNCaP (androgen sensitive) cell line. The cells were treated with anti-androgens which are in clinical use. Cell growth were recorded 24 hours after 10 µM Enzalutamide or 10 µM Abiraterone treatment with and without 1 nM DHT. The results showed that the LNCaP_abl cell line only diminished the growth to control level after Enzalutamide and Abiraterone treatment, while the LNCaP cell line response was more pronounced to the inhibitors. After enzalutamide treatment the LNCaP cell viability decreased approximately by 20% compared to control cells, and the inhibitor completely neutralized the effect of the DHT. With abiraterone we observed similar results: it decreased the viability of cells by 38% and reduced the DHT effect nearly to the level of abiraterone positive, but DHT negative treated cells. These findings suggest that the lead compounds for treatment the castration resistant prostate cancers may have only moderate

effects on some cell subclones, which could become resistant during the therapy in castrated patients.

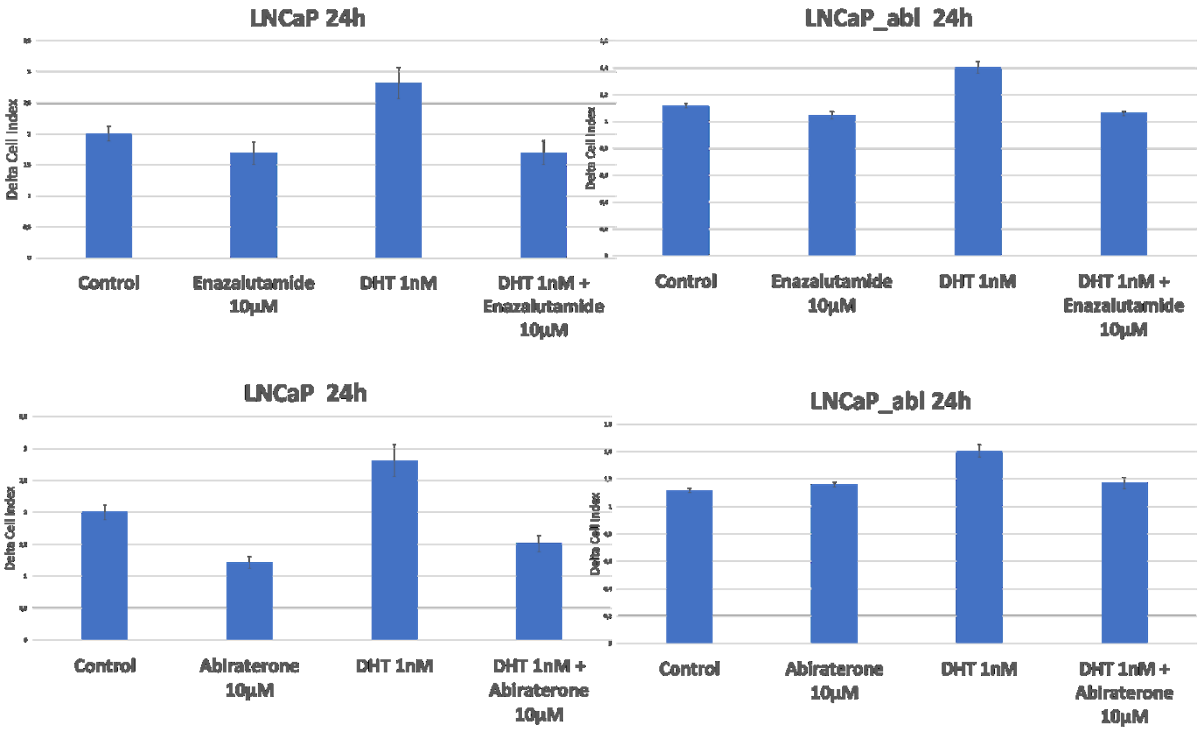


Figure 12 . LNCaP_abl cell responded only slightly to AR inhibition compared to the LNCaP androgen sensitive cell line

4.5 Validation of drugs, proposed from analysis of transcriptional profile of LINCS-L1000 database

Based on the predictions of UKAACHEN, analyzing drug sensitivity screen and transcriptomic data of LINCS-L1000 dataset, 6 compounds were selected for experimental validation. These compounds were predicted to have different toxicity in two prostate cancer cell lines VCaP and PC3. Testosterone, Androstanol, Formestane and CAY-10585 were predicted to be more toxic in VCaP, while Maclocycline and Ornidazole were predicted to be more toxic in PC3 cell lines. Performed cell viability measurements confirmed 4 of these 6 predictions (showing low toxicity / ambiguous results for the remaining 2 cases) (Figure 13).

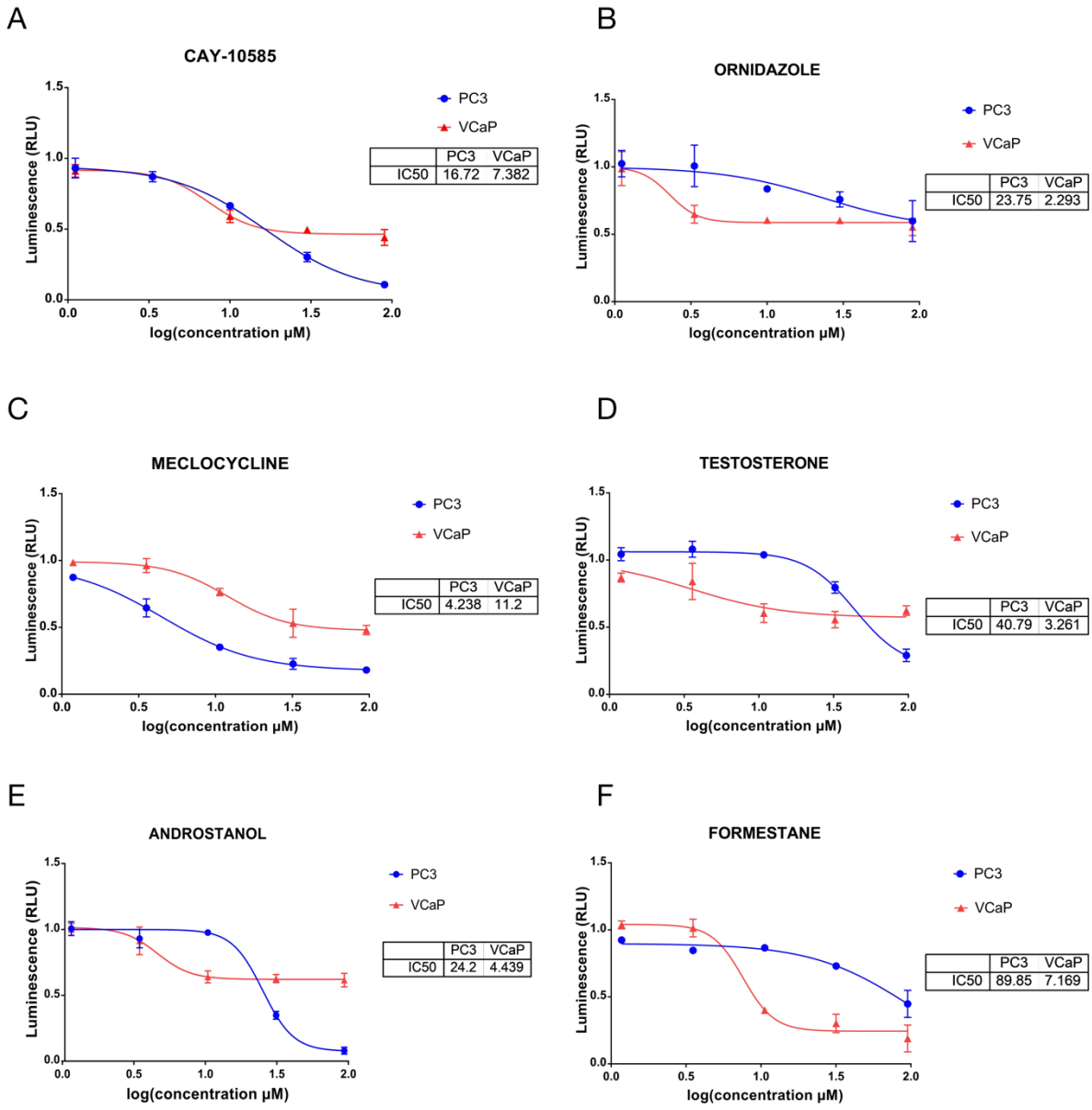


Figure 13 The PC3 and VCaP cell lines were sensitive to different predicted treatment

Chapter 5 Outlook

Other methods for drug action analysis have been proposed by PrECISE partners TUDA and IBM. These methods analyse dynamic response to drug perturbation and the relationship between chemical structure of drug molecule and transcriptomic profiles. Both extend the models, initially proposed in DoA, and address the same objectives: develop or repurpose the drugs that target the proteins and genes causal for cancer development.

5.1 Targeting non-druggable nodes by perturbation of upstream genes

Many of the drugs inhibit tumor growth either by hindering the proliferation or inducing apoptosis. Unfortunately, many of the causal genes or proteins are not directly druggable. However, using knowledge of prostate cancer pathways, we can leverage downstream effects of druggable proteins, in order to perturb non-druggable targets. Tracking these downstream effects in a realistic model becomes quickly computationally infeasible, so we employ a novel fully probabilistic planning method based on variational inference. Details on the variational inference can be found in D4.2 and in [19]. This method is based on a Markov decision process on a graph, where individual nodes represent molecular components connected through a graph given by the enriched interactome used in D4.2. This enriched interactome is based on the logical model provided by CI and has been extended using the ENCODE database.

The optimal planning procedure is defined by the multi-drug therapy π which maximizes the marginal likelihood of the model reaching the desired target state Y – proliferation or apoptosis

$$\pi_{optimal} = \text{argmax}_{\pi}\, \text{P(Y | } \pi \text{)} .$$

However, in order to plan in a realistic scenario, we have to consider the uncertainty over the high-dimensional set of parameters of the interactome. Additionally, many edges cannot be signed rendering individual downstream effects as activation or suppression uncertain. To respect this uncertainty we calculate an approximate marginal likelihood via sampling

$$\pi_{optimal} \approx \text{argmax}_{\pi} \frac{1}{N} \sum_{i=1}^{N} \text{P(Y | } \pi \text{ ,r}_i\text{,G}_i\text{,s}_i\text{)} \ ,$$

where missing graph edges G_i are drawn uniform, signs s_i from a Bernoulli distribution and rates r_i from a truncated exponential distribution. A manuscript on this planning method is currently under review at AISTATS2019.

In the following we display the results of our optimization. Number coding refers to growth condition of cell line (androgen yes/no, EGF yes/no).

In Figure 14 we show the convergence behavior of the marginal likelihood for reaching apoptosis under our optimization scheme. We approximated the marginal likelihood using 200 samples for each condition. The marginal likelihood converges after few iterations.

In Figure 15 we display optimal therapies to reach apoptosis state. For druggable nodes we employ the color-coding back/red, for non-druggable target nodes yellow/green for down/up-regulation.

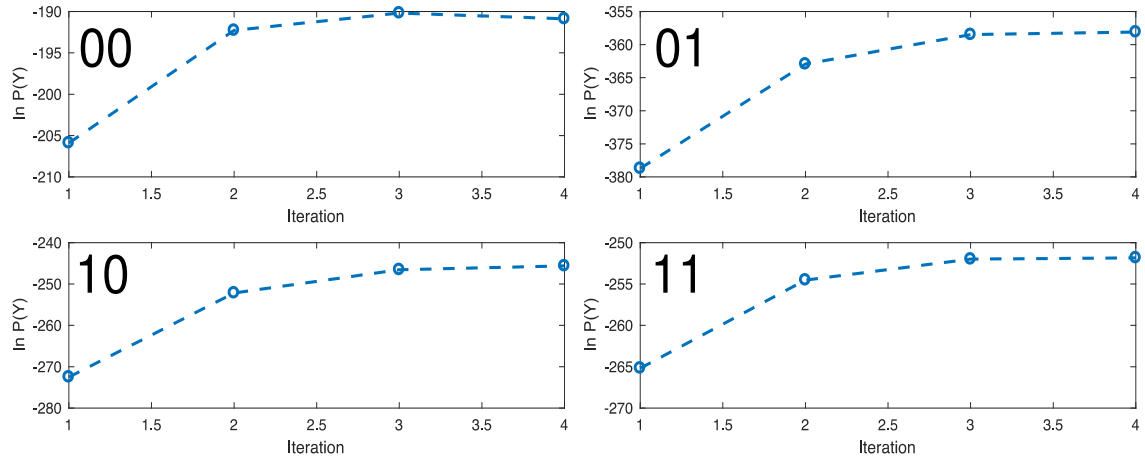


Figure 14 Convergence of marginal likelihood of apoptosis. Convergence happens in all cases after few iterations.

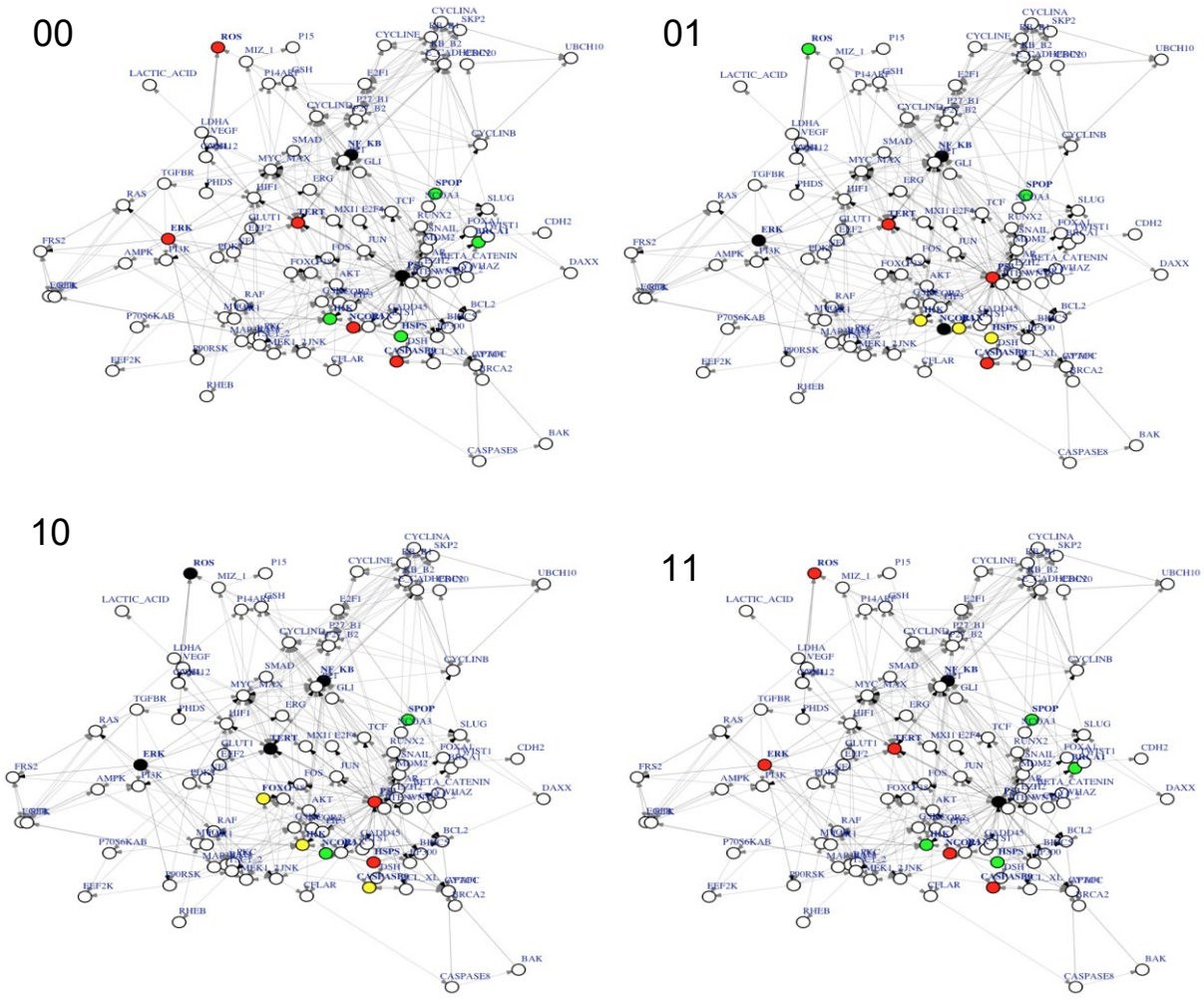


Figure 15 Optimal multi drug-therapies to reach apoptosis goal-state. We plotted only interactome components connected to either druggable nodes or targets to avoid visual clutter.

5.2 Prediction of drug sensitivity based on atomic structure of the drug and transcriptomic profiles.

There is strong evidence that the response to anticancer therapy depends on the tumor genomic and transcriptomic makeup, resulting in heterogeneity in patient clinical response to anticancer drugs. Most of previous work focuses on prediction of drug sensitivity in cancer cells [14,15], however, only few models have proposed the integration of two different data modalities, e.g., genomic features and chemical descriptors [16]. Here we employed the gene expression and drug IC50 data publicly available as part of the Genomics of Drug Sensitivity in Cancer (GDSC) database [17].

Partners IBM and UKAACHEN have developed an approach for the prediction of anticancer compound sensitivity using multi-modal attention-based neural networks (PaccMann) [18]. This approach integrates three key pillars of drug sensitivity: 1) the molecular structure of compounds, 2) transcriptomic profiles of cancer cells and 3) prior knowledge about interactions among proteins within cells. The models ingest a drug-cell pair consisting of SMILES encoding of a compound and the gene expression profile of a cancer cell and predicts an IC50 sensitivity value. Gene expression profiles are encoded using an attention-based encoding mechanism that assigns higher weights to informative genes. The devised models are compared against a baseline model that ingests engineered fingerprints to represent the molecular structure. We show that proposed attention-based encoders surpass the baseline model. This also enables us to identify genes, bonds and atoms that were used by the network to make a prediction: for example, we demonstrate that the Tipifarnib underline the role of EIF2A, a key gene for tumor initiation (see Figure 16). Thus, the use of attention-based encoders enhance interpretability.

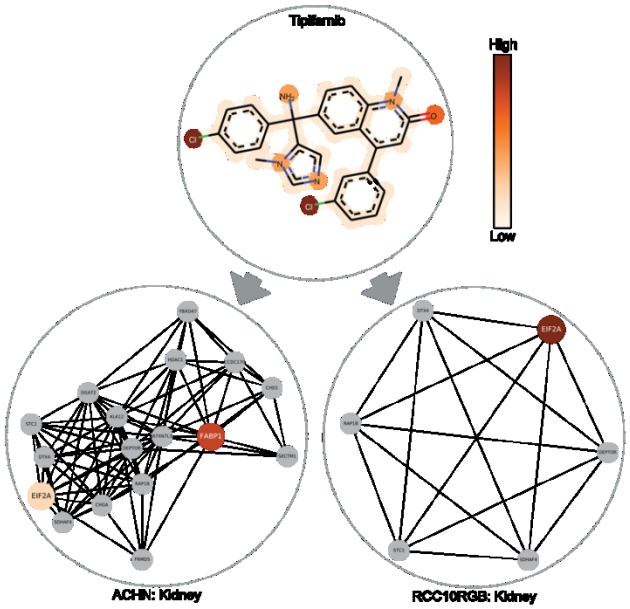


Figure 16 PaccMann highlights the relevant chemical features of the Tipifarnib. Importantly, the application of the compound on cell lines coming from the same tissue highlights similar key genes.

PaccMann represents an effective way to perform high-throughput drug screenings that can be used in anti-cancer compound design and can help generating new hypothesis for targeted experiments.

Chapter 6 Summary and Conclusion

The validation assays successfully confirmed findings of WP5 (D5.4). Specifically, in accordance with ETH's phosphoproteomic findings we could confirm that cell perturbation induced by PI3K and MEK kinase inhibitors was androgen independent. Furthermore, the androgen independent cell line, LNCaP_abl was more sensitive to PI3K inhibitors pictilisib and PI-103. According to our results these drugs could be also used in castration resistant prostate cancer patients.

Additionally, we tested the protein target HSP90, which was predicted by logical model of CI. Two different Hsp90 inhibitors were tested on both cell lines using end-point and real-time cellular assays. Like in the case of PI3K inhibition, similar differential sensitivity was recorded: the androgen independent cell line LNCaP_abl was more sensitive than normal LNCaP cells. Additionally, Hsp90 inhibitors are very promising drugs in anticancer therapy.

Other targets suggested by WP5 gene expression data based predictions, such as Foxo1, telomerase and MEK 1/2 kinase were also studied in our assays using specific inhibitors with and without androgen treatment. No significant differences in cytotoxic effects could be detected in case of Foxo1 and telomerase inhibitors between the different prostate cell lines used in the assays. However, slight difference in cytotoxicity could be detected in case of all the three applied MEK inhibitors in LNCaP cell line. This could underline the possible therapeutic potential of MEK inhibitors in androgen sensitive prostate cancer patients, but not in castration resistant cases.

The validations also confirmed cell line selective toxicity of testosterone, androstanol and formestane in VCaP cell line and meclocycline in PC3 cell line. The usability of androgen receptor agonists has been proposed previously, so our results confirm these therapeutic directions, but also highlights the different sensitivities of castration resistant disease model cell lines. Meclocycline can be an interesting drug in prostate cancer, as meclocycline is an antibacterial antibiotic with low adverse effect profile.

The cell line viability assays, described in this deliverable, are used to validate the models (Table 2), developed within WP5 "Logic models of prostate cancer patients: predicting personalized drug therapies". However, the drug therapies can be identified also by other approaches. Using methods, developed in WP3 and WP4, partners TUDA have proposed to identify new drug targets by reconstructing networks from SWATH-MS data using cluster variational approximation to Bayesian networks (T3.4 and T4.2, by TUDA) and by exploring network sub-modules using multi-modal attention-based neural network (T4.2, IBM and UKAACHEN). These models extend the work, initially planned for T6.3 and therefore validation of these models will have to be continued after PrECISE project.

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Chapter 7 List of Abbreviations

AR	Androgen receptor
CI	Cell index
DHT	Dihydrotestosterone
Dx.x	Deliverable x.x
FBS	Fetal Bovine Serum
WP	Work Package
IC50	inhibitory concentration, 50% of the maximal inhibition

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