Tracking the Trace: Analysis of Circulating Tumour Cells and mRNA for Androgen Receptors and Splice Variants in Prostate Cancer

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Background

- Prostate cancer (PC) constitutes the **most common malignancy in men** and accounts for **13% of** all cancer-related **deaths**
- **Conventional anti-androgens** are used in **hormone-sensitive PC**
- However, almost all patients with PC ultimately become **resistant to androgens** and develop distant **metastases** (=metastatic castration-resistant PC, mCRPC)
- In mCRPC, more potent **novel anti-androgens** can be used (*Enzalutamide, Abiraterone*)
- Whilst *Enzalutamide* directly blocks the androgen-receptor (AR), *Abiraterone* inhibits an enzyme required for synthesis of androgens
- To overcome this blockage, tumour cells may **express splice variants** of the AR, most commonly AR-V7
- The herein **changed structure of the novel AR impairs bondage** of *Enzalutamide* to the receptor
- **Liquid biopsies** constitute a new way of gaining information on **tumour progression** via minimally- to non-invasive approaches

Aims

- The aim of this prospective study is to **identify AR-V7** in mCRPC-patients **refractory to treatment** with *Enzalutamide* or *Abiraterone*
- **Different liquid biopsy-techniques** are used to obtain patient blood and saliva samples
- **Experimental findings** will finally be correlated with clinical outcome of mCRPC-patients

Patients and Methods

Patients			
	Test Cohort	**Validation Cohort**	**Control group**
Planned	20	40	20
Recruited	20	2	-

- **Blood and saliva samples** are analysed by *ViennaLab* with **pre-amp** and **rt-PCR** (real-time polymerase chain reaction)
- **Circulating tumour cells** (CTCs) released from GILUPI-wire (preserved in TRIzol) are subsequently tested for AR-V7 by *ViennaLab*
- CTCs attached to the GILUPI-wire are analysed by in situ ***padlock probe technology*** at the *Gottfried Schatz Research Centre, Cell Biology, Histology and Embryology* (*Medical University of Graz*)

Materials		
Blood Samples	**CTCs**	**Saliva***
1 x STECK RNA Tube*	2 x GILUPI wires	1 x PureSAL
2 x EDTA Tubes		
1 x PAXgene RNA Tube		
1 x PAXgene cfDNA Tube		
**Not collected any longer*		

Results

GILUPI CellCollectors – Cell Based Approach

- **Candidate CTCs** were detectable in **60%** of GILUPI-wires deriving from **patients of the test cohort** (12/20) by applying ***padlock probe technology***
- In **55% of patients**, **AR-V7** splice variant was **detected** (11/20)
- Additionally, **32%** were **positive** for **AR-full length** and **26%** positive for **prostate-specific antigen** (PSA)
- GILUPI-wires of 20 patients were additionally analysed with the TRIzol extraction protocol, but AR-V7 signals could not be detected

Tubes – Circulating Cell Free Nucleic Acid Approach

- **RNA fragments** could **not** be **extracted** from **saliva** samples, wherefore collection of these probes was **prematurely stopped**
- Moreover, analysis of ***STRECK* tubes** for nucleic acids proved **ineffective**, whereas new tubes (*PAXgene*) were introduced in clinical practice
- In **5 out of 9 patients**, AR-V7 could be detected in *PAXgene RNA* tubes, whilst *PAXgene ccfDNA* tubes were all negative (**Figure 1**).

		Ct Values - PreAmp + rt-PCR				CellCollector
ID	**Sample**	**GUS**	**AR-V7**	**AR-FL**	**KLK3 (PSA)**	**AR-V7*(1)**
12	PAX CBMED 12	20,7	33,6	26,7	39,8	1
13	PAX CBMED 13	19,4	33,2	27,8	34,7	11
14	PAX CBMED 14	18,6	32,3	26,8	35,5	10
15	PAX CBMED 15	20	35,9	28	26,4	4
16	PAX CBMED 16	19,4	-	31,7	34,4	0
17	PAX CBMED 17	19,3	-	27	29,8	1
18	PAX CBMED 18	18,6	32,9	26,1	28,1	7
19	PAX CBMED 19	18,9	-	28	34,9	0
20	PAX CBMED 20	18,2	-	26,6	33,8	3
13	PAX ccfDNA/ccfRNA CBMED 13	31,6	-	35,2	35,8	11
14	PAX ccfDNA/ccfRNA CBMED 14	30,1	-	31,7	34,5	10
15	PAX ccfDNA/ccfRNA CBMED 15	36,7			29,3	4
16	PAX ccfDNA/ccfRNA CBMED 16	33,3	-	-	34,6	0
17	PAX ccfDNA/ccfRNA CBMED 17	32	-	-	34,5	1
18	PAX ccfDNA/ccfRNA CBMED 18	30,7	-	31,7	36	7
19	PAX ccfDNA/ccfRNA CBMED 19	33,3	-	33	37	0
20	PAX ccfDNA/ccfRNA CBMED 20	30,9	-	32,7	35,7	3

Figure 1. *AR-V7 positivity as seen with the circulating cell-free nucleic acid approach via preAMP and rt-PCR as well as the cell-based approach using the GILUPI CellCollecotr. *Number of cells positive for AR-V7. rt-PCR was considered positive if Ct values <34*

Conclusions

- **Identification** of CTCs **on GILUPI-wires** (± AR-V7 signals) is **promising** using the padlock probe-technology and in line with literature
- However, **additional approaches** are **needed** to confirm these results
- There is an **overlap of 71.4%** regarding AR-V7 positivity using the cell-based and cell-free nucleic acid approach
- **AR-V7** can be **detected** by applying **liquid biopsy** in patients resistant to *Enzalutamide* or *Abiraterone*
- **Correlation of experimental findings** with the **clinical progression** of mCRPC-patients will help to **clarify** the role of AR-V7 as a **prognostic** and **predictive biomarker**

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