ESMO Translational Research Fellowship
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FINAL REPORT

Host Institute: The Institute of Cancer Research, London, United Kingdom
Mentor: Dr Gerhardt Attard
Project title: Developing novel biological profiles of sensitivity and/or resistance to Abiraterone and/or Enzalutamide in patients with castration-resistant prostate cancer

Home Institute: Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Meldola, Italy.

Introduction

Inhibition of androgen receptor (AR) signalling with abiraterone or enzalutamide is now standard treatment at emergence of castration-resistant prostate cancer (CRPC), often first in a sequence followed by chemotherapy. However, the duration of response is variable and overall survival in unselected patients is modest despite some patients having responses that last several years. There is therefore an urgent need to develop biomarker strategies to *a priori* identify CRPC patients who will derive minimal benefit from *AR* targeting and offer them an alternative treatment paradigm. Recently, many studies have advocated the utility of "liquid biopsies" to develop biological profiles of resistance to novel hormonal drugs in different types of cancer patients. The use of circulating tumor DNA (ctDNA), obtained from plasma through a minimally invasive blood test, has been demonstrated to have the capacity to monitor tumor clone dynamics and disease evolution and identify genomic aberrations that emerge with drug resistance.

Rationale and Aim

1. To evaluate androgen receptor (*AR)* gene status using a minimally invasive assay in plasma samples from patients treated with abiraterone or enzalutamide before and after chemotherapy.
2. To characterize candidate genomic alterations (*AR* copy number variations and/or point mutations) that associate with sensitivity or resistance to abiraterone and enzalutamide.
3. To associate *AR* gene status at baseline with clinical outcome.

Experimental design

We collected plasma samples at two institutions [Royal Marsden (RM), London and IRST, Meldola, Italy] from 268 CRPC patients (97 patients in 2015 and 171 patients in 2016) treated with abiraterone 1g/od and prednisolone 5 mg/bid or enzalutamide 160 mg/od and who provided informed consent to participate in molecular characterization protocols aimed to identify predictive biomarkers (REC04/Q0801/6 for RM and REC2192/2013 for IRST). Sample collection has been performed at different time points (before starting treatment, on treatment, and at progression disease).

We performed DNA extraction and quantification from peripheral blood and then we performed droplet digital PCR (ddPCR) and Next Generation Sequencing (NSG) on plasma samples. A multiplex ddPCR assay was optimized for the evaluation of *AR* copy number (CN), using the reference genes: ZXDB, NSUN3, ElF2C1,

and AP3B1, or *AR* somatic point mutations. We performed targeted-NGS by Ion Torrent PGM and Proton, using a gene panel including AR, CYP17A1, FOXA1, PTEN, and SPOP.

We then aimed to use our test in a second cohort of 94 chemotherapy-naïve patients treated with enzalutamide in the prospective biomarker PREMIERE study of 17 institutions (EudraCT: 2014-003192-28, NCT02288936).

The association between plasma *AR* aberrations and progression-free/overall survival (PFS/OS) was evaluated by the Kaplan-Meier method and log-rank test. All these data have been analyzed to identify molecular stratification and treatment selection of CRPC patients.

Results, Conclusions and Future Perspectives

In line with proposed timeline and thanks to the collaboration of experts, approximately 400 plasma samples of CRPC patients treated with abiraterone and enzalutamide at RM, London and IRST, Meldola, have been analyzed in these two years by using targeted-NGS and ddPCR. In the last year, we validated our data by using plasma samples from chemotherapy-naïve patients treated with enzalutamide in Spain.

I started my project from the preliminary data of my Italian Group (1,2) showing a significant correlation between copy number variations of circulating cell-free *AR* by using Taqman copy number assays and the clinical outcome of metastatic CRPC patients treated with abiraterone or enzalutamide.

During my fellowship, we performed circulating DNA extraction using the QIAamp Circulating Nucleic Acid kit or QIAamp DNA kit and quantified using the Quant-iT double-stranded DNA Assay Kit. We used a custom Ampliseq panel and a total DNA input of 10 ng. Computational analysis estimating the plasma DNA tumor content, *AR* CN quantitation and point mutation detection (with a sensitivity of 98-99% depending on position and coverage) was performed. In addition, ddPCR was performed on a QX200 ddPCR system (Bio-Rad). For each individual sample the estimated *AR* CN and rare mutation [2105T>A (p.L702H), 2632A>G (T878A), and 2626T>C (p.F876L)] detection assays were performed for using a custom-made single nucleotide polymorphism (SNP) genotyping assay (Life Technologies) with a sensitivity of 98%.

The most important results of my project evidenced a strong correlation between plasma *AR* aberrations (copy number gain or point mutation) and clinical outcome (3,4) (PSA response, overall survival, and progression-free survival). Consequently, plasma *AR* can be considered as a minimally invasive genetic biomarker of clinical outcome and resistance to abiraterone or enzalutamide, and can identify *a priori* men who will not benefit from anti-A*R* therapies.
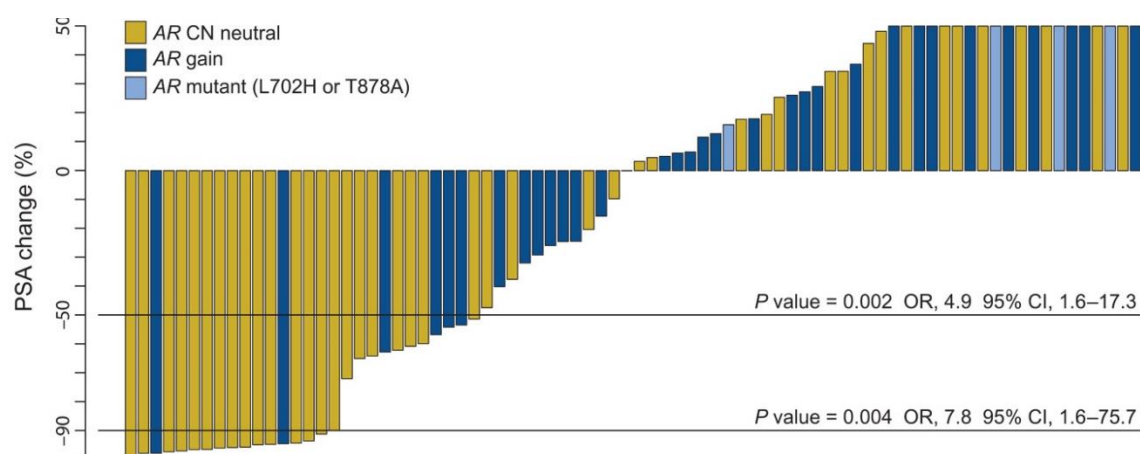

Figure 1. Association between plasma *AR* status and PSA response in CRPC patients treated with abiraterone. Waterfall plot showing the magnitude of PSA decline in patients with *AR* gain, *AR* point mutation, or *AR* copy number neutral. The odds ratios for *AR* copy number neutral having a ≥50 or ≥90% decline in PSA were calculated using Fisher's exact test *(Romanel A, Gasi Tandefelt D, Conteduca V, et al. Science Transl Med 2015)*
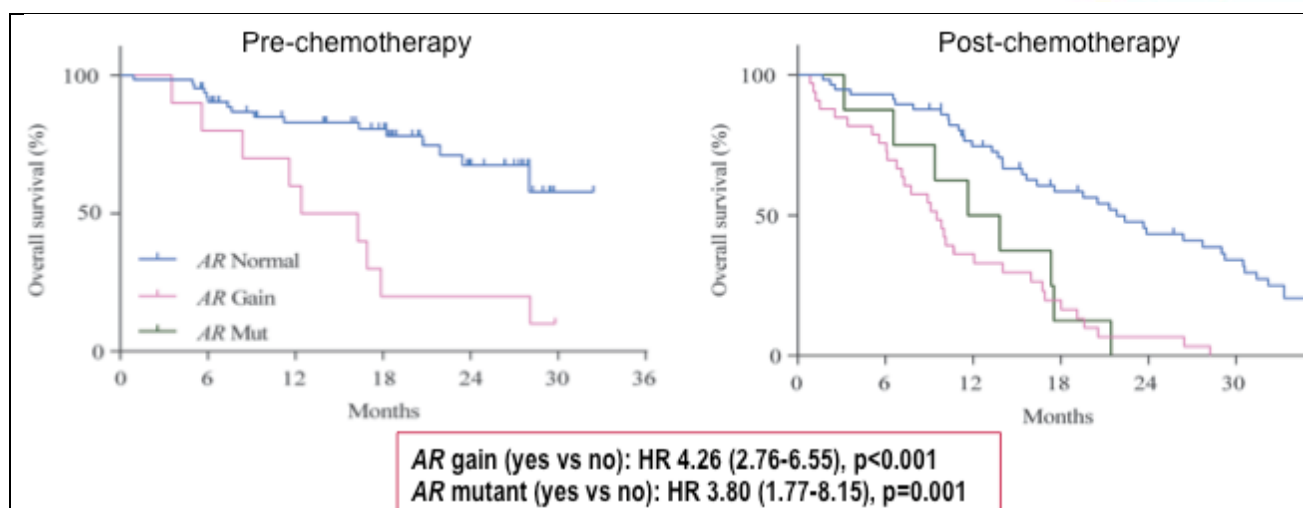
Figure 2. Association between plasma AR status and overall survival in chemotherapy-naïve and docetaxel-treated patients with abiraterone or enzalutamide *(Conteduca V et al. Ann Oncol 2017).*

During my ESMO project, I performed additional studies aimed to associate cell-free *AR* status with different factors to identify potential prognostic groups for a better treatment selection of CRPC patients (5-16). Furthermore, I attended the ECCO-AACR-EORTC-ESMO Flims Workshop on Methods in Clinical Cancer, Flims, Switzerland, in 2015 and I took care of writing the protocol myself and developed an investigator-initiated phase-2 trial with sequential therapy between docetaxel and radium-223 in castration-resistant prostate cancer (CRPC) (RAPSON study, clinical trial number: NCT03230734). Thanks to this study, I will have the opportunity to identify potential circulating predictive/prognostic biomarkers (involving *AR* and other targets) in patients treated with other different drugs for CRPC.

Future prospective studies should consider stratifying patients by plasma *AR* status in view of the differences in clinical outcome and treatment response and trials selecting patients for treatment based on plasma *AR* are now warranted to provide level one evidence to enable a change in clinical care.

References

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2. Salvi S, Casadio V, Conteduca V, et al. Circulating cell-free AR and CYP17A1 copy number variations may associate with outcome of metastatic castration-resistant prostate cancer patients treated with abiraterone. Br J Cancer. 2015;112(10):1717-24.

3. Conteduca V, et al. Androgen receptor gene status in plasma DNA associates with worse outcome on enzalutamide or abiraterone for castration-resistant prostate cancer: a multi-institution correlative biomarker study. Ann Oncol 2017;28(7):1508-1516.

4. Romanel A, Gasi Tandefelt D, Conteduca V, et al. Plasma AR and abiraterone-resistant prostate cancer. Sci Transl Med. 2015;7(312):312re10.

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6. Re: Marzia Del Re, Elisa Biasco, Stefania Crucitta, et al. The Detection of Androgen Receptor Splice Variant 7 in Plasma-derived Exosomal RNA Strongly Predicts Resistance to Hormonal Therapy in

Metastatic Prostate Cancer Patients. Eur Urol 2017;71:680-7. De Giorgi U, Conteduca V, Scarpi E. Eur Urol. 2017 Aug 5. pii: S0302-2838(17)30661-9.

7. Conteduca V, et al. Long-term clinical impact of PSA surge in castration-resistant prostate cancer patients treated with abiraterone. Prostate 2017;77(9):1012-1019.

8. Pietri E, Conteduca V, et al. Androgen receptor signaling pathwaysas a target for breast cancer treatment. Endocr Relat Cancer. 2016;23(10):R485-98.

9. ESMO congress, Madrid, September 2017: "Conteduca V, et al. Combining functional imaging with circulating biomarker analysis to improve prognostication of metastatic castration-resistant prostate cancer" (poster discussion)

10. ESMO congress, Madrid, September 2017: "Metabolic Syndrome and Inflammation in Castration Resistant Prostate Cancer (CRPC) patients (pts) treated with Abiraterone (abi) and Enzalutamide (enza)" (poster discussion)

11. ASCO congress, Chicago, June 2017: "Conteduca V, et al. Association of androgen receptor (AR) status in plasma DNA with outcome on enzalutamide (enza) or abiraterone (abi) for castration resistant prostate cancer (CRPC)" (poster discussion, 2017 Conquer Cancer Foundation Merit Award).

12. ASCO congress, Chicago, June 2017: "Grande E, et al. Association of androgen receptor (AR) gene status in plasma DNA with outcome on enzalutamide in chemotherapy-naive metastatic castration-resistant prostate cancer (mCRPC): Exploratory results from the PREMIERE trial. On behalf of SOGUG" (poster discussion).

13. ASCO Genito-Urinary congress, Orlando, February 2017: "Conteduca V, et al. Circulating Androgen Receptor and Serum Chromogranin A in castration-resistant prostate cancer (CRPC) patients treated with abiraterone and enzalutamide" (poster discussion).

14. Molecular Analysis for Personalised therapy (MAP) conference, London, September 2016: "Conteduca V, et al. Clinical utility of tracking circulating AR aberrations in castration resistant-prostate cancer (CRPC)" (poster discussion).

15. ESMO congress, Copenhagen, October 2015: "Conteduca V, et al. Increased choline uptake in androgen receptor (AR) copy number gain castration-resistant prostate cancers (CRPC)" (poster discussion).

16. ESMO congress, Vienna, September 2015: "Conteduca V, et al. The correlation between circulating cell-free AR and CYP17A1 copy number variations and tumor burden estimated by 18F-fluorocholine PET/CT in metastatic castration-resistant prostate cancer patients treated with abiraterone" (poster discussion).