# Letter to the editor

## Tailoring treatment of salivary duct carcinoma (SDC) by liquid biopsy: ARv7 expression in circulating tumor cells

We read with interest the paper by Fushimi et al. [1] prospectively reporting for the first time activity and acceptable safety profile by combined androgen blockade (CAB) in patients with metastatic, androgen receptor (AR) positive, salivary duct carcinoma (SDC). Fushimi et al. found no correlations between treatment outcomes and clinicobiologic factors/biomarkers. Here we report our preliminary findings demonstrating that circulating tumor cells (CTCs) isolated in patients with SDC can be used to guide treatment with androgen blockade, thus offering a way to improve treatment outcomes by exploiting blood borne biomarkers in a paradigm similar to prostate cancer (PC) [2].

Androgen blockade is hindered by the onset of resistance mechanisms linked, among others, to expression of AR splice-variants lacking the hormone binding domain and acting as ligand-independent transcription factors [3, 4]. The most frequently expressed splice variant in SDC, the ARv7, is associated with resistance to enzalutamide/bicalutamide in PC [2]. Since in the advanced settings tissue from the primary or metastatic disease is not always available for molecular analyses, liquid biopsy could be a potential tool to explore the molecular profile of SDC and investigate the androgen-resistance mechanisms.

Liquid biopsy holds promise in the arena of precision medicine and CTCs offer the unique opportunity of obtaining samples that can be used for comprehensive molecular analyses. This concept was challenged in a single patient treated within the EORTC 1206 trial (NCT01969578) and receiving CAB from July 2016 to April 2017 with a stable disease as best response. At progression (new brain lesions) in May 2017 he was enrolled in the phase II trial with abiraterone (NCT02867852). After having carried out whole brain radiotherapy (total dose = 30 Gy/10 fx), the first and second radiologic evaluations at 2 and 4 months showed stable disease, whereas the last imaging carried out at 6 months showed bone and liver PD.

Using a commercial kits for enrichment (ProstateCancerSelect, QIAGEN, Hilden, Germany), detection (BreastCancerDetect kit QIAGEN) and molecular characterization (AR-V7 assay RT PCR, Bird, Monteriggioni, Italy), we report for the first time the presence of CTCs and the expression of full-length AR and of the splicing variant ARv7 in the CTCs isolated in blood collected before treatment with abiraterone. Both the full length AR and the splicing variant ARv7 were highly expressed, 344 and 77 copies/ml of blood, respectively in the CTC isolated before treatment. Although no comparative data are available for SDC, the reported ARv7 levels were definitely higher than the median ARv7 levels (35.5 copies/ml blood) observed in castration resistant PC patients undergoing progression under enzalutamide or abiraterone in our institutional experience (unpublished data). In keeping with this, in our SDC patient the CTC ARv7 expression predicted the onset of resistance with an anticipation of six months.

Using in parallel an unbiased CTC-enrichment method (Parsortix) coupled with single-CTC identification and recovery by the DEPArray, which allows also identification of non-conventional CTC (ncCTC) lacking epithelial markers [5], three

**A** **CTC detection with the AdnaBreastDetect kit**

| CTC-specific gene | EPCAM | MUC1 | ERBB2 |
|---|---|---|---|
| Amplicon size | 395 | 299 | 265 |
| Expression ng/µl | 2.57 | 1.09 | 0.79 |
| CTC status | pos | pos | pos |

**B** **Genome-wide copy number profiles in single non conventional CTCs isolated from venous blood by enrichment with Parsortix and single-cell recovery with DEPArray**

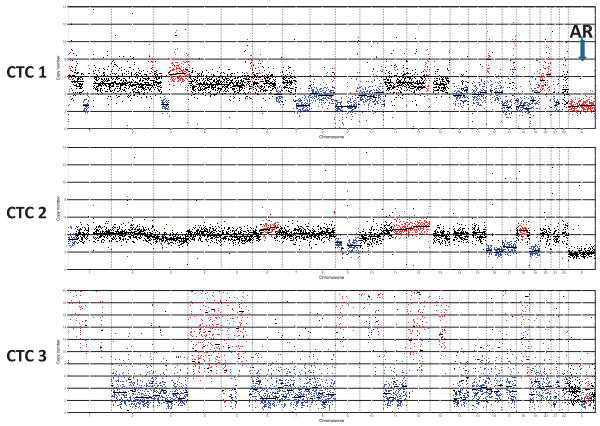


**Figure 1.** (A) *EPCAM*, *MUC1* and *ERBB2* gene expression levels (ng/µl) were evaluated by RT-PCR on mRNA isolated from CTC fractions enriched from whole blood with the AdnaProstateSelect kit using the AdnaBreastDetect kit (QIAGEN) to indirectly allow CTC detection. For all the tested genes expression levels were above the positivity threshold levels defined using blood from healthy donors, thus suggesting the positive CTC status. The expression of *ERBB2*, above our cutoff level (0.20 ng/µL) is consistent with the p185 positivity observed by immunohistochemistry (3+) in the fine needle aspirate obtained from the primary tumor 1 year before CTC detection. (B) Patterns of copy number aberrations and chromosomal aneuploidies in the entire genome for three single CTCs isolated by DEPArray after unbiased CTC enrichments from whole blood with the Parsortix. The estimated copy number is reported on the *y*-axis; red and blue spots represent genomic gains and losses, respectively. The arrow highlights a gain in the X-chromosome region containing the *AR* gene.

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ncCTC harboring numerous genetic aberrations were identified (Figure 1). CNA-profiles suggested genomic heterogeneity among CTCs and interestingly a gain could be observed in the chromosome X region harboring the *AR* gene in keeping with the over-expression of AR observed by immunohistochemistry in the primary tumor.

These findings support exploitation of liquid biopsy-based approach in clinical practice to improve management of SDC patients, by the early detection of androgen-resistance molecular mechanisms.

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## Disclosure

The authors have declared no conflicts of interest.

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