

The androgen antagonist seviteronel inhibits triple negative breast cancer progression by targeting cancer stem cells

Beatriz P San Juan^{1,2,3*}, Soroor Hadiyah-Zadeh⁴, Laura Rangel^{1,2,3}, Vanina Rodriguez^{1,3}, Heloisa H Milioli^{1,2,3}, Felix Kohane^{6,1}, Lesley Castillo¹, Abigail Bunkum¹, Anie Kurumlilian¹, Carley A Purcell^{1,2,3}, Dharmesh Elgene Lim^{1,2}, Vinod Ganju⁸, Rachel Dear², Sandra O'Toole¹, Cristina Vargas⁹, Theresa E Hickey¹⁰, Leonard D Goldstein^{1,2,3}, John G Lock⁶, Melissa J Davis^{4,5,6,*} and Christine L Chaffer^{1,2,3,*}

1. Garvan Institute of Medical Research, Darlinghurst, NSW, Australia
2. St Vincent's Clinical School, UNSW Medicine, UNSW Sydney, NSW, Australia
3. The Kinghorn Cancer Centre, Darlinghurst, NSW, Australia
4. Walter and Eliza Hall Institute of Medical Research, Parkville, VIC, Australia
5. Department of Medical Biology, University of Melbourne, Parkville, VIC, Australia
6. Department of Biochemistry, Faculty of Medicine, Dentistry and Health Sciences, University of Melbourne, Parkville, VIC, Australia
7. Department of Pathology, UNSW Medicine, UNSW Sydney, NSW, Australia
8. Monash University, Monash, VIC, Australia
9. Douglas Hanley-Moir, Pathology Lab, Macquarie, Sydney, NSW, Australia
10. Dame Roma Mitchel Cancer Research Laboratories, Adelaide Medical School, University of Adelaide, Adelaide, SA, Australia

Correspondence: *Christine Chaffer: c.chaffer@garvan.org.au*
Melissa Davis: m.davis@wehi.com.au
Beatriz Pérez San Juan: b.perez@garvan.org.au

Therapeutic strategies that improve survival outcomes for advanced-stage breast cancers have proven a major clinical challenge. This is in part due to the lack of understanding of the underlying biology of disease progression. Cancer stem cells (CSCs), which reside in different mesenchymal states, drive tumour initiation, metastatic spread and chemoresistance, hence identifying strategies to eradicate these cells should lead to effective therapies. The aggressive triple-negative breast cancer (TNBC) subtype is enriched in CSC subpopulations, as non-CSC cells readily covert into CSCs through activation of non-genetic molecular mechanisms, including EMT. Through transcriptomic analysis of non-CSC and CSC enriched cell subpopulations, we have defined the signalling network that governs the maintenance and de novo formation of TNBC CSCs, and determined that the androgen receptor (AR) signalling is a major driver. In response to chemotherapy, AR activation switches cells into a mesenchymal/cancer stem cell-like state, while AR antagonism suppress cell state-switching and cancer stem cell function. In vivo, the dual AR antagonist, seviteronel (VT-464/INO-464) enhances chemotherapy efficacy, inhibiting tumour growth and metastasis development, while preventing acquisition of chemoresistance. Interestingly, we found that cytoplasmic AR is highly expressed in metastases and xenografted tumours with a CSC profile. Analysis of 3 independent TNBC patient cohorts identified that cytoplasmic AR, which is not currently scored in the clinic, prognosticates poor survival in treatment-naïve patients and predicts poor response to chemotherapy. In line with our findings, retrospective analysis of a Phase II clinical trial data, showed that seviteronel treatment, alone or followed by chemotherapy, improves survival for cytoplasmic-AR+ TNBC patients. Together, our work has shed light on the mechanisms that drive cancer stem cell biology, identifying a novel biomarker and therapeutic strategy with the potential to

change clinical practice and improve TNBC patient's outcome. A clinical trial derived from this work has begun in Australia (NCT04947189).

We show that in response to chemotherapy, androgen receptor activation pushes cells into a cancer stem cell state, while androgen receptor antagonism suppresses chemotherapy-induced cell state switching and cancer stem cell function. Using xenograft and PDX TNBC models, we have validated that the novel AR antagonist, seviteronel (VT-464/INO-464), blocks primary and metastatic tumour growth and strongly enhances chemotherapy effectiveness. Interestingly, xenograft tumours matching a CSC profile show high cytoplasmic AR, which is not currently scored in the clinic. Analysis of 2 independent treatment-naïve TNBC patient cohorts identified that cytoplasmic, but not nuclear, AR expression prognosticates poor survival. Furthermore, staining an independent cohort, containing biopsies collected prior, during and post chemotherapy treatment, we defined that high cytoplasmic-AR expression at baseline predicts lack of therapeutic response. Retrospective analysis of data derived from a Phase II clinical trial showed improved survival outcomes for cytoplasmic AR+ TNBC patients treated with seviteronel followed by chemotherapy. These data demonstrate that blocking AR sensitises cytoplasmic AR+ tumours to standard-of-care chemotherapy, evidencing a new targeted therapeutic strategy for triple-negative breast cancer that could prove transformative in the clinic.

Therapeutic strategies that improve survival outcomes for advanced-stage breast cancers have proven a major clinical challenge. Here, we define the signalling network that governs the maintenance and de novo formation of triple-negative breast cancer (TNBC) stem cells, and determine that the androgen receptor (AR) signalling is a major driver. In response to chemotherapy, AR activation switches cells into a cancer stem cell state, while AR antagonism suppress cell-state switching and cancer stem cell function. In vivo, we validate that the AR antagonist, seviteronel (VT-464/INO-464) significantly improves chemotherapy-mediated inhibition of primary and metastatic tumour growth. Analysing 3 independent TNBC patient cohorts we identify that cytoplasmic-AR expression prognosticates poor survival in treatment-naïve patients and predicts poor response to chemotherapy. Additionally, seviteronel treatment followed by chemotherapy shows in phase-II clinical trial data improved survival for cytoplasmic-AR+ TNBC patients. Hence, AR inhibition and - chemotherapy combination represents a promising therapeutic strategy for TNBC.