MOLECULAR ONCOLOGY XXX (2015) 1–13

available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/molonc

Robust *BRCA1*-like classification of copy number profiles of samples repeated across different datasets and platforms

Philip C. Schouten[a], *Anita Grigoriadis*[b], *Thomas Kuilman*[c], *Hasan Mirza*[b], *Johnathan A. Watkins*[b], *Saskia A. Cooke*[b], *Ewald van Dyk*[d], *Tesa M. Severson*[a], *Oscar M. Rueda*[e], *Marlous Hoogstraat*[a,d,f,g], *Caroline Verhagen*[h], *Rachael Natrajan*[i], *Suet-Feung Chin*[e], *Esther H. Lips*[a], *Janneke Kruizinga*[j], *Arno Velds*[j], *Marja Nieuwland*[j], *Ron M. Kerkhoven*[j], *Oscar Krijgsman*[c], *Conchita Vens*[h], *Daniel Peeper*[c], *Petra M. Nederlof*[k], *Carlos Caldas*[e,l,m], *Andrew N. Tutt*[b], *Lodewyk F. Wessels*[d,n], *Sabine C. Linn*[a,o,p,*]

[a]*Department of Molecular Pathology, Netherlands Cancer Institute, Amsterdam, The Netherlands*
[b]*Breakthrough Breast Cancer Research Unit, Department of Research Oncology, Guy's Hospital, King's College London School of Medicine, London, United Kingdom*
[c]*Division of Molecular Oncology, Netherlands Cancer Institute, Amsterdam, The Netherlands*
[d]*Department of Molecular Carcinogenesis, Netherlands Cancer Institute, Amsterdam, The Netherlands*
[e]*Cancer Research UK Cambridge Research Institute, Li Ka Shing Centre, Cambridge, UK*
[f]*Department of Medical Oncology, University Medical Center Utrecht, Utrecht, The Netherlands*
[g]*Netherlands Center for Personalized Cancer Treatment, Utrecht, The Netherlands*
[h]*Division of Biological Stress Response, Netherlands Cancer Institute, Amsterdam, The Netherlands*
[i]*The Breakthrough Breast Cancer Research Centre, The Institute of Cancer Research, London, UK*
[j]*Genomics Core Facility, Netherlands Cancer Institute, Amsterdam, The Netherlands*
[k]*Department of Pathology, Netherlands Cancer Institute, Amsterdam, The Netherlands*
[l]*Department of Oncology, University of Cambridge, Addenbrooke's Hospital, Cambridge, UK*
[m]*Cambridge Experimental Cancer Medicine Centre and NIHR Cambridge Biomedical, Research Centre, Cambridge University Hospitals NHS, Cambridge, UK*
[n]*Faculty of Electrical Engineering, Mathematics and Computer Science, Delft University of Technology, Delft, The Netherlands*
[o]*Department of Pathology, University Medical Center Utrecht, Utrecht, The Netherlands*
[p]*Division of Medical Oncology, Netherlands Cancer Institute, Amsterdam, The Netherlands*

Abbreviations: aCGH, array Comparative Genomic Hybridization; BAC, Bacterial Artificial Chromosome; BAC32K, Bacterial Artificial Chromosome aCGH, 32K platform; BAC3K, Bacterial Artificial Chromosome aCGH, 3.2K platform; *BRCA1*, Breast Cancer Early Onset 1; CN, Copy number; DNA, Deoxyribonucleic acid; dsDNA, double-stranded DNA; FFPE, Formalin Fixed Paraffin Embedded; hg 18, human reference genome version 18; hg19, human reference genome version 19; MIP, Molecular Inversion Probe; NG135, Nimblegen 135k oligonucleotide aCGH; NG720, Nimblegen 720K oligonucleotide aCGH; NGS, Low coverage next generation sequencing; SNP6, Affymetrix SNP6 array; SNR, Signal to Noise Ratio; VN, Variance of the Noise.

* *Corresponding author*. Departments of Medical Oncology and Molecular Pathology at Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands. Tel.: +31 20 512 2951.
E-mail address: s.linn@nki.nl (S.C. Linn).

http://dx.doi.org/10.1016/j.molonc.2015.03.002

Please cite this article in press as: Schouten, P.C., et al., Robust *BRCA1*-like classification of copy number profiles of samples repeated across different datasets and platforms, Molecular Oncology (2015), http://dx.doi.org/10.1016/j.molonc.2015.03.002

ARTICLE INFO

Article history:
Received 26 November 2014
Received in revised form
1 March 2015
Accepted 11 March 2015
Available online ■

Keywords:
BRCA1
Breast cancer
Classification
Copy number aberration profiles

ABSTRACT

Breast cancers with *BRCA1* germline mutation have a characteristic DNA copy number (CN) pattern. We developed a test that assigns CN profiles to be '*BRCA1*-like' or 'non-*BRCA1*-like', which refers to resembling a *BRCA1*-mutated tumor or resembling a tumor without a *BRCA1* mutation, respectively. Approximately one third of the *BRCA1*-like breast cancers have a *BRCA1* mutation, one third has hypermethylation of the *BRCA1* promoter and one third has an unknown reason for being *BRCA1*-like. This classification is indicative of patients' response to high dose alkylating and platinum containing chemotherapy regimens, which targets the inability of *BRCA1* deficient cells to repair DNA double strand breaks. We investigated whether this classification can be reliably obtained with next generation sequencing and copy number platforms other than the bacterial artificial chromosome (BAC) array Comparative Genomic Hybridization (aCGH) on which it was originally developed.

We investigated samples from 230 breast cancer patients for which a CN profile had been generated on two to five platforms, comprising low coverage CN sequencing, CN extraction from targeted sequencing panels (CopywriteR), Affymetrix SNP6.0, 135K/720K oligonucleotide aCGH, Affymetrix Oncoscan FFPE (MIP) technology, 3K BAC and 32K BAC aCGH. Pairwise comparison of genomic position-mapped profiles from the original aCGH platform and other platforms revealed concordance. For most cases, biological differences between samples exceeded the differences between platforms within one sample. We observed the same classification across different platforms in over 80% of the patients and kappa values of at least 0.36. Differential classification could be attributed to CN profiles that were not strongly associated to one class. In conclusion, we have shown that the genomic regions that define our *BRCA1*-like classifier are robustly measured by different CN profiling technologies, providing the possibility to retro- and prospectively investigate *BRCA1*-like classification across a wide range of CN platforms.

1. Introduction

Breast cancer arising in patients with a germline *BRCA1* mutation are thought to be genomically unstable due to the impairment of error-free homologous recombination DNA repair in which BRCA1 has a role (Venkitaraman, 2009; Vollebergh et al., 2012). DNA copy number (CN) profiles provide a snapshot of a result of genomic instability in cancer, namely the CN aberrations. The copy number profiles of patients with a *BRCA1* mutation have specific gains and losses (Alvarez et al., 2005; Tirkkonen et al., 1997; Wessels et al., 2002). We previously developed a shrunken centroids classifier which uses 371 genomic regions to assign a CN profile to the *BRCA1*-like (sharing characteristics of *BRCA1* mutated breast cancer) or non-*BRCA1*-like phenotype (Vollebergh et al., 2011). This classifier not only identifies germline *BRCA1*-mutated cases (approximately 1/3 of the *BRCA1*-like tumors) but also enriches for tumors with other mechanisms of BRCA1 inactivation, for example promoter hypermethylation (approximately 1/3 of the *BRCA1*-like tumors, mutually exclusive with *BRCA1* mutation) (Joosse et al., 2011; Vollebergh et al., 2011; Lips et al., 2011) which can confer to non-familial cases a tumor phenotype that is similar to *BRCA1* mutation carriers. Alternative modes of *BRCA1* inactivation and similarity of these tumors to *BRCA1*-mutated tumors have been observed in other datasets as well (Turner et al., 2004; Esteller et al., 2000; Alvarez et al., 2005; Tung et al., 2010; Cancer Genome Atlas Network, 2012) and has been referred to as 'BRCAness' (Turner et al., 2004). The cases with unknown cause for being classified as *BRCA1*-like may thus be subject to *BRCA1* dysfunction due to yet unidentified causes, or reflect a broader pathway dysfunction. Subsequently, we demonstrated that *BRCA1*-like patients benefit significantly more from high dose DNA double strand break-inducing chemotherapy, containing both platinum and alkylating agents, than from a conventional second generation chemotherapy regimen (Vollebergh et al., 2011). Two follow-up studies with different chemotherapy regimens demonstrated that *BRCA1*-like patients benefit also from tandem high dose (both including alkylating agents, one including platinum) compared to conventional, and from tandem high dose compared to dose dense chemotherapy, underlining the clinical relevance of the *BRCA1*-like profile (Schouten et al., 2015, 2014, 2013b.). Technological advances in experimental platforms have provided many datasets to study *BRCA1*-like profiles next to those generated on the original BAC (BAC3K) platform and 135k oligonucleotide aCGH (NG135), on which we reported in a previous manuscript (Schouten et al., 2013a). Given this reported reproducibility between different CN profiling platforms, we investigated whether *BRCA1*-like classification of CN profiles of repeated samples could be reliably obtained across multiple platforms (Baumbusch et al., 2008; Curtis et al., 2009; Hester et al., 2009; Krijgsman et al., 2012; Schouten et al., 2013a; Wicker et al., 2007). For this study we compared data from samples for which data from at least two of the following platforms were available: low coverage genome-wide sequencing,

Please cite this article in press as: Schouten, P.C., et al., Robust *BRCA1*-like classification of copy number profiles of samples repeated across different datasets and platforms, Molecular Oncology (2015), http://dx.doi.org/10.1016/j.molonc.2015.03.002