MOLECULAR ONCOLOGY 8 (2014) 656–668

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# Molecular correlates of platinum response in human high-grade serous ovarian cancer patient-derived xenografts

*Monique D. Topp[a,b], Lynne Hartley[a], Michele Cook[a], Valerie Heong[a,c,d], Emma Boehm[a], Lauren McShane[a], Jan Pyman[c], Orla McNally[c], Sumitra Ananda[c], Marisol Harrell[e], Dariush Etemadmoghadam[f,g,h], Laura Galletta[f], Kathryn Alsop[f], Gillian Mitchell[f], Stephen B. Fox[f,h], Jeffrey B. Kerr[b], Karla J. Hutt[b,i], Scott H. Kaufmann[j], Australian Ovarian Cancer Study[f,k,l,m], Elizabeth M. Swisher[e], David D. Bowtell[f,g,n], Matthew J. Wakefield[a,d,o,1], Clare L. Scott[a,c,d,*,1]*

[a]*The Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria 3052, Australia*
[b]*Department of Medicine and Health Sciences, Monash University, Clayton, Victoria 3168, Australia*
[c]*Royal Women's Hospital, Parkville, Victoria 3052, Australia*
[d]*Department of Medical Biology, University of Melbourne, Parkville, Victoria 3052, Australia*
[e]*University of Washington, Seattle, WA, USA*
[f]*Peter MacCallum Cancer Centre, East Melbourne, Victoria 8006, Australia*
[g]*Sir Peter MacCallum Department of Oncology, University of Melbourne, Melbourne, Victoria 3010, Australia*
[h]*Department of Pathology, University of Melbourne, Melbourne, Victoria 3010, Australia*
[i]*Prince Henry's Institute of Medical Research, Clayton, Victoria 3168, Australia*
[j]*Mayo Clinic Cancer Centre, Rochester, MN, USA*
[k]*Westmead Institute for Cancer Research, University of Sydney at Westmead Millennium Institute, Westmead Hospital, NSW, Australia*
[l]*Department of Gynaecological Oncology, Westmead Hospital, NSW, Australia*
[m]*Queensland Institute of Medical Research, Brisbane, QLD, Australia*
[n]*Department of Biochemistry and Molecular Biology, University of Melbourne, Melbourne, Victoria 8006, Australia*
[o]*Department of Genetics, University of Melbourne, Melbourne, Victoria 8006, Australia*

ARTICLE INFO

*Article history:*
Received 7 October 2013
Received in revised form
20 December 2013
Accepted 14 January 2014
Available online 24 January 2014

*Keywords:*
Serous ovarian cancer

ABSTRACT

*Introduction:* Improvement in the ability to target underlying drivers and vulnerabilities of high-grade serous ovarian cancer (HG-SOC) requires the development of molecularly annotated pre-clinical models reflective of clinical responses.
*Methods:* We generated patient-derived xenografts (PDXs) from consecutive, chemotherapy-naïve, human HG-SOC by transplanting fresh human HG-SOC fragments into subcutaneous and intra-ovarian bursal sites of NOD/SCID IL2Rγ$^{null}$ recipient mice, completed molecular annotation and assessed platinum sensitivity.
*Results:* The success rate of xenografting was 83%. Of ten HG-SOC PDXs, all contained mutations in *TP53*, two were mutated for *BRCA1*, three for *BRCA2*, and in two, *BRCA1* was methylated.

*Abbreviations:* MLPA, multiplex ligation-dependent probe amplification; PFA, paraformaldehyde; FCS, fetal calf serum; FFPE, Formalin Fixed Paraffin Embedded; PBS, phosphate buffered saline; d, day.

* *Corresponding author*. Walter and Eliza Hall Institute of Medical Research, 1G Royal Parade, Parkville, Victoria 3052, Australia. Tel.: +61 3 9345 2498; fax: +61 3 9347 0852.
E-mail addresses: scottc@wehi.edu.au, scott.clare90@gmail.com (C.L. Scott).

[1] MW and CLS share equal authorship.


http://dx.doi.org/10.1016/j.molonc.2014.01.008

Platinum
Xenograft
DNA repair
BRCA1
BRCA2

*In vivo* cisplatin response, determined as platinum sensitive (progression-free interval ≥100 d, $n = 4$), resistant (progression-free interval <100 d, $n = 3$) or refractory ($n = 3$), was largely consistent with patient outcome. Three of four platinum sensitive HG-SOC PDXs contained DNA repair gene mutations, and the fourth was methylated for *BRCA1*. In contrast, all three platinum refractory PDXs overexpressed dominant oncogenes (*CCNE1*, *LIN28B* and/or *BCL2*).

*Conclusions:* Because PDX platinum response reflected clinical outcome, these annotated PDXs will provide a unique model system for preclinical testing of novel therapies for HG-SOC.



## 1. Introduction

Epithelial ovarian cancer (OC) remains the most lethal of gynecological malignancies, with over 70% of cases resulting in mortality. Improvement in our ability to target the underlying drivers and vulnerabilities of sub-groups of OC requires the development of molecularly annotated pre-clinical models that reflect clinical responses and can be utilized to test novel therapies (Bookman, 2011). Recently, however, five of the most frequently utilized ovarian cancer cell lines, which are generally employed to model the most common and aggressive OC subtype, high-grade serous OC (HG-SOC), were shown to lack the common genetic features of HG-SOC (Domcke et al., 2013). These observations highlight the urgent need to develop and characterize new HG-SOC models that more accurately reflect the biology of this neoplasm.

HG-SOC has been characterized as a disease of genomic instability, with relatively few recurrent somatic mutations or dominantly acting oncogenes (TCGA (2011)). At present, standard-of-care therapy for women with advanced OC includes cytoreductive surgery and platinum/taxane-based chemotherapy. Factors underlying response and resistance to platinum-based therapy in HG-SOC remain incompletely understood. Progress in the development of novel therapeutics in OC has been hampered by lack of biomarkers that permit appropriate targeting of new agents. Access to serial biopsies of human OC, before and after treatment, is recommended as a central component of clinical trial design (Vaughan et al., 2011) but has been slow to occur.

At present, response to platinum chemotherapy is the strongest predictor of survival for women with HG-SOC (Bookman et al., 2009). Patients with platinum-sensitive OC, defined as clinical response for at least six months following cessation of primary platinum therapy, have a better overall survival than patients with either platinum-resistant disease (relapse <6 months following cessation of primary platinum therapy) or platinum-refractory disease (progressive disease while on primary platinum therapy). These variations in response appear to reflect, at least in part, differences in integrity of the homologous recombination (HR) DNA repair pathway, which contains mutations in up to 50% of HG-SOCs (2011). In particular, HR-compromising *BRCA1* or *BRCA2* mutations occur in 18–21% of HG-SOC (TCGA, 2011; Walsh et al., 2011) and are associated with improved response to DNA damaging chemotherapy (Alsop et al., 2012; Kaye et al., 2012; TCGA, 2011) as well as PARP inhibitors (Audeh et al., 2010; Ledermann et al., 2012). Conversely, secondary somatic mutations or "reversions" that restore *BRCA1/2* (Edwards et al., 2008; Sakai et al., 2008) in OC from women with germline *BRCA1/2* mutations predict acquired resistance to platinum or PARP inhibitor therapy (Barber et al., 2013; Norquist et al., 2011). Despite the clinical importance of *BRCA1/2* mutations, established human OC cell lines contain *BRCA1* and *BRCA2* mutations only rarely (Domcke et al., 2013; Stordal et al., 2013), further highlighting the limitations of current pre-clinical OC models for therapeutic studies.

Additional changes with the potential to effect platinum sensitivity have also been observed in OC. *CCNE1* has been shown to be associated with resistance to platinum drugs based on an empiric screen of platinum resistant HG-SOC (Etemadmoghadam et al., 2010) and was the most common amplification identified by TCGA (TCGA (2011)). The MYCN pathway was the oncogenic pathway associated with the proliferative subgroup of HG-SOC, identified by hierarchical clustering (Helland et al., 2011). Overexpression or amplification of pro-survival oncogenic members of the *BCL2* family has been observed (Beroukhim et al., 2010) and has been associated with diminished efficiency of targeted therapeutics as well as classical cytotoxics (Cragg et al., 2009).

Previous reports of molecularly annotated PDXs derived from unmanipulated HG-SOC for pre-clinical evaluation are extremely limited. Most OC animal models have been generated from murine ovarian surface epithelial cell lines (ID8 cells) (Roby et al., 2000) or by xenografting established human HG-SOC cell lines, which are often of poorly-defined origin and have been in culture for many years (Domcke et al., 2013). In contrast, even though PDXs from primary HG-SOC retain pathological and immunohistochemical features of the primary tumor (Lee et al., 2005) and have been used to study tumor-initiating cell frequency (Stewart et al., 2011), the response of these PDXs to conventional or targeted therapeutics has only been described for a handful of individual PDX models (e.g., 1–2 independent HG-SOC PDX per report (Faratian et al., 2011; Kortmann et al., 2011; Press et al., 2008; Sims et al., 2012)) in the context of molecular annotation or patient outcome data.

To determine i) whether the major genetic changes observed in HG-SOCs are retained in PDXs, ii) compare the responses of PDXs and the clinical tumors in the same patients, and iii) develop an *in vivo* model for preclinical studies that more closely represents the biology of HG-SOC, we transplanted consecutive chemotherapy-naive fresh human HG-SOC fragments without prior *in vitro* culture and drove platinum resistance with first- and subsequent-line cisplatin