THE UNIVERSITY of EDINBURGH

Edinburgh Research Explorer

Clinical and molecular characterization of ovarian carcinoma displaying isolated lymph node relapse

Citation for published version:
Hollis, RL, Carmichael, J, Meynert, AM, Churchman, M, Hallas-Potts, A, Rye, T, MacKean, M, Nussey, F, Semple, CA, Herrington, CS & Gourley, C 2019, 'Clinical and molecular characterization of ovarian carcinoma displaying isolated lymph node relapse', *American Journal of Obstetrics and Gynecology*. https://doi.org/10.1016/j.ajog.2019.04.035

Digital Object Identifier (DOI):
10.1016/j.ajog.2019.04.035

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Peer reviewed version

Published In:
American Journal of Obstetrics and Gynecology

General rights
Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.

OPEN ACCESS

Download date: 23. Jul. 2021

Accepted Manuscript

Clinical and molecular characterization of ovarian carcinoma displaying isolated lymph node relapse

Robert L. Hollis, Juliet Carmichael, Alison M. Meynert, Mr Michael Churchman, Miss Amelia Hallas-Potts, Mrs Tzyvia Rye, Melanie Mackean, Fiona Nussey, Colin A. Semple, C. Simon Herrington, Charlie Gourley

PII: S0002-9378(19)30620-9

DOI: https://doi.org/10.1016/j.ajog.2019.04.035

Reference: YMOB 12664

To appear in: *American Journal of Obstetrics and Gynecology*

Received Date: 1 February 2019

Revised Date: 18 April 2019

Accepted Date: 26 April 2019

Please cite this article as: Hollis RL, Carmichael J, Meynert AM, Churchman M, Hallas-Potts A, Rye T, Mackean M, Nussey F, Semple CA, Herrington CS, Gourley C, Clinical and molecular characterization of ovarian carcinoma displaying isolated lymph node relapse, *American Journal of Obstetrics and Gynecology* (2019), doi: https://doi.org/10.1016/j.ajog.2019.04.035.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

ACCEPTED MANUSCRIPT

Clinical and molecular characterization of ovarian carcinoma displaying isolated lymph node relapse

Robert L HOLLIS[1], Juliet CARMICHAEL[1], Alison M MEYNERT[2], (Mr) Michael CHURCHMAN[1], (Miss) Amelia HALLAS-POTTS[1], (Mrs) Tzyvia RYE[1], Melanie MACKEAN[3], Fiona NUSSEY[3], Colin A SEMPLE[2], C. Simon HERRINGTON[1] and Charlie GOURLEY[1]

[1]Nicola Murray Centre for Ovarian Cancer Research, Cancer Research UK Edinburgh Centre, MRC Institute of Genetics and Molecular Medicine, University of Edinburgh, UK

[2]MRC Human Genetics Unit, MRC Institute of Genetics and Molecular Medicine, University of Edinburgh, UK

[3]Edinburgh Cancer Centre, Western General Hospital, Edinburgh, UK.

Correspondence: Robert Hollis, Nicola Murray Centre for Ovarian Cancer Research, Cancer Research UK Edinburgh Centre, MRC Institute of Genetics and Molecular Medicine, University of Edinburgh. Crewe Road South, Edinburgh, EH4 2XU, UK. 0044 131 651 8579; email: robb.hollis@ed.ac.uk

Counts: Abstract 340 words. Main text 2720 words. 2 figures, 3 tables.
Supplement material: 9 pages; 3 supplementary figures; 6 supplementary tables.

Funding: This study was supported by an MRC PhD Studentship and MRC-funded Research Fellowship awarded to RLH, and by funding from The Nicola Murray Foundation.

Conflicts of interest: Conflicts of interest: RLH, none. JC, none. AMM, none. MC, none. AHP, none. TR, none. MM: honoraria from Tesaro, BristolMyersSquibb and Roche. FN: non-personal interests in AstraZeneca and Tesaro. CAS, none. CSH, none. CG discloses: research funding from AstraZeneca, Aprea, Nucana, Tesaro and Novartis; honoraria/consultancy fees from Roche, AstraZeneca, Tesaro, Nucana, Clovis, Foundation One, Cor2Ed and Sierra Oncology; named on issued/pending patents related to predicting treatment response in ovarian cancer outside the scope of the work described here.

Authors' contributions: Conceptualisation: RLH, JC, MC, CSH, CG. Data collection: RLH, JC, AHP, TR, MM, FN. Data analysis: RLH, AM, JC. Data interpretation: RLH, JC, MC, CAS, CSH, CG. Supervision: CG, CSH, CAS. Visualisation and original manuscript draft: RLH. Manuscript review and editing: RLH, JC, AMM, MC, AHP, TR, MM, FN, CAS, CSH, CG.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Condensation: Ovarian carcinoma patients demonstrating isolated lymph node relapse display favorable outcome and greater tumour-infiltrating T-cell burden at diagnosis compared to extra-nodal relapse cases

Short Title: Clinical and molecular characterisation of ILNR ovarian carcinoma

AJOG at a glance: A. A number of investigators have reported a relatively indolent disease course in ovarian carcinoma patients experiencing isolated lymph node relapse (ILNR). However, none have systematically compared these to extra-nodal relapse or performed molecular characterisation of cases that go on to experience this distinct pattern of recurrence.

B. ILNR patients demonstrated significantly prolonged overall and post-relapse survival compared to extra-nodal relapse cases. ILNR cases demonstrated greater tumor-infiltrating lymphocyte burden at diagnosis, but did not demonstrate significant enrichment or depletion of *BRCA1/2* mutation or gain of *CCNE1*, both known to be prognostic in OC.

C. This is the first report demonstrating significantly improved clinical outcome in ILNR ovarian carcinoma when compared directly to extra-nodal relapse, and represents the first study to perform molecular characterisation of cases that go on to experience ILNR.

Key words: cancer recurrence, isolated lymph node relapse, ovarian cancer, survival, tumor-infiltrating lymphocytes

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

ABSTRACT

Background: Disease relapse is the primary cause death from ovarian carcinoma (OC). Isolated lymph node relapse (ILNR) is a rare pattern of OC recurrence, with a reported median post-relapse survival (PRS) of 2.5-4 years. To date, investigations have not compared ILNR OC directly to a matched extra-nodal relapse (ENR) cohort or performed molecular characterization of cases that subsequently experience ILNR.

Objective(s): Here we seek to compare the clinical outcome, tumour-infiltrating lymphocyte burden and frequency of known prognostic genomic events in ILNR OC versus ENR OC.

Study design: 49 ILNR OC patients were identified and matched to 49 ENR cases using the Edinburgh Ovarian Cancer Database, from which the clinical data for identified patients were retrieved. Matching criteria were disease stage, histological subtype and grade, extent of residual disease following surgical debulking and age at diagnosis. Clinicopathological factors and survival data were compared between the ILNR and ENR cohorts. Genomic characterization of tumor material from diagnosis was performed using panel-based high throughput sequencing and tumor-infiltrating T-cell burden was assessed using immunohistochemistry for CD3+ and CD8+ cells.

Results: ILNR cases demonstrated significantly prolonged PRS and overall survival (OS) versus ENR upon multivariable analysis (HR^{multi}=0.52[0.33-0.84] and 0.51[0.31-0.84]). Diagnostic specimens from high grade serous (HGS) OCs that subsequently displayed ILNR harboured significantly greater CD3+ and CD8+ cell infiltration compared to ENR cases (P=0.001 and P=0.009, Bonferroni-adjusted P=0.003 and 0.019). ILNR HGS OC cases did not show marked enrichment or depletion of cases with *BRCA1/2* mutation or *CCNE1* copy number gain when compared to their ENR counterparts (24.4% vs 19.4% and 18.2% vs 22.6%, P=0.865 and P=0.900).

Conclusion(s): ILNR OC represents a distinct clinical entity with favorable outcome compared to ENR. There was no clear enrichment or depletion of *BRCA1/2* mutation or *CCNE1* gain in the ILNR OC

cohort compared with ENR cases, suggesting that these known prognostic genomically-defined subtypes of disease do not display markedly altered propensity for ILNR. Diagnostic tumor material from ILNR patients demonstrated greater CD3+ and CD8+ cell infiltration, indicating stronger tumor engagement by T-cell populations, which may contribute to the more indolent disease course of ILNR.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

INTRODUCTION

Ovarian carcinoma (OC) is the most lethal gynaecological malignancy, accounting for over 180,000 deaths per year worldwide [1]. OC is now recognised to comprise five core histological subtypes: high grade serous (HGS), endometrioid, clear cell, low grade serous (LGS) and mucinous OC – each displaying distinct molecular landscapes and clinical behaviour [2]. Within HGS cases, homologous recombination deficiency by virtue of *BRCA1* or *BRCA2* mutation has been associated with favorable outcome, greater sensitivity to platinum-based chemotherapy and marked benefit from poly (ADP-ribose) polymerase (PARP) inhibitors [3-6]. Conversely, *CCNE1* copy number gain has been associated with chemoresistance and poorer survival in this group [3, 7].

While most OC cases – particularly HGS OCs - are typically sensitive to chemotherapy in the first-line setting, the majority of patients will experience disease relapse which acquires resistance to chemotherapy [8, 9]. The most common sites of recurrence are the pelvis and peritoneum [10]. Involvement of lymph nodes (LNs) at relapse is common; however, recurrence confined solely to LNs is a rare event, accounting for ≤5% of relapsed OCs [11, 12]. These isolated LN relapse (ILNR) cases have been described as a unique clinical disease entity and are thought to experience a relatively indolent disease course, with a reported median post-relapse survival (PRS) and overall survival (OS) of around 2.5-4 and >5 years, respectively [11-18].

A number of previous studies have reported on the clinical outcome of apparent ILNR OC (summarised in Table 1) [11-18]. Many of these studies have reported only a small number of cases [11, 13, 17, 18], with a minority reporting larger numbers identified from multiple centres [14, 16]. To our knowledge, none of these studies have compared outcome directly to a matched extra-nodal relapse (ENR) cohort. Furthermore, they have not performed contemporary histological subtyping or molecular characterization in order to identify potential subgroups of disease with a propensity to experience this distinct pattern of disease relapse.

Here, we report clinical and molecular characterization of a matched ILNR and ENR cohort with contemporary pathology review to compare the clinical outcome and molecular landscape of ILNR and ENR OC.

METHODS

ILNR patient identification

ILNR OC cases were identified from the Edinburgh Ovarian Cancer Database (Supplementary Figure S1), wherein the clinical variables, treatment details and follow-up data of OC patients treated within the Edinburgh Cancer Centre are collected prospectively as part of routine care. Potential ILNR cases were identified using the search terms “lymph node” or “groin node” as the dominant site of relapse, yielding 161 results. Non-epithelial tumors (n=1), tumors of borderline malignancy (n=1), and primary LN serous carcinomas (n=2) were excluded. Patients with concurrent extra-nodal disease (n=50), lack of cross-sectional imaging to confirm sole ILNR (n=13) or coexistence of other malignancies leading to uncertain origin of LN disease (n=2) were excluded. Patients with residual disease (RD) after completion of first-line treatment (n=19) or insufficient clinical data for eligibility assessment (n=24) were also excluded, leaving 49 ILNR cases.

Matching of ILNR to ENR

ILNR cases were electronically matched to ENR cases with complete response to first-line therapy using the Edinburgh Ovarian Cancer Database. Matching criteria were: (i) diagnostic histological subtype and grade, (ii) stage at diagnosis, (iii) extent of RD following debulking surgery and (iv) closest age at diagnosis following matching of (i)-(iii). Criteria were relaxed to facilitate matching of all ILNR cases as detailed in Supplementary Table S1.

Ethical approval and tissue collection

Clinical research access and ethical approval for correlation of molecular data to clinicopathological features and clinical outcome in OC was obtained via NHS Lothian Research and Development (reference ID 2007/W/ON/29). Ethical approval for the use of human tumor material in translational research was obtained from South East Scotland Human Annotated Bioresource (Lothian NRS Bioresource Ethics Reference 15/ES/0094-SR831). Tumor material was available for 75.5% (74 of 98) of cases (77.6%, 38 of 49 ILNR and 73.5%, 36 of 49 ENR).

Histological subtyping of ovarian carcinomas

Contemporary pathology review of ILNR and matched ENR cases was performed by an expert gynaecologic pathologist (CSH). Where appropriate (n=9), immunohistochemistry (IHC) for WT1 and P53 was performed to aid histological subtyping [19]. WT1 IHC was performed using 1:1000 dilutions of antibody M3561 clone 6F-H2 (Dako, Agilent Technologies). p53 staining was performed using 1:50 dilutions of antibody M7001 clone DO-7 (Dako, Agilent Technologies). Both stains were performed using the Leica Bond III Autostainer (Leica Biosystems).

Nucleic acid isolation

Up to ten 10µm FFPE sections, macrodissected using marked H&E-stained slides as a guide to enrich for tumor purity (supplementary table S2), were used for DNA extraction. DNA was extracted using the QIAamp DNA FFPE Tissue Kit and Deparaffinization Solution (Qiagen).

Panel-based sequencing of BRCA and non-BRCA homologous recombination deficiency genes

High throughput sequencing was performed using an 83-gene custom Integrated DNA Technologies (IDT) gene capture panel with unique molecular indices (UMIs) as described in Appendix A. Gene targets, centred around the homologous recombination DNA repair pathway, are detailed in Supplementary Table S3. The median per-sample mean target coverage achieved was 386X.

Assessment of CCNE1 copy number

Copy number variants in *CCNE1* were characterised by TaqMan Genotyping qPCR Copy Number Assays (Applied Biosystems, Thermo Fisher Scientific) as detailed in Appendix B.

Assessment of tumor infiltrating lymphocyte density

Tumor infiltrating lymphocytes (TILs) were assessed using 4µm FFPE sections of diagnostic tumor material from first-line cytoreductive surgery. IHC for CD3 and CD8 was performed using Bond ready-to-use CD8-4B11 and CD3-LN10 antibodies (Leica Biosystems) on the Leica Bond III Autostainer. Human tonsil was used as a positive control for both markers. Stained slides were digitized and marker-positive cells were quantified using QuPath [20] in eight randomly selected tumor-containing 500µm by 500µm fields per sample. Tumor area was marked as a region of interest (supplementary figure S2) and marker-positive cells were quantified using the positive cell detection protocol as a percentage of the total cell number demonstrating marker positivity.

TIL scoring validation was performed by manual counting of marker-positive cells by two human observers (RLH and AHP), in a randomly selected validation cohort representing 15% of samples for each marker. The correlation of marker-positive cell counts (observer 1 vs observer 2 vs QuPath) demonstrated excellent agreement for both markers (Spearman's rho>0.95, P<0.0001 for all comparisons).

Statistical analyses

Statistical analyses were performed using R version 3.5.1. Disease-free interval (DFI) was calculated as time from end of first-line chemotherapy to disease recurrence. Comparisons of OS and PRS were conducted using Cox proportional hazards regression models within the Survival R package [21] and presented as hazard ratios (HRs) alongside their 95% confidence intervals (CIs). Frequency comparisons were made using the Chi-squared test and Fisher's exact test as appropriate. Comparisons of TIL density were made using the Mann Whitney-U test. Analyses were adjusted for multiplicity of testing using the Bonferroni correction, where specified.

RESULTS

Cohort Characteristics

Demographics of the ILNR and ENR cohorts are summarised in Table 2. There was no significant difference in age at diagnosis, RD following primary surgical debulking, histology or grade of disease at diagnosis, or disease stage at diagnosis between the ILNR and ENR groups. These data indicate good fidelity of the ILNR-ENR matching process. Patterns of ILNR are described in Table 3.

Clinical outcome in ILNR versus ENR

ILNR patients displayed prolonged OS and PRS compared to the ENR cohort (HR=0.55 [0.34-0.87], P=0.011 and HR=0.50 [0.31-0.80], P=0.004) (Figure 1A and Figure 1B). The median OS and PRS in the ILNR cohort was 72.9 (95% CI 62.2-96.5) and 32.0 (95% CI 23.3-53.3) months, compared to 41.1 (95% CI 30.0-58.8) and 14.9 (95% CI 12.9-23.7) months in the ENR cohort. The length of the disease-free interval (DFI) prior to ILNR or ENR was not significantly different between the two cohorts (HR=0.86 [0.60-1.29], P=0.473).

Multivariable analysis for OS accounting for extent of RD following primary debulking, FIGO stage and age at diagnosis identified significantly prolonged OS in the ILNR cohort (HR^{multi}=0.51 [0.31-0.84], P=0.008) (Supplementary Table S4). Multivariable analysis of PRS, accounting for DFI and age identified prolonged PRS in ILNR cases (HR^{multi}=0.52 [0.33-0.84], P=0.007) (Supplementary Table S5).

Significantly prolonged OS (HR^{multi} for OS=0.53 [0.29-0.99], P=0.046) and PRS (HR^{multi} for PRS=0.54 [0.31-0.96], P=0.037) was demonstrated for ILNR OC when considering HGS cases specifically (34 ILNR HGS OCs, 31 ENR HGS OCs).

Longer DFI is associated with prolonged PRS in ILNR OC

The importance of DFI on clinical outcome in ILNR OC remains controversial, with some authors reporting no association between DFI length and PRS or OS in this setting [11, 16, 18] and others reporting significant associations [12, 14, 15]. Within the ILNR cohort, DFI ≥12 months was associated with markedly prolonged PRS when accounting for patient age (HR^{multi}=0.38 [0.19-0.78], P=0.008), with median PRS of 47.3 versus 20.1 months in those with DFI ≥12 months and DFI <12 months, respectively (Figure 1C).

Impact of ILNR pattern on outcome

There was no clear differential PRS between multi-region ILNR and single-region ILNR (2 regions versus single site HR=1.06 [0.49-2.30], P=0.890; ≥3 sites versus single site HR=0.94 [0.36-1.43], P=0.898).

Six ILNR cases (12.2%) involved supraclavicular LN sites. While these cases demonstrated an apparent trend for inferior PRS (HR=2.52 [0.95-6.69], P=0.064) (Supplementary Figure S3), there was no significant difference after accounting for DFI and age (HR^{multi}=1.63 [0.58-4.60], P=0.359). Other specific LN sites were not associated with apparent differential PRS (Supplementary Table S6).

Molecular landscape of ILNR HGS OC

64 HGS OC cases (33 ILNR, 31 ENR) were successfully characterised for HR gene mutations and *CCNE1* copy number. Frequencies of genomic abnormalities are outlined in Figure 2A and Supplementary Table S3. Within HGS OC cases, there was no significant difference in the rate of *CCNE1* copy number gain (18.2%, 6/33 versus 22.6%, 7/31, P=0.900) or *BRCA1/2* mutation (24.4%, 8/33 vs 19.4%, 6/31, P=0.865) between the ILNR and ENR cohorts (Figure 2A).

The CD3+ and CD8+ TIL burden was greater in diagnostic tumor specimens from HGS OC patients who went on to experience ILNR when compared to their ENR counterparts (median CD3+ cell density 1.94% vs 1.13%, P=0.001 and median CD8+ cell density 0.90% vs 0.45%, P=0.009; Bonferroni-adjusted P=0.003 and P=0.019) (Figure 2B).

COMMENT

Principle findings

The principle findings of this study are: (i) ILNR represents a distinct pattern of OC relapse with prolonged survival versus ENR cases; (ii) longer DFI prior to ILNR is associated with prolonged PRS in ILNR; (iii) ILNR OC do not demonstrate significantly differential composition of known genomic subtypes associated with prognosis, namely *BRCA1/2* mutation or gain of *CCNE1*; (iv) cases that go on to experience ILNR demonstrate greater TIL burden at diagnosis compared to ENR cases.

Study strengths and limitations

A key strength of this study is the direct comparison of ILNR OC to matched ENR cases: a number of studies have reported ILNR as a distinct pattern of OC relapse with a relatively indolent disease course, but have not systematically compared ILNR cases directly to a matched ENR cohort [11-18].

Moreover, these studies did not perform pathology review of identified cases, precluding the ability to characterise ILNR outcome in the context of contemporary OC histotypes, which are now known to display markedly differential clinical outcome [22]. Critically, we characterise ILNR OC following contemporary histological subtyping to facilitate investigation of ILNR in a histotype-specific manner.

The majority of previous studies investigating ILNR have identified fewer than twenty OC cases of serous histology that go on to experience this rare relapse pattern; moreover, previous reports have not performed molecular characterisation of OC cases that demonstrate ILNR [11-18]. We identified 49 ILNR OC patients treated within the Edinburgh Cancer Centre, including 34 cases reviewed as HGS OC. This study represents the largest ILNR OC series from a single centre and the only report investigating the molecular landscape of ILNR OC to date.

While this study does represent one of the largest reported ILNR OC cohorts, case numbers were still restricted due to the rarity of ILNR OC. In particular, power to detect differential outcome between distinct patterns of ILNR was limited, and we could not perform meaningful analysis comparing rates of rare genomic events present in both ILNR and ENR cohort, including mutational events in *RB1*, *NF1* and *PTEN*, as well as gene-specific analysis of *BRCA1* and *BRCA2*. Other limitations of this study include heterogeneous treatment of OC patients across the time period in which these cases were diagnosed, though diagnosis periods were comparable between the ILNR and ENR cohorts (Table 2).

Clinical outcome in ILNR OC

The median PRS and OS of ILNR cases was approximately 2.7 and 6 years, consistent with previous reports of ILNR OC [11-18]. ILNR cases displayed significantly prolonged OS and PRS compared to their ENR counterparts upon multivariable analysis (HR^{multi}=0.51 and 0.52 for OS and PRS). Critically, this difference was maintained in a histotype-specific analysis of HGS cases, which account for the majority of OCs. To our knowledge, this is the first report directly demonstrating a significant difference in outcome between ILNR and ENR OC.

Only half of the reports investigating the impact of DFI length on ILNR outcome to date have identified associations with OS or PRS [12, 14, 15]. Here, we demonstrate that DFI ≥12 months is associated with a substantial PRS benefit (median PRS approximately 3.9 versus 1.7 years), largely reflective of established associations in unselected OC cases [23]. While this contradicts reports from some investigators [11, 16, 18], two of these studies reported specifically in the context of ILNR undergoing secondary debulking [16, 18] and the other compared cases using a cut-off DFI of 24 months, rather than 12 months as described here [11], potentially explaining this discrepancy. Notably, the intervals considered in our study are akin to those used clinically to define platinum sensitivity in unselected relapsed OC [23].

We show no significant difference in clinical outcome between patients with ILNR at multiple sites versus those with single site ILNR, or between distinct patterns of ILNR. While univariable analysis suggested that supraclavicular LN involvement may confer inferior PRS, this trend was not apparent when accounting for DFI and patient age, suggesting that this is not a genuine phenomenon of supraclavicular ILNR. Notably, the number of patients with supraclavicular LN involvement was low (n=6). Together, these data support the consideration of ILNR OC as a single disease entity, regardless of the number and location of involved sites.

The genomic landscape of ILNR OC

Until now, the molecular landscape of ILNR has been completely uncharacterised. It has therefore been unclear as to whether OC cases that go on to experience ILNR demonstrate enrichment of tumors belonging to known favorable genomic subgroups. Within unselected cohorts of HGS OC, inactivation of *BRCA1* or *BRCA2* has been associated with favorable outcome [3, 4], while copy number gain of *CCNE1* has been associated with poor survival and chemoresistance [3, 7]. Genomic characterization of this cohort did not identify significant depletion or enrichment of these molecular events in ILNR HGS OC cases versus their ENR counterparts. These data suggest that the survival benefit of ILNR OC is not underpinned by large scale enrichment for *BRCA1/2*-mutant cases

with favourable prognosis or absence of *CCNE1*-gained cases which have poorer prognosis, and suggest that these genomic subgroups do not display markedly differential propensity for ILNR.

Greater TIL burden at diagnosis in patients who subsequently experience ILNR

Intriguingly, assessment of the CD3+ and CD8+ cell burden in ILNR and ENR tumor material – reflective of whole T-cell and cytotoxic T cell populations – uncovered significantly greater TIL burden in diagnostic tissue from patients who subsequently experienced ILNR (2-fold enrichment for CD8+ cells, approximately 1.7-fold enrichment for CD3+ cells). These data suggest that active engagement of the immune system at diagnosis impacts upon the nature of disease at relapse, and that immune-mediated control of cancer cells may contribute to the indolent disease course of ILNR OC. Indeed, these data may well be of interest in relation to the use of immune-directed therapies in cancer treatment [24, 25]. However, while many ILNR cases displayed high TIL burden, some cases demonstrated relatively low levels of TILs, alluding to mechanisms beyond effective T cell engagement at diagnosis underpinning some ILNR cases.

Conclusion

Collectively, the data presented here – supported by previous descriptions of apparent ILNR in the literature – demonstrate that ILNR represents a distinct pattern of OC with favourable clinical outcome when compared to ENR. Cases that go on to experience ILNR harbour greater TIL burden at diagnosis, but do not show marked enrichment or depletion of known genomic subgroups associated with differential outcome.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Acknowledgements: We extend our thanks to the patients who contributed to this study and to the Edinburgh Ovarian Cancer Database from which the clinical data reported here were retrieved. We are thankful to the Wellcome Trust Clinical Research Facility (Western General Hospital, Edinburgh, UK) for their support with the sequencing described here, and to the Nicola Murray Foundation for their generous support of the Nicola Murray Centre for Ovarian Cancer Research.

Conflicts of interest: RLH, none. JC, none. AMM, none. MC, none. AHP, none. TR, none. MM: honoraria from Tesaro, BristolMyersSquibb and Roche. FN: non-personal interests in AstraZeneca and Tesaro. CAS, none. CSH, none. CG discloses: research funding from AstraZeneca, Aprea, Nucana, Tesaro and Novartis; honoraria/consultancy fees from Roche, AstraZeneca, Tesaro, Nucana, Clovis, Foundation One, Cor2Ed and Sierra Oncology; named on issued/pending patents related to predicting treatment response in ovarian cancer outside the scope of the work described here.

Authors' contributions: Conceptualisation: RLH, JC, MC, CSH, CG. Data collection: RLH, JC, AHP, TR, MM, FN. Data analysis: RLH, AM, JC. Data interpretation: RLH, JC, MC, CAS, CSH, CG. Supervision: CG, CSH, CAS. Visualisation and original manuscript draft: RLH. Manuscript review and editing: RLH, JC, AMM, MC, AHP, TR, MM, FN, CAS, CSH, CG.

ACCEPTED MANUSCRIPT

REFERENCES

[1] Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians. 2018;68:394-424.

[2] Prat J. Ovarian carcinomas: five distinct diseases with different origins, genetic alterations, and clinicopathological features. Virchows Archiv : an international journal of pathology. 2012;460:237-49.

[3] Integrated genomic analyses of ovarian carcinoma. Nature. 2011;474:609-15.

[4] Tan DS, Rothermundt C, Thomas K, Bancroft E, Eeles R, Shanley S, et al. "BRCAness" syndrome in ovarian cancer: a case-control study describing the clinical features and outcome of patients with epithelial ovarian cancer associated with BRCA1 and BRCA2 mutations. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2008;26:5530-6.

[5] Fong PC, Boss DS, Yap TA, Tutt A, Wu P, Mergui-Roelvink M, et al. Inhibition of poly(ADP-ribose) polymerase in tumors from BRCA mutation carriers. The New England journal of medicine. 2009;361:123-34.

[6] Moore K, Colombo N, Scambia G, Kim BG, Oaknin A, Friedlander M, et al. Maintenance Olaparib in Patients with Newly Diagnosed Advanced Ovarian Cancer. The New England journal of medicine. 2018.

[7] Patch AM, Christie EL, Etemadmoghadam D, Garsed DW, George J, Fereday S, et al. Whole-genome characterization of chemoresistant ovarian cancer. Nature. 2015;521:489-94.

[8] Ledermann JA, Raja FA, Fotopoulou C, Gonzalez-Martin A, Colombo N, Sessa C. Newly diagnosed and relapsed epithelial ovarian carcinoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Annals of oncology : official journal of the European Society for Medical Oncology. 2013;24 Suppl 6:vi24-32.

[9] Armstrong DK. Relapsed ovarian cancer: challenges and management strategies for a chronic disease. The oncologist. 2002;7 Suppl 5:20-8.

[10] Amate P, Huchon C, Dessapt AL, Bensaid C, Medioni J, Le Frere Belda MA, et al. Ovarian cancer: sites of recurrence. International journal of gynecological cancer : official journal of the International Gynecological Cancer Society. 2013;23:1590-6.

[11] Blanchard P, Plantade A, Pages C, Afchain P, Louvet C, Tournigand C, et al. Isolated lymph node relapse of epithelial ovarian carcinoma: outcomes and prognostic factors. Gynecologic oncology. 2007;104:41-5.

[12] Legge F, Petrillo M, Adamo V, Pisconti S, Scambia G, Ferrandina G. Epithelial ovarian cancer relapsing as isolated lymph node disease: natural history and clinical outcome. BMC cancer. 2008;8:367.

[13] Uzan C, Morice P, Rey A, Pautier P, Camatte S, Lhomme C, et al. Outcomes after combined therapy including surgical resection in patients with epithelial ovarian cancer recurrence(s) exclusively in lymph nodes. Annals of surgical oncology. 2004;11:658-64.

[14] Gadducci A, Cosio S, Zola P, Sostegni B, Ferrero AM, Teti G, et al. The clinical outcome of epithelial ovarian cancer patients with apparently isolated lymph node recurrence: a multicenter retrospective Italian study. Gynecologic oncology. 2010;116:358-63.

[15] Tu H, Huang H, Huang QD, Li Z, Feng YL, Liu JH. [Treatment and prognostic analysis of ovarian cancer patients with isolated region of lymph node recurrence]. Zhonghua fu chan ke za zhi. 2012;47:928-33.

[16] Ferrero A, Ditto A, Giorda G, Gadducci A, Greggi S, Daniele A, et al. Secondary cytoreductive surgery for isolated lymph node recurrence of epithelial ovarian cancer: a multicenter study. European journal of surgical oncology : the journal of the European Society of Surgical Oncology and the British Association of Surgical Oncology. 2014;40:891-8.

ACCEPTED MANUSCRIPT

[17] Santillan A, Karam AK, Li AJ, Giuntoli R, 2nd, Gardner GJ, Cass I, et al. Secondary cytoreductive surgery for isolated nodal recurrence in patients with epithelial ovarian cancer. Gynecologic oncology. 2007;104:686-90.
[18] Fotiou S, Aliki T, Petros Z, Ioanna S, Konstantinos V, Vasiliki M, et al. Secondary cytoreductive surgery in patients presenting with isolated nodal recurrence of epithelial ovarian cancer. Gynecologic oncology. 2009;114:178-82.
[19] Kobel M, Rahimi K, Rambau PF, Naugler C, Le Page C, Meunier L, et al. An Immunohistochemical Algorithm for Ovarian Carcinoma Typing. International journal of gynecological pathology : official journal of the International Society of Gynecological Pathologists. 2016;35:430-41.
[20] Bankhead P, Loughrey MB, Fernández JA, Dombrowski Y, McArt DG, Dunne PD, et al. QuPath: Open source software for digital pathology image analysis. Scientific reports. 2017;7:16878.
[21] Therneau T. A Package for Survival Analysis in S. . 2015.
[22] Hollis RL, Gourley C. Genetic and molecular changes in ovarian cancer. Cancer Biol Med. 2016;13:236-47.
[23] Ushijima K. Treatment for Recurrent Ovarian Cancer—At First Relapse. Journal of Oncology. 2010;2010.
[24] Postow MA, Chesney J, Pavlick AC, Robert C, Grossmann K, McDermott D, et al. Nivolumab and ipilimumab versus ipilimumab in untreated melanoma. The New England journal of medicine. 2015;372:2006-17.
[25] Muro K, Chung HC, Shankaran V, Geva R, Catenacci D, Gupta S, et al. Pembrolizumab for patients with PD-L1-positive advanced gastric cancer (KEYNOTE-012): a multicentre, open-label, phase 1b trial. The Lancet Oncology. 2016;17:717-26.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Figure legends

Figure 1. Clinical outcome of ILNR OC. (A) OS in ILNR vs ENR OC; (B) PRS in ILNR vs ENR OC; (C) PRS in ILNR OC by DFI length.

Figure 2. Molecular landscape of ILNR and ENR OC. (A) Genomic events in ILNR and ENR cases; (B) TIL burden of ILNR and ENR HGS OCs.

Supplementary figure legends

Figure S1. ILNR cohort identification from the Edinburgh Ovarian Cancer Database.

Figure S2. Automated marker-positive cell quantification by QuPath

Figure S3. PRS of ILNR OC with and without supraclavicular LN involvement

Supplementary table legends

Table S1. Tolerances for electronic matching of ENR to ILNR

Table S2. Cellularity of specimens used for DNA extraction

Table S3. Frequency of patients with detrimental mutations in genes sequenced on IDT gene capture NGS panel

Table S4. Multivariable analysis for OS in ILNR vs ENR in OC

Table S5. Multivariable analysis of time to ILNR as a predictor of PRS in ILNR OC

Table S6. Univariable analyses of specific LN site involvement and association with PRS

ACCEPTED MANUSCRIPT

Table 1. Previous reports of ILNR OC.

		Ferrero (16)	Tu (15)	Gadducci (14)	Fotiou (18)	Legge (12)	Santillan (17)	Blanchard (11)	Uzan (13)	Summary
ILNR cases	**N**	73	38	69	21	32	25	27	12	Few reports of ≥40 cases
Age at diagnosis	**Years**	median 54	24 (63%) >50; 14 (37%) ≤ 50	median 58	mean 50	median 60	mean 58	mean 59	median 51	Largely unremarkable compared to unselected OC cohorts
	Range	29-73		34-78	36-67	45-76	41-82	41-85	42-71	
Stage at diagnosis	**I**	14 (19%)	0	11 (16%)	3 (14%)	0	2 (8%)	4 (15%)	5 (42%)	
	II	4 (6%)	15 (39%)	6 (9%)	3 (14%)	1 (3%)	5 (20%)	5 (18%)	1 (8%)	
	III	51 (70%)	23 (61%)	46 (67%)	14 (67%)	29 (91%)	15 (60%)	15 (56%)	6 (50%)	
	IV	4 (6%)	0	6 (9%)	1 (5%)	2 (6%)	3 (12%)	3 (11%)	0	
RD following first-line debulking	**0cm / <0.5cm**	57 (78%)	17 (45%)	22 (32%)	8 (38%)	14 (44%)	18 (72%)	NA	7 (58%)	
	≤1cm	10 (14%)	10 (26%)	11 (16%)	7 (33%)	6 (19%)	5 (20%)	NA	4 (33%)	
	<2cm	4 (6%)	11 (29%)	36 (52%)	4 (19%)		0	NA		
	>2cm	2 (3%)			2 (10%)	12 (38%)	2 (8%)	NA	1 (8%)	
Grade at diagnosis	**I**	4 (6%)	7 (18%)	3 (4%)	0	9 (32%)	25 (100%) high grade	NA	NA	
	II	5 (7%)	14 (37%)	13 (19%)	8 (38%)					
	III	64 (88%)	17 (45%)	54 (78%)	13 (62%)	19 (68%)				
	NA	-	-	-	-	4	-			
Reported histological subtype at diagnosis	**Serous**	53 (73%)	19 (50%)	52 (75%)	16 (76%)	26 (81%)	19 (76%)	17 (62%)[a]	8 (67%)	Predominantly serous / HGS cases, as with unselected OC cohorts
	Endometrioid	11 (15%)	9 (24%)	12 (17%)	5 (24%)	2 (6%)	2 (8%)	3 (11%)	3 (25%)	
	Clear Cell	0	0	1 (1%)		0	0	0	0	
	Mucinous	1 (1%)	1 (3%)	0		1 (3%)	0	3 (11%)	0	
	Other	8 (11%)	9 (24%)	4 (6%)		3 (9%)	4 (16%)	4 (15%)	1 (8%)	
DFI / time to ILNR[b]	**Median months**	18	18	44 (62%) >12 months	21	17.5	16	26 months from diagnosis	21	Median 1.5-2 years DFI
	Range	6-192	9-96		8-156	1-134	6-40	1-159	6-72	
ILNR site(s)	**Para-aortic only**	37 (51%)	10 (26%)	23 (33%)	8 (38%)	14 (44%)	15 (60%)	9(33%) retro. alone, 6(22%) retro. + other. Supraclavicular, mediastinal, iliac and inguinal involvement in 7(26%), 4(15%), 4(15%) and 3(11%) cases.	5 (42%)	Most commonly involves pelvic and/or para-aortic sites
	Pelvic only	21 (29%)	15 (39%)	12 (17%)	4 (19%)	1 (3%)	3 (12%)		4 (33%)	
	Para-aortic & pevlic	9 (12%)	7 (18%)	6 (9%)	4 (19%)	9 (28%)	1 (4%)		1 (8%)	
	Inguinal only	3 (4%)	2 (5%)	12 (17%)	4 (19%)	2 (6%)	5 (20%)		1 (8%)	
	Other combinations	3 (4%)	4 (11%)	16 (23%)	1 (5%)	6 (19%)	1 (4%)		1 (8%)	
ILNR pattern	**Single region**	61 (84%)	27 (71%)	47 (77%)	17 (81%)	20 (63%)	24 (96%)	17 (63%)	10 (83%)	Most commonly localised to a single region
	Multi-region	12 (16%)	11 (29%)	14 (23%)	4 (19%)	9 (28%)	1 (4%)	10 (37%)	2 (17%)	
	NA	-	-	8	-	3	-	-	-	
PRS	**Median months**	5-yr PRS 64%; 5-yr OS ~80%	5-yr PRS 66.5%	32.1	47	37	37	26	5-yr PRS 71%	Median 2-4 years
OS	**Median months**			62.9	66	109	61	68		Median >5 years
Surgery for ILNR	**Yes**	73 (100%)	19 (50%)	24 (35%)	21 (100%)	12 (38%)	25 (100%)	8 (30%)	12 (100%)	Heterogeneous management, typically involving chemotherapy
	No	0	19 (50%)	45 (65%)	0	20 (63%)	0	19 (70%)	0	
ILNR	**Chemo alone**	0	5 (13%)	44 (64%)	0	19 (59%)	0	8 (30%)	0	

ACCEPTED MANUSCRIPT

intervention: regime	**Surgery alone**	3 (4%)	0	1 (1%)	0	1 (3%)	2 (8%)	2 (7%)	0	
	Surgery-chemo combination	70 (96%)	19 (50%)	22 (32%)	17 (81%)	11 (34%)	15 (60%)	5 (19%)	10 (83%)	
	Radio alone	0	0	1 (1%)	0	0	0	2 (7%)	0	
	No intervention	0	0	0	0	1 (3%)	0	7 (26%)	0	
	Other	0	14 (37%)	1 (1%)	4 (19%)	0	8 (32%)	3 (11%)	2 (17%)	

Chemo, chemotherapy; DFI, disease-free interval; ENR, extra-nodal relapse; ILNR, isolated lymph node relapse; OS, overall survival; PRS, post-relapse survival; radio, radiotherapy; RD, residual disease; retro, retroperitoneal; 5-yr, 5-year; NA, not available. [a]Includes 5 cases described as papillary; [b]from end of first-line chemotherapy

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Table 2. Demographics of ILNR and ENR OC cohorts.

		ILNR, n=49		ENR, n=49		ILNR vs ENR
Factor	Class	N	%/range	N	%/range	P-value
Stage at Diagnosis	I	5	10.6	5	10.2	1.000[a]
	II	10	21.3	11	22.4	
	III	27	57.4	28	57.1	
	IV	5	10.6	5	10.2	
	NA	2		0		
Histology at Diagnosis	Serous	25	51.0	33	67.3	.502[b]
	Endometrioid	12	24.5	11	22.5	
	Clear Cell	1	2.0	1	2.0	
	Mixed histology	8	16.3	4	8.2	
	Unclassified Adenocarcinoma	3	6.1	0	0.0	
Grade at Diagnosis	I	0	0.0	1	2.0	1.000[c]
	II	6	13.0	6	12.2	
	III	40	87.0	42	75.7	
	NA	3		0		
Contemporary Histological Classification	HGS	34	89.5	31	86.1	.733[d]
	Endometrioid	2	5.3	3	8.3	
	LGS	2	5.3	1	2.8	
	Mixed HGS /endometrioid	0	0.0	1*	2.8	
	No specimen available	11		13		
Surgical debulking status	RD <2cm	34	75.6	33	70.2	.733[e]
	RD 2-5cm	7	15.6	8	17.0	
	RD ≥5cm	4	8.9	6	12.8	
	NA	4		2		
First-line chemotherapy	Platinum	21	42.9	17	34.7	.693[f]
	Platinum combination	25	51.0	28	57.1	
	Other	3	6.1	4	8.2	
Neoadjuvant first-line chemotherapy	Yes	2	4.1	1	2.0	1.000[g]
	No	47	95.9	48	98.0	
Decade of Diagnosis	≤1999	23	46.9	21	42.9	.667[f]
	2000-2005	19	38.8	23	46.9	
	2006-2010	7	14.3	5	10.2	
Age at Diagnosis	Median years	61	41-80	62	41-80	.339[h]
Specimen from diagnosis	Primary site	33	91.7	29	80.6	.307[i]
	Omentum	2	5.6	6	16.7	
	Other	1	2.8	1	2.8	
	NA	2		0		

ACCEPTED MANUSCRIPT

	No specimen available	11		13		

NA, not available; RD, residual disease. [a]Chi-squared test, stage I/II vs stage III/IV; [b]Chi-squared test, Serous/mixed versus other; [c]Chi-squared test, grade I/II vs grade III; [d]Fisher's exact test, HGS versus non-HGS; [e]Chi-squared test, RD <2cm vs ≥2cm; [f]Chi-squared test; [g]Fisher's exact test; [h]Welch two-sample T test; [i]Fisher's exact test, primary site vs omentum/other. *****This tumour had two morphologically distinct components with different immunophenotypes

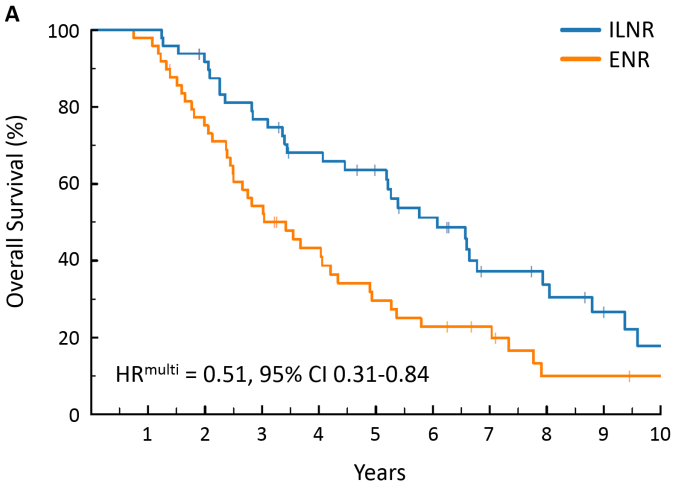
ACCEPTED MANUSCRIPT

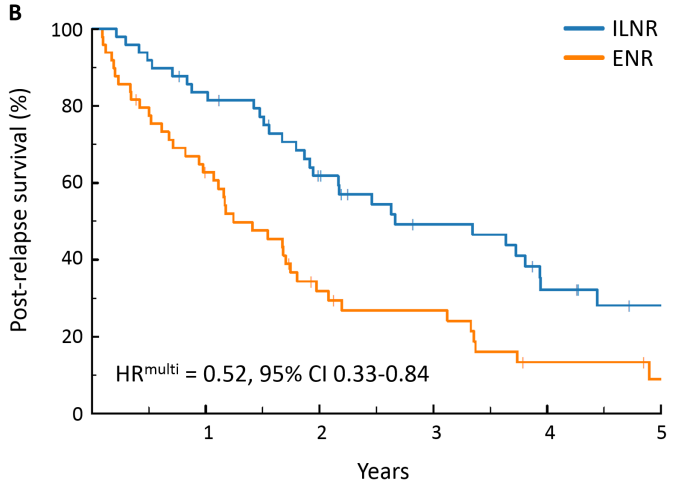
ACCEPTED MANUSCRIPT

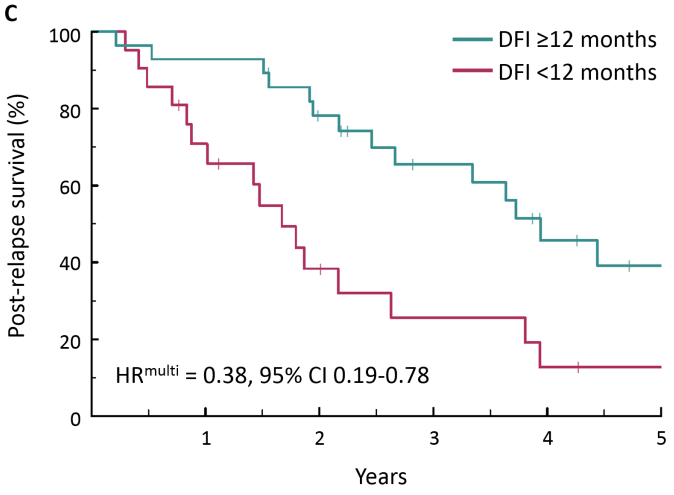
Table 3. Patterns of ILNR OC

	Cases	Proportion of cases (%)
ILNR Pattern		
Single site	22	44.9
Multi-regional		
2	17	34.7
3	8	16.3
4	2	4.1
ILNR Sites		
Para-aortic only	16	32.7
Pelvic only	4	8.2
Inguinal only	2	4.1
Pelvic & para-aortic	6	12.2
Supraclavicular & other sites	6	12.2
Pelvic, para-aortic & other(s)	6	12.2
Other combinations	9	18.4

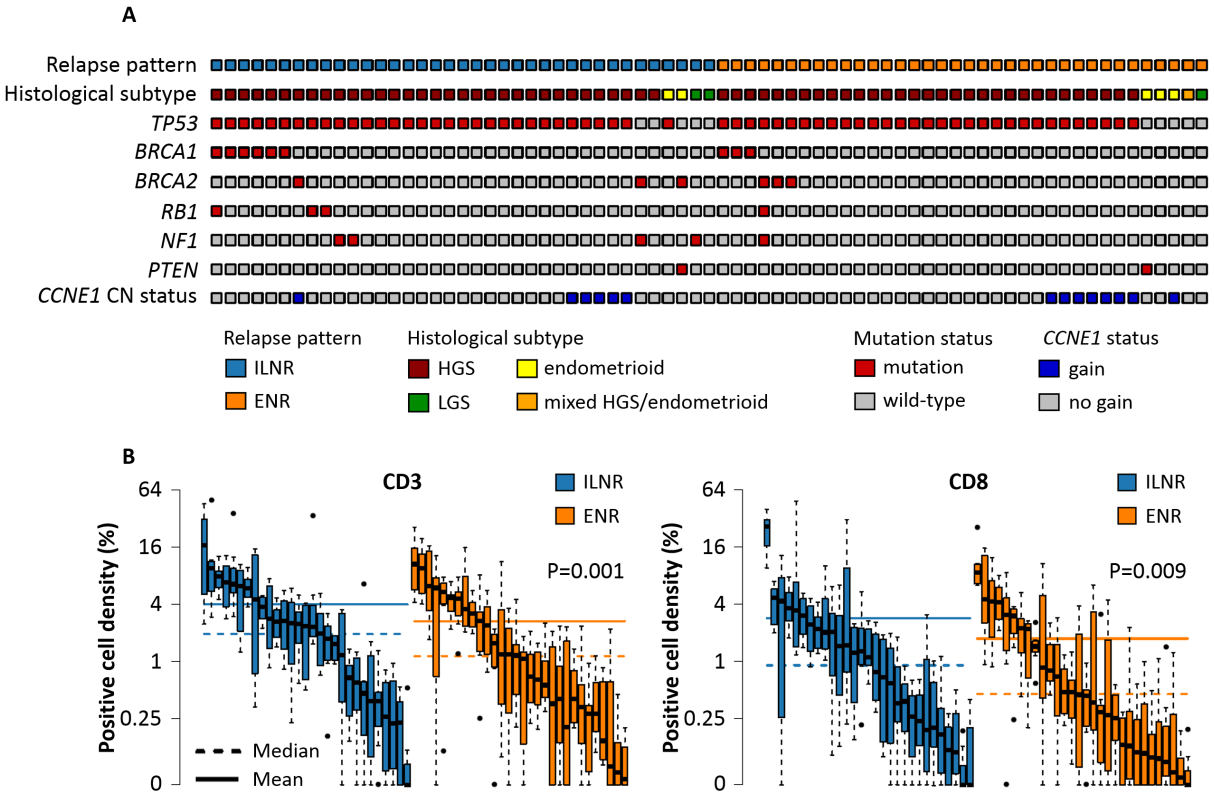
ACCEPTED MANUSCRIPT


A
ILNR
ENR
Overall Survival (%)
100
80
60
40
20
0
HR^multi = 0.51, 95% CI 0.31-0.84
1
2
3
4
5
6
7
8
9
10
Years


B
ILNR
ENR
Post-relapse survival (%)
100
80
60
40
20
0
HR^multi = 0.52, 95% CI 0.33-0.84
1
2
3
4
5
Years


C
DFI ≥12 months
DFI <12 months
Post-relapse survival (%)
100
80
60
40
20
0
HR^multi = 0.38, 95% CI 0.19-0.78
1
2
3
4
5
Years

ACCEPTED MANUSCRIPT



ACCEPTED MANUSCRIPT

APPENDIX A – high throughput sequencing of FFPE DNA

Whole genome libraries were generated using 200ng input DNA as determined by Qubit Fluorimetry (Invitrogen, ThermoFisher Scientific). One sample failed library preparation. Libraries were pooled in groups of 16 (100ng generated library per sample) for gene capture and sequenced by the Wellcome Trust Clinical Research Facility (Western General Hospital, Edinburgh, UK) using an Illumina NextSeq. Following alignment and consensus read generation using UMIs (as detailed below), the median per-sample mean target coverage achieved was 386X (range 102X – 970X).

Sequence reads were processed using the bcbio v1.0.6 pipeline (https://github.com/chapmanb/bcbio-nextgen) and reads aligned against hg38 with bwa v0.7.17, sorted, then duplicate-marked with bamsormadup (biobambam v2.0.79) (1, 2). UMIs were added as tags by umis v0.9.0b0, and files were indexed after conversion to BAM format using samtools v1.6 (3). Consensus reads were called and filtered with fgbio v0.4.0 (https://github.com/fulcrumgenomics/fgbio) following read grouping by UMIs, then extracted with bamtofastq (biobambam), re-aligned, sorted and indexed. The aligned consensus reads underwent base quality score recalibration with the GATK v3.8 (4).

Variant calling was performed using a multicaller approach: variants were called with GATK Mutect2, Freebayes (v1.1.0.46) (https://github.com/ekg/freebayes) and VarDict (Java v1.5.1) (5), then decomposed and normalized with vt v2015.11.10 (https://github.com/atks/vt). VarDict variants were annotated with vcfanno and bcftools v1.6 (6, 7). Freebayes variants were annotated with GATK VariantAnnotator. A majority vote system was used for curating high confidence calls with minimum 10% variant allele frequency, with a 2/3 caller majority needed for inclusion in the final callset.

Called variants were annotated using the Ensembl Variant Effect Predictor v90.9 against Ensembl release 90 (8). Variants documented as benign (as annotated by Ensembl VEP) were discarded, while documented pathogenic were retained. Remaining nonsense mutations, frameshifting indels and splice site variants were retained as likely function variants. Remaining synonymous variants, non-

coding variants and missense variants of undocumented significance were discarded as variants of uncertain significance.

TP53 mutations were classified independently with additional reference to the UMD *TP53* variation database (9). Manual review of aligned sequence read for the supposed *TP53* wild-type OCs (6 HGS, 4 endometrioid, 3 LGS, 1 mixed serous/endometrioid) was performed owing to the known high *TP53* mutation rate in OC (particularly in HGS OCs). These analyses revealed further high confidence pathogenic mutations affecting splice sites in 4 HGS OC cases, two of which were present in the callset from a single caller and hence didn't qualify for the ensemble callset. The apparent poorer sensitivity to splice site events was attributed to the proximity of these variants to read ends, compounded by the relatively reduced coverage at exon-intron boundaries owing to the capture design being targeted toward coding regions.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

APPENDIX B – *CCNE1* copy number assays

Copy number (CN) variants in *CCNE1* were identified by TaqMan Genotyping qPCR Copy Number Assays using the StepOne Plus Real-Time PCR System (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA) and StepOne Software Version 2.3 (Life Technologies, Thermo Fisher Scientific, Waltham, MA, USA). VIC dye–labelled RNAseP TaqMan™ Copy Number Reference Assay was used as a reference assay, alongside FAM dye-labelled Hs07158517_cn targeting *CCNE1*. Target amplicons were chosen to ensure FFPE compatibility (87bp product for *RNaseP*, 91bp product for *CCNE1*).

Amplification efficiency was assessed using serial dilutions of NA12878 genome in a bottle DNA (10) in triplicate. Efficiency was calculated using the gradient of the line of best fit for Ct value against the logarithm (base 10) of ng DNA input (efficiency = -1 + 10-1/slope of Ct versus log10 of input DNA) (11), yielding assay efficiencies of 103.4%, 97.0% and 103.3% for *CCNE1*, *EMSY* and RNaseP (*RPPH1*), respectively. These data indicate excellent amplification efficiency in all assays.

CN assays for FFPE-derived DNA were performed alongside NA12878 DNA and FUOV1 cell line DNA controls, representing DNA with normal CN and *CCNE1* CN gain, respectively (10, 12). CN variants were called using CopyCaller v2.0 (Life Technologies, Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA) using NA12878 as calibrator sample (CN=2). *CCNE1* CN ≥4 was considered CN gain.

Supplementary references

1. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics (Oxford, England). 2009;25(14):1754-60.
2. Tischler G, Leonard S. biobambam: tools for read pair collation based algorithms on BAM files. Source Code for Biology and Medicine. 2014;9:13-.
3. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, et al. The Sequence Alignment/Map format and SAMtools. Bioinformatics (Oxford, England). 2009;25(16):2078-9.
4. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome research. 2010;20(9):1297-303.
5. Lai Z, Markovets A, Ahdesmaki M, Chapman B, Hofmann O, McEwen R, et al. VarDict: a novel and versatile variant caller for next-generation sequencing in cancer research. Nucleic acids research. 2016;44(11):e108.

ACCEPTED MANUSCRIPT

6. Pedersen BS, Layer RM, Quinlan AR. Vcfanno: fast, flexible annotation of genetic variants. Genome biology. 2016;17(1):118.
7. Danecek P, McCarthy SA. BCFtools/csq: haplotype-aware variant consequences. Bioinformatics (Oxford, England). 2017;33(13):2037-9.
8. McLaren W, Gil L, Hunt SE, Riat HS, Ritchie GR, Thormann A, et al. The Ensembl Variant Effect Predictor. Genome biology. 2016;17(1):122.
9. Hamroun D, Kato S, Ishioka C, Claustres M, Beroud C, Soussi T. The UMD TP53 database and website: update and revisions. Human mutation. 2006;27(1):14-20.
10. Zook JM, Chapman B, Wang J, Mittelman D, Hofmann O, Hide W, et al. Integrating human sequence data sets provides a resource of benchmark SNP and indel genotype calls. Nature biotechnology. 2014;32(3):246-51.
11. Kubista M, Andrade JM, Bengtsson M, Forootan A, Jonak J, Lind K, et al. The real-time polymerase chain reaction. Molecular aspects of medicine. 2006;27(2-3):95-125.
12. Domcke S, Sinha R, Levine DA, Sander C, Schultz N. Evaluating cell lines as tumour models by comparison of genomic profiles. Nature communications. 2013;4:2126.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Supplementary Table S1. Tolerances for electronic matching of ENR to ILNR

Feature	ILNR OC case documented diagnostic characteristic	Matched ENR OC case documented diagnostic characteristic	Patients
Stage at Diagnosis	Inadequate information to stage	Stage II, n=1 Stage IIIC, n=1	3
	Stage IIB	Stage IIA, n=1	
Documented Histology at Diagnosis	Adenocarcinoma	Serous papillary, n=3	9
	Endometrioid	Mixed serous/endometrioid, n=1	
	Mixed histology	Serous papillary, n=5	
Grade at Diagnosis	NA	Grade I, n=1 Grade III, n=2	3
RD following debulking	<2cm	NA, n=1	4
	NA	2-5cm, n=1 >5cm, n=2	

Note: The matching criteria were relaxed for 2 fields for 2 patients and 3 fields for 1 patient*****. NA, not available; RD, residual disease

*****1 x stage IIB grade III mixed histology carcinoma with RD <2cm matched to stage IIA grade III serous carcinoma with RD <2cm

1 x stage IV unclassified adenocarcinoma of unknown grade and RD <2cm matched to stage IV grade III serous carcinoma with RD <2cm

1 x stage IV unclassified adenocarcinoma of unknown grade and RD not available matched to stage IV grade III serous carcinoma with RD >5cm

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Supplementary Table S2. Cellularity of specimens used for DNA extraction

Tumour cellularity of macrodissected area	Number of cases
<20%	3 (4.1%)
20-39%	7 (9.5%)
40-59%	9 (12.2%)
60-79%	26 (35.1%)
≥80%	29 (39.2%)

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Supplementary Table S3. Frequency of patients with detrimental mutations in genes sequenced on IDT gene capture NGS panel

Gene	ILNR OC with mutation	ENR OC with mutation
ABCB1	1	0
ARID1A	1	2
ATM	2	0
ATR	1	0
BRCA1	6	3
BRCA2	3	3
CTNNB1	1	1
FANCC	0	1
KRAS	1	1
MSH2	1	1
NF1	4	1
PIK3CA	2	1
PRKDC	1	0
PTEN	1	1
RB1	3	1
SLX4	0	1
TP53	32	31
Genes with no detected mutations: *ATRX, BAP1, BARD1, BCL2L1, BLM, BRAF, BRIP1, C11orf65, CCNE1, CDK12, CHD4, CHEK1, CHEK2, EGFR, EMSY, ERBB2, ERCC4, EZH2, FANCA, FANCB, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCL, FANCM, GNAS, KIT, MAD2L2, MDM2, MLH1, MRE11, MSH6, MUS81, MUTYH, NBN, NDUFB2, NF2, NRAS, PALB2, PARP1, PARP2, PAXIP1, PDGFRA, PER3, PMS2, PPP2R1A, PPP2R2A, RAD50, RAD51, RAD51B, RAD51C, RAD54L, RNASEH2A, RNASEH2B, RNASEH2C, RPA1, RUNDC3B, SHFM1, SLC25A40, SLFN11, TOE1, TP53BP1, UBE2T, VRK2*		

OC, ovarian carcinoma

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Supplementary Table S4. Multivariable analysis for OS in ILNR vs ENR in OC

Factor	Class	N	HR^{multi}	Low 95% CI	Upper 95% CI	P
Relapse type	ILNR	49	0.51	0.31	0.84	0.008
	ENR	49	Ref	Ref	Ref	Ref
Stage at Diagnosis	Early (I/II)	31	0.41	0.17	1.02	0.055
	III	55	0.45	0.19	1.03	0.060
	IV	10	Ref	Ref	Ref	Ref
	NA	2	-	-	-	-
Surgical debulking status	RD < 2cm	67	0.60	0.32	1.12	0.109
	RD ≥ 2cm	25	Ref	Ref	Ref	Ref
	NA	6	-	-	-	-
Age at Diagnosis	Years		1.03	1.00	1.05	0.050

HR, hazard ratio; CI, confidence interval.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Supplementary Table S5. Multivariable analysis of time to ILNR as a predictor of PRS in ILNR OC

Factor	Class	N	HR^{multi}	Low 95% CI	Upper 95% CI	P
Relapse type	ILNR	49	0.53	0.33	0.84	0.007
	ENR	49	Ref	Ref	Ref	Ref
DFI	≥12 months	46	0.47	0.29	0.75	0.006
	<12 months	52	Ref	Ref	Ref	Ref
Age	Years		1.03	1.01	1.06	0.006

HR, hazard ratio; CI, confidence interval; DFI, disease-free interval.

ACCEPTED MANUSCRIPT

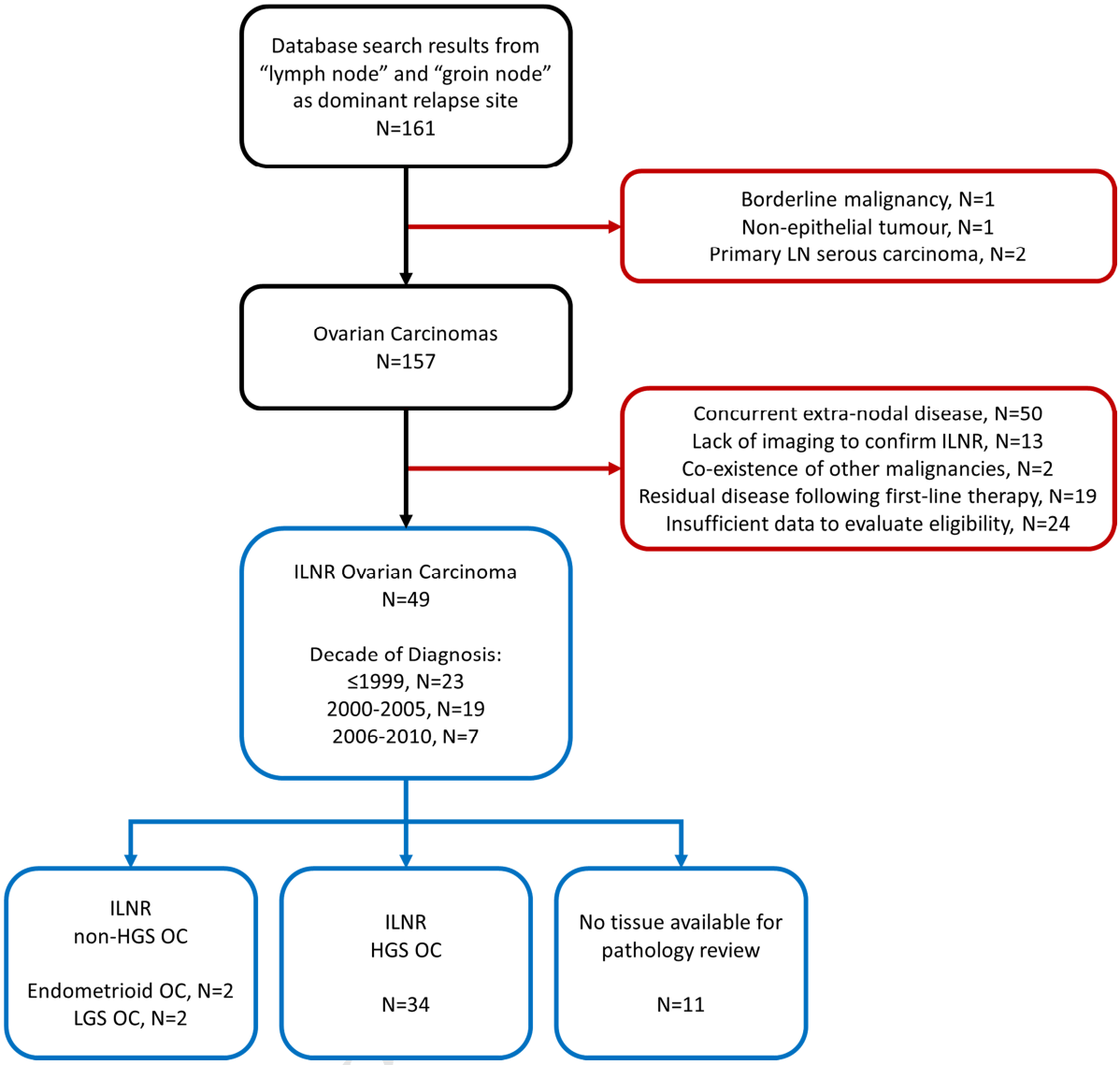
ACCEPTED MANUSCRIPT

Supplementary Table S6. Univariable analyses of specific LN site involvement and association with PRS

Factor	Class	N	HR	Low 95% CI	Upper 95% CI	P
Supraclavicular LN involvement	Yes	6	2.52	0.95	6.69	0.064
	No	43	Ref	Ref	Ref	Ref
Pelvic LN involvement	Yes	20	0.73	0.35	1.51	0.393
	No	29	Ref	Ref	Ref	Ref
Inguinal LN involvement	Yes	9	0.72	0.28	1.87	0.502
	No	40	Ref	Ref	Ref	Ref
Para-aortic LN involvement	Yes	36	1.10	0.48	2.56	0.818
	No	13	Ref	Ref	Ref	Ref

HR, hazard ratio; CI, confidence interval; LN, lymph node

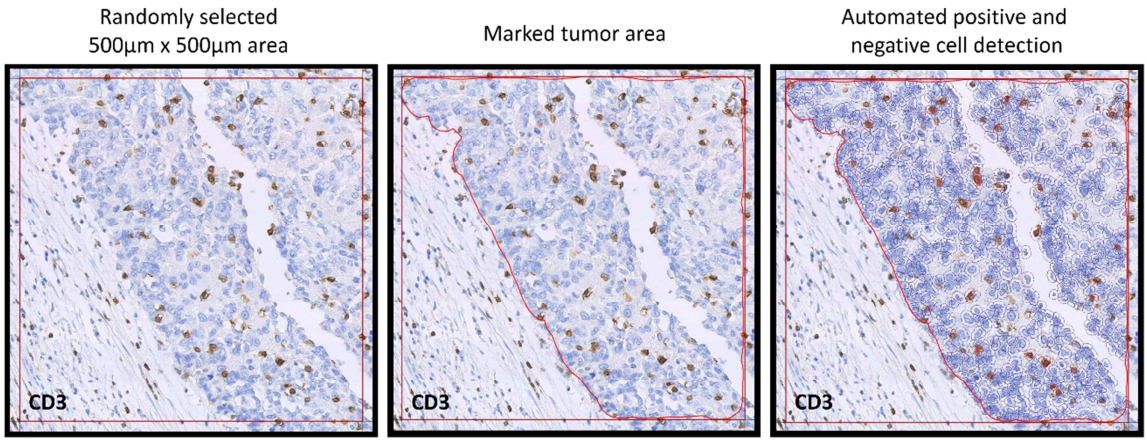
ACCEPTED MANUSCRIPT



Supplementary Figure S1 ILNR cohort identification from the Edinburgh Ovarian Cancer Database.

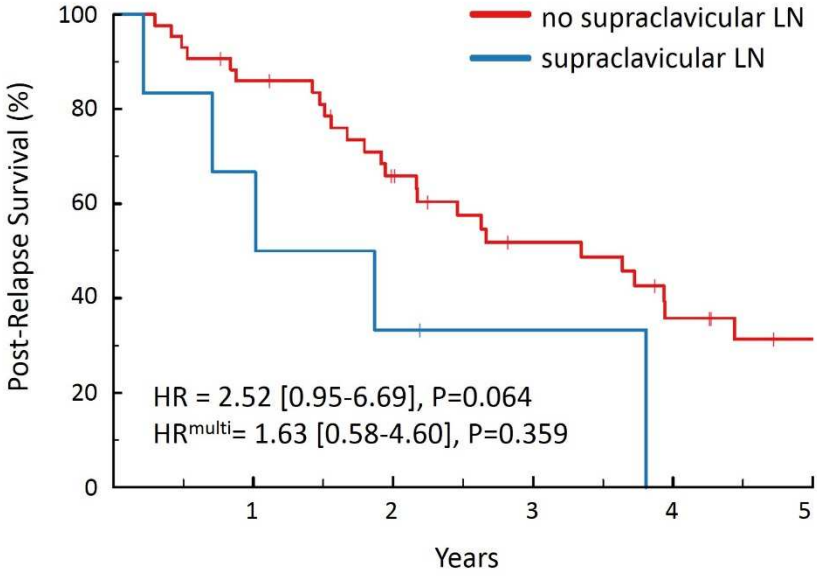
ACCEPTED

ACCEPTED MANUSCRIPT



Supplementary Figure S2. Automated marker-positive cell quantification by QuPath.

ACCEPTED MANUSCRIPT



Supplementary Figure S3. PRS of ILNR OC with and without supraclavicular LN involvement

ACCEPTED MANUSCRIPT