Establishing a framework for the clinical translation of germline findings in precision oncology

Katherine Dixon, MSc, Sean Young, PhD, Yaoqing Shen, PhD, My Linh Thibodeau, MD, MSc, Alexandra Fok, MSc, Erin Pleasance, PhD, Eric Zhao, PhD, Martin Jones, PhD, Geraldine Aubert, PhD, Linlea Armstrong, MD, Alice Virani, PhD, Dean Regier, PhD, Karen Gelmon, MD, Dan Renouf, MD, Stephen Chia, MD, Ian Bosdet, PhD, S. Rod Rassekh, MD, Rebecca J. Deyell, MD, Stephen Yip, MD, PhD, Ana Fisic, BScN, Emma Titmuss, MSc, Shirin Abadi, PharmD, MBA, Steven J.M. Jones, PhD, Sophie Sun, MD, Aly Karsan, MD, Marco Marra*, PhD, Janessa Laskin*, MD, Howard Lim*, MD, Kasmintan A. Schrader*, MBBS, PhD

*Co-senior authors

Affiliations of authors: Department of Medical Genetics, University of British Columbia, Vancouver, British Columbia, Canada (KD, MLT, AV, SJMJ, MM, KAS); Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, British Columbia, Canada (SY, IB, SY); Canada's Michael Smith Genome Sciences Centre, BC Cancer, Vancouver, British Columbia, Canada (YS, AF, EP, EZ, MJ, ET, SJMJ, AK, MM); Terry Fox Laboratory, BC Cancer Research Centre, Vancouver, British Columbia, Canada (GA); Provincial Medical Genetics Program, Children's & Women's Health Centre of British Columbia, Vancouver, British Columbia, Canada (LA); Ethics Service, Provincial Health Service of Authority of BC, Vancouver, British Columbia, Canada (AV); Canadian Centre for Applied Research in Cancer Control, Cancer Control Research, BC Cancer, Vancouver, British Columbia, Canada (DR); School of Population and Public Health, University of British Columbia, Vancouver, British Columbia, Canada (DR); Division of Medical Oncology, BC Cancer, Vancouver, British Columbia, Canada (KG, DR, SC, AF, SS, JL, HL); Cancer Genetics and Genomics Laboratory, BC Cancer, Vancouver, British Columbia, Canada (SY, IB); BC

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Children's Hospital Research Institute, Vancouver, British Columbia, Canada (SRR, RJD); Division of Hematology/Oncology and BMT, Department of Pediatrics, University of British Columbia, Vancouver, British Columbia, Canada (SRR, RJD); Department of Pharmacy, BC Cancer, Vancouver, British Columbia, Canada (SA); Hereditary Cancer Program, BC Cancer, Vancouver, British Columbia, Canada (SS, KAS); and Department of Molecular Oncology, BC Cancer, Vancouver, British Columbia, Canada (KAS).

**Corresponding author:**

Kasmintan Schrader
Hereditary Cancer Program
BC Cancer
600 West 10th Avenue
Vancouver, British Columbia, V5Z 4E6
Telephone: 604-877-6000 ext. 672198
Email: ischrader@bccancer.bc.ca
Abstract

Inherited genetic variation has important implications for cancer screening, early diagnosis, and disease prognosis. A role for germline variation has also been described in shaping the molecular landscape, immune response, microenvironment, and treatment response of individual tumours. However, there is a lack of consensus on the handling and analysis of germline information that extends beyond known or suspected cancer susceptibility in large-scale cancer genomics initiatives. As part of BC Cancer's Personalized OncoGenomics program, we performed whole-genome and transcriptome sequencing in paired tumour and normal tissues from advanced cancer patients to characterize the molecular tumour landscape and identify putative targets for therapy. Overall, our experience supports a multi-disciplinary and integrative approach to germline data management. This includes a need for broader definitions and standardized recommendations regarding primary and secondary germline findings in precision oncology. Here we propose a framework for identifying, evaluating, and returning germline variants of potential clinical significance that may have indications for health management beyond cancer risk reduction or prevention in patients and their families.
Background

Characterizing hereditary genetic variation in high- and moderate-penetrance cancer predisposition genes may have implications for cascade carrier testing, cancer risk-reduction and screening interventions in individuals at risk of having inherited a causal germline variant. Individual variability in disease prognosis and treatment response also occurs within the context of heterogeneous genetic backgrounds, including rare variants associated with cancer susceptibility and common polymorphisms in other cancer-related genes\(^1\)–\(^4\). However, standards for the clinical translation of genetic information relevant to cancer susceptibility, pathogenesis, prognosis and treatment, as well as secondary or incidental genetic information unrelated to cancer, is inconsistent across cancer genomics programs\(^5\). This may result from varying regional policies regarding the return of research results, clinician preference or genetic literacy.

The Personalized OncoGenomics (POG) program is a precision medicine initiative in British Columbia (BC), Canada that was established to identify clinically actionable molecular events in adult metastatic cancer patients and pediatric patients with poor prognosis cancers (NCT02155621). Whole-genome sequencing (WGS) and RNA sequencing (RNA-seq) of fresh frozen tumour biopsies performed with WGS of paired normal tissues has helped identify somatic alterations and inherited genetic variants that shape tumour progression\(^6\)–\(^9\). This and similar projects, such as the Cancer Genome Atlas and International Cancer Genome Consortium, have provided important resources for understanding the morbid human genome\(^10,11\). At the same time, the rate of data production has far outpaced the development of evidence-based guidelines for managing germline findings. Here we advocate for a broader understanding of what defines primary germline findings in oncology and propose a framework for identifying, evaluating and reporting research germline findings within the clinical infrastructure of a publicly-funded provincial health authority.

Early years of the POG program
Since its establishment in 2012, the POG program has continued to develop effective, collaborative and transparent data management and reporting strategies⁶. These have allowed the accommodation of advancements in technology, computational pipelines, therapeutic developments, and biological and clinical knowledge. Before standard recommendations for variant interpretation were published by the American College of Medical Genetics and Genomics (ACMG) and Association for Molecular Pathology (AMP) in 2015, germline data was assessed on an ad hoc basis to identify high-penetration variants relevant to inherited cancer susceptibility or treatment¹². Any research findings were discussed with treating oncologists as part of the POG Tumour Board, but this approach was not broadly or consistently actionable in a maturing program. This was complicated by conflicting interpretations of variant pathogenicity and/or differing opinions regarding the return of results, indicating the need for a standardized procedure for germline assessment. Given the ethical challenges of germline data analysis within the oncology setting, the POG Ethics and Germline Working Group was created in 2014. Using autonomy, beneficence, non-maleficence, and justice as guiding principles, the mandate of the Ethics and Germline Working Group includes addressing ongoing issues related to the management and clinical translation of germline findings. The group meets monthly and consists of a multi-disciplinary team of oncologists, medical and molecular geneticists, pathologists, bioinformaticians and other scientists, an ethicist, and lawyers.

**Transparency and standardization of informed consent**

The Ethics and Germline Working Group reached a consensus that informed consent or assent should include by default the reporting of any clinically actionable cancer-related germline genetic information for both adults and children with cancer. This mandate keeps with the primary goal of the POG program: to characterize the complete genomic architecture of an individual cancer while maintaining data transparency and allowing opportunities for clinical translation. Cancer-related germline findings of clinical significance are currently returned to the
research participant, designee, or next-of-kin by their oncologist, and a referral is made to BC Cancer's provincial Hereditary Cancer Program (HCP) for genetic counseling and clinical variant confirmation. For both tumour-only and tumour-normal sequencing, an appropriate consent procedure includes a detailed review of family history and pre-test counseling regarding the potential risks and benefits of germline findings. This is critical for ensuring ethical justification of studies involving the analysis of hereditary genetic variation.

An opt-in procedure for the return of germline findings of clinical significance unrelated to cancer has been adopted by the POG program, consistent with recommendations based on patient and public preference studies\textsuperscript{13}. While these findings are not routinely sought in cancer genomics analyses, 97.9\% of participants enrolled in the program between 2012 and July 2019 (\(n = 993\)) opted for the return of incidental germline findings unrelated to cancer. These observations suggest that the level of potential risk in learning about inherited genetic variation, including risks related to cancer susceptibility, disease carrier status, or paternity, is acceptable to patients and has not resulted in a decline in participation in the program. The potential for learning about genetic cancer predisposition in particular should be an essential part of the patient’s education and communicated to them at the time of diagnosis.

**Germline variant curation, validation and return**

Pathogenic and likely pathogenic germline variants in moderate- to high-penetrance cancer predisposition genes underlie 5-10\% of all cancers, with a prevalence of up to 20\% in certain cancer types\textsuperscript{14,15}. In collaboration with HCP and the Cancer Genetics and Genomics Laboratory (CGL), the Ethics and Germline Working Group developed standard procedures for germline variant prioritization and evaluation (Figures 1 & 2). Genome-wide variant calling is performed in parallel pipelines for small variants, including single nucleotide variants and small insertions and deletions, and structural variants (SVs), including copy number variants and balanced genomic rearrangements (Figure 1). SV calling through short-read-based next-
generation sequencing in particular has inherent technical and computational challenges due to low-complexity sequences in the human reference genome. Therefore, using multiple computational tools that employ complementary variant calling methods is preferred in order to improve the sensitivity of SV detection. Following gene- and function-based filtering, candidate germline variants are manually reviewed in a genome browser to flag putative technical or sequence artifacts prior to clinical review.

To allow rapid return of results for variants with established clinical actionability, a tiered list of cancer susceptibility genes is analyzed according to the following guidelines. Known pathogenic and likely pathogenic variants in curated variant databases, such as ClinVar and local CGL database, and novel predicted loss-of-function variants (e.g. deletion, frameshift, nonsense, canonical splice site and stop loss) are prioritized for curation in known cancer predisposition genes (Table S1). For several genes associated with high-penetrance syndromes, variants of uncertain significance (VUS) and rare variants in coding and splice regions are further prioritized for review. Variant classification is performed by a clinical molecular geneticist from CGL and certified by the Canadian College of Medical Genetics and Genomics or ACMG according to updated guidelines from the ACMG, AMP and Clinical Genome Resource (ClinGen). This approach integrates clinical expertise and laboratory protocols in variant classification and confirmatory genetic testing, respectively, allowing seamless translation for patients referred to HCP (Figure 2). Final recommendations for return of information and referral for genetic counselling and clinical testing are made based on consensus among the Ethics and Germline Working Group. In exceptional cases, germline variants may be subject to expedited review by a core expert panel, including a clinical molecular geneticist, medical geneticist, and oncologist, to allow immediate return of information and clinical referral (Table S2). Clinical genetics expertise and group consultation are thus integral parts of germline assessment in the POG program to ensure reliability, consistency and transparency.
Evolving variant curation guidelines indicate the need for dynamic computational pipelines that integrate updated variant information from population and clinical databases and encourage efficient retrospective analysis\textsuperscript{16,17}. To aid in large-scale variant identification and classification, special consideration should also be given to variants with founder effects, specific modes of disease inheritance and variants with low to moderate cancer risk. Founder mutations in common cancer predisposition genes such as \textit{BRCA1}, \textit{BRCA2}, \textit{CHEK2}, and \textit{MUTYH} should be excluded from global allele frequency thresholds used in automated variant filtering. For example, over 1\% of patients in the POG program are carriers for pathogenic \textit{MUTYH} variants, reflecting a strong representation of individuals of East and Southeast Asian descent in BC\textsuperscript{9,18}. In such cases, we recommend developing highly curated internal databases with known pathogenic and likely pathogenic germline variants to reduce the incidence of false negative findings. Furthermore, variants in Mendelian disease genes underlying cancer predisposition syndromes inherited in autosomal recessive or X-linked recessive patterns, such as \textit{MUTYH}-associated polyposis (MAP) and dyskeratosis congenita, respectively, also require exceptional committee review. The position of the Ethics and Germline Working Group is to evaluate the risks and benefits of returning carrier status for autosomal recessive cancer susceptibility genes on a gene- and case-specific basis. Finally, identification of low- to moderate-penetrance cancer predisposition variants may present an additional challenge due to limited evidence-based guidelines regarding effective clinical management and cancer risk reduction strategies. Personal and family medical history should be carefully considered in these cases to determine the appropriateness of variant disclosure for cancer susceptibility.

For individuals with phenotypic indications of high-penetrance cancer predisposition syndromes and uninformative clinical genetic testing, evaluation of tumour WGS and RNA-seq may improve genetic diagnosis through the resolution of potential splicing variants, non-coding variants in regulatory regions, or structural variants\textsuperscript{9,19}. Tumour data may also inform possible roles for VUS or autosomal recessive gene variants in pathogenesis, recently demonstrated in a
patient with biallelic variants in MUTYH (p.Gly286Glu and p.Ser346Ser) with tumour evidence supporting global deficiency in base excision repair\textsuperscript{7–9}. The specific types of molecular data that could be considered in whole-genome and transcriptome studies include genome-wide mutation burden, simple somatic mutations and mutational signatures, copy number alterations, loss-of-heterozygosity, structural variants and structural variant signatures, expression outliers, expression-based classifications, and alternative splicing (Table S3). However, recommendations for incorporating tumour data into variant classification are still evolving, and recent guidelines recommend cautious variant interpretation based on this evidence alone without corresponding data from \textit{in vitro} or \textit{in vivo} functional studies\textsuperscript{20}. In the POG program, VUS are not reclassified on the basis of tumour data alone without ClinVar classifications or published phenotypic and functional evidence supporting pathogenicity. For patients with relevant personal or family history, VUS may be returned to the treating clinician with a recommendation for referral to HCP if the individual is eligible for publicly-funded index genetic testing. The Ethics and Germline Working Group does not support clinical decision-making based on the presence of VUS alone, and stresses the importance of consultation with core members of a clinical genetics team to ensure appropriate clinical follow-up when indicated.

**Implications for germline genetic variation beyond cancer susceptibility**

Germline variation and somatic alterations both have potential clinical significance in precision oncology, and integrated analysis of tumour-normal sequencing may identify roles for germline variation that extend beyond cancer susceptibility. Therefore, our framework for germline data management defines primary germline findings as any variants with relevance to tumour biology or treatment. This includes variants with implications for estimating cancer risk, determining the landscape of somatic alterations or tumour evolution, modifying the immune response and microenvironment, and predicting overall response or adverse reactions to treatment (Figure 3). This broad definition is based on findings from large tumour sequencing
projects that have allowed functional characterization of inherited genetic variation other than rare coding variants in known disease genes. These include a number of recent studies that have identified roles for common and non-coding variants in tumourigenesis that may ultimately guide treatment interventions or development of targeted therapeutics. Identifying germline variation with potential tumour relevance is thus an important part of characterizing the complex molecular architecture of individual tumours and may have immediate or future clinical implications.

**Framework for the clinical translation of germline findings**

Based on our experience during seven years of the POG program, we developed an integrated schema for the management of germline genetic information. We propose a non-mutually exclusive categorical framework for evaluating clinically actionable germline variants that aims to identify inherited cancer susceptibility, characterize treatment indications, and provide opportunities for the discovery of novel genetic associations (Table 1). This includes pathogenic and likely pathogenic variants in known cancer predisposition genes with defined cancer risk estimates and screening recommendations, and may include VUS in cases where the patient’s phenotype supports pathogenicity. In the POG program, known and novel pathogenic and likely pathogenic variants in known cancer predisposition genes are reviewed by the Ethics and Germline Working Group, and these are then disclosed to the Tumour Board and case oncologist with a general recommendation for return to the patient with a referral to HCP for follow-up. Secondary germline findings in the 59 genes defined by the ACMG should consistently be returned to patients if informed consent for return of such findings was given at the time of study enrolment.

With an inclusive definition of primary germline findings in the context of cancer genomics, pharmacogenomic variants with known drug associations, polymorphic alleles with putative immune response associations, variants in genes with potential tumour relevance
based on histological or molecular characteristics, and other variants with potential clinical relevance as requested by the case oncologist could also be analyzed. The Ethics and Germline Working Group supports routine reporting of germline variants predictive of immune response, such as HLA class I genotypes, but recommends caution in reporting pharmacogenomic variants with limited or conflicting scientific evidence that may prevent use of important supportive medications\textsuperscript{27}. Clinicians and scientists should refer to public curated databases such as PharmGKB for updated variant reviews and clinical guidelines\textsuperscript{28}. In practice, a curated variant database relevant for chemotherapy and other cancer therapy with established pharmacogenomic associations, such as gastrointestinal and bone marrow toxicity in carriers of the \textit{UGT1A1*28} polymorphism, should be prioritized during routine germline analysis unless otherwise requested by the case clinician\textsuperscript{29}.

Pharmacogenomic variants will be an important aspect of cancer treatment in the era of precision medicine. The potential benefits of profiling pharmacogenomic variants as part of the POG program was demonstrated in a patient with a gastrointestinal stromal tumour (GIST) and prolonged QT interval during a high-dose course of imatinib. Further genomic profiling was performed at the request of the case clinician, and a common polymorphism in \textit{SCN5A} (H558R) that has been previously reported as a genetic modifier in cardiac arrhythmia syndromes was identified\textsuperscript{30,31}. Metabolic and cardiovascular gene variants that are known to modulate drug metabolism or mediate adverse events in response to treatment have immediate clinical utility in treatment selection. Thus prior knowledge of variants that can impact treatment choice may provide indications for or against the use of certain drugs. In the case described above, this may have indicated an avoidance of specific tyrosine kinase inhibitors associated with prolonged QT intervals\textsuperscript{32,33}.

Novel relationships between genetic alterations and histological or molecular phenotypes could also be investigated through an agnostic analysis of germline variation. Mutation and expression comparisons using public datasets is an invaluable tool for
characterizing the individual tumour genome and in validating putative molecular associations. However, these must be interpreted cautiously given expected differences between sample handling and preparation protocols, sequencing chemistries and platforms, and computational pipelines. With a secondary goal of discovery, deleterious germline variants in known cancer genes defined in the Catalog of Somatic Mutations in Cancer (COSMIC) Cancer Gene Census or genes in other disease-related pathways could be prioritized but not limiting when investigating novel associations with outlier tumour phenotypes (Table S4)\textsuperscript{34,35}. It should be noted that germline findings with unconfirmed implications for disease pathogenesis, prognosis, or treatment are purely the result of research and, due to their uncertain nature, are strongly discouraged from being returned to the patient unless indicated by the Tumour Board for treatment indications. If germline findings are determined to be of clinical importance, notice should be given to the case clinician to ensure banking of a clinical DNA sample and patient and/or family referral for genetic counselling and variant confirmation. Disclosure of germline variants to the POG Tumour Board and case clinician is encouraged in cases with established implications for clinical management, including for cancer risk reduction, screening, or treatment indications. In the future, larger studies and agnostic analyses may uncover additional roles for these variants in disease pathogenesis that may be predictive or prognostic of overall survival and treatment outcomes.

**Concluding remarks**

Hereditary cancer susceptibility is commonly observed in unselected cohorts of cancer patients that do not meet current clinical testing guidelines\textsuperscript{36,37}. In precision oncology, the utility of exploring inherited genetic variation is achieved through the implementation of cancer prevention and screening strategies and by the use of targeted therapies. However, personnel and financial resources in jurisdictions with universal health care may be a significant barrier to service accessibility as genetic testing becomes more common and additional germline variants
with clinical significance are discovered. These issues were strongly considered by the Ethics and Germline Working Group in assessing the benefits of disclosure for certain variants, but these guidelines must be re-evaluated moving forward to help inform cost-effective patient-centred care. Based on our experience in the POG program, genome-wide analysis of inherited genetic variation should allow for the examination of normal and disease-causing variation that may affect tumour evolution, response to therapy, immune function, predict adverse events, and/or be relevant to non-cancer disease risk that may be meaningful to the patient and their care.
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Notes

Affiliations of authors: Department of Medical Genetics, University of British Columbia, Vancouver, British Columbia, Canada (KD, MLT, LA, AV, SJMJ, MM, KAS); Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, British Columbia, Canada (SY, IB, SY); Canada's Michael Smith Genome Sciences Centre, BC Cancer, Vancouver, British Columbia, Canada (YS, AF, EP, EZ, MJ, RR, ET, SJMJ, AK, MM); Terry Fox Laboratory, BC Cancer Research Centre, Vancouver, British Columbia, Canada (GA); Provincial Medical Genetics Program, Children's & Women's Health Centre of British Columbia, Vancouver, British Columbia, Canada (LA); Ethics Service, Provincial Health Service of Authority of BC, Vancouver, British Columbia, Canada (AV); Canadian Centre for Applied Research in Cancer Control, Cancer Control Research, BC Cancer, Vancouver, British Columbia, Canada (DR); School of Population and Public Health, University of British Columbia, Vancouver, British Columbia, Canada (DR); Division of Medical Oncology, BC Cancer, Vancouver, British Columbia, Canada (KG, DR, SC, AF, SS, JL, HL); Cancer Genetics and Genomics Laboratory, BC Cancer, Vancouver, British Columbia, Canada (SY, IB); BC Children's Hospital Research Institute, Vancouver, British Columbia, Canada (SRR, RJD); Division of Hematology/Oncology and BMT, Department of Pediatrics, University of British
Columbia, Vancouver, British Columbia, Canada (SRR, RJD); Department of Pharmacy, BC Cancer, Vancouver, British Columbia, Canada (SA); Hereditary Cancer Program, BC Cancer, Vancouver, British Columbia, Canada (SS, KAS); and Department of Molecular Oncology, BC Cancer, Vancouver, British Columbia, Canada (KAS).

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References

1. Copson ER, Maishman TC, Tapper WJ, et al. Germline BRCA mutation and outcome in young-onset breast cancer (POSH): a prospective cohort study. *Lancet Oncol.* 2018;19:169-180. doi:10.1016/S1470-2045(17)30891-4

2. Robson M, Im S-A, Senkus E, et al. Olaparib for Metastatic Breast Cancer in Patients with a Germline *BRCA* Mutation. *N Engl J Med.* 2017;377(6):523-533. doi:10.1056/NEJMoa1706450

3. Ryu JS, Hong YC, Han HS, et al. Association between polymorphisms of ERCC1 and XPD and survival in non-small-cell lung cancer patients treated with cisplatin combination chemotherapy. *Lung Cancer.* 2004;44(3):311-316. doi:10.1016/j.lungcan.2003.11.019

4. Musolino A, Naldi N, Bortesi B, et al. Immunoglobulin G Fragment C Receptor Polymorphisms and Clinical Efficacy of Trastuzumab-Based Therapy in Patients With HER-2/neu-Positive Metastatic Breast Cancer. 2008. doi:10.1200/JCO.2007.14.8957

5. Mandelker D, Zhang L. The emerging significance of secondary germline testing in cancer genomics. *J Pathol.* 2018;244(5):610-615. doi:10.1002/path.5031

6. Laskin J, Jones S, Aparicio S, et al. Lessons learned from the application of whole-genome analysis to the treatment of patients with advanced cancers. *Cold Spring Harb Mol case Stud.* 2015;1(1):a000570. doi:10.1101/mcs.a000570

7. Zhao EY, Shen Y, Pleasance E, et al. Homologous Recombination Deficiency and Platinum-Based Therapy Outcomes in Advanced Breast Cancer. *Clin Cancer Res.* 2017;23(24):7521-7530. doi:10.1158/1078-0432.CCR-17-1941

8. Wong H-L, Yang KC, Shen Y, et al. Molecular characterization of metastatic pancreatic neuroendocrine tumors (PNETs) using whole-genome and transcriptome sequencing. *Cold Spring Harb Mol case Stud.* 2018;4(1). doi:10.1101/mcs.a002329

9. Thibodeau ML, Zhao EY, Reisle C, et al. Base excision repair deficiency signatures implicate germline and somatic MUTYH aberrations in pancreatic ductal adenocarcinoma
and breast cancer oncogenesis. *Cold Spring Harb Mol case Stud.* 2019;5(2):a003681. doi:10.1101/mcs.a003681

10. Weinstein JN, Collisson EA, Mills GB, et al. The cancer genome atlas pan-cancer analysis project. *Nat Genet.* 2013;45(10):1113-1120. doi:10.1038/ng.2764

11. Hudson TJ, Anderson W, Aretz A, et al. International network of cancer genome projects. *Nature.* 2010;464(7291):993-998. doi:10.1038/nature08987

12. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med.* 2015;17(5):405-423. doi:10.1038/gim.2015.30

13. Regier DA, Peacock SJ, Pataky R, et al. Societal preferences for the return of incidental findings from clinical genomic sequencing: a discrete-choice experiment. *Can Med Assoc J.* 2015;187(6):E190-E197. doi:10.1503/cmaj.140697

14. Rahman N. Realizing the promise of cancer predisposition genes. *Nature.* 2014;505(7483):302-308. doi:10.1038/nature12981

15. Huang K, Mashl RJ, Wu Y, et al. Pathogenic Germline Variants in 10,389 Adult Cancers. *Cell.* 2018;173(2):355-370.e14. doi:10.1016/j.cell.2018.03.039

16. Landrum MJ, Lee JM, Benson M, et al. ClinVar: improving access to variant interpretations and supporting evidence. *Nucleic Acids Res.* 2018;46(D1):D1062-D1067. doi:10.1093/nar/gkx1153

17. Li Q, Wang K. InterVar: Clinical Interpretation of Genetic Variants by the 2015 ACMG-AMP Guidelines. *Am J Hum Genet.* 2017;100(2):267-280. doi:10.1016/j.ajhg.2017.01.004

18. Statistics Canada. British Columbia [Province] and Canada [Country] (table). Census Profile. Statistics Canada Catalogue no. 98-316-X2016001. https://www12.statcan.gc.ca/census-recensement/2016/dp-pd/prof/index.cfm?Lang=E. Published 2017. Accessed July 14, 2019.
19. Cummings BB, Marshall JL, Tukiainen T, et al. Improving genetic diagnosis in Mendelian disease with transcriptome sequencing Genotype-Tissue Expression Consortium. 2017. http://stm.sciencemag.org.ezproxy.library.ubc.ca/content/scitransmed/9/386/eaal5209.full.pdf. Accessed September 9, 2017.

20. Walsh MF, Ritter DI, Kesserwan C, et al. Integrating somatic variant data and biomarkers for germline variant classification in cancer predisposition genes. *Hum Mutat.* 2018;39(11):1542-1552. doi:10.1002/humu.23640

21. Middlebrooks CD, Banday AR, Matsuda K, et al. Association of germline variants in the APOBEC3 region with cancer risk and enrichment with APOBEC-signature mutations in tumors. *Nat Genet.* 2016;48(11):1330-1338. doi:10.1038/ng.3670

22. Carter H, Marty R, Hofree M, et al. Interaction Landscape of Inherited Polymorphisms with Somatic Events in Cancer. *Cancer Discov.* 2017;7(4):410-423. doi:10.1158/2159-8290.CD-16-1045

23. Lim YW, Chen-Harris H, Mayba O, et al. Germline genetic polymorphisms influence tumor gene expression and immune cell infiltration. *Proc Natl Acad Sci U S A.* 2018;115(50):E11701-E11710. doi:10.1073/pnas.1804506115

24. Lin P-C, Yeh Y-M, Wu P-Y, Hsu K-F, Chang J-Y, Shen M-R. Germline susceptibility variants impact clinical outcome and therapeutic strategies for stage III colorectal cancer. *Sci Rep.* 2019;9(1):3931. doi:10.1038/s41598-019-40571-0

25. Green RC, Berg JS, Grody WW, et al. ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing. *Genet Med.* 2013;15(7):565-574. doi:10.1038/gim.2013.73

26. Kalia SS, Adelman K, Bale SJ, et al. Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2016 update (ACMG SF v2.0): a policy statement of the American College of Medical Genetics and Genomics. *Genet Med.* 2017;19(2):249-255. doi:10.1038/gim.2016.190
27. Chowell D, Morris LGT, Grigg CM, et al. Patient HLA class I genotype influences cancer response to checkpoint blockade immunotherapy. *Science*. 2018;359(6375):582-587. doi:10.1126/science.aao4572

28. Whirl-Carrillo M, McDonagh EM, Hebert JM, et al. Pharmacogenomics knowledge for personalized medicine. *Clin Pharmacol Ther*. 2012;92(4):414-417. doi:10.1038/clpt.2012.96

29. Iyer L, Das S, Janisch L, et al. UGT1A1*28 polymorphism as a determinant of irinotecan disposition and toxicity. *Pharmacogenomics J*. 2002;2(1):43-47. http://www.ncbi.nlm.nih.gov/pubmed/11990381. Accessed July 15, 2019.

30. Ye B, Valdivia CR, Ackerman MJ, Makielski JC. A common human *SCN5A* polymorphism modifies expression of an arrhythmia causing mutation. *Physiol Genomics*. 2003;12(3):187-193. doi:10.1152/physiolgenomics.00117.2002

31. Matsumura H, Nakano Y, Ochi H, et al. H558R, a common *SCN5A* polymorphism, modifies the clinical phenotype of Brugada syndrome by modulating DNA methylation of *SCN5A* promoters. *J Biomed Sci*. 2017;24(1):91. doi:10.1186/s12929-017-0397-x

32. Strevel EL, Ing DJ, Siu LL. Molecularly targeted oncology therapeutics and prolongation of the QT interval. *J Clin Oncol*. 2007;25(22):3362-3371. doi:10.1200/JCO.2006.09.6925

33. Kloth JSL, Pagani A, Verboom MC, et al. Incidence and relevance of QTc-interval prolongation caused by tyrosine kinase inhibitors. *Br J Cancer*. 2015;112(6):1011-1016. doi:10.1038/bjc.2015.82

34. Futreal PA, Coin L, Marshall M, et al. A census of human cancer genes. *Nat Rev Cancer*. 2004;4(3):177-183. doi:10.1038/nrc1299

35. Tate JG, Bamford S, Jubb HC, et al. COSMIC: the Catalogue Of Somatic Mutations In Cancer. *Nucleic Acids Res*. 2019;47(D1):D941-D947. doi:10.1093/nar/gky1015

36. Schrader KA, Cheng DT, Joseph V, et al. Germline Variants in Targeted Tumor Sequencing Using Matched Normal DNA. *JAMA Oncol*. 2016;2(1):104-111.
37. Mandelker D, Zhang L, Kemel Y, et al. Mutation Detection in Patients With Advanced Cancer by Universal Sequencing of Cancer-Related Genes in Tumor and Normal DNA vs Guideline-Based Germline Testing. *JAMA*. 2017;318(9):825.
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Figure Legends

Figure 1. Approach for germline variant calling, annotation and filtering in tumour-normal whole-genome sequencing. In the POG program, parallel pipelines are implemented for the analysis of small variants and structural variants (SVs). Low-complexity regions, strong GC bias and repetitive elements limit the accuracy of SV calling through short-read (50-300 bp) sequencing. Consequently, complementary read depth-, flanking read-, split read- and contig-based computational approaches are incorporated to increase the sensitivity of SV detection. Germline variants with known or putative clinical significance are prioritized by clinical annotation, functional effect prediction and population frequency in 98 cancer predisposition genes. All candidate variants are reviewed in a genome browser to flag possible technical artifacts, and this information is included in an integrated germline report along with relevant tumour data.

Figure 2. Standard procedure for the review, reporting and clinical translation of germline variants in the Personalized OncoGenomics program. Given an integrated germline analysis report, a Clinical Molecular Geneticist at the Cancer Genetics and Genomics Laboratory (CGL) curates all germline variants with known or potential clinical significance in cancer predisposition genes undergoing prioritized review. The Ethics and Germline Working Group determine by consensus final recommendations for variant reporting and whether a referral to the Hereditary Cancer Program (HCP) should be made for patient counseling and clinical genetic testing. In the absence of functional evidence supporting pathogenicity, variants of uncertain significance are disclosed only when the patient's personal or family history is suggestive of hereditary cancer susceptibility. Clinically actionable variants that occur in areas of the reference genome flagged as low-complexity or repetitive regions will be validated at CGL prior to return of results.
If false positive variants are identified, an updated bioinformatics pipeline is implemented to flag these variants in future cases.

**Figure 3. Extending the clinical significance of integrated molecular analysis of tumour and normal tissues beyond cancer susceptibility.** SSM: simple somatic mutations; CNV: copy number variants; LOH: loss-of-heterozygosity; SSV: somatic structural variants.
Table 1. Framework for the return of germline findings in precision oncology.

| Tier                  | Clinical indication                                      | Recommended categories for consideration                                                                 | Examples                                                                                     | PEWG recommendations for disclosure† |
|-----------------------|----------------------------------------------------------|-----------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------|----------------------------------------|
| **Primary**           | Cancer susceptibility                                    | Known or novel pathogenic and likely pathogenic variants associated with moderate- to high-penetrance cancer susceptibility* | CHEK2 c.1100delC (p.Thr367Metfs)                                                            | Return to patient with referral to HCP† |
|                       |                                                          | Known or novel pathogenic and likely pathogenic variants associated with low-penetrance cancer susceptibility* | CHEK2 c.470T>C (p.Ile157Thr)                                                               | Return to patient with referral to HCP on a case-by-case basis |
|                       |                                                          | Autosomal or X-linked carrier status for known or novel pathogenic and likely pathogenic variants associated with low-, moderate- or high-penetrance cancer susceptibility* | ERCC5 c.529-1G>A                                                                            | Return to patient with referral to HCP on a case-by-case basis |
|                       |                                                          | VUS in cancer predisposition genes with clinical and molecular indications for pathogenicity                | MUTYH c.996G>A (p.Ser332Ser)                                                               | Return to patient with referral to HCP on a case-by-case basis |
|                       | Disease pathogenesis, prognosis or treatment            | Known or novel variants in cancer predisposition genes understood to contribute to tumour phenotype and evolution | Biallelic loss through combined germline and somatic aberrations: NTHL1 carrier status MUTYH carrier status | Return to patient with referral to HCP on a case-by-case basis and if clinical significance has been established |
|                       |                                                          | Deleterious variants in known or novel cancer-related genes                                               | Deletion polymorphisms in APOBEC3A and APOBEC3B                                             | Return to patient if clinical significance has been established |
|                       |                                                          | Pharmacogenomic variants with established or potential cancer treatment associations                      | UGT1A1*28                                                                                  | Return to patient if clinical significance has been established |
|                       |                                                          | Alleles associated with immune response                                                                    | HLA class I genotypes                                                                       | Return to patient if clinical significance has been established |
|                       |                                                          | Variants in gene(s) reviewed at the request of the case clinician given patient consent                   | SCN5A c.1673A>G (p.His558Arg)                                                              | Return to patient if clinical significance has been established |
| **Secondary**         | Mendelian disease risk or carrier status                | Deleterious variants in genes without known implications for cancer prevention, cancer screening or treatment | Pathogenic and likely pathogenic variants in Mendelian disease genes defined by the ACMG†     | Return to patient with referral to Medical Genetics program if patient preference for non-cancer related information is indicated in consent |

*Implications for cancer susceptibility should be considered in the context of variant zygosity and the typical mode of inheritance observed for a given gene. In particular, this includes variants in autosomal recessive genes or in X-linked genes conferring recessive disease risk that may have differing indications for XY males, XX females and XO females.

†ACMG, American College of Medical Genetics and Genomics; HCP: Hereditary Cancer Program; PEWG, POG Ethics and Germline Working Group
Establishing a framework for the clinical translation of germline findings in precision oncology

Katherine Dixon, MSc, Sean Young, PhD, Yaoqing Shen, PhD, My Linh Thibodeau, MD, MSc, Alexandra Fok, MSc, Erin Pleasance, PhD, Eric Zhao, PhD, Martin Jones, PhD, Geraldine Aubert, PhD, Linlea Armstrong, MD, Alice Virani, PhD, Dean Regier, PhD, Karen Gelmon, MD, Dan Renouf, MD, Stephen Chia, MD, Ian Bosdet, PhD, S. Rod Rassekh, MD, Rebecca J. Deyell, MD, Stephen Yip, MD, PhD, Ana Fisic, BScN, Emma Titmuss, MSc, Shirin Abadi, PharmD, MBA, Steven J.M. Jones, PhD, Sophie Sun, MD, Aly Karsan, MD, Marco Marra*, PhD, Janessa Laskin*, MD, Howard Lim*, MD, Kasmintan A. Schrader*, MBBS, PhD

*Co-senior authors

Affiliations of authors: Department of Medical Genetics, University of British Columbia, Vancouver, British Columbia, Canada (KD, MLT, LA, AV, SJMJ, MM, KAS); Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, British Columbia, Canada (SY, IB, SY); Canada’s Michael Smith Genome Sciences Centre, BC Cancer, Vancouver, British Columbia, Canada (YS, AF, EP, EZ, MJ, ET, SJMJ, AK, MM); Terry Fox Laboratory, BC Cancer Research Centre, Vancouver, British Columbia, Canada (GA); Provincial Medical Genetics Program, Children’s & Women’s Health Centre of British Columbia, Vancouver, British Columbia, Canada (LA); Ethics Service, Provincial Health Service of Authority of BC, Vancouver, British Columbia, Canada (AV); Canadian Centre for Applied Research in Cancer Control, Cancer Control Research, BC Cancer, Vancouver, British Columbia, Canada (DR); School of Population and Public Health, University of British Columbia, Vancouver, British Columbia, Canada (DR); Division of Medical Oncology, BC Cancer, Vancouver, British Columbia, Canada (KG, DR, SC, AF, SS, JL, HL); Cancer Genetics and Genomics Laboratory, BC Cancer, Vancouver, British Columbia, Canada (SY, IB); BC
Children's Hospital Research Institute, Vancouver, British Columbia, Canada (SRR, RJD);
Division of Hematology/Oncology and BMT, Department of Pediatrics, University of British Columbia, Vancouver, British Columbia, Canada (SRR, RJD); Department of Pharmacy, BC Cancer, Vancouver, British Columbia, Canada (SA); Hereditary Cancer Program, BC Cancer, Vancouver, British Columbia, Canada (SS, KAS); and Department of Molecular Oncology, BC Cancer, Vancouver, British Columbia, Canada (KAS).

**Corresponding author:**

Kasmintan Schrader

Hereditary Cancer Program

BC Cancer

600 West 10th Avenue

Vancouver, British Columbia, V5Z 4E6

Telephone: 604-877-6000 ext. 672198

Email: ischrader@bccancer.bc.ca
Abstract

Inherited genetic variation has important implications for cancer screening, early diagnosis, and disease prognosis. A role for germline variation has also been described in shaping the molecular landscape, immune response, microenvironment, and treatment response of individual tumours. However, there is a lack of consensus on the handling and analysis of germline information that extends beyond known or suspected cancer susceptibility in large-scale cancer genomics initiatives. As part of BC Cancer’s Personalized OncoGenomics program, we performed whole-genome and transcriptome sequencing in paired tumour and normal tissues from advanced cancer patients to characterize the molecular tumour landscape and identify putative targets for therapy. Overall, our experience supports a multi-disciplinary and integrative approach to germline data management. This includes a need for broader definitions and standardized recommendations regarding primary and secondary germline findings in precision oncology. Here we propose a framework for identifying, evaluating, and returning germline variants of potential clinical significance that may have indications for health management beyond cancer risk reduction or prevention in patients and their families.
Characterizing hereditary genetic variation in high- and moderate-penetration cancer predisposition genes may have implications for cascade carrier testing, cancer risk-reduction and screening interventions in individuals at risk of having inherited a causal germline variant. Individual variability in disease prognosis and treatment response also occurs within the context of heterogeneous genetic backgrounds, including rare variants associated with cancer susceptibility and common polymorphisms in other cancer-related genes. However, standards for the clinical translation of genetic information relevant to cancer susceptibility, pathogenesis, prognosis and treatment, as well as secondary or incidental genetic information unrelated to cancer, is inconsistent across cancer genomics programs. This may result from varying regional policies regarding the return of research results, clinician preference or genetic literacy.

The Personalized OncoGenomics (POG) program is a precision medicine initiative in British Columbia (BC), Canada that was established to identify clinically actionable molecular events in adult metastatic cancer patients and pediatric patients with poor prognosis cancers (NCT02155621). Whole-genome sequencing (WGS) and RNA sequencing (RNA-seq) of fresh frozen tumour biopsies performed with WGS of paired normal tissues has helped identify somatic alterations and inherited genetic variants that shape tumour progression. This and similar projects, such as the Cancer Genome Atlas and International Cancer Genome Consortium, have provided important resources for understanding the morbid human genome. At the same time, the rate of data production has far outpaced the development of evidence-based guidelines for managing germline findings. Here we advocate for a broader understanding of what defines primary germline findings in oncology and propose a framework for identifying, evaluating and reporting research germline findings within the clinical infrastructure of a publicly-funded provincial health authority.

Early years of the POG program
Since its establishment in 2012, the POG program has continued to develop effective, collaborative and transparent data management and reporting strategies\(^6\). These have allowed the accommodation of advancements in technology, computational pipelines, therapeutic developments, and biological and clinical knowledge. Before standard recommendations for variant interpretation were published by the American College of Medical Genetics and Genomics (ACMG) and Association for Molecular Pathology (AMP) in 2015, germline data was assessed on an ad hoc basis to identify high-penetrance variants relevant to inherited cancer susceptibility or treatment\(^12\). Any research findings were discussed with treating oncologists as part of the POG Tumour Board, but this approach was not broadly or consistently actionable in a maturing program. This was complicated by conflicting interpretations of variant pathogenicity and/or differing opinions regarding the return of results, indicating the need for a standardized procedure for germline assessment. Given the ethical challenges of germline data analysis within the oncology setting, the POG Ethics and Germline Working Group was created in 2014. Using autonomy, beneficence, non-maleficence, and justice as guiding principles, the mandate of the Ethics and Germline Working Group includes addressing ongoing issues related to the management and clinical translation of germline findings. The group meets monthly and consists of a multi-disciplinary team of oncologists, medical and molecular geneticists, pathologists, bioinformaticians and other scientists, an ethicist, and lawyers.

**Transparency and standardization of informed consent**

This POG program is approved by the University of British Columbia Research Ethics Committee, and written informed consent or assent is obtained for all patients involved in this research. The Ethics and Germline Working Group reached a consensus that informed consent or assent should include by default the reporting of any clinically actionable cancer-related germline genetic information for both adults and children with cancer. This mandate keeps with the primary goal of the POG program: to characterize the complete genomic architecture of an
individual cancer while maintaining data transparency and allowing opportunities for clinical translation. Cancer-related germline findings of clinical significance are currently returned to the research participant, designee, or next-of-kin by their oncologist, and a referral is made to BC Cancer’s provincial Hereditary Cancer Program (HCP) for genetic counseling and clinical variant confirmation. For both tumour-only and tumour-normal sequencing, an appropriate consent procedure includes a detailed review of family history and pre-test counseling regarding the potential risks and benefits of germline findings. This is critical for ensuring ethical justification of studies involving the analysis of hereditary genetic variation.

An opt-in procedure for the return of germline findings of clinical significance unrelated to cancer has been adopted by the POG program, consistent with recommendations based on patient and public preference studies\textsuperscript{13}. While these findings are not routinely sought in cancer genomics analyses, 97.9\% of participants enrolled in the program between 2012 and July 2019 (\(n = 993\)) opted for the return of incidental germline findings unrelated to cancer. These observations suggest that the level of potential risk in learning about inherited genetic variation, including risks related to cancer susceptibility, disease carrier status, or paternity, is acceptable to patients and has not resulted in a decline in participation in the program. The potential for learning about genetic cancer predisposition in particular should be an essential part of the patient’s education and communicated to them at the time of diagnosis.

**Germline variant curation, validation and return**

Pathogenic and likely pathogenic germline variants in moderate- to high-penetrance cancer predisposition genes underlie 5-10\% of all cancers, with a prevalence of up to 20\% in certain cancer types\textsuperscript{14,15}. In collaboration with HCP and the Cancer Genetics and Genomics Laboratory (CGL), the Ethics and Germline Working Group developed standard procedures for germline variant prioritization and evaluation (Figures 1 & 2). Genome-wide variant calling is performed in parallel pipelines for small variants, including single nucleotide variants and small
insertions and deletions, and structural variants (SVs), including copy number variants and balanced genomic rearrangements (Figure 1). SV calling through short-read-based next-generation sequencing in particular has inherent technical and computational challenges due to low-complexity sequences in the human reference genome. Therefore, using multiple computational tools that employ complementary variant calling methods is preferred in order to improve the sensitivity of SV detection. Following gene- and function-based filtering, candidate germline variants are manually reviewed in a genome browser to flag putative technical or sequence artifacts prior to clinical review.

To allow rapid return of results for variants with established clinical actionability, a tiered list of cancer susceptibility genes is analyzed according to the following guidelines. Known pathogenic and likely pathogenic variants in curated variant databases, such as ClinVar and local CGL database, and novel predicted loss-of-function variants (e.g. deletion, frameshift, nonsense, canonical splice site and stop loss) are prioritized for curation in known cancer predisposition genes (Table S1). For several genes associated with high-penetrance syndromes, variants of uncertain significance (VUS) and rare variants in coding and splice regions are further prioritized for review. Variant classification is performed by a clinical molecular geneticist from CGL and certified by the Canadian College of Medical Genetics and Genomics or ACMG according to updated guidelines from the ACMG, AMP and Clinical Genome Resource (ClinGen). This approach integrates clinical expertise and laboratory protocols in variant classification and confirmatory genetic testing, respectively, allowing seamless translation for patients referred to HCP (Figure 2). Final recommendations for return of information and referral for genetic counselling and clinical testing are made based on consensus among the Ethics and Germline Working Group. In exceptional cases, germline variants may be subject to expedited review by a core expert panel, including a clinical molecular geneticist, medical geneticist, and oncologist, to allow immediate return of information and clinical referral (Table S2). Clinical genetics expertise and group consultation are thus
integral parts of germline assessment in the POG program to ensure reliability, consistency and transparency.

Evolving variant curation guidelines indicate the need for dynamic computational pipelines that integrate updated variant information from population and clinical databases and encourage efficient retrospective analysis\textsuperscript{16,17}. To aid in large-scale variant identification and classification, special consideration should also be given to variants with founder effects, specific modes of disease inheritance and variants with low to moderate cancer risk. Founder mutations in common cancer predisposition genes such as \textit{BRCA1}, \textit{BRCA2}, \textit{CHEK2}, and \textit{MUTYH} should be excluded from global allele frequency thresholds used in automated variant filtering. For example, over 1\% of patients in the POG program are carriers for pathogenic \textit{MUTYH} variants, reflecting a strong representation of individuals of East and Southeast Asian descent in BC\textsuperscript{9,18}. In such cases, we recommend developing highly curated internal databases with known pathogenic and likely pathogenic germline variants to reduce the incidence of false negative findings. Furthermore, variants in Mendelian disease genes underlying cancer predisposition syndromes inherited in autosomal recessive or X-linked recessive patterns, such as \textit{MUTYH}-associated polyposis (MAP) and dyskeratosis congenita, respectively, also require exceptional committee review. The position of the Ethics and Germline Working Group is to evaluate the risks and benefits of returning carrier status for autosomal recessive cancer susceptibility genes on a gene- and case-specific basis. Finally, identification of low- to moderate-penetrance cancer predisposition variants may present an additional challenge due to limited evidence-based guidelines regarding effective clinical management and cancer risk reduction strategies. Personal and family medical history should be carefully considered in these cases to determine the appropriateness of variant disclosure for cancer susceptibility.

For individuals with phenotypic indications of high-penetrance cancer predisposition syndromes and uninformative clinical genetic testing, evaluation of tumour WGS and RNA-seq may improve genetic diagnosis through the resolution of potential splicing variants, non-coding
variants in regulatory regions, or structural variants\(^9,19\). Tumour data may also inform possible roles for VUS or autosomal recessive gene variants in pathogenesis, recently demonstrated in a patient with biallelic variants in \(MUTYH\) (p.Gly286Glu and p.Ser346Ser) with tumour evidence supporting global deficiency in base excision repair\(^7\)–\(^9\). The specific types of molecular data that could be considered in whole-genome and transcriptome studies include genome-wide mutation burden, simple somatic mutations and mutational signatures, copy number alterations, loss-of-heterozygosity, structural variants and structural variant signatures, expression outliers, expression-based classifications, and alternative splicing (Table S3). However, recommendations for incorporating tumour data into variant classification are still evolving, and recent guidelines recommend cautious variant interpretation based on this evidence alone without corresponding data from \textit{in vitro} or \textit{in vivo} functional studies\(^20\). In the POG program, VUS are not reclassified on the basis of tumour data alone without ClinVar classifications or published phenotypic and functional evidence supporting pathogenicity. For patients with relevant personal or family history, VUS may be returned to the treating clinician with a recommendation for referral to HCP if the individual is eligible for publicly-funded index genetic testing. The Ethics and Germline Working Group does not support clinical decision-making based on the presence of VUS alone, and stresses the importance of consultation with core members of a clinical genetics team to ensure appropriate clinical follow-up when indicated.

**Implications for germline genetic variation beyond cancer susceptibility**

Germline variation and somatic alterations both have potential clinical significance in precision oncology, and integrated analysis of tumour-normal sequencing may identify roles for germline variation that extend beyond cancer susceptibility. Therefore, our framework for germline data management defines primary germline findings as any variants with relevance to tumour biology or treatment. This includes variants with implications for estimating cancer risk, determining the landscape of somatic alterations or tumour evolution, modifying the immune
response and microenvironment, and predicting overall response or adverse reactions to treatment (Figure 3). This broad definition is based on findings from large tumour sequencing projects that have allowed functional characterization of inherited genetic variation other than rare coding variants in known disease genes. These include a number of recent studies that have identified roles for common and non-coding variants in tumourigenesis that may ultimately guide treatment interventions or development of targeted therapeutics\textsuperscript{21–24}. Identifying germline variation with potential tumour relevance is thus an important part of characterizing the complex molecular architecture of individual tumours and may have immediate or future clinical implications.

**Framework for the clinical translation of germline findings**

Based on our experience during seven years of the POG program, we developed an integrated schema for the management of germline genetic information. We propose a non-mutually exclusive categorical framework for evaluating clinically actionable germline variants that aims to identify inherited cancer susceptibility, characterize treatment indications, and provide opportunities for the discovery of novel genetic associations (Table 1). This includes pathogenic and likely pathogenic variants in known cancer predisposition genes with defined cancer risk estimates and screening recommendations, and may include VUS in cases where the patient's phenotype supports pathogenicity. In the POG program, known and novel pathogenic and likely pathogenic variants in known cancer predisposition genes are reviewed by the Ethics and Germline Working Group, and these are then disclosed to the Tumour Board and case oncologist with a general recommendation for return to the patient with a referral to HCP for follow-up. Secondary germline findings in the 59 genes defined by the ACMG should consistently be returned to patients if informed consent for return of such findings was given at the time of study enrolment\textsuperscript{25,26}.
With an inclusive definition of primary germline findings in the context of cancer genomics, pharmacogenomic variants with known drug associations, polymorphic alleles with putative immune response associations, variants in genes with potential tumour relevance based on histological or molecular characteristics, and other variants with potential clinical relevance as requested by the case oncologist could also be analyzed. The Ethics and Germline Working Group supports routine reporting of germline variants predictive of immune response, such as HLA class I genotypes, but recommends caution in reporting pharmacogenomic variants with limited or conflicting scientific evidence that may prevent use of important supportive medications\(^{27}\). Clinicians and scientists should refer to public curated databases such as PharmGKB for updated variant reviews and clinical guidelines\(^{28}\). In practice, a curated variant database relevant for chemotherapy and other cancer therapy with established pharmacogenomic associations, such as gastrointestinal and bone marrow toxicity in carriers of the \textit{UGT1A1*28} polymorphism, should be prioritized during routine germline analysis unless otherwise requested by the case clinician\(^{29}\).

Pharmacogenomic variants will be an important aspect of cancer treatment in the era of precision medicine. The potential benefits of profiling pharmacogenomic variants as part of the POG program was demonstrated in a patient with a gastrointestinal stromal tumour (GIST) and prolonged QT interval during a high-dose course of imatinib. Further genomic profiling was performed at the request of the case clinician, and a common polymorphism in \textit{SCN5A} (H558R) that has been previously reported as a genetic modifier in cardiac arrhythmia syndromes was identified\(^{30,31}\). Metabolic and cardiovascular gene variants that are known to modulate drug metabolism or mediate adverse events in response to treatment have immediate clinical utility in treatment selection. Thus prior knowledge of variants that can impact treatment choice may provide indications for or against the use of certain drugs. In the case described above, this may have indicated an avoidance of specific tyrosine kinase inhibitors associated with prolonged QT intervals\(^{32,33}\).
Novel relationships between genetic alterations and histological or molecular phenotypes could also be investigated through an agnostic analysis of germline variation. Mutation and expression comparisons using public datasets is an invaluable tool for characterizing the individual tumour genome and in validating putative molecular associations. However, these must be interpreted cautiously given expected differences between sample handling and preparation protocols, sequencing chemistries and platforms, and computational pipelines. With a secondary goal of discovery, deleterious germline variants in known cancer genes defined in the Catalog of Somatic Mutations in Cancer (COSMIC) Cancer Gene Census or genes in other disease-related pathways could be prioritized but not limiting when investigating novel associations with outlier tumour phenotypes (Table S4)\textsuperscript{34,35}. It should be noted that germline findings with unconfirmed implications for disease pathogenesis, prognosis, or treatment are purely the result of research and, due to their uncertain nature, are strongly discouraged from being returned to the patient unless indicated by the Tumour Board for treatment indications. If germline findings are determined to be of clinical importance, notice should be given to the case clinician to ensure banking of a clinical DNA sample and patient and/or family referral for genetic counselling and variant confirmation. Disclosure of germline variants to the POG Tumour Board and case clinician is encouraged in cases with established implications for clinical management, including for cancer risk reduction, screening, or treatment indications. In the future, larger studies and agnostic analyses may uncover additional roles for these variants in disease pathogenesis that may be predictive or prognostic of overall survival and treatment outcomes.

**Concluding remarks**

Hereditary cancer susceptibility is commonly observed in unselected cohorts of cancer patients that do not meet current clinical testing guidelines\textsuperscript{36,37}. In precision oncology, the utility of exploring inherited genetic variation is achieved through the implementation of cancer
prevention and screening strategies and by the use of targeted therapies. However, personnel and financial resources in jurisdictions with universal health care may be a growing barrier to service accessibility as genetic testing becomes more common and additional germline variants with clinical significance are discovered. These issues were strongly considered by the Ethics and Germline Working Group in assessing the benefits of disclosure for certain variants, but these guidelines must be re-evaluated moving forward to help inform cost-effective patient-centred care. Based on our experience in the POG program, genome-wide analysis of inherited genetic variation should allow for the examination of normal and disease-causing variation that may affect tumour evolution, response to therapy, immune function, predict adverse events, and/or be relevant to non-cancer disease risk that may be meaningful to the patient and their care.

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References

1. Copson ER, Maishman TC, Tapper WJ, et al. Articles Germline BRCA mutation and outcome in young-onset breast cancer (POSH): a prospective cohort study. Lancet Oncol. 2018;19:169-180. doi:10.1016/S1470-2045(17)30891-4

2. Robson M, Im S-A, Senkus E, et al. Olaparib for Metastatic Breast Cancer in Patients with a Germline BRCA Mutation. N Engl J Med. 2017;377(6):523-533. doi:10.1056/NEJMoa1706450

3. Ryu JS, Hong YC, Han HS, et al. Association between polymorphisms of ERCC1 and XPD and survival in non-small-cell lung cancer patients treated with cisplatin combination chemotherapy. Lung Cancer. 2004;44(3):311-316. doi:10.1016/j.lungcan.2003.11.019

4. Musolino A, Naldi N, Bortesi B, et al. Immunoglobulin G Fragment C Receptor Polymorphisms and Clinical Efficacy of Trastuzumab-Based Therapy in Patients With HER-2/neu-Positive Metastatic Breast Cancer. 2008. doi:10.1200/JCO.2007.14.8957

5. Mandelker D, Zhang L. The emerging significance of secondary germline testing in cancer genomics. J Pathol. 2018;244(5):610-615. doi:10.1002/path.5031
6. Laskin J, Jones S, Aparicio S, et al. Lessons learned from the application of whole-genome analysis to the treatment of patients with advanced cancers. *Cold Spring Harb Mol case Stud.* 2015;1(1):a000570. doi:10.1101/mcs.a000570

7. Zhao EY, Shen Y, Pleasance E, et al. Homologous Recombination Deficiency and Platinum-Based Therapy Outcomes in Advanced Breast Cancer. *Clin Cancer Res.* 2017;23(24):7521-7530. doi:10.1158/1078-0432.CCR-17-1941

8. Wong H-L, Yang KC, Shen Y, et al. Molecular characterization of metastatic pancreatic neuroendocrine tumors (PNETs) using whole-genome and transcriptome sequencing. *Cold Spring Harb Mol case Stud.* 2018;4(1). doi:10.1101/mcs.a002329

9. Thibodeau ML, Zhao EY, Reisle C, et al. Base excision repair deficiency signatures implicate germline and somatic MUTYH aberrations in pancreatic ductal adenocarcinoma and breast cancer oncogenesis. *Cold Spring Harb Mol case Stud.* 2019;5(2):a003681. doi:10.1101/mcs.a003681

10. Weinstein JN, Collisson EA, Mills GB, et al. The cancer genome atlas pan-cancer analysis project. *Nat Genet.* 2013;45(10):1113-1120. doi:10.1038/ng.2764

11. Hudson TJ, Anderson W, Aretz A, et al. International network of cancer genome projects. *Nature.* 2010;464(7291):993-998. doi:10.1038/nature08987

12. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med.* 2015;17(5):405-423. doi:10.1038/gim.2015.30

13. Regier DA, Peacock SJ, Pataky R, et al. Societal preferences for the return of incidental findings from clinical genomic sequencing: a discrete-choice experiment. *Can Med Assoc J.* 2015;187(6):E190-E197. doi:10.1503/cmaj.140697

14. Rahman N. Realizing the promise of cancer predisposition genes. *Nature.* 2014;505(7483):302-308. doi:10.1038/nature12981
15. Huang K, Mashl RJ, Wu Y, et al. Pathogenic Germline Variants in 10,389 Adult Cancers. *Cell*. 2018;173(2):355-370.e14. doi:10.1016/j.cell.2018.03.039

16. Landrum MJ, Lee JM, Benson M, et al. ClinVar: improving access to variant interpretations and supporting evidence. *Nucleic Acids Res*. 2018;46(D1):D1062-D1067. doi:10.1093/nar/gkx1153

17. Li Q, Wang K. InterVar: Clinical Interpretation of Genetic Variants by the 2015 ACMG-AMP Guidelines. *Am J Hum Genet*. 2017;100(2):267-280. doi:10.1016/j.ajhg.2017.01.004

18. Statistics Canada. British Columbia [Province] and Canada [Country] (table). Census Profile. Statistics Canada Catalogue no. 98-316-X2016001. https://www12.statcan.gc.ca/census-recensement/2016/dp-pd/prof/index.cfm?Lang=E. Published 2017. Accessed July 14, 2019.

19. Cummings BB, Marshall JL, Tukiainen T, et al. Improving genetic diagnosis in Mendelian disease with transcriptome sequencing Genotype-Tissue Expression Consortium. 2017. http://stm.sciencemag.org.ezproxy.library.ubc.ca/content/scitransmed/9/386/eaal5209.full.pdf. Accessed September 9, 2017.

20. Walsh MF, Ritter DI, Kesserwan C, et al. Integrating somatic variant data and biomarkers for germline variant classification in cancer predisposition genes. *Hum Mutat*. 2018;39(11):1542-1552. doi:10.1002/humu.23640

21. Middlebrooks CD, Banday AR, Matsuda K, et al. Association of germline variants in the APOBEC3 region with cancer risk and enrichment with APOBEC-signature mutations in tumors. *Nat Genet*. 2016;48(11):1330-1338. doi:10.1038/ng.3670

22. Carter H, Marty R, Hofree M, et al. Interaction Landscape of Inherited Polymorphisms with Somatic Events in Cancer. *Cancer Discov*. 2017;7(4):410-423. doi:10.1158/2159-8290.CD-16-1045

23. Lim YW, Chen-Harris H, Mayba O, et al. Germline genetic polymorphisms influence tumor gene expression and immune cell infiltration. *Proc Natl Acad Sci U S A*. 

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2018;115(50):E11701-E11710. doi:10.1073/pnas.1804506115

24. Lin P-C, Yeh Y-M, Wu P-Y, Hsu K-F, Chang J-Y, Shen M-R. Germline susceptibility variants impact clinical outcome and therapeutic strategies for stage III colorectal cancer. 
*Sci Rep.* 2019;9(1):3931. doi:10.1038/s41598-019-40571-0

25. Green RC, Berg JS, Grody WW, et al. ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing. *Genet Med.* 2013;15(7):565-574. doi:10.1038/gim.2013.73

26. Kalia SS, Adelman K, Bale SJ, et al. Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2016 update (ACMG SF v2.0): a policy statement of the American College of Medical Genetics and Genomics. *Genet Med.* 2017;19(2):249-255. doi:10.1038/gim.2016.190

27. Chowell D, Morris LGT, Grigg CM, et al. Patient HLA class I genotype influences cancer response to checkpoint blockade immunotherapy. *Science.* 2018;359(6375):582-587. doi:10.1126/science.aao4572

28. Whirl-Carrillo M, McDonagh EM, Hebert JM, et al. Pharmacogenomics knowledge for personalized medicine. *Clin Pharmacol Ther.* 2012;92(4):414-417. doi:10.1038/clpt.2012.96

29. Iyer L, Das S, Janisch L, et al. UGT1A1*28 polymorphism as a determinant of irinotecan disposition and toxicity. *Pharmacogenomics J.* 2002;2(1):43-47. http://www.ncbi.nlm.nih.gov/pubmed/11990381. Accessed July 15, 2019.

30. Ye B, Valdivia CR, Ackerman MJ, Makielski JC. A common human SCN5A polymorphism modifies expression of an arrhythmia causing mutation. *Physiol Genomics.* 2003;12(3):187-193. doi:10.1152/physiolgenomics.00117.2002

31. Matsumura H, Nakano Y, Ochi H, et al. H558R, a common SCN5A polymorphism, modifies the clinical phenotype of Brugada syndrome by modulating DNA methylation of SCN5A promoters. *J Biomed Sci.* 2017;24(1):91. doi:10.1186/s12929-017-0397-x
32. Strevel EL, Ing DJ, Siu LL. Molecularly targeted oncology therapeutics and prolongation of the QT interval. J Clin Oncol. 2007;25(22):3362-3371. doi:10.1200/JCO.2006.09.6925

33. Kloth JSL, Pagani A, Verboom MC, et al. Incidence and relevance of QTc-interval prolongation caused by tyrosine kinase inhibitors. Br J Cancer. 2015;112(6):1011-1016. doi:10.1038/bjc.2015.82

34. Futreal PA, Coin L, Marshall M, et al. A census of human cancer genes. Nat Rev Cancer. 2004;4(3):177-183. doi:10.1038/nrc1299

35. Tate JG, Bamford S, Jubb HC, et al. COSMIC: the Catalogue Of Somatic Mutations In Cancer. Nucleic Acids Res. 2019;47(D1):D941-D947. doi:10.1093/nar/gky1015

36. Schrader KA, Cheng DT, Joseph V, et al. Germline Variants in Targeted Tumor Sequencing Using Matched Normal DNA. JAMA Oncol. 2016;2(1):104-111. doi:10.1001/jamaoncol.2015.5208

37. Mandelker D, Zhang L, Kemel Y, et al. Mutation Detection in Patients With Advanced Cancer by Universal Sequencing of Cancer-Related Genes in Tumor and Normal DNA vs Guideline-Based Germline Testing. JAMA. 2017;318(9):825. doi:10.1001/jama.2017.11137
Table 1. Framework for the return of germline findings in precision oncology.

| Tier                        | Clinical indication                          | Recommended categories for consideration                                                                 | Examples                                                                 | PEWG recommendations for disclosure† |
|-----------------------------|---------------------------------------------|-----------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------|---------------------------------------|
| **Primary**                 |                                             |                                                                                                            |                                                                        |                                       |
| Cancer susceptibility       | Known or novel pathogenic and likely pathogenic variants associated with moderate- to high-penetrance cancer susceptibility* | CHEK2 c.1100delC (p.Thr367Metfs)                                                                         | Return to patient with referral to HCP†                                |                                       |
|                             | Known or novel pathogenic and likely pathogenic variants associated with low-penetrance cancer susceptibility* | CHEK2 c.470T>C (p.Ile157Thr)                                                                            | Return to patient with referral to HCP on a case-by-case basis          |                                       |
|                             | Autosomal or X-linked carrier status for known or novel pathogenic and likely pathogenic variants associated with low-, moderate- or high-penetrance cancer susceptibility* | ERCC5 c.529-1G>A                                                                                       | Return to patient with referral to HCP on a case-by-case basis          |                                       |
|                             | VUS in cancer predisposition genes with clinical and molecular indications for pathogenicity | MUTYH c.996G>A (p.Ser332Ser)                                                                          | Return to patient with referral to HCP on a case-by-case basis          |                                       |
| Disease pathogenesis, prognosis or treatment | Known or novel variants in cancer predisposition genes understood to contribute to tumour phenotype and evolution | Biallelic loss through combined germline and somatic aberrations: NTHL1 carrier status MUTYH carrier status | Return to patient with referral to HCP on a case-by-case basis and if clinical significance has been established |                                       |
|                             | Deleterious variants in known or novel cancer-related genes | Deletion polymorphisms in APOBEC3A and APOBEC3B                                                           | Return to patient if clinical significance has been established         |                                       |
|                             | Pharmacogenomic variants with established or potential cancer treatment associations | UGT1A1*28                                                                                                | Return to patient if clinical significance has been established         |                                       |
|                             | Alleles associated with immune response   | HLA class I genotypes                                                                                   | Return to patient if clinical significance has been established         |                                       |
|                             | Variants in gene(s) reviewed at the request of the case clinician given patient consent | SCN5A c.1673A>G (p.His558Arg)                                                                          | Return to patient if clinical significance has been established         |                                       |
| **Secondary**               | Mendelian disease risk or carrier status    | Deleterious variants in genes without known implications for cancer prevention, cancer screening or treatment | Pathogenic and likely pathogenic variants in Mendelian disease genes defined by the ACMG† | Return to patient with referral to Medical Genetics program if patient preference for non-cancer related information is indicated in consent |                                       |

*Implications for cancer susceptibility should be considered in the context of variant zyosity and the typical mode of inheritance observed for a given gene. In particular, this includes variants in autosomal recessive genes or in X-linked genes conferring recessive disease risk that may have differing indications for XY males, XX females and XO females.†ACMG, American College of Medical Genetics and Genomics; HCP: Hereditary Cancer Program; PEWG, POG Ethics and Germline Working Group
Figure Legends

Figure 1. Approach for germline variant calling, annotation and filtering in tumour-normal whole-genome sequencing. In the POG program, parallel pipelines are implemented for the analysis of small variants and structural variants (SVs). Low-complexity regions, strong GC bias and repetitive elements limit the accuracy of SV calling through short-read (50-300 bp) sequencing. Consequently, complementary read depth-, flanking read-, split read- and contig-based computational approaches are incorporated to increase the sensitivity of SV detection. Germline variants with known or putative clinical significance are prioritized by clinical annotation, functional effect prediction and population frequency in 98 cancer predisposition genes. All candidate variants are reviewed in a genome browser to flag possible technical artifacts, and this information is included in an integrated germline report along with relevant tumour data.

Figure 2. Standard procedure for the review, reporting and clinical translation of germline variants in the Personalized OncoGenomics program. Given an integrated germline analysis report, a Clinical Molecular Geneticist at the Cancer Genetics and Genomics Laboratory (CGL) curates all germline variants with known or potential clinical significance in cancer predisposition genes undergoing prioritized review. The Ethics and Germline Working Group determine by consensus final recommendations for variant reporting and whether a referral to the Hereditary Cancer Program (HCP) should be made for patient counseling and clinical genetic testing. In the absence of functional evidence supporting pathogenicity, variants of uncertain significance are disclosed only when the patient's personal or family history is suggestive of hereditary cancer susceptibility. Clinically actionable variants that occur in areas of the reference genome flagged as low-complexity or repetitive regions will be validated at CGL prior to return of results.
If false positive variants are identified, an updated bioinformatics pipeline is implemented to flag these variants in future cases.

Figure 3. Extending the clinical significance of integrated molecular analysis of tumour and normal tissues beyond cancer susceptibility. SSM: simple somatic mutations; CNV: copy number variants; LOH: loss-of-heterozygosity; SSV: somatic structural variants.
Small variant calling

SAMtools

Variant annotation and functional prediction

SnpEff, dbSNP, ClinVar, COSMIC

Gene-based filtering

variants in genes of interest

Clinical annotation and variant effect filtering

known pathogenic/likely pathogenic
predicted loss-of-function

Population frequency filtering

rare coding and splicing variants

Copy number and structural variants

Read depth

Control-FREEC

Paired reads, split reads, contigs

DELLY, Manta, Trans-ABySS

Merging and annotation

MAVIS

Region-based filtering

variants overlapping target genes

Quality filtering

filter recurrent technical artifacts

Gene-based filtering

variants in genes of interest

Figure 1

SNVs and indels

integration germline tumour data
Integrated germline report

Germline variants with known or predicted clinical significance in cancer predisposition genes with relevant tumour data

Clinical molecular geneticist review

Pathogenic or likely pathogenic

Variant of uncertain significance

Benign/likely benign

Does personal or family history suggest hereditary cancer susceptibility?

No

Do not report variant and no further follow up is required

Yes

Does this variant occur in a low-complexity region or in a region with low coverage?

No

Report variant to Tumour Board and case clinician for return to patient

Benign/likely benign

Variant of uncertain significance

Does personal or family history suggest hereditary cancer susceptibility?

No

Do not report variant and no further follow up is required

Yes

Validation possible technical artifacts by Sanger sequencing

HCP referral

Genetic counseling and clinical confirmation

Update bioinformatics pipeline to flag technical artifacts
immune competence

drug metabolism

off-target effects

treatment indications and response

gene fusions

splicing alterations

mRNA expression

mutational signatures

neoantigens

molecular subtypes

cancer prognosis and tumour evolution

personal and family cancer risk and screening

germline genetic variation

Figure 3
Small variant calling
SAMtools

Variant annotation and functional prediction
SnpEff, dbSNP, ClinVar, COSMIC

Gene-based filtering
variants in genes of interest

Clinical annotation and variant effect filtering
known pathogenic/likely pathogenic
predicted loss-of-function

Population frequency filtering
rare coding and splicing variants

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SNVs and indels

Copy number and structural variants

Figure 1
Integrated germline report

germline variants with known or predicted clinical significance in cancer predisposition genes with relevant tumour data

Clinical molecular geneticist review

Pathogenic or likely pathogenic

Variant of uncertain significance

Benign/likely benign

Does personal or family history suggest hereditary cancer susceptibility?

No

Yes

No

Do not report variant and no further follow up is required

No

Yes

Report variant to Tumour Board and case clinician for return to patient

Validate possible technical artifacts by Sanger sequencing

Update bioinformatics pipeline to flag technical artifacts

Genetic counseling and clinical confirmation

HCP referral

Figure 2--FINAL

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