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Molecular Case Studies

PRECISION MEDICINE IN PRACTICE

Lessons learned from the application of whole-genome analysis to the treatment of patients with advanced cancers

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Abstract Given the success of targeted agents in specific populations it is expected that some degree of molecular biomarker testing will become standard of care for many, if not all, cancers. To facilitate this, cancer centers worldwide are experimenting with targeted "panel" sequencing of selected mutations. Recent advances in genomic technology enable the generation of genome-scale data sets for individual patients. Recognizing the risk, inherent in panel sequencing, of failing to detect meaningful somatic alterations, we sought to establish processes to integrate data from whole-genome analysis (WGA) into routine cancer care. Between June 2012 and August 2014, 100 adult patients with incurable cancers consented to participate in the Personalized OncoGenomics (POG) study. Fresh tumor and blood samples were obtained and used for whole-genome and RNA sequencing. Computational approaches were used to identify candidate driver mutations, genes, and pathways. Diagnostic and drug information were then sought based on these candidate "drivers." Reports were generated and discussed weekly in a multidisciplinary team setting. Other multidisciplinary working groups were assembled to establish guidelines on the interpretation, communication, and integration of individual genomic findings into patient care. Of 78 patients for whom WGA was possible, results were considered actionable in 55 cases. In 23 of these 55 cases, the patients received treatments motivated by WGA. Our experience indicates that a multidisciplinary team of clinicians and scientists can implement a paradigm in which WGA is integrated into the care of late stage cancer patients to inform systemic therapy decisions.

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INTRODUCTION

Cancer is a complex biological process. Historically, cancers have been classified according to their anatomic site of origin (e.g., lung, breast, liver, colon), but within these groupings there are multiple subtypes with differences in response to treatment and overall behavior. Oncologists traditionally base their treatment recommendations on the reports of clinical trials or on their personal anecdotal experiences that may, or may not, reflect the individual characteristics of the patient sitting before them. The consequence of this decision-making process is that many patients, including the majority with advanced cancers, receive toxic and expensive chemotherapy treatments that may convey limited or no clinical benefit.

In a few subtypes of cancers, specific mutations or other aberrations have been identified and successfully targeted using novel systemic therapy agents (Druker et al. 2001; Slamon et al. 2001; Lynch et al. 2004; Paez et al. 2004; Pao et al. 2004). The now well-established mutational, spatial, and temporal heterogeneities of malignancies imply that assessment of the biology of individual cancers may result in better alignment of treatment choices with individual patient characteristics (Dienstmann et al. 2013). One widely used approach amenable to improved characterization of individual cancers is DNA sequencing (MacConaill 2013).

Currently, there are a number of DNA sequence–based tests that are used in cancer medicine, ranging from single gene tests for mutations with well characterized prognostic and predictive significance, such as epidermal growth factor receptor (*EGFR*)–activating mutations in lung cancer, to more expansive panels which might include hundreds of defined cancer genes and mutational "hot spots" (Dias-Santagata et al. 2010). Technological advances have now brought the possibility of more extensive interrogation of the genome through exome, transcriptome, and whole-genome sequencing to a clinical reality (Garraway 2013; Andre et al. 2014). Given the rapid advances of genome technologies, the gap between what science and technology can deliver and its application to clinical practice has never been wider, presenting important challenges and opportunities as we endeavor to navigate and close that gap.

In 2010, we were the first to publish an example of clinical treatment decision-making based on whole-genome analysis (WGA) of a rare tumor (Jones et al. 2010). Tumor and normal genome sequences were used together with RNA sequence data to successfully "personalize" a treatment plan for an adult patient with an adenocarcinoma of the tongue with lung metastases. Motivated by the success of this effort, and enabled by technology advances and concomitant decreasing costs, we have expanded beyond this experience to a broader patient population reflecting the province-wide mandate of the BC Cancer Agency. This expansion has involved the assembly of a network of clinicians, pathologists, and researchers committed to the use of "rapid" whole-genome and transcriptome analysis to aid treatment decision-making for advanced pediatric, adolescent, and adult cancers in our Personalized OncoGenomics (POG) program. Although not currently required by Canadian regulations, all sample processing and sequencing was performed in a CAP-accredited facility within our sequencing center. As part of assessing the feasibility of scaling the POG program to the population level, we have collated the experience gained from the enrollment of the first 100 patients and the WGA of 78 patients. This collective experience has been used to address critical questions regarding the practical application of POG in a broad clinical context, and has resulted in the evolution of a consultative multidisciplinary "tumor group" structure that meets weekly at our tertiary care cancer hospital, frequently including participants joining by video conference. Our perception is that there is a growing momentum in the oncology community to develop similar clinical sequencing programs (Meric-Bernstam et al. 2013) and, motivated by the opportunities for global collaborations, partnerships, and a desire to

promote advances in the area, we seek to share our perspectives in this report. We thus present an overview of the POG program with particular emphasis on the clinical challenges we face and solutions we have implemented.

Setting the Stage for Translational Oncogenomics

The British Columbia Cancer Agency (BCCA) coordinates the provincial cancer program for a population of more than 4.6 million through six regional cancer centers in the province of British Columbia, Canada. The clinical aspects of this study are coordinated through the Vancouver Cancer Centre which is a tertiary care cancer center adjacent to another BCCA facility, Canada's Michael Smith Genome Sciences Centre (GSC), which presently has the capacity to sequence more than 600 trillion raw bases annually, an amount of DNA approximately equivalent to ~6000 30× human genomes per year.

The history of close collaboration between the clinicians at the BCCA and scientists at the GSC paved the way for POG's creation and expansion. The POG clinical team includes the majority of medical oncologists at the Vancouver Centre (18 of 22) as well as pediatric oncologists; a group of motivated interventional radiologists and surgeons for tissue procurement; and medical geneticists, ethicists, and pathologists who are critical to the analysis, interpretation and clinical communication of whole-genome data sets from patients.

Approach to Recruiting Patient Participants

In the POG program, medical oncologists recruited patients from their general oncology clinics with the intention of sampling a variety of cancer histologies. The enthusiasm of patients and participating oncologists for POG is tempered by the realization that whole-genome data are complex and, as applied to cancer treatment planning, somewhat controversial and, at present, experimental. Therefore, we chose to include in these early stages of POG only those patients with incurable cancers, typically patients who had limited or no standard treatment options available. This cohort could thus be considered a "phase 1 trial eligible" population.

Recruitment of potential patients has not been a limiting factor particularly because of the increasing global media attention and the marketing of cancer panels directly to patients. It is now common that the request to have "cancer sequencing" performed is initiated by patients or families. Sophisticated marketing techniques from private enterprises across the world have created a sense among many patients that personalized cancer treatments are possible and beneficial for management of their disease.

The POG project launched in July 2012 and enrolled one patient per month for the first 5 months and scaled up accrual once process refinements were implemented. The study schema is outlined in Figure 1.

As of August 2014, 100 adult and six pediatric patients had been consented to the study, representing 30 different cancer types. Patients donated fresh tumor biopsy and blood samples, the latter to act as a source of normal DNA for identification of somatic alterations. Of these 106 cases, we have completed sequencing and analysis of 85 patients (78 adults) (Fig. 2). Twenty-one patients were not sequenced or were removed from study for a variety of reasons including the following: biopsy was not technically possible, biopsy tumor content was inadequate for sequencing, patient withdrawal, or unexpected death. Table 1 outlines patient demographics and baseline characteristics of the adult patients.

The local Clinical Research Ethics Board approved this protocol. One of the most obvious differences between the POG protocol and other clinical trials is the fact that we could not predict which, if any, systemic therapy agents might be identified based on the genomic profiling. Therefore, the protocol and ethics approval does not include information about

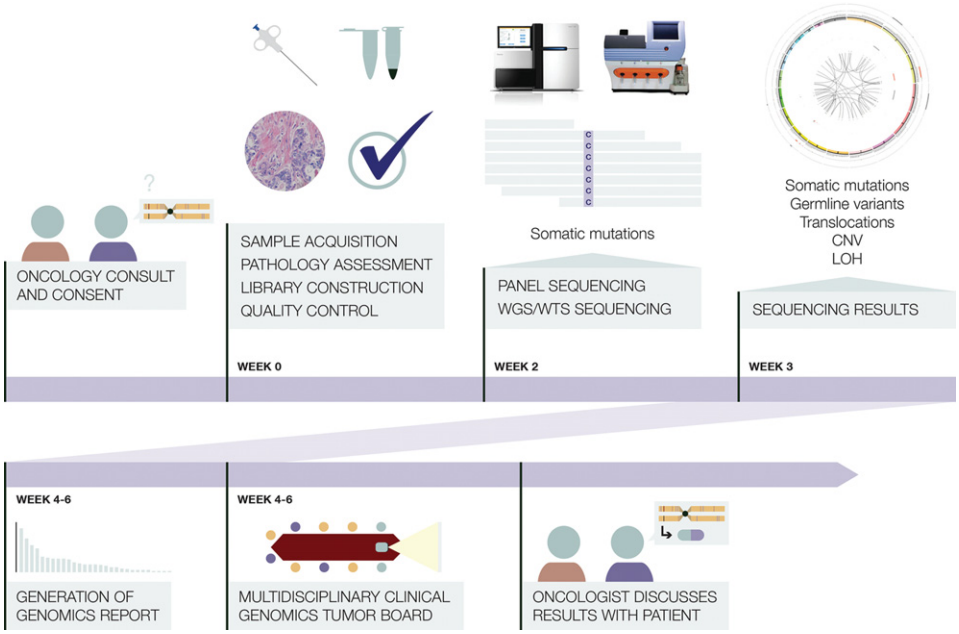


Figure 1. Schema outlining a high-level model of the process from the time of patient consent to the generation of a Personalized OncoGenomics report and discussion with the patient.

specific treatments and it stipulates that any treatments given to a patient based on the POG information should be considered experimental and should not replace standard of care or any potentially curative therapies.

Approach to WGA

Recognizing the diversity of genes and mutations that have been linked to cancer pathways, our intention from the outset was to assess the clinical feasibility and potential utility of WGA of patient tumors rather than panel or exome approaches. The primary question we sought to address was whether we could design a system that would make WGA as clinically accessible as single gene or panel tests. Although the cost of WGA is higher than that of panels,

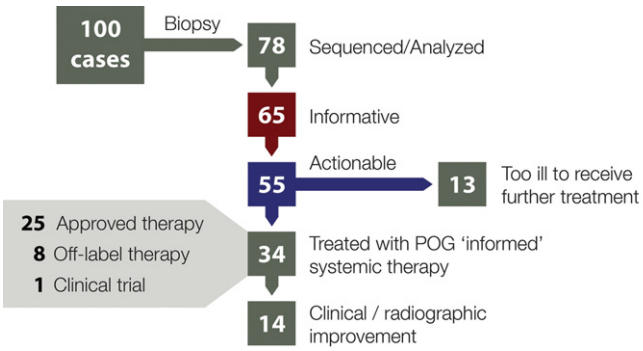


Figure 2. CONSORT diagram of the 100 adult patients consented into the Personalized OncoGenomics (POG) study.

Table 1. Basic demographics

Adult cohort	
Gender ($N = 100$)	
Male	17
Female	83
Median age at consent	52
Average (mean) number of lines of chemotherapy received before POG (range)	3 (0–9)
Primary cancer	
Breast	38
GI (includes pancreas)	14
Lung	11
Gynecologic	10
Head and neck/sarcoma/primary unknown	4/4/4
Peritoneal mesothelioma	3
Adrenal/hematologic/skin	2/2/2
Other	6
Pediatrics cohort	
Gender ($N = 6$)	
Male	4
Female	2
Median age at consent	10
Primary cancer	
Neuroblastoma	3
Neurofibroma	1
Sarcoma	1
Melanoma	1

POG, Personalized OncoGenomics; GI, gastrointestinal.

the technology continues to advance and costs continue to decline while offering the opportunity to capture the full spectrum of somatic and germline abnormalities already linked to cancers. Further, WGA offered the opportunity to discover new lesions in our cohort. The comprehensive characterization of genomes and transcriptomes, including de novo assembly approaches, had a number of important advantages, including the identification of structural variants such as translocations and inversions, loss of heterozygosity, and copy number variants, all of which are known to play roles in oncogenesis.

Our emphasis on the integrative analysis of genomes and transcriptomes in a clinical context distinguishes our efforts from those of others and provides a valuable resource of comprehensively characterized tumors with associated clinical data that will be used to compare future patient samples to aid in their interpretation. We envision the expansion of such a resource through our continued efforts will help change the way advanced cancers are treated in BC and beyond.

Approach to Tissue Acquisition, Processing, and Requirements

The transcriptome data were required for identifying and evaluating dysregulated gene expression, confirming genomic alterations such as single nucleotide variants, indels, and gene fusions, which were further validated by alternate approaches such as targeted sequencing and fluorescence in situ hybridization (FISH) where appropriate, and identifying candidate

drug targets for verification using standard immunohistochemistry. As the quality of transcriptome data from paraffin-embedded tissue was generally inferior to data obtained using fresh biopsy material, our process was linked to the availability of fresh or fresh-frozen tissue. To obtain fresh biopsy material, we included in our team colleagues in diagnostic imaging, surgery and dermatology who routinely perform biopsies.

Biopsy materials and blood samples were immediately delivered to a participating pathologist to confirm the diagnosis, ensure that there was sufficient viable tumor present in the biopsy and mark the areas most suitable for extraction of nucleic acid. The material was then sent for DNA and RNA extraction. Aliquots of nucleic acid were used for analysis on the Ion Torrent AmpliSeq Cancer panel (Singh et al. 2013; Snuderl et al. 2014) and for whole genome and transcriptome analysis on the Illumina HiSeq platform as previously described.

The blood samples generally served as sources of "normal" (or "germline") DNA to allow identification of somatic variants. Considering both costs and a level of sensitivity adequate to detect dominant clones in the cancer sample, we elected to sequence the patient germline derived DNA to ~40-fold redundant sequence coverage and the tumor sample to exceed 80-fold redundant coverage. Tumor and blood DNA samples were also subjected to sequence analysis using a commercially available AmpliSeq Cancer panel, which provided the opportunity for comparison of whole genome versus focused approaches for treatment planning purposes. In particular, we wished to assess the relative uptake of the results from these approaches among members of the clinical team.

RNA-seq was used to profile gene expression in tumor material to an average depth of 200 million reads. This depth of coverage was chosen to maintain sensitivity (i.e., in heterogeneous tumors [Shah et al. 2012; The Cancer Genome Atlas Research Network 2014]) at costs affordable to the POG project.

The POG process demands a significant investment of resources. In an effort to ensure meaningful results, library construction was restricted to those specimens most likely to yield high-quality genome and transcriptome data. To this end, core needle and surgical biopsy tissues were the preferred method for tissue procurement. Pathologists with expertise in molecular genetic pathology reviewed all specimens for overall cellularity and tumor content. Early experience showed that specimens with tumor content determined by the sequencing data to be below 45% resulted in reduced sensitivity for detection of mutations as well as increased uncertainty in interpretation of gene expression information. Furthermore, due to the different approaches in measuring tumor content we typically observed inconsistencies between tumor content estimations as determined by pathologists and from that determined from the sequencing data. To take this into account, a 55% tumor content as estimated by pathology became the cut-off tumor content for samples to be sequenced. The stringency of the tissue requirements may exclude many patients from analysis and therefore borderline cases with tumor contents of >30% were reviewed by the team for adequacy; the pathologist who performed this second review was blinded to the actual cut-offs for proceeding with library construction.

An average of five core needle biopsies were required to obtain an adequate amount of material for DNA and RNA sequencing. Table 2 outlines our experience with tissue procurement. Overall, 6% of consented cases required more than one biopsy to obtain sufficient material, and 16% could not be sequenced due to low tumor content. Based on these findings, the team did not support tissue acquisition via fine needle aspirates including those from endoscopy or bronchoscopy, or fluid samples from the pleural or abdominal space, as these specimens tended to have inadequate tumor content for whole-genome sequencing. In addition it was noted that samples from patients who were on or had just completed chemotherapy tended to have low tumor content and thus obtaining biopsies from such patients was discouraged when possible.

Table 2. Summary of types of biopsies performed on the adult population, the average tumor content as estimated by a pathologist, and the frequency with which each technique yielded tumor content more than the 55% threshold we required to proceed with sequencing

Type of biopsy	*N* = 100	Average tumor content (estimated by pathologist) (%)	Frequency in which the sample had tumor content >55% (%)
Ultrasound guided core	54	64	76
CT-guided core	7	57	57
Surgery	9	69	78
Excisional or incisional	16	70	88
Bite or punch biopsy	4	70	100
Bronchoscopic/ endoscopic core biopsy	3	67	100
Endoscopy or broncoscopy or EBUS	4	44	25
Blood draw	1	N/A	100
Thoracentesis	2	50	48

N/A, not applicable; CT, computed tomography; EBUS, endobrachial ultrasound.

Approach to WGA and Reporting

Our approach to data analysis included the identification of somatic single nucleotide and copy number variants, indels, gene fusions, genome rearrangements, and dysregulated gene expression patterns. Such features were identified using a purpose-built data analysis pipeline that included whole genome and transcriptome assembly, and detailed annotation. The focus of the analysis was (1) to integrate information from various data types to infer the genes and pathways that were possibly functionally critical to individual malignancies and to identify candidate therapeutic vulnerabilities; and (2) to rationalize treatment planning. For RNA analysis, the tumor transcriptome was compared with a compendium of data available in the public domain (primarily The Cancer Genome Atlas [TCGA] data set). For each case three expression metrics were generated: a fold change in gene expression compared with a compendium of normal tissues (Illumina Body Map 2.0), a percentile ranking of gene expression within similar tumor types (TCGA) and a within-sample expression rank of each gene. To assess differential gene expression relative to normal tissues, tumor transcriptome reads per kilobases per million mapped reads (RPKM) data were compared with a compendium of 16 different tissue samples (Illumina Body Map 2.0). For each gene a fold-change in RPKM between normal versus tumor sample was calculated. In cases in which there was no direct tissue match to the primary site of the disease the fold change was calculated against the average RPKM of the entire data set. In addition to the fold-change calculation a percentile value was placed on the individual gene expression with reference to a tumor sample data set. To do this TCGA (level 3) gene expression data for 5976 tumor samples (representing 25 cancer types) was downloaded (https://tcga-data.nci.nih.gov/tcga/) and analyzed to calculate RPKM values. Tumor RPKM data were then ranked within the data by tumor type or the average of the entire data set. Expression outliers were evaluated case-by-case, taking into account supporting evidence from other data sources and in relation to other potentially relevant genomic events.

Syntheses of the analyzed data were compiled into a standardized report format, designed by a committee including pathologists and medical oncologists. Individual case reports included a list of potentially actionable or informative features emerging from the data analysis. Analysis results were also delivered in conference-style format to our

interdisciplinary genomics tumor group, in which summaries of the somatic alterations most relevant to treatment planning were presented. Included in the presentation were candidate therapies reported in the literature to impact molecules and pathways identified in the analysis. The levels of evidence to support relationships between somatic alterations and candidate drug classes were included in these reports.

The purpose of the report was to provide information to the clinician to use at their discretion in treatment planning and in discussing treatment options with the patient. The data were not considered prescriptive, but rather as offering additional information to consider in the management of individual cancer patients. We noted that clinicians were most comfortable when several abnormalities coalesced around a pathway, (e.g., evidence of both "activating" copy number changes and high gene expression levels), and only rarely used single somatic alterations to inform a treatment decision.

Communication of Genome Analysis Results

The application of whole-genome analysis to cancer treatment planning required a strategy for the communication of WGA to oncologists and pathologists. Our approach to communication involved the formation of a new multidisciplinary Clinical Genomics Tumour Board. The creation of the Board provided a venue for case reporting in which discussion of every case involved an introduction to the case by the treating oncologist, followed by a report from the pathologist assigned to the case and finally a review of the WGA data presented by a bioinformatician. The bioinformatics report focused the identification of candidate driver mutations and pathways, and possible therapeutic strategies to target these. Most of the discussion revolved around pathway diagrams in which somatic alterations and gene expression patterns were integrated in an effort to depict inter-pathway interactions. Medical oncologists participating in the Board discussion then sought to achieve a consensus opinion for treatment options, which was then documented for the primary oncologist to take back to their patient for discussion. These Tumor Board meetings served to expose and educate both researchers and clinicians in the interpretation and application of WGA as a treatment decision aid.

Patients were given the option to opt in for return of germline variants. As a part of the consent process, subjects were made aware that germline findings could potentially be reported to their treating oncologists and used to inform their treatment options. All germline variants identified were reviewed and classified. Potentially pathogenic variants were reported for expedited review to an ethics subcommittee consisting of molecular pathologists, medical geneticists, oncologists and bioinformaticians. Variants deemed to be ACMG Class 4 or 5 (likely pathogenic or pathogenic) were reported to the case clinician. All germline variants of uncertain significance were communicated to the Clinical Genetics Laboratory (CGL) for independent evaluation, the results of which were then reported to the ethics subcommittee for final determination of whether the variant in question was of sufficient clinical relevance to be reported back to the treating clinician. Where appropriate, patients were also offered referral to the Hereditary Cancer Program for genetic counseling.

Essential to the evolution of our process was the reciprocal exchange of information between medical professionals and genome scientists. This reciprocal knowledge transfer, conducted weekly in the form of our Tumor Board discussions, appears to be a necessary feature of our program and served to inform the process; assess the impact or success of WGA as applied to treatment planning, and avoid possible inappropriate interpretation of WGA and attendant risks as we seek to expand the application of WGA into standard cancer care.

To establish and evolve processes and guidelines for the management and discussion of incidental germline findings and variants of uncertain significance (Lim et al. 2014), we established a POG Ethics and Policy subcommittee. This subcommitte included an

ethicist, genetic counselors, medical geneticists, pathologists, bioinformaticians, and medical oncologists.

Turnaround Time for Whole-Genome Analysis

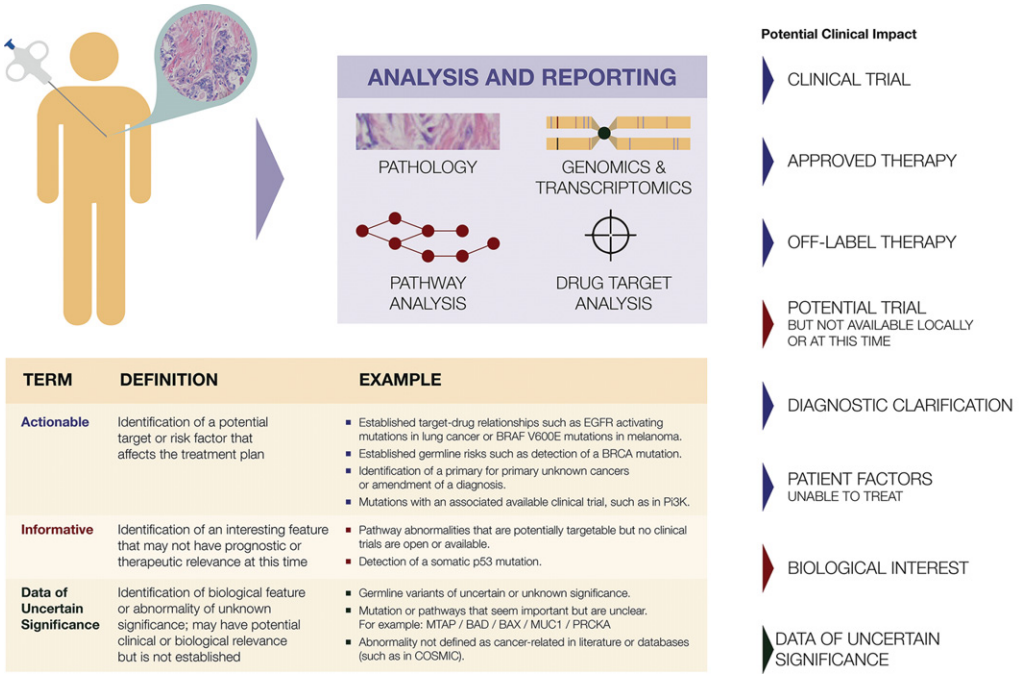
Lengthy turnaround times (TATs) for the production and analysis of whole-genome data has hampered its clinical application in cancer, where rapid treatment decisions are frequently required. Advances in sequencing technology and computational approaches have reduced TATs generally, and so we sought to evaluate TAT in the context of our POG effort. In particular, we wished to understand whether an individual whole-genome analysis could be produced and interpreted in a time frame that was relevant to patient treatment planning. Most patients and physicians would find 10–14 d an acceptable amount of time to wait for results that might inform treatment decisions and this is our goal going forward. For the first 100 POG patients, the median time from biopsy to presentation of the report was 58 d. However, this TAT was skewed by the lack of automated analysis routines and other procedures at the start of the project. The evolution of POG process routines including automation that, when implemented, had a significant impact on TAT, decreasing it from 80 d for the first set of patients to 50 d over time. Although our panel results were delivered on average in 7 working days, the majority of the time panel results did not yield information that changed clinical management. Of 81 patients with panel results, 73% carried mutations that were classed as informative but not actionable, the majority of which were mutations in *TP53* (55%). In the remaining 27% of cases no variants were detected using the panel. The overall time frames for the POG process are included in Figure 1.

The POG team has thus worked to establish and implement efficient standard operating procedures to automate many aspects of whole-genome analysis. Most challenging to automate, however, and where whole-genome analysis seems poised to significantly contribute to treatment planning, is the final stage of data analysis that links somatic alterations and gene expression patterns to drug information. This process is currently manually intensive, involving PhD level scientists engaged in substantial literature review and interpretation for each case. Achieving these linkages is challenging because the relevant literature base is constantly evolving, and the quality and utility of the literature to our application varies hugely. However, because each possible therapeutic recommendation we have generated has been carefully evaluated in the context of the literature available at the time the case was analyzed, our data have established a framework for comparison of automated approaches. For example, we can now imagine evaluating the specificity and sensitivity of automation against the 78 cases we have analyzed manually.

Frequency of Informative or Actionable Information

One of the most important questions driving the POG project centered around the frequencies of informative or actionable variants and gene expression patterns. Key to addressing this question was a range of opinions of what "informative" and "actionable" meant, and thus the need to erect local definitions for these terms.

The Tumor Board meeting served as an important venue to articulate opinions regarding the extent to which particular observations were informative or actionable, and it was within Board Discussions that a basis for locally relevant definitions emerged (Fig. 3). Briefly, we defined the term "informative" to mean "identification of an interesting feature that may not have prognostic or therapeutic relevance at this time" and the term "actionable" to mean "identification of a potential target or risk factor that affects the treatment plan." The local definitions were important because, for example, candidate drugs that might be obtained in an alternate setting, but not locally, were considered informative but not actionable by our clinicians. Similarly, the detection of very common mutations such as *TP53* was not



TERM	DEFINITION	EXAMPLE
Actionable	Identification of a potential target or risk factor that affects the treatment plan	▪ Established target-drug relationships such as EGFR activating mutations in lung cancer or BRAF V600E mutations in melanoma. ▪ Established germline risks such as detection of a BRCA mutation. ▪ Identification of a primary for primary unknown cancers or amendment of a diagnosis. ▪ Mutations with an associated available clinical trial, such as in PI3K.
Informative	Identification of an interesting feature that may not have prognostic or therapeutic relevance at this time	▪ Pathway abnormalities that are potentially targetable but no clinical trials are open or available. ▪ Detection of a somatic p53 mutation.
Data of Uncertain Significance	Identification of biological feature or abnormality of unknown significance; may have potential clinical or biological relevance but is not established	▪ Germline variants of uncertain or unknown significance. ▪ Mutation or pathways that seem important but are unclear. For example: MTAP / BAD / BAX / MUC1 / PRCKA ▪ Abnormality not defined as cancer-related in literature or databases (such as in COSMIC).

Figure 3. Working definitions for genomics output and potential clinical impact of this data.

considered to be actionable. This landscape is of course a changing one, as new trials accessible to our patient population could result in a reclassification of observations as actionable. Other considerations around the extent to which observations were considered actionable included: availability of a drug in a local clinical trial, off-label use of an approved therapy, when a therapy was available but the patient was too unwell to receive treatment, and when the observation aided in the diagnosis of classification of the cancer. Figure 3 presents these definitions and the variety of clinical implications we encountered.

Data on treatments that were informed by our POG process, and outcome data, were recorded and are outlined in Table 3 and Figure 2. Seventy-eight of the adult patients had biopsies yielding sufficient tissue for sequencing and analysis. In 83% of these cases, the clinician who was making treatment decisions with the specific patient indicated that the data information were informative. In five of these cases the diagnosis was either changed to a different primary tumor than originally diagnosed or a primary was suggested in primary unknown cases (Jamshidi et al. 2014). Given the fragile nature of these phase 1–like patients it was encouraging that 71% of patients received a POG-informed treatment and 62% of those treated achieved some disease control.

It should be noted that this feasibility study of 100 patients did not specify tumor measurements or the timing of assessments and as such the response data are not comprehensive. Furthermore the significant number of cases with the primary diagnosis of breast cancer (38 of 100) has the potential to skew the response assessment toward that expected for a refractory breast cancer cohort. Clinician-assessed response to POG-informed therapy was recorded as the best response: progressive disease, some disease control or reduction in size, or remarkable or unexpected disease control (usually compared with the patient's response to prior regimens or the median response seen in that patient population) (Von Hoff et al. 2010; Tsimberidou et al. 2012). This aspect of response to POG-informed therapy is of critical importance to study in future protocols but it was not the objective or design of this initial program.

Table 3. Data for the adult Personalized OncoGenomics (POG) population describing how often the data was felt to be informative or actionable by the treating medical oncologist and the clinician-assessed response to any POG-informed treatment that was delivered

	N = 100 adults	Percentage
Sufficient tissue for POG analysis	78	78%
Insufficient tissue for POG analysis		
Biopsy content too low for sequencing	16	16%
Unable to biopsy due to specific patient factors	6	6%
Informative	65	65% total; 83% when sequencing was possible
Actionable	55	55% total; 71% when sequencing was possible
Patients received POG-informed treatment	34	34% total; 44% when sequencing was possible; 62% when there was something actionable identified
Well patients eligible for POG-informed treatment who did not receive therapy	8	8%
No treatment available	2	2%
Not eligible for identified clinical trial	2	2%
Already on an alternative clinical trial	1	1%
Unknown	3	3%
Clinician-assessed clinical or radiographic improvement in cancer (including stable disease)	14	41% (14 of 34)
Actionable target identified but the patient was too unwell or death before POG-informed therapy could be offered	13	24% (13 of 55)
Amended or clarified the diagnosis or primary site	5	5% total; 6% when sequencing possible

The difficulty of designing and conducting an *N* of 1 series clinical trial with hard response or survival endpoints is challenging (Sleijfer et al. 2013; Andre et al. 2014) and beyond the scope of the feasibility study we report here.

DISCUSSION

Oncologists are becoming rapidly educated about the range of genomic platforms that exist and there is an established comfort level with the development of drugs linked to companion diagnostic tests. Although POG has been an extremely successful first step in evaluating the utility of whole-genome analysis in cancer treatment planning using a multidisciplinary approach, significant work remains to be done as we imagine scaling the approach to larger populations.

The smooth integration of genomic information into the standard of care will demand a TAT from consent to report of <3 wk. Over the course of 100 cases, we were able to

significantly reduce our TAT from ~80 to 50 d. However additional improvements are required. One approach to reduce TAT is to create a biopsy process around a team of radiologists and surgeons devoted to the activity. Another way forward is to refine the process by which high-quality sequencing can be done on archival tumor specimens for both DNA and RNA. If feasible and reliable this would potentially allow for sequencing of existing tumor banks as well as shorten the current time frame for patient participants.

Another challenge that needs to be addressed on several fronts is the ability to act on the findings of the genomic analysis with an available drug. Most actionable findings from POG are targeted chemotherapy agents either approved or in clinical trials. For genomics to be truly integrated as a decision-making tool these treatments need to be available and thus an active clinical trials program is critical. An ideal schema for genomic studies is the "basket"-like trial in which a broad spectrum of cancer patients who may harbor one or more of a defined cluster of potential genomic biomarkers can gain access to novel targeted therapies. This not only provides drug access to individual patients but can also greatly contribute to the drug development knowledge-bases within the pharmaceutical industry and/or collaborative groups. In parallel with the clinical trial initiatives there needs to be the recognition that off-label use of some targeted agents may be an appropriate strategy to use and a process could be developed to obtain such agents, at least on a trial basis. Perhaps with the ongoing genomic and biomarker education of regulatory authorities it may come to pass that a patient may only need to have an identifiable target or biomarker(s), and not necessarily a specific tumor type, to gain access to a targeted systemic therapy.

The clinical feedback that is an integral component of the POG program is critical to the emerging success of WGA in a clinical setting. When an individual on POG receives a systemic therapy treatment and either responds or does not respond, this information is fed back to the genome analytic team and entered into a database that will highlight this observation in future analyses. In reality, most genetic variants are of unknown clinical utility and how best to use sequence data to guide cancer treatment choices is still within the realm of academic research. There is a global need to continuously update the relationships between genomic alterations, cancer biology and the drugs that target these potential drivers. It is clear that a cooperative effort, including data sharing between institutions nationally and internationally, will help create more accurate and efficient analytic systems.

CONCLUSION

Based on the success of targeted agents in specific populations it is expected that some degree of molecular biomarker testing will be the standard of care for many, if not all cancers in the near future. There is no longer a question of whether genome sequencing should or can be done, it is a question of who should do it, on whom, at what time points, on which specimens and with what tools.

The optimal use of next-generation sequencing technology beyond the research setting is a critical question that needs to be explored and developed in an academic setting, or industry and private enterprise will define its use for us; we have a responsibility to educate ourselves, our colleagues, and our patients to best integrate this powerful tool into health care.

In a clinical oncology hospital, POG has demonstrated the feasibility of this approach, addressed these issues, identified challenges, and outlined a path forward. Ultimately, the eventual accumulation of thousands of comprehensive genome and transcriptome sequences, matched to therapies and relevant clinical outcomes, will change cancer medicine and provide both more effective and possibly less toxic therapeutic options to our patients.

ADDITIONAL INFORMATION

Ethics Statement

The study was approved by the University of British Columbia Research Ethics Committee (REB# H12-00137) and written informed consent was obtained from each patient prior to genomic profiling. Patient identity was anonymized within the research team and an identification code was assigned to the case for communicating clinically relevant information to physicians. The patients consented to potential publication of findings. Raw sequence data and downstream analytics were maintained within a secure computing environment.

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Competing Interest Statement

The authors have declared no competing interest.

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