PANOPLY: Omics-guided drug prioritization method tailored to an individual patient

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ABSTRACT

Purpose The majority of cancer patients receive treatments that are minimally informed by omics data. Our goal was to develop a precision medicine computational framework (PANOPLY**:** **P**recision c**a**ncer ge**no**mic re**p**ort: single samp**l**e inventor**y**) to identify and prioritize drug targets and cancer therapy regimens.

Methods The PANOPLY approach integrates clinical data with germline and somatic features obtained from multi-omics platforms, and apply machine learning, and network analysis approaches in the context of the individual patient and matched controls. The PANOPLY workflow employs four steps (i) selection of matched controls to the case of interest (ii) identification of case-specific genomic events (iii) identification of suitable drugs using the driver-gene network and random forest analyses and (iv) provide an integrated multi-omics case report of the patient with prioritization of anti-cancer drugs.

Results The PANOPLY workflow can be executed on a stand-alone virtual machine and is also available for download as an R package. We applied the method to an institutional breast cancer neoadjuvant chemotherapy study which collected clinical and genomic data as well as patient-derived xenografts (PDXs) to investigate the prioritization offered by PANOPLY. In a chemotherapy-resistant PDX model, we found that that the prioritized drug, olaparib, was more effective than placebo at treating the tumor ($P < 0.05$). We also applied PANOPLY to in-house and publicly accessible multi-omics tumor datasets with therapeutic response or survival data available.

Conclusion In summary, PANOPLY prioritizes drugs based on both clinical and multi-omics data, and it can aid oncologists in their decision-making to effectively treat an individual patient.

INTRODUCTION

There has been substantial progress in the fight against cancer; however, cancer remains the second leading cause of death in the United States [1]. A major focus of cancer research has been the identification of oncogenic drivers and the development of drugs that selectively target those driver events. This approach has led to the development of agents that have been shown to successfully target the driver mutational events such as: trastuzumab, which targets *HER2+* breast cancer [2,3], imatinib, which inhibits the *BCR-ABLl* tyrosine kinase produced by the Philadelphia translocation in chronic myelogenous leukemia [4], vemurafenib for the treatment of *BRAF* V600E mutant malignant melanoma [5], agents targeting *EGFR* mutations non-small cell lung carcinoma and [6], and crizotinib for non-small cell lung cancer with *ALK* rearrangements [7].

The mapping of the human genome has opened the door to the exploration of the tumor and environmental features to uncover the drivers of cancer and its resistance to treatment. A number of commercial gene sequencing platforms (e.g., Foundation One, Ambry Genetics) have been developed to identify tumor mutations used in clinical decision making. Most of these platforms are focused on detecting a limited number of gene abnormalities in specific genes and do not include comprehensive "multi-omics" data analysis. For many tumor types, this leads to the inability to link mutational drivers with druggable targets. A pressing need exists for better approaches to identify right drugs for an individual patient utilizing multi-omics data. While most of the studies utilize single omics data type to predict drug response, there are algorithms that use two or more genomic features to predict drug response in cancer cell lines [8-10]and in The Cancer Genome Atlas (TCGA) subsets[11]. There are databases such as MD Anderson's Personalized Cancer Therapy [12], Vanderbilt's My Cancer Genome [13], the Broad Institute's TARGET [14], TCGA [15], and the Catalogue of Somatic Mutations in Cancer (COSMIC) [16] containing information on the frequency of alterations in thousands of patients with cancer. Programs such as DriverNet [17], IntOGen [18], analyze a single type of omics data, such as somatic mutations, to identify potential driver genes. Other programs, such as XSeq [19], OncoRep [20], OncoIMPACT [21], and iCAGES [22] integrate on somatic mutations and/or copy number

alterations (CNAs), and/or gene expression. Although integrating these data types represents substantial progress toward the full molecular characterization needed for precision cancer care, no comprehensive method for integrating clinical and multi-omics data has yet been developed and validated for selecting the most compatible agents for a given patient's –omics profile. Thus, we have developed a workflow called PANOPLY (**P**recision c**a**ncer ge**no**mic re**p**ort: single samp**l**e inventor**y**) that identifies molecular alterations that are unique to a cancer patient compared to matched-controls with similar disease characteristics who had a favorable clinical course and then performs a comprehensive, integrated multi-omics analysis to identify druggable genomic events for an individual's tumor. The results are summarized in a report which the patient's medical oncology team can use the data to choose the particular agent to be administered.

In brief, PANOPLY uses machine learning and knowledge-driven network analysis to analyze patient-specific alterations (CNA, germline and somatic alterations from DNA, and RNA gene expression and expressed mutations) driving oncogenesis and prioritizes drugs which target the networks and pathways associated with these cancer-driving alterations. We describe the workflow and provide examples using both institutional and publicly-available datasets where PANOPLY was used to identify drugs for individual patients and subgroups of patients whose disease is resistant to standard chemotherapy. We validated these drugs in a patient with chemo-resistant triple-negative breast cancer (TNBC) using patient-derived xenografts (PDXs) by testing the top drugs for that patient.

MATERIALS AND METHODS

Data Sources

Simulation Data: Data was simulated from multivariate normal (MVN) distribution for samples using the mean and covariance structure based on 20 non-cancer tissue samples. The samples were collected by the Susan G. Komen Tissue Bank (phs000644.v1.p1) and SOLID RNA-Seq data was normalized and provided by Dr. Milan Radovich from Indiana University[23].

TCGA with a clinical follow-up: Clinical and processed multi-omics data for breast and colon cancers were downloaded from Synapse and CGHub [24] and other TCGA repositories. Specifically in this study, we utilized 1) 94 breast cancer normal-adjacent samples gene expression data 2) Normalized copy number and gene expression TCGA colon cancer (COAD) data (https://www.synapse.org/#!Synapse:syn274420). We restricted the COAD data to male patients age 45-70 with Stage II or higher disease. We selected a patient who died within 155 days of initial diagnosis of colon cancer and compared with five disease-free matched-controls.

Mayo Clinic Neo-Adjuvant Triple Negative Breast Cancer (TNBC) Patients (BEAUTY Study): We utilized 36 basal TNBC patients from a neoadjuvant clinical trial [25] for whom DNA, RNA sequencing and drug response to chemotherapy was available. As described in Goetz et al.2017, there were 19 chemo-sensitive and 17 chemo-resistant TNBC patients. PDX models were established for 16 TNBC patients before chemotherapy[26], which were used for experimental validation of PANOPLY.

Curated Knowledge Datasets for PANOPLY Workflow: We used the three sources to curate cancer-specific knowledge networks in PANOPLY; briefly, 844 cancer genes [27-29] described in Figure_S1, and gene-gene interactions [30] and cancer drug-gene interactions [31] are described in the Supplementary Methods.

PANOPLY workflow

The PANOPLY workflow is available for download as an R package from http://bioinformaticstools.mayo.edu/research/panoply/. A high-level overview of the workflow is shown in Figure 1, which we briefly describe below. The details of each step are provided in the Supplementary Methods.

Input: The minimum input required for PANOPLY is normalized gene expression data for a patient and for a set of clinically similar cancer patients who differ in regards to the phenotype of interest (e.g., response or survival time). Multi-omics data for patients such as CNA, somatic mutations, and/or germline variants, and expressed variants can be provided as additional inputs to PANOPLY.

Steps:

1. *Selection of matched controls for a case:* For a patient of interest, the user selects a matched set of controls defined by the phenotype of interest. Matching algorithms are not provided within the workflow, but we provide examples of how we constructed for our studies.
2. *Patient-Specific Genomic Events*: Using the pre-processed data provided (mandatory: expression data, and optional: somatic, germline, copy number, and/or expressed alterations), patient-specific genomic alterations that are different from the matched-controls are identified.

3. *Identification of suitable drugs using driver-gene networks and random forest analyses:* We apply two network-based tests for drugs to be prioritized for the patient: Drug Network Test (DNT) and Drug Meta Test (DMT). The sum of the negative log10 p-values from these tests is provided as a score for each drug (P.score). In addition, we apply random forest method to identify multi-omics features that distinguish the case from the matched-controls, which we aggregate by drugs that target those events into a score (RF.score). These tests are detailed in the Supplementary Methods.
4. Integrated Multi-Omics Case Report: The integrated multi-omics and drug prioritization data are presented as actionable information to a clinician.

False Positive Rate (FPR) Simulations

We used 94 adjacent-normal breast tissue expression from TCGA, and also simulated 500 MVN datasets from normal breast tissues to examine the false discovery rate (FDR) of drug selection against an incremental matched set of random 'matched-controls' (M=2, 4, 8, or 16) within each set (Supplementary Methods for details). We evaluated FPR under three scenarios of varying levels of correlation between the drug-gene networks and the gene-gene networks: Sc1) all gene-gene and drug-gene networks; Sc2) reduced gene-gene networks, complete drug-gene networks; and Sc3) reduced gene-gene and reduced drug-gene networks. We describe the justification for these scenarios in more detail in the Supplementary Methods, where the binary clustering of the gene-gene and drug-gene networks are illustrated in Figures S2 and S3, respectively.

True Positive Rate (TPR) Simulations

Using the same MVN simulation framework as the FPR simulations, we changed the mean expression level for a subset of genes for the cases, but not the matched-controls. Here, we chose three subsets of genes that are targeted differently by the Olaparib drug-gene network: Set-A) the

complete drug-gene network for Olaparib; Set-B) a small network of BRCA2-specific genes, and Set-C) a mixture of genes that are sub-networks of the ATM/ATR networks, which are larger than the BRCA2 network (Details in Supplementary Methods).

Validation of PANOPLY's Drug Predictions With PDXs

To validate PANOPLY's drug predictions, we tested PDX models obtained from BEAUTY study (BC_051_1_1) for a TNBC patient whose tumor did not respond to neoadjuvant paclitaxel and anthracycline/cyclophosphamide treatment. PDX tumors created with pre- and post-treatment samples were studied in female NOD-SCID mice. The tumors were grown to 200–250 mm^3, and then the mice were randomized (7 per group) into olaparib (15 mg/kg, once daily) or vehicle treatment groups. Mouse tumor size and body weight were measured twice per week, and mice were euthanized after 12 days of treatment. The difference in volume between drug- and vehicle-treated tumors was assessed using Wilcoxon rank-sum tests.

Mining multiple patient reports

We performed PANOPLY on a group of patients and applied clustering techniques to the results from the patient reports to identify a subset of patients and drugs that could fit into an on-going bucket trial. We applied non-negative matrix factorization (NMF) (38) to prioritized drugs for the cases using percentile ranking based on the combination of the RF.score and P.score. The clusters were evaluated by assessing the cophenetic and average silhouette scores, and drugs are assessed using Kim's method (38) to select the most delineating drugs. A word cloud of genes is generated using the genomic targets of the clusters of drugs associated with the top 10% of delineating drugs.

RESULTS

Statistical Performance of PANOPLY Using Simulated Data

We evaluated the FDR of PANOPLY's network tests using simulated data constructed following the mean and covariance structure of 20 healthy breast samples. Baseline performance was estimated using the complete curated-cancer network; while we evaluated reduced networks to establish the underlying bias in the network, arising from drug labeling and target annotation which result in redundant gene targets of anti-cancer therapies.

FPR (Type I error): We evaluated FPR by using both simulated and TCGA non-cancer tissue samples. We observe in Supplementary Figure S4 the FPR for DNT, DMT, and P.score are controlled near the nominal $\alpha = 0.05$ and 0.01 levels under a typical analysis with PANOPLY. We evaluated three scenarios (Sc1-Sc3) with varying correlation of the gene-gene and drug-gene networks and with varying matched-control set sizes M=2, 4, 8, and 16. A full explanation of these scenarios (Sc1-Sc3) and results are included in the Supplementary Methods. We show in Table 1A, the results for M=8 for DNT and DMT for all scenarios with the two datasets. The FPRs is slightly higher in the TCGA breast cancer normal-adjacent samples than the simulated MVN set. The DNT error rates are adequate for all three simulations scenarios Sc1-Sc3, but perhaps too conservative in Sc2 and Sc3. The DMT was observed to be higher than the nominal levels in all scenarios Sc1-Sc3 in both datasets but is much closer to the nominal level when the correlation cancer gene networks are reduced in both Sc2 and Sc3. In summary, the suggested setting for running the workflow is scenario Sc2, where only the patient-specific events are considered for testing all 374 drugs.

TPR (Statistical Power): We similarly evaluated the TPR of the Panoply workflow using just the simulated MVN data, by spiking in increased amounts of expression for a subset of genes in the Olaparib gene-drug network. We quantify power as the proportion of simulated datasets for

which the DNT and DMT p-values for Olaparib are significant in two ways: the p-value is less than $\alpha = 0.05$, and the p-value is one of the ten lowest of all drugs. As shown in Table 1B for M=8, the $\alpha = 0.05$ power scores for DNT and DMT are comparable with Gene Set-A while DNT power dramatically drops off with more realistic network-specific gene-gene networks that are activated (Gene Sets B and C), as expected. Complete results for DNT, DMT, and P.score are discussed in the Supplementary Material. Supplementary Figures S5 and S6 show the TPR is robust to the size of the reference sample population (M=2, 4, 8, and 16), the top-10 metric verifies the $\alpha = 0.05$ TPR rates, and that P.score is useful in ranking drugs that perform well across DNT or DMT.

N=1 Case Study

We applied PANOPLY workflow to prioritize drugs for a BEAUTY patient (case BC_051_1_1) who did not respond to neoadjuvant chemotherapy for whom a set of matched controls (n=9) were found among the BEAUTY TNBC patients who had a pathologic complete response (pCR) to neoadjuvant chemotherapy. The PANOPLY report for that patient is available in the Supplementary (file-Supplementary_PANOPLY_BEAUTY_Patient_Report.pdf). Below we have (1) the experimental validation of a PANOPLY-prioritized drug using PDX models and (2) comparison of PANOPLY analysis for a BC_051_1 patient with other methods.

1. *Experiment validation*: Somatic, germline mutations, CNA, gene expression, and expressed mutation data for the case was compared and contrasted with her matched controls using PANOPLY workflow. (Resulting tables for case: BC_051_1_1 are discussed in detail in the Supplementary Results). The PANOPLY results indicated that olaparib was the most promising drug for this patient (Table 2). Figure 2A shows histologic images of the case's tumor and a corresponding PDX, both from the pre-NAC and post-NAC time points. Pre- and post-NAC patient tumor, and its corresponding PDX had similar morphologic features and a triple negative staining pattern (Figure 2A). For both the pre-NAC and post-NAC PDXs, tumor volume at day 12 was significantly lower in the olaparib group than in the vehicle group

(Wilcoxon rank sum test P=0.04 and p <0.001 respectively; Figure 2B). The PDX results show promise that this approach may be successful in identifying an effective therapy for patients.

2. Comparison of PANOPLY with existing methods: Recent bioinformatics software, such as iCages and oncorep, attempt to incorporate a tabulation of anti-cancer drug options targeting observed driver genes. These softwares were developed independently and with their own design assumptions and intent. Table 3 presents a summary of these two software implementations, in comparison to Panoply. We were able to generate a similar iCages report with default settings for case BC_051_1_1, by providing required somatic mutation (VCF format) and the copy number alteration data (BED format). We were not able to configure the current architecture of the Amazon web services, required for the omics_pipe workflow (which precedes the oncorep analysis module).

Extensibility of the Workflow to Cohort Studies and Public Domain Datasets

PANOPLY Drug Predictions for Patients with Chemo-resistant, TNBC cohort: Panoply provides a prioritized drug list for each patient in the cohort. This list corresponds to a unique set of gene targets for each patient, which can be compared and contrasted with similar chemo-resistant patients using existing clustering methods. The genomic characteristics of these clusters can be reverse engineered to find qualifying genomic events which would qualify future patients for drug 'bucket' trials. An illustration of this application of PANAPOLY is provided using the 17 BEAUTY patients with chemoresistant TNBC. NMF clustering [32] was performed with the drug priority scores of these 17 cases. Based on the cophenetic and average silhouette scores, two clusters were selected to be optimal. The percentile ranking of top 10% (35/344) drugs was aggregated per sample cluster using the median score and presented as a heatmap (Figure 3A). The target genes of the drug clusters were collated, and a word cloud was generated with the targets (Figure 3B). As shown in Figure 3A, the cluster 1 consists of nine samples; the patients in that

group primarily consist of kinase-inhibitors as their top prioritized drugs (drugs=16) and the drugs in that cluster can predominantly target the PIK3CA-mTOR-AKT signaling pathways. The other cluster from Figure 3A consists of a set of prioritized drugs (drugs=19) for eight patients, as shown in Figure 3B these drugs can primarily target genes associated with cell cycle control, specifically targeting the histone deacetylases (*HDAC1*) and the Aurora kinases A and B.

PANOPLY drug predictions for public dataset such as TCGA with molecular and clinical data: In here, we present the capability of PANOPLY workflow to be executed with publically available datasets. An example COAD patient's (TCGA-AA-3488) report is discussed briefly below and can be obtained from Supplementary_PANOPLY_TCGA_COAD_Patient_Report.pdf. The case displayed 645 driver genes: 226 CNAs not present in the case; in addition, 419 genes were over-expressed in the case tumor sample relative to the matched-control samples. Of the 226 CNAs, 113 were amplifications, and 113 were deletions. Of those BRAF, CDKN2A, CDKN2B, FGF10, IL7R, INHBA, JAK2, KEL, MAFA, NTRK1, NTRK2, PIK3CA, PRSS1, RBL1, SKIL, SMO, SOX2 and SPTA1 cancer-related genes were both amplified and overexpressed. Of the case's 645 events, 53 genes are differentially expressed between the case and matched-controls and can be targeted by antineoplastic drugs. Based on the network and random forest analysis of driver genes and gene expression data, PANOPLY ranked the following drugs lestaurtinib (JAK2, NTRK1, NTRK2), LY2784544 (JAK2), GDC-0032(PIK3CA), NVP-BGT226 (MTOR, PIK3CA), regorafenib (MAPK11, RAF1, BRAF, KRAS, KIT, FGFR1/2,PDGFRA/B, ARAF, KDR, EPHA2, ABL1, NTRK1, CYP3A4, CYP2C8/9, CYP2B6, CYP2C19,ABCB1, ABCG2, UGT1A1/9,FLT1/4, RET, TEK and DDR2) and ARQ736 (BRAF) as significant for patient TCGA-AA-3488 with significant P.score and RF.score (Table 4).

Similarly, we have also applied PANOPLY to TCGA breast cancer data (BRCA), and the exemplary report was prepared for a TCGA tumor (TCGA-AR-A1AR) sample and is presented in the **Supplementary.**

DISCUSSION

In creating PANOPLY, our goal was to develop a flexible workflow capable of analyzing multiple forms of -omics and clinical data to identify driver genes, their effects on gene networks and the drugs capable of targeting altered gene networks in cancer patients. Using gene expression data, CNA, and DNA variants from publicly available and in-house datasets, we demonstrate that PANOPLY holds promise in identifying agents capable of targeting driver gene–induced changes, both for individual patients and for subgroups of patients that share a cancer subtype and response pattern. This is evident in the example where PANOPLY's prioritization of olaparib as a promising treatment for a patient with chemoresistant TNBC and that agent was found to reduce tumor size when applied to that patient's xenografts. When the same patient's data was run through iCages, the drug with the highest iCageDrugScore (0.52) was Doxorubicin, the drug administered as part of the patient's NAC regimen that failed produce a pCR. While Olaparib scored (1.3×10^{-4}) much lower among potential agents. Doxorubicin intercalates DNA and thereby indirectly targets TP53 (TOP2A), which is observed in a substantial proportion of cancer patients. Co-considering associated genes involved with DNA damage repair and/or active cellular uptake would presumably provide a more accurate prediction of drug efficacy, as implemented by Panoply.

The PANOPLY workflow currently analyzes the patient's molecular and clinical data together along with the knowledge databases such as Reactome, DGI-db, and others for drug-gene network analysis. This represents a substantial advancement relative to existing programs, such as XSeq [19], OncoRep [20], and OncoIMPACT [21], which integrate only molecular data such as somatic mutations or CNAs, and gene expression. Currently, medical oncologists have access to genomics reports generated a limited target panel for decision making. Working closely with clinicians, basic scientists, and pharmacologists, we have developed PANOPLY to integrate molecular, clinical, and drug data to prioritize targets

and facilitate individualized treatment for the patients. Using clinical and molecular profiles of the patient's disease, PANOPLY provides a personalized list of prioritized drugs along with links to literature concerning drug efficacy. The oncologist will still have to go through the list and refine drug selection based on the inherent clinical knowledge, ancillary clinical trials, and insurance coverage availability. Another limitation of PANOPLY is that the method cannot delineate the clinical effectiveness of a similar class of drugs. We plan to accomplish this in future by bringing in drug knowledge such as chemical structure, molecular size, and drug dosage. Our method is dependent on drug-gene target annotations that are heavily biased by product literature and databases. Moreover, clinical translation of PANOPLY results is constrained by the cost-effectiveness in developing PDXs for drug validation.

PANOPLY is a flexible framework that can integrate many other types of –omics data, including protein expression, methylation expression, structural variants, circular RNAs, long non-coding RNAs, and fusion data with modifications to its code. PANOPLY's framework can also be extended to the metastatic setting; additional clinical data such as prior exposure to drugs, features of primary and recurrence disease would be required. Additional genomics data sets are needed to modify the existing approach for metastatic tumors.

As more is learned about the molecular underpinning of cancer using various resources, we plan to expand our knowledge base to improve PANOPLY's predictions using PDX and cell-lines. We have validated PANOPLY's predictions in a single patient at present using PDXs, in future, we plan to validate PANOPLY-predicted drugs in PDXs derived from additional patients. Like several other groups and we have shown, PDX models faithfully represent tumor biology [26,33], so these results should provide insight into PANOPLY's reliability. In conclusion, our results indicate that combining multiple sources of -omics and clinical data to predict promising agents for a patient or groups of patients with cancer is feasible. With further validations, PANOPLY can be a tool to help clinicians in their decision-making process.

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Table 1A. FPR rates for DNT and DMT test p-values at $\alpha = 0.05$ using simulated MVN (500 sets) and TCGA normal-adjacent data (94 sets), for matched control size M=8, and scenarios Sc1-Sc3

Scenario	Drivers	Drugs	Test	False Positive Rate	
				Null	
				MVN	TCGA
Sc1: All Driver Genes and Drugs	429	374	DNT	0.029	0.04
			DMT	0.231	0.208
Sc2: Patient Observed Driver Genes, all Drugs	120	374	DNT	0.031	0.038
			DMT	0.065	0.104
Sc3: Reduced Correlation Drivers and Drugs	175	117	DNT	0.008	0.01
			DMT	0.08	0.088

Table 1B.TPR rates of finding Olaparib as a drug target from DNT and DMT test p-values at $\alpha = 0.05$ using simulated MVN (500 sets) data for matched control size M=8, scenarios Sc1 and Sc2, and up-regulated gene sets A, B, and C.

Simulation Scenario	Drivers	Drugs	Test	True Positive Rate (Power)					
				Set A		Set B		Set C	
				MVN (sd 2)	MVN (sd 3)	MVN (sd 2)	MVN (sd 3)	MVN (sd 2)	MVN (sd 3)
S1: All Driver Genes and Drugs	429	374	DNT	0.582	0.926	0.030	0.062	0.108	0.294
			DMT	0.522	0.588	0.496	0.542	0.498	0.544
S2: Patient Observed Driver Genes, all Drugs	120	374	DNT	0.612	0.942	0.038	0.082	0.132	0.286
			DMT	0.390	0.410	0.320	0.326	0.528	0.590

321

Table 2: Top drugs recommended by PANOPLY for BEAUTY patient (BC_051_1_1) with chemoresistant triple-negative breast cancer and PDX model.

Drug	Case Driver Genes[a]	Number of Pathways[b]	P.score	RF.score
OLAPARIB	ATR,ATM,BRCA2,BRCA1	43	3.009632	0.003743
BMN673	ATR,ATM,BRCA2,BRCA1	32	2.70456	0.004679
AMG900	AURKB,AURKA	8	2.610079	0.001857
AZD7762	CHEK1,CHEK2	15	2.506379	0.001786

[a] Genes on PANOPLY's cancer gene list that are targeted by FDA-approved drugs

[b] Number of pathways that may be affected, based on drug-gene target interactions

Table 3: Comparison of oncorep, iCAGES and Panoply methods

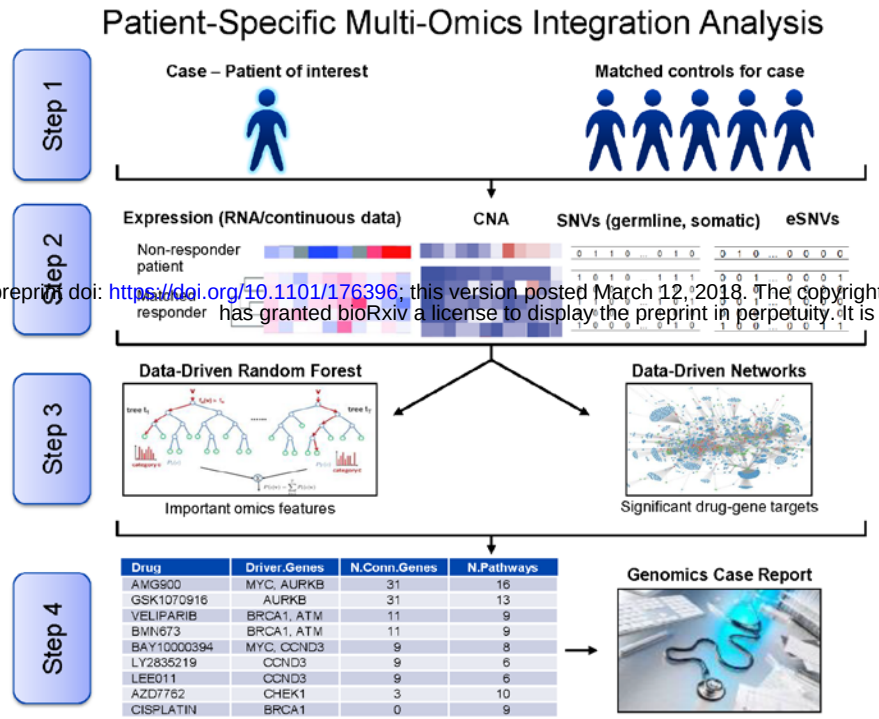
	oncorep	iCAGES	Panoply
Single Patient Analysis	✓	✓	✓
Pre-compiled Reference Population	✓	✓	
Driver Gene Identification	✓	✓	✓
Driver Gene Prioritizatoin		✓	✓
Multiple RNA features (eSNV, fusion, mutations, etc)	Derived		Optionally Provided
Data Input	RNA-Seq	Somatic, Copy number data	Multi-omics
Drug Association	✓	✓	✓
Drug Prioritization		✓	✓
Machine Learning		✓	✓
Summary Report	✓	✓	✓
Pathway Analysis	✓		
Network Analysis		✓	✓
Breast Cancer Specific	✓		
Installation ease	Difficult	Easy	Easy
Extensibility	Moderate	Low	High

Table 4. PANOPLY alternate drug predictions for Colon Cancer TCGA Patient (TCGA-AA-3488) based on molecular data.

Drug	Case Driver Genes[a]	Number of Pathways[b]	P.score	RF.score
LESTAURTINIB	JAK2,NTRK1,NTRK2	25	4.9153	0.0014
LY2784544	JAK2	21	4.2668	0.0017
GDC-0032	PIK3CA	49	4.1257	0.0022
NVP-BGT226	PIK3CA,MTOR	67	3.9725	0.0019
REGORAFENIB	KIT,KRAS,FLT4,NTRK1	166	3.1395	0.0018
ARQ736	BRAF	23	3.0511	0.0025

[a] Genes on PANOPLY's cancer gene list that are targeted by anti-cancer drugs

[b] Number of pathways that may be affected, based on drug-gene target interactions



354

355 **Figure 1. A high-level overview of PANOPLY.** Step 1: A patient of interest is compared with matched-controls.
356 Step 2: For each subject, gene expression data, copy number alteration (CNA), single nucleotide variant (SNV), and
357 expressed single nucleotide variant (eSNV) data will be provided to identify case-specific driver alterations. Step 3:
358 Multi-omics data will be provided to the random forest and network analysis methods to identify the top prioritized
359 drugs to target genes that are driving oncogenesis in the patient. Step 4: A genomics case report listing the drugs to
360 prioritize, based on their ability to target driver mutations and their dysregulated gene networks, is generated for
361 researchers and clinicians.

362

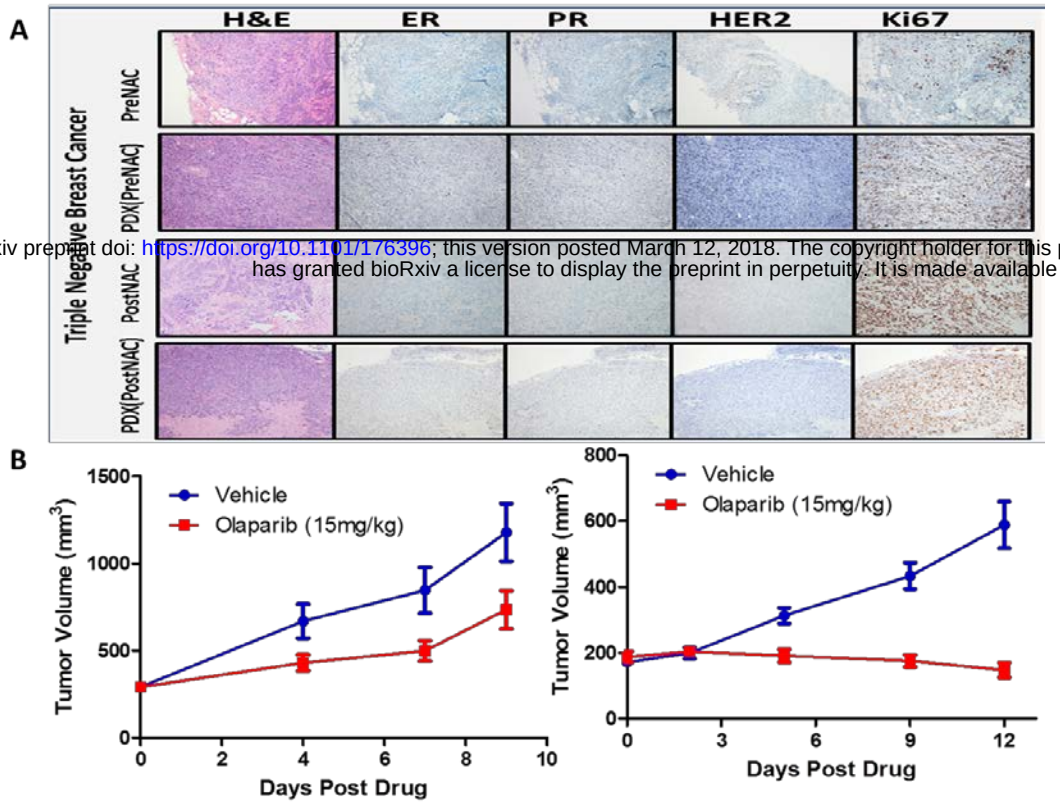
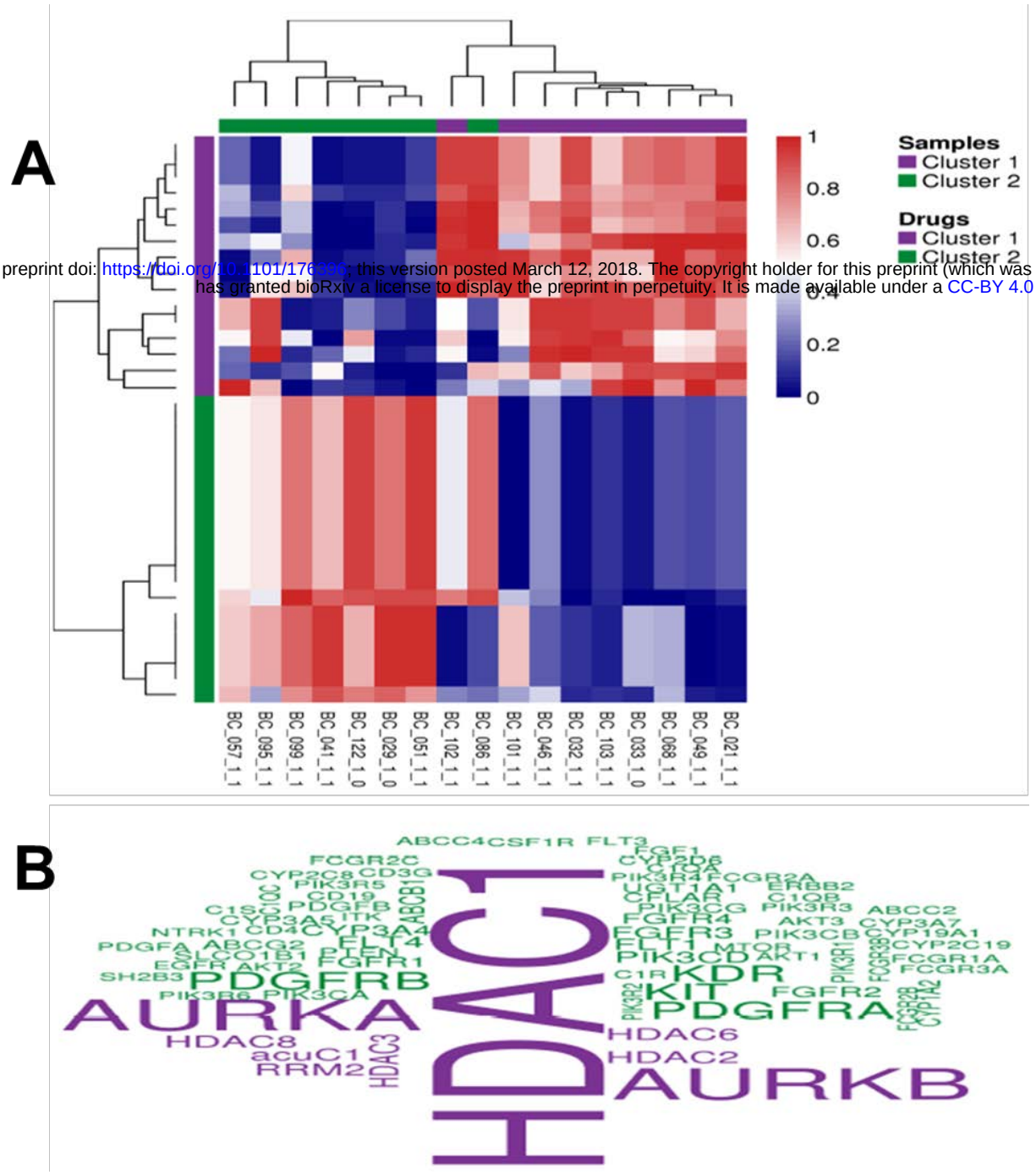


Figure 2. Patient-derived xenografts (PDXs) validate PANOPLY's prediction that olaparib is an effective treatment for a patient with chemoresistant TNBC (BC_051_1_1). A) The top panel shows histological stains of the patient's tumor and PDXs (pre or post-treatment). B) Cytotoxicity data shows the PDXs response to the top predicted drug olaparib compared to the no treatment (the left plot shows the olaparib drug response data from pre-treatment mice, whereas the data from the right shows the data from post-treatment PDX models. Both the datasets were generated using the Vehicle as controls).



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373 **Figure 3**. **Clustering and word cloud plots of 17 TNBC chemotherapy-resistant patients**. A) Two-way
374 hierarchical clustering of the top 10% of the drugs predicted by PANOPLY for 17 basal TNBC patients. The
375 heatmap shows that there are two sample and drug clusters are implicated in the NMF clustering analysis. B) Word
376 cloud of the target genes from the two drug clusters predicted by the NMF analysis.

377