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OncoDynamiX: A Novel Mechanism and Machine Learning Based Integrated Technology Platform for Selection of Cell Lines for Screening Compounds in Cancer Drug Discovery

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Abstract

Background: *Cancer cell lines are fundamental in vitro preclinical models that are used extensively to screen anticancer compounds at early stages of drug discovery. Presently, cell lines are chosen either empirically or based on expression of the target. However, the activity of a drug not only depends on expression of its pharmacological target but also on functional status of upstream regulators and downstream effectors of the target. OncoDynamiX is a novel platform that integrates machine learning with drug target pharmacology networks (DTPNs) and genomics to identify appropriate cell lines for screening.*

Objective: *To demonstrate the selection of appropriate breast cancer cell lines which were sensitive to the Cyclin Dependent Kinase 4/6 (CDK4/6) inhibitor Palbociclib using OncoDynamiX.*

Method: *Forty two breast cancer cell lines were selected from a panel of sixty two that had associated genomic data, and at least one component of the DTPN. A subset of thirty from the forty two cell lines were selected for prediction of sensitivity and resistance to Palbociclib.*

Results: *The predicted results were in agreement with experimental data from published literature. Seventeen cell lines that were sensitive to Palbociclib had combinations of alterations in the DTPN that contributed to the sensitivity to the drug. The biomarkers predictive of sensitivity and resistance to Palbociclib were identified.*

Conclusions: *OncoDynamiX is a versatile platform that can be effectively used in rational selection of appropriate cell lines for compounds in cancer drug discovery, with significant savings in time and resources.*

Key words: *Breast Cancer, Cancer Cell lines, Drug Discovery, Genomics, Machine Learning, Palbociclib*

List of Abbreviations

AP1 = Jun protooncogene, AP1 transcription factor subunit

CDK4 =cyclin dependent kinase 4

CDK6 =cyclin dependent kinase 6

CCND1 = cyclin D1

CCND2 = cyclin D2

CCND3 = cyclin D3

CCLE = Cancer Cell Line Encyclopedia

CNA =Copy number alterations

CDKN2A = cyclin dependent kinase inhibitor 2A

CDKN2B = cyclin dependent kinase inhibitor 2B

CDKN2C = cyclin dependent kinase inhibitor 2C

CDK2 = cyclin dependent kinase 2

CCNA1 = Cyclin A1

CCNE1 =Cyclin E1

CCNE2 = Cyclin E2

CDK1 = cyclin dependent kinase 1

CCNB1 = cyclin B1

DTPN = Drug Target Pharmacology Network

ESR1 =estrogen receptor 1

E2F1 = E2F transcription factor 1

FAT1 = FAT atypical cadherin 1

FOXM1 = forkhead box M1

GOF = Gain of Function

GDSC = Genomics of Drug Sensitivity in Cancer

HER2 = Erb2 receptor tyrosine kinase 2

KD = Knock Down

LOF = Loss of Function

MLE = Maximum Likelihood Estimator

MYC = MYC protooncogene, bHLH transcription factor

NFKB1 = nuclear factor kappa B subunit 1

OE = Over expression

p21 =cyclin dependent kinase inhibitor 1A

RB1 = RB transcriptional corepressor 1

TK1 = thymidine kinase 1

Introduction

Drug discovery and development, particularly in cancer, is a complex process with high attrition rates in late stages of clinical testing **[**1**]**. One of the main reasons for the high attrition is the poor translation of results from preclinical models to the clinic **[**2**]**. At present, significant gaps still exist in the translation of preclinical data to drug efficacy in the clinic. There is an urgent need for predictive preclinical models that can help in better translation to the clinic. Cell lines derived from human cancers serve as basic preclinical models of cancer for evaluating anticancer activities of new chemical entities (NCEs) at early stages of drug discovery such as lead identification **[**3**]**. The basic assumption in using cell line models is that it reflects the molecular aberrations observed in cancer patients, and inhibition activities observed on cell lines are predictive of in vivo efficacy in animal models and in human cancer patients. In the traditional workflow, the selection process is performed either empirically wherein large panels of cell lines are treated with compounds or in cell lines where the target of interest is expressed normally or aberrantly, followed by selection of those cell lines which are most sensitive to treatment. In target based programs,

cell lines which express the target abnormally (for example, hyperactivation due to mutations) or normally are selected to screen compounds to determine their pharmacological activity. The results obtained from the screen form the basis for a) identification of cancer types and indications in which the compound classes are most likely to work b) identification of mechanism of action and biomarkers, c) lead optimisation. This approach yielded clinical candidates that went on to be approved for treatment of different cancers as targeted therapies. Yet, most of the approved targeted therapies show high variability in clinical response and many do not show efficacy in pivotal clinical trials **[**1**]**. Although pharmacokinetics and toxicity are important factors, the variability in efficacy has been attributed to differences observed in molecular alterations in patients which were not accounted for in cell lines **[**4**]**. Thus rational selection of human cancer cell lines in which compounds from a chemical class are most effective is needed for better translation. Many approaches have been described for both assessment and prediction of anticancer activities of test compounds and drugs in cancer cell lines based on genomics, multiomics and machine learning methods **[**5, 6, 7, 8, 9**]**. But none of them address an important aspect of pharmacological action, which is the drug target related pathway itself. We propose that the presence of the target as wild type, activated or over expression is not always adequate for efficacy of the drug, but also on the alterations of upstream regulators, downstream effectors of the target and parallel pathways.

OncoDynamiX is a novel mechanismbased platform that integrates machine learning with drug pharmacological target pathway and genomics to guide rational selection of cancer cell lines for screening of compounds in cancer drug discovery. In this study, we demonstrate the application of OncoDynamix to select the appropriate breast cancer lines for evaluating Palbociclib as an inhibitor of CDK4/6 and highlight up and downstream pathways that determine its pharmacological effect.

Materials & Methods

For the purposes of demonstration of proof of concept of the Onco DynamiX platform to select appropriate breast cancer cell lines, CDK4/6 and Palbociclib (P) were selected as the prognostic target and inhibitor, respectively.

Cyclin dependent kinases (CDKs), a group of serine/threonine kinases, are central to controlling cell cycle activity **[**10, 11**]**. In the normal cell cycle, upon activation by Cyclin D1, the CDKs 4 and 6 facilitate the cellular transition from the G0/G1 checkpoint phase to the S phase. Increased CDK4/6 activity, either by overexpression of Cyclin D1 or loss of negative regulators, leads to pathogenic uncontrolled cellular

division [12]. Inhibitors of CDK 4/6, such as Palbociclib, act by binding to Cyclin D1 and inhibiting the catalytic activity of CDK4/6, thus arresting the cell in G1 phase and inhibiting tumor cell proliferation [13].

OncoDynamix Platform: The workflow of OncoDynamix is shown in figure 1. The details of how it works are described below.

Genomic data processing and functionality prediction: Processing of genomic information from cell lines was done using a combination of data mining, analytics and machine learning to create genomic signature datasets that feeds into the Drug Target Pharmacology Network (DTPN) creation (described later). OncoDynamiX uses a rigorous molecular dynamics based simulation method to create a large volume of energy fluctuation data against a background of known mutation functionality library and uses the energy fluctuation signatures to create a Maximum Likelihood Estimator (MLE) model that predicts functional traits and intensities in mutated proteins. The copy number alterations and methylation signatures, where available, were processed using normalization techniques. Specific biological rules were then applied to filter perturbations that feed into the final genomic finger print of the cell line or patient.

Construction of a Drug Target Pharmacology Network (DTPN) linking Drug Target Pathway Genomics for Palbociclib : The DTPN is a drugspecific pathway constructed around the pharmacological target(s). The DTPN for Palbociclib was constructed using CDK4/6 as drug targets. The DTPN has upstream activators and inhibitors of target, effectors downstream of target, parallel pathways, bypass mechanisms, and pathway crosstalk that potentially influence the activity of the drug (figure 2).

Query Building: Queries are genomic signatures that are known to be significantly correlated with pharmacological response of cell lines or patients to a specific drug. Queries were used to create nD combinations with associated response in cell lines or survival ranking in patients. The ranking was computed as a function of the ratio of median survivals from the stratification program (described later). This created a range of responses from very high to very low and graduations between. For each section of response ranking, the query was performed on all cell lines or patient populations and cell lines matching the queries were grouped. For retrospective studies, the observed response (experimental data from literature) for a group of cell lines was compared with the ranking matrix to check for correlation between the predicted and observed responses to Palbociclib. Genomic alterations (mutation, copy number variations) known to influence the efficacy of Palbociclib on the cell lines were included in the DTPN.

Query for Breast Cancer Cell Lines: An algorithm based selection was carried out to select appropriate breast cancer cell lines from the CCLE (Cancer Cell Line Encyclopedia) [14]. Query genes that had genomic alterations associated with sensitivity (positive biomarkers) and resistance (negative biomarkers) to Palbociclib were identified. They were further classified based on type of alteration, their hierarchy in the DTPN and functional significance of their alteration on activity of CDK4/6 (pharmacological target). Sixty two breast cancer cell lines with genomics data were available in CCLE, out of which forty two cell lines had alterations in at least one component of the DTPN.

Prediction of sensitivity and resistance of breast cancer cell lines to Palbociclib: Sensitivity and resistance scores were predicted for the forty two cell lines based on alteration of genes and their hierarchy (upstream or downstream of drug target) in the DTPN. Positive and negative biomarkers were assigned with positive and negative scores, respectively, with equal weight for all genes (in altered form), to calculate a drug sensitivity score (cumulative score of all the alterations in positive biomarkers) and resistance score (cumulative score of all the alterations in negative biomarkers).

Selection of breast cancer cell lines for in vitro screening: A sub set of thirty cell lines from the forty two cell lines were selected by OncoDynamiX for in vitro screening. The subset was a representation of the global cohort (42 cell lines) in terms of alteration of all genes (part of query) and patterns of alterations of genes (combination of alterations) related to the DTPN. The predicted response (intrinsic sensitivity and resistance scores) of breast cancer cell lines to Palbociclib was compared with the observed response (in vitro potency (IC50) of Palbociclib on cell lines from literature.

Stratification: OncoDynamiX utilized a novel stratification algorithm for identifying genomic signatures that contributed to sensitivity or resistance of cell lines to Palbociclib. The stratification process is an unsupervised feature identification algorithm that looks at statistically significant perturbations in populations associated with a high deviation of output variable in this case the survival function. The indication specific cell lines were queried from the database. The algorithm then identified high frequency mutations and other genomic perturbations, to create nD combinatorial queries on the cell lines. For each unique query, a subset population was identified and the median response was calculated for both the residual and the queried subset. Subgroups of statistical significance, showing high deviation from the background population were selected for query building and biological confirmation tests.

The inverse IC50 values from highthroughput drug sensitivity data of cell lines from Genomics of Drug Sensitivity in Cancer (GDSC) [15] were used to identify mutations, copy number alterations and mRNA

expressions that were associated with drug sensitivity and resistance. Results are shown as response curves, for a population with alterations (mutation, CNA, mRNA expression) versus a population with a wild type gene or protein of interest. All identified statistically significant genomic alterations of genes (efficacy biomarkers) associated with drug sensitivity and resistance, were further filtered and validated based on biological rationale. The hypothetical survival metric for a cell line or patient was calculated as a linear transformation of the inverse of the IC50 for a given therapy. The overall range of the survival was normalized between the values 0 and 1. While the response to a drug is a complex function of many factors, the IC50 (drug potency) is a significant index of therapeutic response. Hence, we used the reciprocal of IC50 as the main determinant for response. The predicted survival curve generated by OncoDynamiX is similar to the KaplanMeier survival function. This was calculated by range normalizing the inverse IC50 values of the total cell line population and determining the subset of the cell line population falling within the specific inverse IC50 range. A higher median value of a subset of cell lines translated to a higher average therapeutic benefit compared to the global cell line dataset.

The algorithm for stratification is shown below.

Algorithm for Stratification

For each cell line: Responsivity = (1/IC50) – (min(1/IC50))/ (max(1/IC50) – (min(1/IC50))

Generating global response curve

R = 0

R_max = 1.0

a = 0.05

for each cell line: remove if (1/IC50 < R+a) > Repeat till R+a = R_max

R = R+a

perc((R+a)) = (total cell lines left / total cell lines) * 100

Repeat for subset

If median(subset) / median(global) > epsilon: significant

epsilon > cutoff factor

Results and Discussion

The DTPN for Palbociclib is shown in Figure 2 and the individual molecular components of the DTPN are summarized in Table 1. Changes in functionality of each component upstream (regulators such as CCND1, CCND2, and CCND3) of the drug target (CDK4/6) or in the drug target itself, as a result of over expression, leads to hyper activation of CDK4/6 leading to increased cell proliferation. Loss of function mutations, deep deletions and hyper methylation affect the functions of CDKN2A, CDKN2B and CDKN2C which negatively regulate CCND1, CCND2, and CCND3 leading to upstream down regulation. Overexpression of transcription factors of CCND1, such as AP1, MYC, NFKB1 and ESR1 also result in hyper activation of CDK4/6. Activation of components in parallel pathways such as CDK2, CCNE1, CCNE2, and CCNA1 because of overexpression can lead to phosphorylation of RB1, the main effector protein for CDK4/6, leading to hyper activation of the main pathway. RB1 inactivation due to loss of function mutations, deep deletions and hyper methylation can lead to CDK4/6 independent activation of the pathway. Over expression of FOXM1, the transcription factor which transcribes genes required for the transition from G2–M phase of cell cycle can form a parallel pathway to activate the CDK4/6 pathway independently. Overexpression of downstream effectors such as CDK1 and CCNB1, both of which are required for mitotic entry, activate the CDK4/6 pathway independently. Overexpression of E2F1 (negatively regulated by RB1), and TK1 (transcribed by E2F1) can also activate the CDK4/6 pathway independently. Taken together, it is evident that the pharmacological activity of Palbociclib not only depends on the presence or hyper activation of CDK4/6 but also on the functional status of the upstream regulators, downstream effectors and parallel pathways. Hyper activation of downstream effectors and parallel pathways independent of CDK4/6 can result in cells becoming resistant to Palbociclib. It is conceivable that breast cancer cell lines can consist of different combinations of molecular alterations in the DTPN of Palbociclib, and thus may respond differently to the drug.

The list of breast cancer cell lines, identified by OncoDynamiX, with at least one alteration in the DTPN is shown in Table 2. Many cell lines showed alterations in regulators and effectors of the DTPN some occurring simultaneously, some exclusively upstream and some exclusively downstream.

OncoDynamiX selected thirty one breast cancer cell lines which were a representation of a global set, in terms of alterations of genes and combination of alterations of genes that are part of the DTPN. The predicted scores for sensitivity and resistance of the cell lines to Palbociclib based on the alterations of genes in the DTPN are summarized in Table 3.

The genes queried in the DTPN and biomarkers predictive for sensitivity and resistance to Palbociclib in breast cancer cell lines are shown in Table 4. The biomarkers predictive of sensitivity of cell lines to Palbociclib were CDK4, CDK6, CCND1, CCND2, CCND3, CDKN2A, CDKN2B, and CDKN2C; and the biomarkers predictive of resistance to Palbociclib were RB1, CCNE1, CCNE2 and FOXM1.

The predicted and observed responses for thirty breast cancer cell lines to Palbociclib are shown in Table 5. A majority of the cell lines with higher predicted sensitive scores were observed to have lower IC50 values (indicating higher drug sensitivity) as against cell lines with high predicted resistance scores, have higher IC50 values (indicating drug resistance). Twenty out of twenty nine cell lines (69%) were predicted to be sensitive to Palbociclib (≤500 nM); nine out of twenty nine cell lines were predicted to be resistant to Palbociclib (>500 nM).

Stratification of cell line selection based on DTPN

The effect of a specific alteration in the DTPN for Palbociclib can significantly affect the sensitivity or resistance of a specific cell line to the drug as shown above. The findings observed in breast cancer cell lines can be extended to breast cancer patients possessing similar alterations in their tumors. Hence, it is possible that the efficacy of Palbociclib in breast cancer patients will be determined based on similar alterations in their DTPN. The stratification algorithm of OncoDynamiX predicted in vivo hypothetical response curves of breast cancer patients carrying individual alterations in their DTPN when treated with Palbociclib (Figure 3). Patients carrying alterations in CCND1 were predicted to show response similar to patients carrying wild type CCND1 (Figure 3A) indicating that this specific alteration upstream of CDK4/6 had no impact on the efficacy of Palbociclib. Significantly, there was no difference in predicted responses to Palbociclib in patients carrying CDK4 (amplified) when compared to patients with wild type CDK4 (Figure 3B) indicating that alteration of CDK4 (the drug target) alone did not have additional benefit when treated with the drug. Deletions in CDKN2A showed significantly improved response to Palbociclib when compared to wild type which suggested that deletions in CDKN2A can influence therapy outcome when compared to wild type (Figure 3C). The deletion of RB1 in patients was predicted to show poor response when compared to wild type carrying patients (Figure 3D) highlighting the important effector role of RB1 in the drug pathway.

The pharmacological action of an anticancer molecule is determined by binding to its molecular target and inhibiting its activity which consequently inhibits the specific pathway in the cancer cell to elicit a

pharmacological effect. In the early stage of drug discovery in cancer, this is done by first testing compounds on the target in a cell free assay and then testing potent compounds in cancer cells expressing the same target to demonstrate proof of concept at the cellular level. As there are more than a thousand cell lines representing different human cancers, it is very important to select appropriate cancer cell lines, expressing the target of interest, in which the compounds are most likely to demonstrate efficacy.

Studies have shown associations between genomic changes (mutations in driver genes) and drug sensitivity of cell lines **[**6, 18, 19, 20**]**; algorithm based predictions of chemo sensitivity of cell lines to drugs (16); deep learning models to predict activity in cell lines based on structures and pathways **[**9**]**; in silico prescriptions matching activated mutations and approved therapies **[**17**]**. The rationale for the approaches mentioned is based on identification of genes or sets of genes associated with dysregulated pathways occurring in cancers: driver mutations (activation mutations in oncogenes, copy number alterations, fusions), loss of function mutations (tumor suppressor genes), epigenetic alterations, and transcriptional changes. The data obtained from these studies have been used for predicting the activity of approved targeted therapies in different cancer types, analysis of translation between preclinical and clinical studies, and identification of therapies for precision medicine.

However, from the drug discovery perspective, these methods have not addressed the important issue of rational selection of cell lines, based on normal or aberrant expression of the target, to test compounds for pharmacological activity. OncoDynamiX was developed to meet this requirement. We hypothesize that the activity of a drug or a test compound on a cancer cell depends not only on the expression of the pharmacological target but also on the molecular components upstream (regulators) and downstream (effectors) of the target. The inhibitory effect of a drug on a particular cancer phenotype (hallmark) will be seen only if all the components of the drug action pathway are functional. In the twenty cell lines which were sensitive to Palbociclib, a high frequency of alterations (various combinations) were observed for CDKN2B, 2A (KD, mutation), CCND1, 2A, 3 (OE), CDK4/6 (OE), ESR1 (OE) and a lower frequency in CCNE2 (OE), FOXM1 (OE), RB1 (KD, Mutation). In the nine cell lines which were resistant to Palbociclib, CDK4/6 was not functionally altered; a high frequency of alterations was seen in CCNE2 (OE), CCNE1 (OE), RB1 (KD), FOXM1 (OE) and a lower frequency in CCND2, 3 (OE), and CDK2NB (KD, Mutation). For example, in MCF7, a cell line sensitive to Palbociclib, ESR1 was over expressed, CDKN2B and CDKN2A were inactivated by deep deletion and CCNE2 was over expressed (Table 2), which together contributed to sensitivity. In contrast, in M DA-M B-4 6 8, a cell line resistant to Palbociclib, RB1 function was lost due to loss of function (deep deletion) causing resistance to Palbociclib

despite the presence of CDK4/6 (Table 2). The predicted response scores by OncoDynamiX were in agreement with the observed in vitro sensitivity of cell lines to Palbociclib [21]. In general the scores for sensitivity were higher than the resistant scores in cell lines in which Palbociclib was most potent and the opposite was true for cell lines resistant to Palbociclib. However the cell line UACC893 was sensitive to Palbociclib although the predicted sensitivity was lesser the resistant score. Whereas in cell line HCC1569 the sensitivity score was higher than the resistant score and it was resistant to Palbociclib in vitro. Cell lines HS578T and M DA-M B-4 3 6 showed equal scores for sensitivity and resistance and the observed response to Palbociclib was lower. In all these cases there could be other mechanisms operating outside the DTPN that affect the activity of Palbociclib. It should be pointed out that the scores estimated by OncoDynamiX do not strictly correlate quantitatively with in vitro potency values. Nevertheless the scores estimated by OncoDynamiX helped in binning cell lines into sensitive and resistant populations.

The individual components of the DTPN also influence the activity of the drug to varying degrees. The stratification algorithm of OncoDynamiX assessed the importance of each component, based on predictions in cell lines, by performing virtual clinical trials in breast cancer patient populations. The predicted responses to Palbociclib in breast cancer patients harbouring alterations, observed in breast cancer cell lines, were found to be consistent with results in breast cancer patients in clinical trials [22, 23]. Overexpression of CDK4 is associated with high tumor cell proliferation and more recently it has been shown that amplification of CDK4 is an important resistance mechanism for CDK4/6 therapy, with overexpression associated with a poorer response to CDK4/6 inhibitors in patients treated with CDK4/6 inhibitors [24]. The stratification algorithm can also help to validate various hypotheses during prescreening pathway analysis, to identify new gene alterations (not a part of initial DTPN) which influence response to drug candidates.

OncoDynamiX selected seventeen cell lines sensitive to Palbociclib from total of sixty breast cancer cell lines based on the DTPN of Palbociclib, identified the most sensitive biomarkers of efficacy and resistance, and finally simulated clinical trials for breast cancer patients with individual alterations. The seventeen cell lines that were sensitive had combinations of alterations in the DTPN that contributed to the sensitivity to the drug. Since cell lines are in vitro models of cancer, similar combinations of alterations can potentially exist in patients and therefore help in selection of patients who will most likely respond to Palbociclib. Identification of reliable biomarkers, predictive of sensitivity or resistance to a drug is a challenge not only for cytotoxic drugs but also for targeted therapies as the presence of drug targets alone are generally poor therapeutic indicators. Although the main objective of this study was to demonstrate

the selection of cell lines using Palbociclib as a tool drug, our findings in this study have clinical implications for it. Breast cancer patients who are ER positive and HER2 negative have shown variable responses to CDK4 targeted therapies including Palbociclib. A main reason for this has been due to lack of reliable biomarkers for selection of patients **[**25**]**. It has also been shown, further supported by our DTPN approach, that presence of CDK4/6 alone is not predictive of clinical response. Biomarkers such as increased CCND expression for sensitivity, loss of RB1, increased Cyclin ECDK2 and FAT expression for resistance have been reported **[**25**]**. However, we suggest that evaluation of individual alterations may not be meaningful; rather all alterations should be evaluated together as they are all related to the pharmacological pathway of Palbociclib. The biomarkers shown in table 4 should be evaluated together for prediction of response for Palbociclib. This study has some limitations. The technology described herein has the ability to identify DTPNs for any existing drug or new pharmacological entity for which the target is known. The main limitation with the process is that many of the pathways identified are not currently druggable, and so it may be argued that if there is no target why identify them. We see this as a positive as it provides biopharmaceutical companies with targets for which they can develop new therapies to "fill in the gaps". Secondly, the selection process was based entirely on genomic data of cell lines. Alterations of individual components of the DTPN of Palbociclib can also be at the levels of transcription, epigenetics and posttranslational modifications. We plan to address this in the next version of OncoDynamiX.

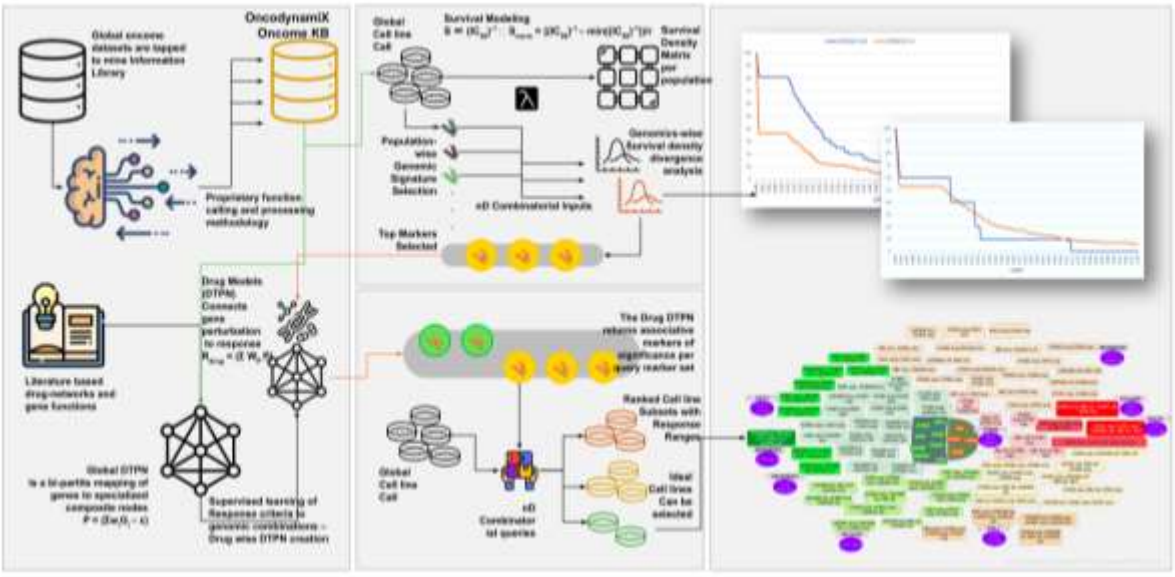

Figure 1: The workflow of OncoDynamix used to select breast cancer cell lines for Palbociclib. See methods for details.

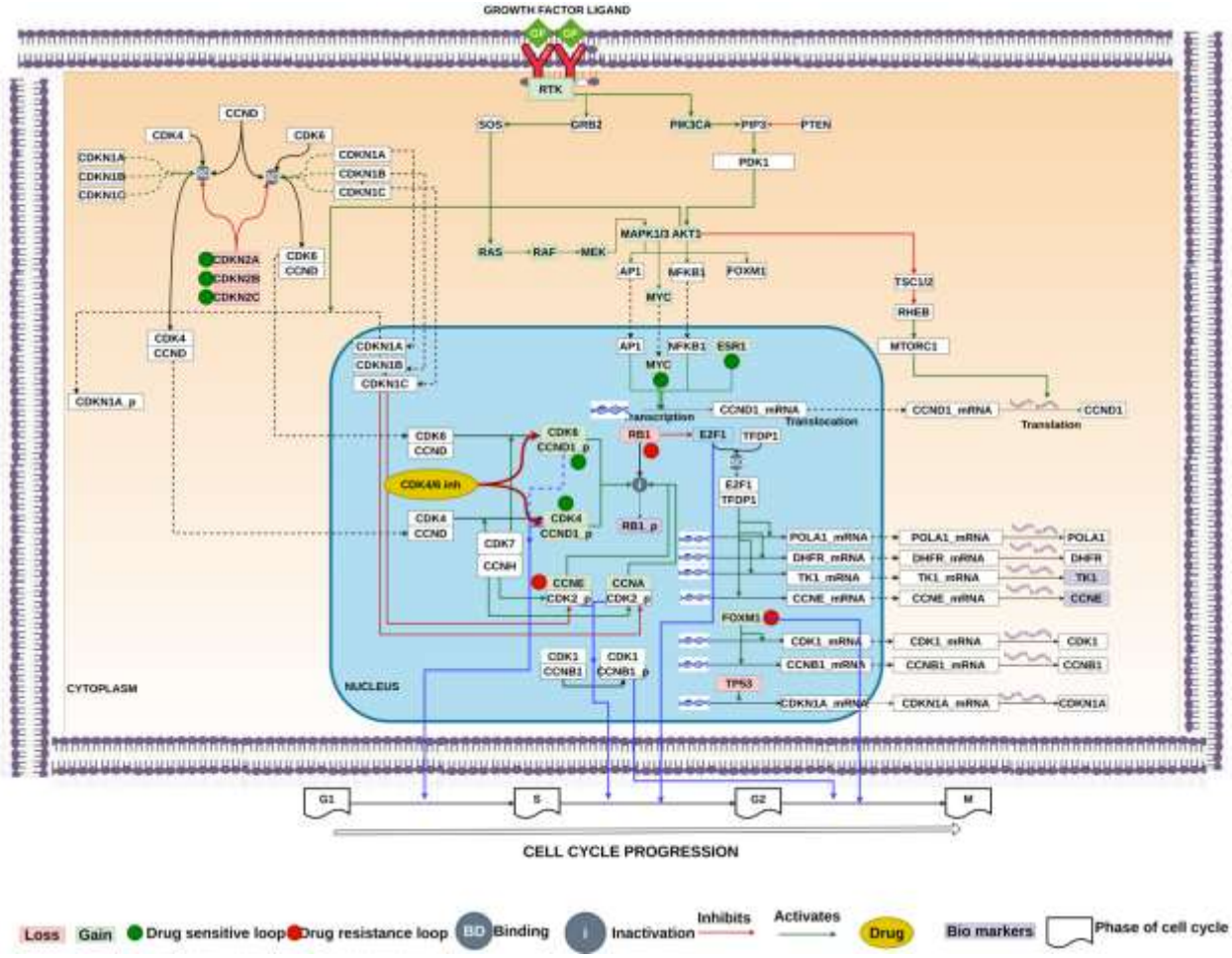

Figure 2: The Drug Target Pharmacology Network (DTPN) for Palbociclib constructed by OncoDynamiX. G1, S, G2 and M represent different stages of the cell cycle. The legends for each symbol are shown below the figure. Abbreviations: DTPN: Drug Target Pharmacology Network; GOF: Gain of Function; KD: Knock Down; LOF: Loss of Function; OE: Over expression; CDK4 : cyclin dependent kinase 4; CDK6 : cyclin dependent kinase 6; CCND1 : cyclin D1; CCND2 : cyclin D2; CCND3 : cyclin D3; HER2 : erbb2 receptor tyrosine kinase 2; RB1 : RB transcriptional corepressor 1; CNA : Copy number alterations; CDKN2A : cyclin dependent kinase inhibitor 2A; CDKN2B : cyclin dependent kinase inhibitor 2B; CDKN2C : cyclin dependent kinase inhibitor 2C; AP1 : Jun protooncogene, AP1 transcription factor subunit ; MYC : MYC protooncogene, bHLH transcription factor ; NFKB1 : nuclear factor kappa B subunit 1; ESR1 : estrogen receptor 1; CDK2 : cyclin dependent kinase 2; CCNA1 : Cyclin A1; CCNE1 : Cyclin E1; CCNE2 : Cyclin E2; FOXM1 : forkhead box M1; CDK1 : cyclin dependent kinase 1; CCNB1 : cyclin B1; TK1 : thymidine kinase 1; E2F1 : E2F transcription factor 1; FAT1 : FAT atypical cadherin 1; p21 : cyclin dependent kinase inhibitor 1A

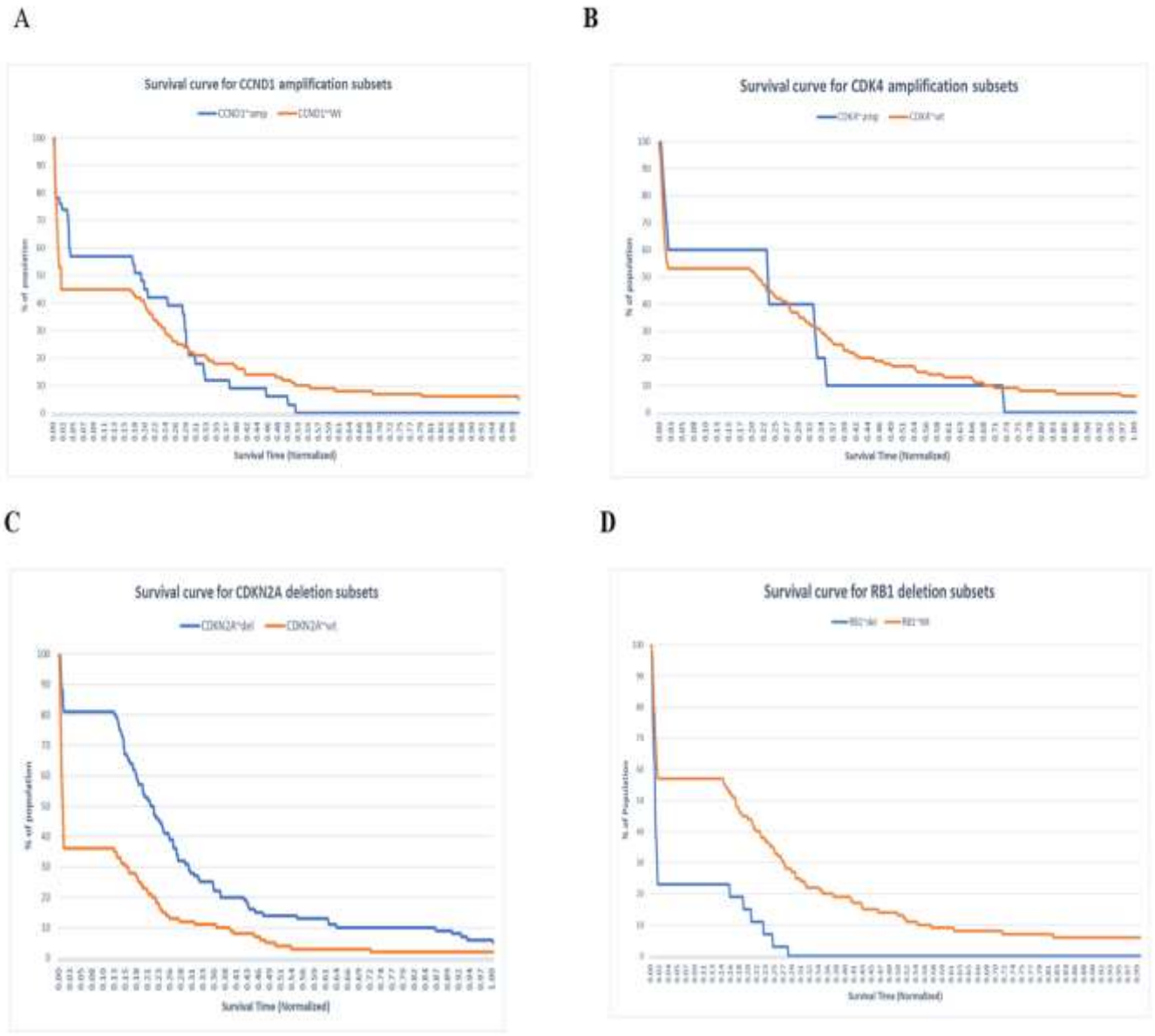

Figure 3: Predicted in vivo response curves for patients treated with Palbociclib carrying alterations in DTPN of Palbociclib. A) Amplification of CCND1; B) Amplification of CDK4; C) Deletion of CDKN2A; D) Deletion of RB1

Gene	Functionality	Location in pathway in relation to drug target	Deregulation in cancer
CDK4	Drug target	Target	Overexpression / upstream mediated hyper activation
CDK6	Drug target	Target	Overexpression / upstream mediated hyper activation
CCND1	Drug target binding (activating) cyclin	Upstream	Overexpression / upstream upregulation
CCND2	Drug target binding (activating) cyclin	Upstream	Overexpression / upstream upregulation
CCND3	Drug target binding (activating) cyclin	Upstream	Overexpression / upstream upregulation
CDKN2A	Negative regulator of CDK4/6 binding to CCND1/2/3	Upstream	Loss of function mutation / deep deletion / hyper methylation / upstream down regulation
CDKN2B	Negative regulator of CDK4/6 binding to CCND1/2/3	Upstream	Loss of function mutation / deep deletion/hyper methylation / upstream down regulation
CDKN2C	Negative regulator of CDK4/6 binding to CCND1/2/3	Upstream	Loss of function mutation / deep deletion /hyper methylation / upstream down regulation
AP1	Transcription factor for CCND1	Upstream	Overexpression / upstream upregulation / hyper activation
MYC	Transcription factor for CCND1	Upstream	Overexpression / upstream upregulation / hyper activation
NFKB1	Transcription factor for CCND1	Upstream	Overexpression / upstream upregulation / hyper activation
ESR1	Transcription factor for CCND1	Upstream	Overexpression / upstream upregulation / hyper activation
CDK2	Bypass mechanism for RB1 phosphorylation (central role of drug target);Cyclindependent kinase for next phase of cell	Bypass /parallel pathway	Overexpression / upstream upregulation / hyper activation

	cycle (G2 M)		
CCNE1	Bypass mechanism for RB1 phosphorylation (central role of drug target); Cyclin for next phase of cell cycle (G2 M)	By pass /parallel pathway	Overexpression / upstream upregulation / hyper activation
CCNE2	Bypass mechanism for RB1 phosphorylation (central role of drug target);Cyclin for next phase of cell cycle (G2 M)	By pass /parallel pathway	Overexpression / upstream upregulation / hyper activation
CCNA1	Bypass mechanism for RB1 phosphorylation (central role of drug target); Cyclin for next phase of cell cycle (G2 M)	Bypass /parallel pathway	Overexpression / upstream upregulation / hyper activation
CDK1	Cyclindependent kinase for mitotic entry	Downstream	Overexpression / upstream upregulation / hyper activation
CCNB1	Cyclin for mitotic entry	Downstream	Overexpression / upstream upregulation / hyper activation
RB1	Downstream effector, inactivated by CDK4/6	Downstream	Loss of function mutation / deep deletion / hyper methylation / upstream down regulation
E2F1	Downstream effector, negatively regulated by RB1	Downstream	Overexpression / upstream upregulation / hyper activation
TK1	Downstream effector, transcribed by E2F1	Downstream	Overexpression / upstream upregulation / hyper activation
FOXM1	Transcription factor transcribes genes required for the transition from G2 – M phase of cell cycle.	Parallel pathway	Over expression / upstream upregulation / hyper activation

Table 1: Individual components of the Drug Target Pharmacology Network (DTPN) of Palbociclib with their location in the pharmacological pathway and their functional status in cancers. See also figure 1

Cell Line	Alteration contributing to sensitivity to Palbociclib				Alteration contributing to resistance to Palbociclib	
CAMA1	CCND3(OE,	CCND1_OE				
HCC202	CCND2_OE	CDK6_OE				
UACC893					CCNE2_OE	FOXM1_OE
EFM19	CDKN2B_KD	CDKN2A_KD				
EFM192A	CDKN2B_KD	CCND1_OE	CDKN2A_KD		CCNE2_OE	
MDAMB361	CDKN2B_KD	CCND1_OE	CDKN2A_KDMUT	CDKN2A_KD		
HCC1500	CDKN2B_KD	CCND1_OE	CDKN2A_KD			
HCC1419	CDKN2B_KD	CDKN2A_KD			CCNE2_OE	
MDAMB415	CCND3_OE					
UACC812	CDKN2B_KDMUT					
ZR751	CDK4_OE	CCND1_OE	CCND2_OE		FOXM1_OE	
MDAMB453	CDKN2B_KD	CCND1_OE	CDKN2A_KD			
MDAMB175VII	CCND1_OE	CDKN2B_KDMUT				
MCF7	ESR1_OE	CDKN2B_KD	CDKN2A_KD		CCNE2_OE	

BT20	CDKN2B_KD	CDKN2A_KD			RB1_KDMUT		
BT474	CDKN2B_KD	CCND1_OE	CDKN2B_KDMUT	CDKN2A_KD			
SKBR3	CDK6_OE						
KPL1	ESR1_OE	CDKN2B_KD	CDKN2A_KD		CCNE2_OE		
MDAMB231	CCND3_OE	CDKN2B_KD	CDKN2A_KD		RB1_KD		
HS578T	CDKN2A_KD				CCNE2_OE		
BT549					CCNE2_OE	RB1_KD	
COLO824					RB1_KDMUT		
DU4475					RB1_KD		
HCC1569	CCND3_OE				CCNE2_OE	CCNE1_OE	
HCC1806	CDKN2B_KD	CDKN2A_KD			CCNE1_OE		
HCC70	CDKN2B_KDMUT				CCNE2_OE	RB1_KDMUT	CCNE1_OE
MDAMB157					RB1_KD	CCNE1_OE	
MDAMB436	CCND2_OE				FOXM1_OE		
MDAMB468					RB1_KD		

AU565	CDK6_OE				
BT483				CCNE2_OE	
CAL120	CDK4_OE			CCNE1_OE	
CAL148	CDKN2A_KDMUT			RB1_KD	
CAL851				RB1_KDMUT	
EVSAT	CCND1_OE	CDK6_OE		CCNE2_OE	RB1_KD
HCC1428	ESR1_OE			CCNE2_OE	FOXM1_OE
HDQP1	CCND1_OE			RB1_KD	
HMC18	CDKN2B_KD	CDKN2A_KD			
JIMT1	CDK4_OE				
MDAMB134VI	CCND1_OE			RB1_KD	
MDAMB330	CDKN2B_KDMUT				
YMB1	CDK4_OE	CCND1_OE	CCND2_OE	FOXM1_OE	

OEOver expression leading to Amplification; KD Knock down due to deep deletion; MUTGOF gain of function mutation; MUTLOF loss of function mutation

Table 2: Breast cancer cell lines retrieved by OncoDynamix with at least one alteration in genes queried using the DTPN of Palbociclib

Cell lines selected	Predicted sensitivity score	Predicted resistance score	Molecular alterations in DTPN of Palbociclib
COLO824	0.00	0.07	RB1_Loss of function mutation
CAL851	0.00	0.07	RB1_Loss of function mutation
MDAMB453	0.27	0.00	CDKN2B_KD, CCND1_Amplification, CDKN2A_KD
HCC1500	0.27	0.00	CDKN2B_deep deletion, CCND1_Amplification, CDKN2A_deep deletion
AU565	0.07	0.00	CDK6_Amplification
HMC18	0.20	0.00	CDKN2B_deep deletion, CDKN2A_deep deletion
EFM19	0.20	0.00	CDKN2B_deep deletion, CDKN2A_deep deletion
BT549	0.00	0.07	CCNE2_Amplification
MDAMB330	0.07	0.00	CDKN2B_Loss of function mutation
UACC812	0.07	0.00	CDKN2B_Loss of function mutation
KPL1	0.20	0.07	CCNE2_Amplification, CDKN2B_deep deletion, CDKN2A_deep deletion
HCC1419	0.20	0.07	CCNE2_Amplification, CDKN2B_deep deletion, CDKN2A_deep deletion
HDQP1	0.07	0.00	CCND1_Amplification
MDAMB134 VI	0.07	0.00	CCND1_Amplification
MDAMB436	0.07	0.07	FOXM1_Amplification, CCND2_Amplification,
HCC1569	0.07	0.13	CCNE2_Amplification, CCND3_Amplification, CCNE1_Amplification,
BT474	0.33	0.00	CDKN2B_deep deletion, CDKN2B_Loss of function mutation, CCND1_Amplification, CDKN2A_deep deletion,
CAL148	0.07	0.00	CDKN2A_Loss of function mutation
JIMT1	0.07	0.00	CDK4_Amplification

ZR751	0.20	0.07	CDK4_Amplification, FOXM1_Amplification, CCND2_Amplification,CCND1_Amplification,
MDAMB157	0.00	0.07	CCNE1_Amplification
MDAMB231	0.27	0.00	CCND3_Amplification, CDKN2B_deep deletion, CDKN2A_deep deletion,
HCC70	0.07	0.20	CCNE2_Amplification, CDKN2B_Loss of function mutation, RB1_Loss of function mutation, CCNE1_Amplification,
MDAMB415	0.13	0.00	CCND3_Amplification, CCND1_Amplification,
HCC1806	0.20	0.07	CDKN2B_deep deletion, CDKN2A_deep deletion, CCNE1_Amplification,
HCC202	0.13	0.00	CCND2_Amplification, CDK6_Amplification,
EFM192A	0.27	0.07	CCNE2_Amplification, CDKN2B_deep deletion, CCND1_Amplification, CDKN2A_deep deletion,
MDAMB175 VII	0.13	0.00	CDKN2B_Loss of function mutation, CCND1_Amplification,
MDAMB361	0.33	0.00	CDKN2B_deep deletion, CDKN2A_Loss of function mutation, CCND1_Amplification, CDKN2A_deep deletion,
EVSAT	0.13	0.07	CCNE2_Amplification, CCND1_Amplification, CDK6_Amplification,

Table 3: Predicted scores, by OncoDynamiX, for sensitivity and resistance to Palbociclib in breast cancer cell lines and the associated molecular alterations in the DTPN

Cell Line	Predicted Sensitivity Score	Predicted Resistance Score	Observed Response IC_{50} (nM)*
CAMA1	0.4	0	8
HCC202	0.4	0	21
UACC893	0	0.4	24
EFM19	0.4	0	27
EFM192A	0.6	0.2	42
MDAMB361	0.8	0	44
HCC1500	0.6	0	45
HCC1419	0.4	0.2	51
MDAMB415	0.4	0	64
UACC812	0.2	0	96
ZR751	0.6	0.2	110
MDAMB453	0.6	0	115
MDAMB175VI	0.4	0	127
MCF7	0.6	0.2	148
BT20	0.4	0.2	177
BT474	0.8	0	240
SKBR3	0.2	0	300
KPL1	0.6	0.2	327
MDAMB231	0.6	0	432
HS578T	0.2	0.2	524
BT549	0	0.4	1000
COLO824	0	0.2	1000
DU4475	0	0.2	1000
HCC1569	0.2	0.4	1000
HCC1806	0.4	0.2	1000
HCC70	0.2	0.6	1000
MDAMB157	0	0.4	1000
MDAMB436	0.2	0.2	1000
MDAMB468	0	0.2	1000

Table 5: Comparison of predicted response scores by OncoDynamiX for thirty breast cancer cell lines to Palbociclib with the observed in vitro responses.

Conclusions

OncoDynamiX is a versatile platform that can be effectively used in rational and mechanism based selection of appropriate cell lines for compounds in early cancer drug discovery, with significant savings in time and resources. It can help in identifying relevant biomarkers associated with drug response that can be of high value in selection of patients in clinical trials for a drug candidate, or indeed select the optimal therapy for existing pharmaceutical agents.

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