Increasing the Potential Targets and Molecularly Targeted Agent Combinations Against Cancer Cell Proliferation

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Abstract

At least four main groups of intracellular signaling pathways or submodules concur in the cell division and proliferation module: those for the control of the cell cycle, those for the metabolism programming, those for cytoskeleton remodeling, and those for DNA replication and repair. Precise signaling pathways that control cell proliferation module require the joint functional collaboration of signaling pathways of cell growth, cell survival, cell differentiation, intracellular senescence and death programs, and appropriate interaction with angiogenesis, cell micro-environment regulation and immunologic system modules. Seeking out actionable aberrations in cancer cells may now selectively targeted by drug compounds to optimize treatment efficacy and minimize toxicity.

This critical review provides an overview of the use of the CDK4/6 inhibitors as the first cell cycle inhibitor that improve the outcomes of patients with HR+ breast cancer. Discusses the connection of different inhibitory agents to modify cell proliferation signaling pathways and sketches the potential use of other molecularly targeted agents in close relationship with proliferation signaling pathways carcinoma cells.

Keywords: Cancer; Cell proliferation signaling pathways; Combination targeted therapies

Introduction

Translational oncology uses molecular profiling of each cancer subtype for both diagnosis and treatment by tumor biomarkers identification in tumor microenvironment or recently in circulating tumor cells, and circulating tumor-free DNA. Translational oncology has obtained early success in clinical practice, but it is far still to obtain the comprehensive complex genomic epigenomic and proteomic patient profile abnormalities that drive the susceptibility, development and progress to cancer.

The translational oncologist needs to analyze and to select the molecular alterations profile of a patient and to organize the use of dynamic and available strategies for streamline personalized therapies similarly to the clinical oncologist practice (in obtaining the clinical profile conditions in susceptibility, development and progress to cancer in a personalized way), or to the anatomopathologist practice (in getting the histopathology tumor analysis profile). Translational oncology had already achieved an outstanding grade of success in patient subgroups on five major cancer types such as colorectal, ovarian, breast, lung and prostate cancers [1].

At present, we know that the majority of cancers arise through a complex series of somatic mutations, and genomic abnormalities, epigenomic and transcriptome alterations, and metabolomic and proteomic deregulations. These chronic molecular variations originate genomic changes temporary-stable that finally provoke the oncogenic reprogramming cellular behavior. These biological interactions vary between individuals, genders and ethnicities, and explain the individual susceptibility on the cancer type´s developing. In aging, specific environmental exposition affects cell functions through direct or indirect mechanisms for specific disease generation [2]. In summary, it is estimated that most of the cancers are explained by the adverse environment effects interacting with genes [3,4].

Neoplastic transformation processes have played a crucial role in the carcinogenesis, where cells with gen-cancer mutations confer increase replicative and survival abilities (classical and no classical hallmarks of cancer underlie the development of a malignant tumor [5-7]. Progressive analysis of the molecular circuits of cancer hallmarks have contributed to identify the signaling mechanisms by which oncogenic mutations preserve the cancer phenotype and thereby are useful to start the identification of rational targets for cancer drugs.

The main hallmarks of cancer cells are self-sufficiency of growth signals, insensitivity to anti-growth signals, limitless replicative potential, evasion of apoptosis, sustained angiogenesis, tissue invasion, metabolic reprograming, genomic instability, and evasion of immune recognition. Each one of the cancer hallmarks disrupts some or all cellular functions that lead, to increased cell proliferation, decreased cell attrition, senescence evasion, disruption of epithelial adhesion and polarity, matrix degradation, gain in motility, defective DNA repair and maintenance surveillance, and deregulation of parenchyma and microenvironment tissue cell interactions. The biological study of each one of cancer hallmarks or oncogenic cellular functions have identified the principal molecular component factors and the main signaling systems altered in transformed cells. Particularly, each type of human cancer is initiated though diverse altered molecular factors and consecutively deregulation of numerous cell signaling pathways. Specific genomic, epigenomic (generated by damaging environmental

factors exposition) changes, and genomic-epigenomic cell interactions in cancer-related genes result in neoplastic formation [8].

Cancer disease emerge from an increase in cells numbers provoked by signaling pathways deregulation that control cell proliferation. Classically, cancer represents a pathological manifestation of uncontrolled cell division. Translational oncology studies have conducted their efforts to target the main molecular factors participating in signaling pathways that control cell proliferation; these studies have flourished in last two decades. Cyclins and cyclin-dependent kinases associated with cell-cycle transitions were in previous years a focus of therapeutic development in cancer. Recently the FDA has approved the palbociclib, a potent and specific oral cyclin-dependent kinase 4/6 inhibitor in combination with two endocrine agents as targets for HR positive-, HER2-negative breast cancer patients [9]. This critical review provides an overview of the use of the CDK4/6 inhibitors, discusses the potential of connecting different inhibitory agents to modify cell proliferation signaling pathways and say anything about other components of the oncogenic signaling pathways associated with biological maintenance signaling pathways in breast carcinoma cells.

Palbociclib is the first cell cycle inhibitor on demonstrate clinic therapeutic activity

Cyclin-dependent kinases (CDKs) are a group of serine/threonine kinases that are sequentially expressed during the cell cycle in normal cells. The cyclin D-cyclin dependent kinase CDK4/6 and their INK4-retinoblastoma inhibitor regulate cellular proliferation by controlling the cell cycle checkpoint of G1 to S phase. Dysregulation of the cyclin D-CDK signaling pathway is a common molecular finding in cancer cells and contributes to cell cycle progression and continued growth [10,11]. Cyclin-dependent kinases, that promote transition through the cell cycle were expected to be key therapeutic targets of many tumorigenic events. After the generally disappointing results seen in clinical trials with non-selective CDK inhibitors, specific CDKs and in appropriate patient selection have turned out cell growth arrest [12]. Preclinal and clinical studies of cyclin-dependent kinase (CDK) 4/6 inhibitors in breast cancer, liposarcoma, mantle cell lymphoma, melanoma and germ cell tumors have demonstrated utility and promising clinical activity [13].

D-CDK 4/6 regulates the phosphorylation state of the normal inhibitor-retinoblastoma (Rb) on transition from G1 to S phase; unphosphorylated-Rb binds and represses the function of the E2 family (E2F) transcription factors. Overexpression of D-CDK4/6 promotes phosphorylation of Rb and of a large number of proteins involved in cell cycle progression, causing transcription of E2F, and leading to cell proliferation and cancer cell growth; this condition can occur by overexpression of D type cyclins, mutation or amplification of CDK4/6, or loss of cyclin D-CDK4/6 negative regulators such as p16INK4A, or by other mechanisms such as epigenetic alterations, amplification/overexpression of cyclin D, and loss of CDKN2A (p16). Also in non-cell-cycle context, CDK4/6 activates the vascular endothelial growth factor (VEGF) transcription, thus promoting angiogenesis and nuclear factor (NF)- κB activation via the p65 transcription factor.

D-CDK4/6 inhibitory activity occurs when cancer cells retain wild-type Rb expression [10]. The Rb-positive cancers are exclusively dependent on CDK4/6 activity for cell proliferation and therefore of their inhibitors. In addition to mitogen-activated protein kinase (MAPK) signaling, other key oncogenic signaling pathways promote cyclin D-CDK4/6 activity, such as phosphadidylinositol 3 kinase (PI3K/AKT/mammalian target of rapamycin-mTOR), WNT/β-catenin, janus kinase (JAK)-signal transducer and activator of transcription (STAT), nuclear factor kappa-light-chain enhancer of activated B cells (NF-κB), and steroid hormone signaling pathways (e.g. estrogen, progesterone and androgen) [11].

Currently, high specific CDK4/6 inhibitors show a selective and potent therapeutic effect and fewer off-target toxicities than pan-CDK inhibitors for the treatment of breast cancer [14,15]. There are three CDK4/6 inhibitors which deactivate these CDK at <40 nM IC50 values: palbociclib, ribociclib and abemaciclib [16]. All three compounds have demonstrated preclinical activity in Rb+ tumor models. Palbociclib recently received Food and Drug Administration approval for the treatment of hormone receptor positive (HR+), HER-negative metastatic breast cancer in combination with letrozole (2015) and in combination with fulvestrant (2016) [9]. Palbociclib is a potent and specific oral D-CDK4/6 inhibitor; strong preclinical data supports its activity in retinoblastoma protein-expressing tumors. The phase 1 trials demonstrated safety, and phase 2 trials have shown single-agent activity with reversible neutropenia as the main toxic effect. The addition of palbociclib to endocrine therapy improves progression-free survival in naive endocrine therapy and resistant metastatic endocrine therapy [15]. The striking clinical activity exhibited by palbociclib in breast cancer announces its additional role in other cancer types. Clinical trials are on the way to explore the synergist effects of CDK4/6 inhibitors and drugs of other classes, such as other hormonal therapies, PI3K/AKT/mTOR pathway inhibitors and RAS/RAF/MEK/ERK pathways inhibitors [11,14,16]. The development and the flourishing of clinical results of the CDK4/6 inhibitors has changed the perception of CDKs as therapeutic targets in cancer. Molecular analysis in-depth of the proliferative tumor cells process alterations may help to identify the patient subgroup most likely to benefit from treatment with CDK4/6 inhibitors and in combination with other molecularly targeted agents and others immuno-oncology agents [9,17,18].

Module and submodules in signaling networks that control cell proliferation

Each cell signaling network has different global components that can be characterized: ligands, receptors, molecular sensors, signals transductor, molecular effectors, targeted genes on/off, target protein and post-translation protein-changes activity and wild-type/altered cell behavior. Also, each cell signaling network has canonical and no-canonical molecular components and pathways, which take part in the wild-type or altered cell behavior of the main type of mammalian cells. Particularly in cancer cell behaviors, there are a huge number of biological components and signaling pathway variants and subvariants.

Control of cell proliferation occurs during G1 phase of eukaryotic division cycle. Many cellular signaling events linked to G1 phase control proliferation, differentiation, cell quiescence, senescence and responses to a variety of stresses. Particularly in cell proliferation, the decision to enter S phase, represents a point of no return, and drives the cells to complete the cell cycle and to divide, what is called the "restriction point". Progression through G1 phase is controlled by the critical role of RB pathways. Phosphorylation of the pRB proteins by CDKs causes pRB to dissociate from E2Fs, allowing the transcription of the genes required for G1/S transition. The principal kinases that phosphorylate pRB family are cyclin-D-dependent CDK4/6 and cyclin-E-dependent CDK2. The cyclins and CDKs functions are

predominantly controlled by transcriptional regulation (by mitogenic signals) and ubiquitin-dependent proteolysis. The canonical RAS-RAF-MEK-ERK mitogen activated protein kinase (MAPK) pathway is the best characterized pathway in the activation of cyclin D-CDK4/6 transcription [19].

Cell division and proliferation module consists at least of four main groups of intracellular signaling pathways or submodules: those for the control of the cell cycle (MAPK/ERK,WNT/PI3K/AKT, E2F/DP1/Rb/D-CDK4/6), those for the metabolism programming (PI3K/AKT/mTOR, HIF-1, Myc/PKM1-2), those for cytoskeleton remodeling (MAPKs/Rho/p38/ROCK/PAK) and those for DNA replication and repair (E2F/E/A-CDK2/DNA pol, ATM/ATR/CHK1-2/WEE/PLK1) [20]. The phenotypic module used for cell progression during the cell division cycle and cell proliferation requires the simultaneous or nearly consecutive participation of the four submodules of intracellular signaling pathways in a manner that these signaling pathways work in closely collaboration. In this way, cyclin D-CDK4/6 is regulated by the MAPK/Ras, β-catenin-Tcf/LEF, PI3K, and Rho/FAK pathways and serve as the molecular cell sensor that integrates extracellular signals with molecular machinery. Additionally, each pathway submodule can transit by different seemed routes, for example different extracellular signals in MAPK pathways transmit and respond by ERK 1/2, ERK 5, JAK or p38/SAPK pathways, and different transcription factors work as signaling molecular effectors, such as AP-1, SP-1, E2F and Oct-4 which are activated by the ERK1/3 ERK5, PI3K/AKT WNT/β-catenin, NF-κB and JAK/STAT signaling pathways.

Interaction of the signaling pathways that control cell proliferation module is an essential condition in human homeostasis, that requires the functional collaboration of the four mentioned signaling pathways submodules [20] (Table 1), but equally it needs a close collaboration of signaling pathways of cell growth, cell survival, cell differentiation, intracellular senescence and death programs, and appropriate interaction with angiogenesis, cell micro-environment regulation and immunologic system modules (Table 1). Particularly, immuno-oncology therapy combinations offer great potential to deliver substantial benefits to patients with a range of different cancers. Better patient selection though the use of more precise predictive biomarkers of response, rational combinations of existing treatments with immunotherapeutic strategies, and the use of immuno-targets that block negative immuno regulators such as anti-CTLA-4 and anti-PD-1, have been shown that cancer immunotherapy induces durable clinical benefit in a fraction of the patients. But a critical issue is to determine how best to combine these immunotherapy agents with existing treatments such as radiotherapy, chemotherapy or molecularly targeted agents. [18,21,22]. Seeking out actionable aberrations in cancer cells can be selectively targeted by a drug or a combination of compounds to optimize treatment efficacy and to minimize toxicity [23].

Breast cancer as model: dissecting the cellular proliferation process components Majority of breast cancers are carcinomas that originate from cells lining milk ducts of the mammary gland. The majority signaling networks of the sporadic breast cancer are partially identified [24]. Two seminal works have classified the sporadic breast cancer subtypes taking into consideration the gene receptor expression patterns, the hormone receptors (estrogen (ER) and progesterone subtypes), and the human epidermal growth factor receptor-2 (HER2) [25,26]. Based in receptor expression profiles, four types were identified: luminal A (hormone receptor positive and HER2 negative),

luminal B (hormone receptor positive and HER-2 positive), luminal HER2 (hormone receptor negative and HER2 positive) and triple-negative breast cancer (TNBCs: hormone receptor negatives and HER2 negative) type, a group class termed basal-like. The general phenotype features of epithelial cell subpopulations correspond to luminal A/B type to mature/terminally differentiated cells, luminal HER2+ type to progenitor cells and triple-negative type with breast cancer stem cells [27]. Luminal subtypes comprise the majority of breast cancers and their expression signature is ESR1, GAT3, FOXA1, XPB1, MYB and cytokeratins 8 and 18, and largely driven by the estrogen/ER pathway deregulation. HER2 subtype has lower expression of ER-related genes, it is a luminal subtype variant and is led by PI3K/AKT and RAS/RAF/MAPK pathway alterations. TNBCs type cancer patients show a high expression of proliferation genes, they have a clinically aggressive prognosis course and express various stem cell-like signaling pathway deregulations such as Notch, WNT/β-catenin and Hedgehog [28].

Targets and molecularly targeted agents against cancer cell proliferation

Signaling network module and submodules that directly control cell proliferation	Signaling network module and submodules in close relationship with proliferation cell
• For the control of the cell cycle (MAPK,/ERK, WNT,PI3K/AKT, E2F/DP1/Rb/D-CDK4/6), such as *CDK4/6 inhibitors* • For the metabolism programming (PI3K/AKT/mTOR, HIF-1,Myc/PKM1-2),such as *PI3K inhibitors* • For cytoskeleton remodeling (MAPKs/Rho/p38/ROCK/PAK), • For DNA replication and repair (E2F/E/A-CDK2/DNA pol, ATM/ATR/CHK1-2/WEE/PLK1), such as *PARP, Chk1, CDKs, ATR, MTH1 inhibitors*	• cell growth, *anti-mitogens (anti-ER)* • cell survival, *YM-155* • cell differentiation, • intracellular senescence and death programs, • angiogenesis, • cell micro-environment regulation, • tissue invasion & metastasis, • appropriate interaction with immunologic system

Table 1: Signaling pathways, targets and molecularly targeted agents against cancer cell proliferation. Right section shows module and submodules that directly control cell cancer proliferation; left sections shows module and submodules in close relationship with proliferation cell. In both sections, main molecularly targeted agents are written in italics words.

Gene expression receptor signatures in breast cancer are currently in clinical use for defining prognosis and for determining the benefit of systemic therapies as chemotherapy or endocrine treatment. Together with breast cancer gene expression, additional characterization of the complementary profile of their ligands, receptors, molecular sensors, signal transductors, molecular effectors, and targeted genes on/off, target protein and post-translation-changes activity and wild-type/altered cell behavior will increase and emphasize the molecular type prognosis. Exposome alterations in type, quantity and interval of estrogen/estradiol and other epi-mitogens levels depends in cells lining the milk-forming ducts of the mammary gland and in the microenvironment tissue cells, which drives to DNA methylation and chromatin modification and lead to alterations in the transcriptome profile that appears to be oncogenic. Also, insulin-like growth factor and epidermal growth factor have been identified as potent endogenous mitogen in mammary tissues, their high levels, together with high estrogen levels are correlated to malignant transformation. Inherited structurally and functionally genomic changes of the receptors of these three ligands have been identified as factors to stimulate the cell transformation of mammary gland. Estrogens exert their actions through both, genomic and non-genomic mechanisms, the non-genomic ER actions result in the phosphorylation and activation of the EGFR, IGF-1R and PI3K receptor kinases, this drives a potent bidirectional cross-talk between ER and growth factor

receptor; the RAS/RAF/MEK/MAPK and RAS/PI3K/AKT/mTOR are oncogenic pathways relevant in breast cancer.

Estrogen receptors are overexpressed in as many as 70% of invasive breast cancers, and estrogen-responsive elements have been found in the promoters of several mitotic genes. The number or ER+ cells expressing proliferation markers increase after menopause [27]. ER may be associated to cell membranes and form functional complexes with the receptor tyrosine kinases of EGFR and IGF-1R. Estrogen can be increased through modifying the activity of growth factor pathways. The ER signaling pathway is a complex network of extensive cross-talk with growth factor signaling pathways, cell cycle control pathways, and protein degradation pathways [29]. Growth-factor receptors, particularly tyrosine kinases receptors may act as ER-dependent and ER-independent drivers of tumor growth and survival. This biological association explains the outstanding participation of estrogens and EGF/IGF/FGF in the proliferation and neoplastic transformation of breast cells, and their use as anti-cancer molecularly targeted agents. A number of agents can inhibit the estrogen signaling by either binding to the receptor itself such as tamoxifen, raloxifene, fulvestrant or by decreasing the production of endogenous estrogen, by means of aromatase inhibitor or ovarian ablation. The delimiting of the EGF/EGFR and IGF-1/IGF-1R, and FGF/FGFR1 overexpression patterns in tumor cells, may allow the development of effective diagnosis and treatment strategies against breast cancer [30,31].

The intracellular signaling alterations cascade are often dysregulated in cancer cells. Approximately 10% of breast cancers have mutation in PI3K/AKT/mTOR or RAS/RAF/MEK/MAPK molecules. As we have mentioned before, the regulation of antigrowth signaling through inhibition of D-CDK4/6 has been shown as a promising therapeutic strategy for human ER+ breast cancer and other tumor types. Compared with other subtypes of breast cancer, HR+ breast cancer is commonly associated with hyperactivation of cyclin D1-CDK4/6 and palbociclib arrests these cell types in G0/G1 cell cycle phase [29,32]. The decision by the FDA to grant accelerate approval to palbociclib in combination with letrozole (2015), and in combination with fulvestrant (2016) was based on the results in the PALOMA-1-2-3 studies [33-35], where the combination of endocrine therapy like a nonsteroidal aromatase inhibitor or and a selective estrogen receptor degrader with a high selective CD4/6 inhibitor, showed impressive improvement in progression-free survival from 7.5 to 26.2 months, and from 4.6 to 9.5 months, respectively [29,34,35]. Ongoing studies are expanding the potential application of these agents beyond the ER-positive advanced breast cancer setting.

Epigenetic alterations that affect DNA methylation, histone modifications and chromatin remodeling patterns in breast cancer cells, are being proved as targets of therapeutic interventions. In combination with fulvestrant, azacitidine is being evaluated in phase II trial in patients with ER+, HER2- breast cancer, and entinostat in combination with azacytidine in TNBC or HR+, HER2- breast cancer.

Each human cell type works by utilizing canonical and no-canonical molecular components, signaling pathways and intracellular signals networks, which are used for the maintenance of the normal or altered cell behavior. Particularly in cancer cell behaviors, there are a number of biological component and pathway variants and subvariants. Overexpression of others cancer-related proteins as cytoplasm molecular sensors, molecular hubs of connected intracellular signaling pathways, molecular hubs of transcription factors, master genes on/off, hub effector proteins activity can modify the altered cell behavior of the main type of cancer cells of the mammary gland. Identification of

these epimutations, mutations and oncogenic pathways have provided opportunities for oncologist to target these patients with specific therapies. Molecular subtyping of tumors may offer additional insight into treatment of early-stage and advanced breast cancer keeping in mind the cancer subtype, temporal profile of molecularly changes, relative tolerability to different drug combinations and the compatibility of routes of administration [29].

Molecular abnormalities individualization leans towards the structure-activity inhibition

The precision cancer medicine paradigm use the molecular data in clinical decision-making at patient diagnosis and treatment. In the last years, advances in precision oncology have acquired a remarkable clinical benefit for patients with BRAF-mutant metastatic melanoma, EGFR-mutant or ALK-mutant non-small-cell lung cancer, and those with BCR-ABL translocation-positive chronic myelogenous leukemia. Different groups of molecular tests currently in use in clinical arena include genome sequencing methods and tumor proteomic and tumor/patient metabolomic analysis. Prominent methods of the first test groups are next generation sequencing or massively parallel sequencing technologies such as tumor sequencing, germline DNA sequencing, array-CGH, array-SNP, multiple ligation dependent probe amplification, and others which will identify genomic and epigenomic abnormalities in RNA-splicing, DNA methylation, chromatin modifications, and in signal transduction kinases. Other tests which evaluate signaling protein pathways have given insights into the independent effects and interactions of co-occurrence on disease-phenotype. The use of most of these techniques and platforms and bioinformatic support is required. These translational analytic tools are the beginning of this modern clinical medical era.

Bioinformatic tools play a great role in evolution of clinical research, not only in helping to analyze the terabytes of genomic data of tumor cells and germ-line genomic and proteomic profiles, but also in the drug discovery and drug development. Structure and ligand based methods are the most commonly used models in the drug discovery field. Identification of drug-targeted interactions though computational models is an important process in drug discovery. Molecular pathway analysis is carried out to identify canonical pathways and their role in different cancers. New technology like molecular docking, molecular dynamics simulation, analyses on the structure and function of proteins and quantitative structure activity are performed to check the potential of small inhibitors of cancer-related biomolecules in faster and easier processes [36-38]. These type of bioinformatic studies should be investigated before clinical trials. Docking and pharmacophore modeling have been widely used in virtual screening studies to identify novel compounds against drug targets. In molecular modeling field, docking is a method which predicts the structure of intermolecular complexes found between two molecules to find the best orientation of ligand which would form a stable complex with overall minimum energy. Computational methods have provided a powerful toolbox for target identification, discovery and optimization of drug candidate molecules. Molecular docking studies are one of the frequently used methods in structure-based drug design, which predicts interactions between three-dimensional structure of drug target and selected ligands [36,38].

To adequately address the immense complexity and heterogeneity underlying oncogenesis and tumor progression, innovative combination strategies will be needed to be customized for patients' unique molecular and immune profiles. Systematic high throughput

biological analysis supported by computational network-based platforms should be utilized to explore the comprehensive understanding of personalized tumor biology, to select molecule-targets and to explore novel therapeutic targets and to identify synergistic or additive drug combinations in early-phase clinical trials. Two meta-analysis of phase 2 clinical trials revealed that in patients with advanced cancers, a personalized strategy was an independent predictor of better outcomes and fewer toxic deaths, on the contrary, non-personalized targeted therapies were associated with significantly poorer outcome; also, response rates were significantly higher with genomic versus protein biomarking [39,40].

In this critical review we have described some of the first steps of the molecular modular architecture and functional changes participating only in early steps of proliferation regulating pathways of a transformed mature luminal-type cells of the milk-forming ducts from mammary gland, and the outstanding clinical results using the CDK4/6 inhibitors as molecularly targeted agents. To take the necessary steps to achieve the promise of precision cancer medicine we need combine strategies to progress in unraveling and understanding the molecular genomic, epigenomic and protein interactome aberrations in each module and submodule of the oncogenic cell behavior of each tumor and of each patient. These molecular findings will contribute to refine our diagnostic, prognostic, and mechanism-based therapeutic approaches. To date, more than 100 molecularly targeted agents have been approved by the US FDA for cancer patients treatment, and more than 3000 clinical trials supported by NCI have been registered, most clinical trials of targeted therapies use immunotherapy/immune-modulation, signals transduction inhibitors, angiogenesis inhibitors and hormone therapy as anti-cancer treatment strategies; the majority of these strategies use drug combinations.

Confict of Interest

No potential conflicts of interest were disclosed

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