Regulation and New Treatment Strategies in Breast Cancer

Rosa-Maria Ferraiuolo[1*], Kay-Uwe Wagner[1]

[1]Karmanos Cancer Institute at Wayne State University School of Medicine, Detroit, MI 48202.

*Correspondence: ferraiur@karmanos.org

Abstract Breast cancer classifications are based on the presence or absence of estrogen receptor and progesterone receptor along with the overexpression or amplification of the Her2 receptor. Although the overall 5-year survival rate of breast cancer patients has increased due to the use of targeted therapies, a subset of patients can acquire resistance over time or are unresponsive when presented in the clinic. Novel therapies focusing on molecular pathways and cell cycle regulation currently being used in the clinic may lead to increased response in this subset of patients.
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Mammary Gland Biology

Breast cancer is a heterogenous disease, with no single cause. Breast cancer can be classified under histological or genetic/molecular factors[1,2]. Female breasts, otherwise known as mammary glands, undergo postnatal growth and development, with cycles of regeneration and development occurring throughout life[3]. Hormones and growth factors tightly regulate the regeneration and development of the mammary glands, promoting the activation or deactivation of various signaling pathways[1,2,3].

A mature female mammary gland is composed of rings of epithelial cells, called alveoli, which produce milk during pregnancy. Multiple alveoli grouped together form lobules that share one lactiferous duct. This duct is responsible for the transport of milk from the lobules to the nipples[4,5]. A two cell layer system is formed from the alveoli and ductal structures and is composed of an inner luminal epithelial layer and an outer myoepithelial layer. Luminal cells can differentiate into milk producing alveolar cells. Myoepithelial cells have higher contractility allowing them to force the milk proteins through the ductal network[4]. The network is enclosed in connective tissue, the extracellular matrix, and the stroma; which contain adipocytes, fibroblasts and inflammatory cells[5]. A large majority of breast cancers will arise in the epithelial cells of the ducts or lobules[5]. Understanding the regulatory cues and the signaling pathways within normal breast development and how they are altered in carcinogenesis is critical for the successful treatment of breast cancer.

Current Molecular Classification of Breast Cancer

The World Health Organization (WHO) classifies breast cancer into 17 histological subtypes, all of which encompass different types and grades of tumors[6]. Initially, breast cancer is classified according to its histology, either grouped into in situ carcinoma or invasive/infiltrative carcinoma. The two groups are further subcategorized depending on where in the tissue the cancer originated; in the ducts or in the lobules[5]. Following histology, pathologists subdivide cancers based on receptor and growth factor status with a specific focus on estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (Her2)[5,7]. Breast cancer can be subdivided into five main subtypes; luminal A, luminal B, Her2-enriched, basal-like, and claudin-low[7].

Luminal A

Luminal A and luminal B breast cancers arise from luminal cells. Luminal A is the most prevalent subtype, making up approximately 40% of breast cancers[5,8-10]. This subtype is characterized as ER/PR positive and Her2 low or

negative. Luminal A has low expression of the proliferation marker Ki67, with only half of the cases having a mutation in the *p53* gene[5,7]. Patients have the best prognosis with the lowest recurrence rates[11,12].

Luminal B

Luminal B breast cancers are characterized as having low ER expression, but positive expression for PR and Her2[5,8-10]. Ki67 expression is normally high and 30% of patients have a mutation in *p53*[5]. This subtype comprises approximately 20% of all breast cancer cases and patients tend to have a worse prognosis than luminal A patients, but still a high survival rate in comparison to Her2-enriched, basal, and claudin-low breast cancers[5,8-11,13].

Treatment of Receptor Positive Breast Cancers

The high survival rates for luminal cancers comes from the ability to target these cancers on their receptors. Early stages can be treated with surgery and radiotherapy. Adjuvant therapy is usually administered to combat recurrence[14,15]. When cancers are positive for ER, treatment strategies include selective estrogen receptor modulators (SERMs), such as the drugs tamoxifen and fulvestrant, which antagonizes the ER to compete with estrogen binding, and aromatase inhibitors (AIs), which reduces the amount of estrogen circulating in the body[16-19].

Her2-enriched

The Her2-enriched subtype comprises approximately 10-15% of all breast cancers. In the clinic, 60% of patients labelled as Her2 positive will fall into this category. Only 30-40% of Her2-enriched patients will present with ER positive tumors, while the majority will have lower expression of ER and PR[7,20]. These tumors are normally characterized with high levels of Ki67, poorer prognosis and a higher and earlier rate of recurrence as well as increased metastasis[7,20,21]. The Her2-enriched subtype does not solely contain tumors where Her2 is amplified or a patient is Her2 positive; in some cases a Her2-negative patient will be categorized within this subtype due to similarity in gene expression profiles.

Her2 is one of four receptors in the ErbB family of receptors. All receptors are categorized as receptor tyrosine kinases (RTKs), which respond to growth factors, cytokines, and other extracellular signaling molecules. Her2 can form homo- and heterodimers with other family members including Her1 or the epidermal growth factor receptor (EGFR)[5,6,21]. Her2 is the preferred binding partner for the other family members. Interestingly, the extracellular domain is always in an open confirmation and it is the only family member without a designated ligand[22-24]; however, patients positive for Her2 can be treated with targeted therapy, such as the drug trastuzumab, which binds to and inhibits the dimerization of Her2 with other receptors[25-27].

Aside from the subfamily of EGFRs, the family of RTKs is comprised of many subfamilies, such as fibroblast growth factor receptors (FGFRs), insulin and insulin-like growth factor receptors (IR and IGFR, respectively), and platelet-derived growth factor receptors (PDGFRs), to name a few[28]. RTKs mediate key signaling pathways, in particular RAS/MAPK (mitogen activated protein kinase) and RAS/PI3K/AKT pathways. 20-25% of breast cancers are found to have mutated RTKs, affecting downstream pathways and resulting in increased proliferation, survival, invasion, and metastasis[29]. RTKs are regulated through internalization, also known as endocytosis[30]. Internalization of RTKs into endosomes is slower than the recycling from endosomes back to the cell surface, causing an accumulation of receptors at the cell surface and easy accessibility of ligands to receptors[31].

Inhibiting the RTK signaling pathway has been a very attractive therapeutic strategy. It has been difficult to date due to the high rate of mutations throughout the pathway and the ability of the cells to adapt to the small molecule inhibitors; thereby, developing resistance through newly acquired mutations[28]. Although beginning with

monotherapy, more research is focusing on targeting multiple components of the RTK pathway as combination therapy. For instance, the inhibitor ONC201 binds to and inhibits the activity of AKT and the extracellular signal-regulated kinase (ERK), which means it has the potential to inhibit both the PI3K and MAPK pathways. Clinical trials utilizing this inhibitor have begun for multiple types of cancer such as glioma, multiple myeloma, and endometrial cancer. Phase II trials on different types of breast cancer (NCT03733119, NCT03394027) were developed based on *in vitro* data showing that ONC201 destroys the mitochondria inside the cells while not affecting the normal cells[32]. Originally, ONC201 was identified as an inducer of transcription of the TNF-related apoptosis-inducing ligand (TRAIL), which kills cancer cells through the activation of TRAIL death receptors[33]. However, further investigation utilizing this small molecule inhibitor on human breast and endometrial cancer cell lines showed this inhibitor induces phosphorylation of AMP-dependent kinase and a loss of ATP. They further showed mitochondrial respiration was inhibited and there was a decrease in mitochondrial DNA[32]. A phase I study showed no serious toxicity to breast cancer patients[34], which shows promise for the active studies utilizing this molecule. Interestingly, in acute myeloid leukemia and mantle cell lymphoma cells, ONC201 did not exert its effects via TRAIL, but rather induced endoplasmic reticulum stress or integrated stress response-related genes[35], which indicates the need to understand the different mechanisms a drug can utilize in different cancers.

Basal-like

Basal-like breast cancers were named based on similar features and cytokeratin expression as basal epithelia of the skin and airways as well as the basal layer of the mammary ducts[7,15,20]. This subtype is characterized by no expression of ER or PR, and no expression or amplification of Her2. However, these cancers do have positive expression of EGFR. This subtype comprises approximately 10-25% of all breast cancer cases. The majority have a *p53* gene mutation and have a high proliferative capacity[7]. Basal-like breast cancers have been labelled as the subtype with the poorest prognosis. Targeted therapies do not exist and are normally treated with chemotherapy and PARP inhibitors. Patients with this subtype usually have high recurrence and metastatic rates and overall survival of patients is low, especially within the first 3 years[7].

Claudin-low

Originally, patients presenting with this subtype were classified as basal-like since they are ER/PR/Her2 negative[36]; however, further development using DNA microarray studies showed that a subset of tumors presented with low levels of the claudin genes, which are required for epithelial cell tight-tight junctions[37]. This subtype represents 5-10% of all breast cancers and have low expression of claudins 3, 4, and 7[38]. E-cadherin, a protein also required for cell-cell junctions, is found to be low in this subtype[7,15,20]. This subtype has shown to have an increase in stem cell features, immune cell infiltration, and have representative features of epithelial-mesenchymal transition (EMT)[36-37]. Patients within this subtype have a poor prognosis and, without any targeted therapy, must rely on chemotherapy as a form of treatment[7].

Cell Cycle and Cancers

All cells are regulated by the cell cycle to regulate the processes of growth, differentiation, senescence, and apoptosis. In cancer, there is a disruption of pathways driving the cell cycle[39]. Various alterations in the cell cycle can impact the growth characteristics of different types of cancers and also determines how the tumor will respond to therapies.

The cell cycle is comprised of interphase (with 3 distinct phases: G1, S, and G2), and mitosis (M). If conditions are not favorable, the cells will enter a state of quiescence (G0). Cells in G0 do not enter S phase and will stay metabolically active until they re-enter the cell cycle[40]. All

phases of the cell cycle are regulated by oscillating accumulation of proteins called cyclins, which are expressed and degraded at different phases[41].

The catalytic partner of a cyclin is called a cyclin dependent kinase (CDK), which is expressed at a constant, but inactive, level. The CDK has an active site where ATP binds, deep within a cleft. In an inactive CDK, a T-loop blocks the active site, suppressing its activity[42,43]. When a cyclin binds, a conformational change occurs, exposing the catalytic cleft for substrate binding[44-47]. Binding of a cyclin to its CDK partner does not fully activate the CDK. For full activation, the CDK requires posttranslational modifications. The CDK activating kinase (CAK) phosphorylates a threonine on the T-loop, which flattens the T-loop and moves it near the cyclin. This conformational change creates a binding site that contains the consensus sequence ((S/T)PX(K/R))[43,44,46,48,49]. Cyclins have a hydrophobic patch composed of an MRAIL motif. This motif binds to a CDK substrate if the substrate has a complementary RXL sequence. This interaction increases the binding affinity of the kinase with its substrate[45, 49-54]. Further action required for activation of the CDK includes the removal of inhibitory phosphorylation of threonine 14 and tyrosine 15 removed by Cdc25 phosphatases[49, 52-54]. Specific formation of cyclin-CDK complexes and their activation govern each phase of the cell cycle; G1 phase is controlled by Cyclin D-CDK4/6 (early to mid-G1) as well as Cyclin E-CDK2 complexes (late G1 and entry into S phase), S phase is controlled by Cyclin A-CDK2, and G2 phase is controlled Cyclin A-CDK1 and Cyclin B-CDK1[40, 55-58].

Normally, the cell cycle is tightly regulated. Different phases of the cell cycle can be transiently inhibited by CDK inhibitors (CKIs). G1/S phase can be inhibited by the Cip/Kip family composed of $p21^{Cip1}$, $p27^{Kip1}$, and $p57^{Kip1}$, which inhibit Cyclin E-CDK2 and Cyclin A-CDK2. These CKIs bind to both the MRAIL motif on the cyclin and a domain on the CDK. When bound, this alters the confirmation of the cyclin-CDK complex, providing limited access to the catalytic cleft[58,59]. The second family of CKIs is the Ink4 family and is specific for CDK4/6. This family consists of $p16^{Ink4a}$ or its alternate reading frame (ARF) $p14^{ARF}$, $p15^{Ink4b}$, $p18^{Ink4c}$, $p19^{Ink4d}$, and $p19^{ARF}$ [59-61]. This family binds to monomeric CDK4 and CDK6, causing a conformational change, which will inhibit the ability of Cyclin D from binding to and activating the CDK[59]. Proper regulation of the cell cycle by these complexes have an important role in the initiation and progression of various cancers. Different therapies alter the cell cycle in order to promote apoptosis of tumor cells.

Chemotherapies

With over 100 different chemotherapies currently in use, overall survival of breast cancer patients has been significantly increased. Chemotherapies are designed to target rapidly dividing cells to inhibit the cell cycle and promote apoptosis. A major disadvantage of this treatment is the inability of the drugs to distinguish between a cancerous and a normal cell[62].

Alkylating Agents

Alkylating agents, such as cyclophosphamide, will directly damage the DNA by adding an alkyl group to guanine base of DNA, which will form a cross-link. Cross-linked DNA will maintain a coiled position unable to separate, thereby, preventing DNA synthesis[63]. A major disadvantage of alkylating agents is their ability to cause long-term damage to the bone marrow of a patient, which can lead to acute leukemia[64,65].

Platinum based drugs, such as cisplatin, carboplatin, and oxaliplatin, are also considered a form of alkylating agents since they have a similar mechanism to damage the DNA[66,67]. The difference between platinum drugs and alkylating agents is platinum drugs do not have an alkyl group. When administered, one of the chloride ligands in the platinum drug is displaced by water, allowing the platinum molecule to bind to DNA bases, preferably guanine. This

interaction forms a DNA adduct, which then displaces the second chloride ligand, followed by binding of a second platinum molecule[68]. Platinum-DNA adducts interfere with cell division, triggering the DNA repair machinery, activating the apoptotic pathway if the damage cannot be fixed. These adducts do not get metabolized into harmful by-products, which provides a less toxic effect on normal cells and less likelihood of developing leukemia in the future[66,67,69].

Anti-tumor Antibiotics

Anti-tumor antibiotics include anthracyclines, such as doxorubicin. These drugs have the ability to work in all cell cycle phases; however, they have a preference in interfering with DNA replication enzymes. Therefore, they prefer to exert their effects in S-phase[70-72]. Unlike alkylating agents, anthracyclines have 4 mechanisms of action: I) they can inhibit DNA and RNA synthesis through intercalation[72], II) they inhibit topoisomerase II, which is responsible for DNA separation[71], III) they generate free oxygen radicals to damage DNA, protein, and cell membranes[72], and IV) they promote histone eviction from chromatin to activate DNA damage repair or activation of apoptosis[73]. The disadvantage of this class of drugs is the possible permanent heart damage or an increased risk of developing a second cancer, such as myelogenous leukemia[70,72].

Mitotic Inhibitors

As indicated by the name of this class, these inhibitors inhibit the M-phase of the cell cycle[74]. Most of these inhibitors are derived from plant alkaloids[75]. Paclitaxel, derived from the Pacific yew tree bark, is one of the most common types of mitotic inhibitors. Paclitaxel gets metabolized into 6-α-hydroxypaclitaxel, which then stabilizes microtubules and stops polymers from disassembly[75,76]. This causes a defect in spindle assembly, segregation of chromosomes, and cell division by blocking mitosis and delaying the spindle assembly checkpoint (SAC), which promotes apoptosis. A disadvantage of this class is the potential for peripheral nerve damage[65,74,77,78].

Synthetic CDK Inhibitors in Breast Cancer Treatment

During cancer progression, many CKIs become deregulated and their targets, cyclin-CDKs, become elevated. This elevation contributes to treatment resistance to chemotherapy and even targeted hormone therapies[61,79-81]. Developing drugs that can reinstate the control CKIs have on the cell cycle is a beneficial treatment strategy, and is currently a hot topic in the clinical field. Synthetic CKIs are purine-based drugs that have been designed to mimic $p21^{Cip1}$ and $p27^{Kip1}$. These synthetic CKIs competitively bind to the ATP-binding site of CDKs, inhibiting its kinase activity[82]. Synthetic CKI selectivity is challenging due to the similarity of the active sites of the CDKs[86].

CDK4/6 Inhibitors

The first documented clinical trial using CDK4/6 inhibitors where they stratified patients based on molecular signature of the patient has been completed in ER-positive breast cancer[87]. This trial looked specifically for an amplification of Cyclin D1, a loss of p16, or both[87,88]. The stratification of the patients resulted in improved responses to the CDK4/6 inhibitor palbociclib (PD0332991)[87,88]. *In vitro* studies determined this inhibitor was able to inhibit the phosphorylation of the tumor suppressor retinoblastoma (RB) protein in MDA-MB-435 cells, a melanoma-like cell line, as well as inhibiting the proliferation of human breast, colon, lung, and leukemia cell lines[89-91]. Further research has eluted to the anti-tumor immunity promoted by CDK4/6 inhibitors, confirmed through transcriptomic analysis of human biopsies from clinical trials. Treatment with CDK4/6 inhibitors suppresses the proliferation of regulatory T-cells; which will increase tumor immunogenicity and blocks immune checkpoints[92].

A phase II neoadjuvant trial assessing the addition of palbociclib to anti-estrogen

treatment anastrozole showed a significantly higher complete cell cycle arrest rate in comparison to anastrozole alone. Cell cycle control was significantly enhanced with the addition of palbociclib regardless of whether the patient was luminal A or B subtype. Resistance from this trial was only seen in patients with nonluminal subtypes[94]. Similarly, in a follow-up study to a phase II trial to improve the outcomes of first-line treatment of ER-positive but Her2-negative breast cancers treated with endocrine therapy and palbociclib, a phase III trial was conducted and found addition of palbociclib with letrozole resulted in longer progression-free survival in comparison to letrozole alone[95].

The majority of the research performed on CDK4/6 inhibitors focuses on luminal subtypes; however, it was recently discovered, through the use of Her2-overexpressing transgenic mouse models, cell lines, and clinical specimens, that recurrence of this disease is driven by the Cyclin D-CDK4/6 pathway[92,93]. The first line of treatment for Her2-overexpressing or amplified tumors is monoclonal antibody therapy, such as trastuzumab[25,26]. During times of recurrence and trastuzumab resistance, the tumors were found to have higher levels of Cyclin D1, but lower levels of p16. Resistance can be overcome when CDK4/6 inhibitors, abemaciclib or lapatinib, were used. Combination of abemaciclib with trastuzumab had a significant effect in survival in comparison to abemaciclib alone, which suggests that inhibiting CDK4/6 will re-sensitize the cells to trastuzumab[96]. Utilizing these inhibitors for more aggressive cancers could be a novel therapy that may have a role in increasing sensitivity and reducing recurrence.

Similarly, recent research has shed some light on the effect on triple negative breast cancer (TNBC). TNBCs are characterized as being negative for ER, PR, and Her2[7,20]. All claudin-low breast cancers are TN, while over 50% of all TNBC cases are basal-like. TNBCs are less differentiated, have a poor prognosis, a significantly increased rate of relapse within the first 3 years, and an increased proliferative capacity[5,7,20,36]. When palbociclib was tested *in vitro* on ER-positive (T47D) and TNBC (BT-549 and MDA-MB-231) cells, proliferation of the ER-positive cell lines was inhibited, whereas the inhibitor had no effect on TNBC cell proliferation[97]. Although these results were not unexpected, the inhibitor did reduce cell migration of the MDA-MB-231 cells. Patient-derived xenograft (PDX) models implanted with human breast tumor biopsies showed, when untreated, metastatic lesions could be found in the liver, lung and ovary; however, after administering palbociclib, there was a significant decrease in both the liver and lung metastases[97]. These data suggest inhibiting CDK4/6 will not affect the size and proliferation of the primary tumor, but could inhibit metastases of tumor cells and be a novel therapeutic for metastatic TNBC.

CDK1 and CDK2 Inhibitors

Breast cancer contains a plethora of tumorigenic events that trigger proliferation through the recruitment of CDKs. Ample evidence has shown the need for CDK2 in the regulation of hormone positive breast cancers at the G1/S transition[98,99]. Recent research is now beginning to investigate the role of CDKs in hormone-negative breast cancers, such as TNBCs. Initial synthetic CKIs were designed to inhibit multiple CDKs (pan-CKIs). The first generated synthetic CKI, flavopiridol, was found to induce cell cycle arrest in both G1 and G2 phases *in vitro*; however, significant cytotoxic effects were seen *in vivo* due to its ability to inhibit CDK7 and CDK9[100]. Combination therapy of flavopiridol with trastuzumab has shown cytotoxic synergy in cells overexpressing Her2[101]. Since flavopiridol may be working through Ras-dependent pathways, further work has investigated combination therapy of flavopiridol with Sorafenib, which is a multi-kinase small molecule inhibitor that can disrupt Ras-MAPK signaling. Flavopiridol potentiated cytotoxicity induced by Sorafenib, especially in cell lines containing EGFR/Her2 overexpression, constitutive activation of the Ras-MAPK pathway, or KRAS-BRAF mutations[102]. Pre-clinical and clinical trials

utilizing flavopiridol as monotherapy have observed little success and significant toxic effects on normal cells[83,85]. Similarly, another pan-CKI, roscovitine, has been shown to accumulate cells in the G2/M phase of the cell cycle[103, 104] and potentiate the anti-tumor effects of the chemotherapy drug, doxorubicin[105]. Although it predominately inhibits CDK2, it can also inhibit CDK1, CDK5, CDK7 and CDK9[82,105,106] and has entered phase II clinical trials[82,106]. As combination therapy with various chemotherapies, pan-CKIs have not shown promising benefits to breast cancer patients, but have shown to be promising in phase II clinical trials in relapsed and refractory multiple myeloma[107].

A major limitation of synthetic CDK inhibitors *in vivo* and *in vitro* is the compensation of its activity by other CDKs[108]. This compensation can be partly attributed to the conservation of the CDK active site between different CDKs[87]. Second and third generation synthetic CKIs have started selectively targeting specific CDKs in pre-clinical studies; however, clinical trials utilizing specific synthetic CKIs is lacking. As previously mentioned, most clinical trials have focused on pan-CKIs and the majority focus on hormone positive breast cancer patients; however, dual inhibition of CDK1 and CDK2 has become an area of interest, especially to combat the compensation effect of kinases. Dinaciclib is a potent dual CDK1 and CDK2 inhibitor that is currently being used in many clinical trials for many types of cancers, including TNBC (NCT01624441). Although *in vitro* data has shown promising results, it has been seen to have severe toxicities in combination with the anthracyclin epirubicin in clinical trials[109]. Whether combination of dinaciclib with other chemotherapies will have similar toxic effects still remains to be elucidated. Currently, another clinical trial is testing its efficacy with the monoclonal antibody pembrolizumab in patients with low levels of ER and PR and those not overexpressing Her2 (NCT01676753). Although this trial is ongoing, the future results will add another piece to a very intricate and elaborate puzzle in determining effective therapeutic strategies for aggressive breast cancers. Without a complete understanding of how these synthetic CKIs function *in vivo* and their off target or compensatory effects, utilizing these molecules in clinical trials will require further investigation at the molecular level.

Conclusions

Breast cancer remains the second leading cause of death among women. With the gross heterogeneity and multiple subtypes, treatment regimens are highly dependent on multiple factors including their molecular and histopathological characteristics of the cancer. As research has explored various molecular pathways, new developments are combining these pathways with cell cycle regulators. Cell cycle properties of breast cancer tumors/cells is a highly important aspect in understanding whether a specific treatment regimen will work and how altering the cell cycle could not only be a novel form of therapy, but could enhance the efficacy of current therapies. Reintroduction of lost/downregulated CKIs in cancers through the use of synthetic CKIs is a newly emerging form of therapy[87]. If proven effective, synthetic CKIs can be an invaluable tool to promote a homeostatic state by triggering apoptosis in cancer cells. Currently, a lack of understanding in the exact mechanism of CKI activity and the CDKs they inhibit is a major pitfall for the use of these molecules. As more research of the cell cycle and its regulators develops, a better understanding of the proper combinations and timing of therapies can be discovered.

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Competing Interests

The authors declare that they have no competing interests.

Abbreviations

AI – aromatase inhibitor

ARF – alternate reading frame
BC – breast cancer
CAK – cyclin activating kinase
CDK – cyclin dependent kinase
CKI – cyclin dependent kinase inhibitor
EGFR – epidermal growth factor receptor
EMT – epithelial-mesenchymal transition
ER – estrogen receptor
ErbB – erythroblastic oncogene B
ERK – extracellular signal-related kinase
FGFR – fibroblast growth factor receptor
Her2 – human epidermal growth factor receptor 2
IGFR – insulin-like growth factor receptor
IR – insulin receptor
MAPK – mitogen activated protein kinase
PDGFR – platelet-derived growth factor receptor
PDX – patient-derived xenograft
PI3K – phosphatidylinositide 3-kinase
PR – progesterone receptor
RB – retinoblastoma protein
RTK – receptor tyrosine kinase
SAC – spindle assembly checkpoint
SERMs – selective estrogen receptor modulators
TNBC – triple negative breast cancer
TRAIL – TNF-related apoptosis-inducing ligand
WHO – World Health Organization

References

1. The mammary gland: a unique organ for the study of development and tumorigenesis. Medina, D. 1996, J Mammary Gland Biol Neoplasia, Vol. 1, pp. 5-19. https://doi.org/10.1007/BF02096299 PMid:10887477

2. Essential role of endogenous estrogen in directly stimulating mammary growth demonstrated by implants containing pure antiestrogens. Silberstein GB, Van Horn K, Shyamala G, Daniel CW. 1994, Endocrinology, Vol. 134, pp. 84-90. https://doi.org/10.1210/endo.134.1.8275973 PMid:8275973

3. Mammary stem cells and the differentiation hierarchy: current status and perspectives. Visvader JE, Stingl J. 2014, Genes Dev, Vol. 28, pp. 1143-1158. https://doi.org/10.1101/gad.242511.114 PMid:24888586 PMCid:PMC4052761

4. Keeping abreast of the mammary epithelial hierarchy and breast tumorigenesis. Visvader. 2009, Genes Dev, Vol. 23, pp. 2563-2577.https://doi.org/10.1101/gad.1849509 PMid:19933147 PMCid:PMC2779757

5. Histological, molecular, and functional subtypes of breast cancers. Malhotra GK, Zhao X, Band H, Band V. 2010, Cancer Biol Ther, Vol. 10, pp. 955-960. https://doi.org/10.4161/cbt.10.10.13879 PMid:21057215 PMCid:PMC3047091

6. Histological types of breast cancer: how special are they? Weigelt B, Geyer FC, Reis-Filho JS. 2010, Mol Oncol, Vol. 4, pp. 192-208. https://doi.org/10.1016/j.molonc.2010.04.004 PMid:20452298 PMCid:PMC5527938

7. Molecular portraits of human breast tumours. Perou CM, Sorlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, Pollack JR, Ross DT, Johnsen H, Akslen LA, et al. 2000, Nature, Vol. 406, pp. 747-752. https://doi.org/10.1038/35021093 PMid:10963602

8. Differential isolation of normal luminal mammary epithelial cells and breast cancer cells from primary and metastatic sites using selective media. Ethier SP, Mahacek ML, Gullick WJ, Frank TS, Weber BL. 1993, Cancer Res, Vol. 53, pp. 627-635.

9. Luminal breast cancer metastases and tumor arousal from dormancy are promoted by direct actions of estradiol and progesterone on the malignant cells. Ogba N, Manning NG, Bliesner BS, Ambler SK, Haughian JM, Pinto MP, Jedlicka P, Joensuu K, Heikkila P, Horwitz KB. 2014, Breast Cancer Res, Vol. 16, p. 489. https://doi.org/10.1186/s13058-014-0489-4 PMid:25475897 PMCid:PMC4303198

10. Gene expression abnormalities in histologically normal breast epithelium from

patients with luminal type of breast cancer. Zubor P, Hatok J, Moricova P, Kajo K, Kapustova I, Mendelova A, Racay P, Danko J. 2015, Mol Biol Rep, Vol. 42, pp. 977-988. https://doi.org/10.1007/s11033-014-3834-x PMid:25407308

11. Patterns of Recurrence and outcome according to breast cancer subtypes in lymph node-negative disease: results from international breast cancer study group trials VIII and IX. Metzger-Filho O, Sun Z, Viale G, Price KN, Crivellari D, Snyder RD, Gelber RD, Castiglione-Gertsch M, Coates AS, Goldhirsch A, Cardoso F. 2013, J Clin Oncol, Vol. 31, pp. 3083-3090. https://doi.org/10.1200/JCO.2012.46.1574 PMid:23897954 PMCid:PMC3753700

12. A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. Paik S, Shak S, Tang G, Kim C, Baker J, Cronin M, Baehner FL, Walker MG, Watson D, Park T, et al. 2004, N Engl J Med, Vol. 351, pp. 2817-2826. https://doi.org/10.1056/NEJMoa041588 PMid:15591335

13. The prognostic contribution of clinical breast cancer subtype, age, and race among patients with breast cancer brain metastases. Anders CK, Deal AM, Miller CR, Khorram C, Meng H, Burrows E, Livasy C, Fritchie K, Ewend MG, Perou CM, Carey LA. 2011, Cancer, Vol. 117, pp. 1602-1611. https://doi.org/10.1002/cncr.25746 PMid:21472708 PMCid:PMC4265570

14. Breast cancer stem cells: treatment resistance and therapeutic opportunities. Al-Ejeh F, Smart CE, Morrison BJ, Chenevix-Trench G, Lopez JA, Lakhani SR, Brown MP, Khanna KK. 2011, Carcinogenesis, Vol. 32, pp. 650-658. https://doi.org/10.1093/carcin/bgr028 PMid:21310941

15. Deconstructing the molecular portraits of breast cancer. Prat A, Perou CM. 2011, Mol Oncol, Vol. 5, pp. 5-23. https://doi.org/10.1016/j.molonc.2010.11.003 PMid:21147047 PMCid:PMC5528267

16. Hormonal effects of aromatase inhibitors: focus on premenopausal effects and interaction with tamoxifen. Dowsett M, Haynes BP. 2003, J Steroid Biochem Mol Biol, Vol. 86, pp. 255-263. https://doi.org/10.1016/S0960-0760(03)00365-0

17. A role for estrogen phosphorylation in the resistance to tamoxifen. de Leeuw R, Neefjes J, Michalides R. 2011, Int J Breast Cancer, Vol. 2011, p. 232435. https://doi.org/10.4061/2011/232435 PMid:22295213 PMCid:PMC3262574

18. Circumventing tamoxifen resistance in breast cancers using antiestrogens that induce unique conformational changes in the estrogen receptor. Connor CE, Norris JD, Broadwater G, Willson TM, Gottardis MM, Dewhirst MW, McDonnell DP. 2001, Cancer Res, Vol. 61, pp. 2917-2922.

19. Treatment of estrogen receptor-positive breast cancer. Lumachi F, Brunello A, Maruzzo M, Basso U, Basso SM. 2013, Curr Med Chem, Vol. 20, pp. 596-604. https://doi.org/10.2174/09298671380499303 PMid:23278394

20. Molecular stratification of triple-negative breast cancers. Perou. 2010, The Oncologist, Vol. 16, pp. 39-48. https://doi.org/10.1634/theoncologist.2010-S5-39 PMid:21138954

21. Age/race differences in HER2 testing and in incidence rates for breast cancer triple subtypes: a population-based study and first report. Lund MJ, Butler EN, Hair BY, Ward KC, Andrews JH, Oprea-Ilies G, Bayakly AR, O'Regan RM, Vertino PM, Eley JW. 2010, Cancer, Vol. 116, pp. 2549-2559.

22. Structure of the extracellular region of HER2 alone and in complex with the Herceptin Fab. Cho HS, Mason K, Ramyar KX, Stanley AM, Gabelli SB, Denney DW Jr, Leahy DJ. 2003, Nature, Vol. 421, pp. 756-760.

https://doi.org/10.1038/nature01392
PMid:12610629

23. The crystal structure of a truncated ErbB2 ectodomain reveals an active conformation, poised to interact with other ErbB receptors. Garrett TP, McKern NM, Lou M, Elleman TC, Adams TE, Lovrecz GO, Kofler M, Jorissen RN, Nice EC, Burgess AW, Ward CW. 2003, Mol Cell, Vol. 11, pp. 495-505. https://doi.org/10.1016/S1097-2765(03)00048-0

24. ErbB-2, the preferred heterodimerization partner of all ErbB receptors, is a mediator of lateral signaling. Graus-Porta D, Beerli RR, Daly JM, Hynes NE. 1997, EMBO J, Vol. 16, pp. 1647-1655.
https://doi.org/10.1093/emboj/16.7.1647
PMid:9130710 PMCid:PMC1169769

25. Her2-positive breast cancer: herceptin and beyond. Dean-Colomb W, Esteva FJ. 2008, Eur J Cancer, Vol. 44, pp. 2806-2812. https://doi.org/10.1016/j.ejca.2008.09.013
PMid:19022660

26. Herceptin (trastuzumab): adjuvant and neoadjuvant trials. Yaal-Hahoshen N, Safra T. 2006, Isr Med Assoc J, Vol. 8, pp. 416-421.

27. Effects of Herceptin on circulating tumor cells in HER2 positive early breast cancer. Zhang JL, Yao Q, Chen YWJH, Wang H, Fan Q, Ling R, Yi J, Wang L. 2015, Genet Mol Res, Vol. 14, pp. 2099-2103.
https://doi.org/10.4238/2015.March.20.20
PMid:25867356

28. Targeting RTK Signaling Pathways in Cancer. Regad. 2015, Cancers, Vol. 7, pp. 1758-1784. https://doi.org/10.3390/cancers7030860
PMid:26404379 PMCid:PMC4586793

29. Receptor tyrosine kinases: role in cancer progression. Sangwan V, Park M. 2006, Curr Oncol, Vol. 13, pp. 191-193.

30. Endocytosis of Receptor Tyrosine Kinases. Goh LK, Sorkin A. 2013, Cold Spring Harb Perspect Biology, Vol. 5, p. a017459. https://doi.org/10.1101/cshperspect.a017459
PMid:23637288 PMCid:PMC3632065

31. Effects of Membrane Trafficking on Signaling by Receptor Tyrosine Kinases. Miaczynska. 2013, Cold Spring Harb Perspect Biol, Vol. 5, p. a009035. https://doi.org/10.1101/cshperspect.a009035
PMid:24186066 PMCid:PMC3809584

32. ONC201 kills breast cancer cells in vitro by targeting mitochondria. Greer YE, Porat-Shiliom N, Nagashima K, Stuelten C, Crooks D, Koparde VN, Gilbert SF, Islam C, Ubaldini A, Ji Y, Gattinoni L, Soheilian F, Wang X, Hafner M, Shetty J, Tran B, Jailwala P, Cam M, Lang M, Voeller D, Reinhold WC, Rajapakse V, et al. 2018, Oncotarget, Vol. 9, pp. 18454-18479. https://doi.org/10.18632/oncotarget.24862

33. Dual inactivation of Akt and ERK by TIC10 signals Foxo3a nuclear translocation, TRAIL gene induction, and potent antitumor effects. Allen JE, Krigsfeld G, Mayes PA, Patel L, Dicker DT, Patel AS, Dolloff NG, Messaris E, Scata KA, Wang W, Zhou JY, Wu GS, El-Deiry WS. 2013, Sci Transl Med, Vol. 5, p. 171ra17. https://doi.org/10.1126/scitranslmed.3004828
PMid:23390247 PMCid:PMC4535715

34. First-in-Human Clinical Trial of Oral ONC201 in Patients with Refractory Solid Tumors. Stein MN, Bertino JR, Kaufman HL, Mayer T, Moss R, Silk A, Chan N, Malhotra J, Rodriguez L, Aisner J, Aiken RD, Haffty BG, DiPaola RS, et al. 2017, Clin Cancer Res, Vol. 23, pp. 4163-4169. https://doi.org/10.1158/1078-0432.CCR-16-2658 PMid:28331050

35. ATF4 induction through an atypical integrated stress response to ONC201 triggers p53-independent apoptosis in hematological malignancies. Ishizawa J, Kojima K, Chachad D, Ruvolo P, Ruvolo V, Jacamo RO, Borthakur G, Mu H, Zeng Z, Tabe Y, Allen JE, Wang Z, Ma W, et al. 2016, Sci Signal., Vol. 9, p. ra17. https://doi.org/10.1126/scisignal.aac4380
PMid:26884599 PMCid:PMC4815038

36. Claudin-low breast cancers: clinical, pathological, molecular and prognostic characterization. Sabatier R, Finetti P, Guille A, Adelaide J, Chaffanet M, Viens P, Birnbaum D, Bertucci F. 2014, Mol Cancer, Vol. 13, p. 228.

https://doi.org/10.1186/1476-4598-13-228
PMid:25277734 PMCid:PMC4197217

37. Phenotypic and molecular characterization of the claudin-low intrinsic subtype of breast cancer. Prat A, Parker JS, Karginova O, Fan C, Livasy C, Herschkowitz JI, He X, Perou CM. 2010, Breast Cancer Res., Vol. 12, p. R68. https://doi.org/10.1186/bcr2635
PMid:20813035 PMCid:PMC3096954

38. Claudin-Low Breast Cancer; Clinical & Pathological Characteristics. Dias K, Dvorkin-Gheva A, Hallett RM, Wu Y, Hassell J, Pond GR, Levine M, Whelan T, Bane AL. 2017, PLoS One, Vol. 12, p. e0168669. https://doi.org/10.1371/journal.pone.0168669
PMid:28045912 PMCid:PMC5207440

39. The cell cycle and cancer. Collins K, Jacks T, Pavletich NP. 1997, Proc Natl Acad Sci USA, Vol. 94, pp. 2776-2778. https://doi.org/10.1073/pnas.94.7.2776
PMid:9096291 PMCid:PMC34145

40. Cell cycle control of mammalian neural stem cells: putting a speed limit on G1. Salomoni P, Calegari F. 2010, Trends Cell Biol, Vol. 20, pp. 233-243. https://doi.org/10.1016/j.tcb.2010.01.006
PMid:20153966

41. Activation of the various cyclin/cdc2 protein kinases. Solomon. 1993, Curr Opin Cell Biol, Vol. 5, pp. 180-186. https://doi.org/10.1016/0955-0674(93)90100-5

42. Cell cycle regulation of CDK2 activity by phosphorylation of Thr160 and Tyr15. Gu Y, Rosenblatt J, Morgan DO. 1992, EMBO J, Vol. 11, pp. 3995-4005. https://doi.org/10.1002/j.1460-2075.1992.tb05493.x PMid:1396589
PMCid:PMC556910

43. Mechanism of CDK activation revealed by the structure of a cyclinA-CDK2 complex. Jeffrey PD, Russo AA, Polyak K, Gibbs E, Hurwitz J, Massague J, Pavletich NP. 1995, Nature, Vol. 376, pp. 313-320. https://doi.org/10.1038/376313a0
PMid:7630397

44. Crystal structure and mutational analysis of the human CDK2 kinase complex with cell cycle regulatory protein CksHs1. Bourne Y, Watson MH, Hickey MJ, Holmes W, Rocque W, Reed SI, Tainer JA. 1996, Cell, Vol. 84, pp. 863-874. https://doi.org/10.1016/S0092-8674(00)81065-X

45. The structural basis for specificity of substrate and recruitment peptides for cyclin-dependent kinases. Brown NR, Noble ME, Endicott JA, Johnson LN. 1999, Nat Cell Biol, Vol. 1, pp. 438-443. https://doi.org/10.1038/15674
PMid:10559988

46. The role of Thr160 phosphorylation of Cdk2 in substrate recognition. Holmes JK, Solomon MJ. 2001, Eur J Biochem, Vol. 268, pp. 4647-4652. https://doi.org/10.1046/j.1432-1327.2001.02392.x PMid:11532001

47. Substrate recruitment to cyclin-dependent kinase 2 by a multipurpose docking site on cyclin A. Schulman BA, Lindstrom DL, Harlow E. 1998, Proc Natl Acad Sci USA, Vol. 95, pp. 10453-10458. https://doi.org/10.1073/pnas.95.18.10453
PMid:9724724 PMCid:PMC27915

48. Phosphorylation at Thr167 is required for Schizosaccharomyces pombe p34cdc2 function. Gould KL, Moreno S, Owen DJ, Sazer S, Nurse P. 1991, EMBO J, Vol. 10, pp. 3297-3309. https://doi.org/10.1002/j.1460-2075.1991.tb04894.x PMid:1655416
PMCid:PMC453056

49. Regulation of CDKs by phosphorylation. Solomon MJ, Kaldis P. 1998, Results Probl Cell Differ, Vol. 22, pp. 79-109. https://doi.org/10.1007/978-3-540-69686-5_4
PMid:9670320

50. The cyclin box and C-terminus of cyclins A and E specify CDK activation and substrate specificity. Horton LE, Templeton DJ. 1997,

Oncogene, Vol. 14, pp. 491-498. https://doi.org/10.1038/sj.onc.1200851 PMid:9053846

51. Cyclin specificity in the phosphorylation of cyclin-dependent kinase substrates. Loog M, Morgan DO. 2005, Nature, Vol. 434, pp. 104-108. https://doi.org/10.1038/nature03329 PMid:15744308

52. p107wee1 is a dual-specificity kinase that phosphorylates p34cdc2 on tyrosine 15. Parker LL, Atherton-Fessler S, Piwnica-Worms H. 1992, Proc Natl Acad Sci USA, Vol. 89, pp. 2917-2921. https://doi.org/10.1073/pnas.89.7.2917 PMid:1372994 PMCid:PMC48774

53. Regulation of the human WEE1Hu CDK tyrosine 15-kinase during the cell cycle. Watanabe N, Broome M, Hunter T. 1995, EMBO J, Vol. 14, pp. 1878-1891. https://doi.org/10.1002/j.1460-2075.1995.tb07180.x PMid:7743995 PMCid:PMC398287

54. How tyrosine 15 phosphorylation inhibits the activity of cyclin-dependent kinase 2-cyclin A. Welburn JP, Tucker JA, Johnson T, Lindert L, Morgan M, Willis A, Noble ME, Endicott JA. 2007, J Biol Chem, Vol. 282, pp. 3173-3181. https://doi.org/10.1074/jbc.M609151200 PMid:17095507

55. Cyclin activation of p34cdc2. Solomon MJ, Glotzer M, Lee TH, Philippe M, Kirschner MW. 1990, Cell, Vol. 63, pp. 1013-1024. https://doi.org/10.1016/0092-8674(90)90504-8

56. The decision to enter mitosis: feedback and redundancy in the mitotic entry network. Lindqvist A, Rodriguez-Bravo V, Medema RH. 2009, J Cell Biol, Vol. 185, pp. 193-202. https://doi.org/10.1083/jcb.200812045 PMid:19364923 PMCid:PMC2700378

57. Cdks, cyclins and CKIs: roles beyond cell cycle regulation. Lim S, Kaldis P. 2013, Development, Vol. 140, pp. 3079-3093. https://doi.org/10.1242/dev.091744 PMid:23861057

58. The crystal structure of cyclin A. Brown NR, Noble ME, Endicott JA, Garman EF, Wakatsuki S, Mitchell E, Rasmussen B, Hunt T, Johnson LN. 1995, Structure, Vol. 3, pp. 1235-1247. https://doi.org/10.1016/S0969-2126(01)00259-3

59. Cip/Kip cyclin-dependent kinase inhibitors: brakes of the cell cycle engine during development. Nakayama. 1998, Bioessays, Vol. 20, pp. 1020-1029. https://doi.org/10.1002/(SICI)1521-1878(199812)20:12<1020::AID-BIES8>3.0.CO;2-D

60. Induction of Cip/Kip and Ink4 cyclin dependent kinase inhibitors by interferon-alpha in hematopoietic cell lines. Sangfelt O, Erickson S, Einhorn S, Grander D. 1997, Oncogene, Vol. 14, pp. 415-423. https://doi.org/10.1038/sj.onc.1200832 PMid:9053838

61. Loss of p21 expression is associated with p53 mutations and increased cell proliferation and p27 expression is associated with apoptosis in maxillary sinus squamous cell carcinoma. Bandoh N, Hayashi T, Takahara M, Kishibe K, Ogino T, Katayama A, Imada M, Nonaka S, Harabuchi Y. 2005, Acta Otolaryngol, Vol. 125, pp. 779-785. https://doi.org/10.1080/00016480410023056 PMid:16012042

62. Resistance to chemotherapy: new treatments and novel insights into an old problem. Raguz S, Yague E. 2008, Br J Cancer, Vol. 99, pp. 387-391. https://doi.org/10.1038/sj.bjc.6604510 PMid:18665178 PMCid:PMC2527800

63. Phase I and pharmacologic study of the alkylating agen modulator novobiocin in combination with high-dose chemotherapy for the treatment of metastatic breast cancer. Kennedy MJ, Armstrong DK, Huelskamp AM, Ohly K, Clarke BV, Colvin OM, Grochow LB, Chen TL, Davidson NE. 1995, J Clin Oncol, Vol. 13, pp. 1136-1143. https://doi.org/10.1200/JCO.1995.13.5.1136 PMid:7738619

64. Aldehyde dehyrogenase activity as the basis for the relative insensitivity of murine pluripotent hematopoietic stem cells to oxazaphosphorines. Kohn FR, Sladek NE. 1985, Biochem Pharmacol, Vol. 34, pp. 3465-3471. https://doi.org/10.1016/0006-2952(85)90719-1

65. The problem of permanent bone marrow damage after cytotoxic drug treatment. Lohrmann. 1984, Oncology, Vol. 41, pp. 180-184. https://doi.org/10.1159/000225819 PMid:6374556

66. Platinum compounds: a new class of potent antitumour agents. Rosenberg B, VanCamp L, Trosko JE, Mansour VH. 1969, Nature, Vol. 222, pp. 385-386. https://doi.org/10.1038/222385a0 PMid:5782119

67. Cellular processing of platinum anticancer drugs. Wang D, Lippard SJ. 2005, Nat Rev Drug Discov, Vol. 4, pp. 307-320. https://doi.org/10.1038/nrd1691 PMid:15789122

68. Cisplatin: mode of cytotoxic action and molecular basis of resistance. Siddik. 2003, Oncogene, Vol. 22, pp. 7265-7279. https://doi.org/10.1038/sj.onc.1206933 PMid:14576837

69. Participation of Omi Htra2 serine-protease activity in the apoptosis induced by cisplatin on Sw480 colon cancer cells. Pruefer FG, Lizarraga F, Maldonado V, Melendez-Zajgla J. 2008, J Chemother, Vol. 20, pp. 348-354. https://doi.org/10.1179/joc.2008.20.3.348 PMid:18606591

70. Anthracyclines: molecular advances and pharmacologic developments in antitumor activity and cardiotoxicity. Minotti G, Menna P, Salvatorelli E, Cairo G, Gianni L. 2004, Pharmacol Rev, Vol. 56, pp. 185-229. https://doi.org/10.1124/pr.56.2.6 PMid:15169927

71. DNA topoisomerases and their poisoning by anticancer and antibacterial drugs. Pommier Y, Leo E, Zhang H, Marchand C. 2010, Chem Biol, Vol. 17, pp. 421-433. https://doi.org/10.1016/j.chembiol.2010.04.012 PMid:20534341

72. The anthracyclines: will we ever find a better doxorubicin? Weiss. 1992, Semin Oncol, Vol. 19, pp. 670-686.

73. Drug-induced histone eviction from open chromatin contributes to the chemotherapeutic effects of doxorubicin. Pang B, Qiao X, Janssen L, Velds A, Groothuis T, Kerkhoven R, Nieuwland M, Ovaa H, Rottenberg S, van Tellingen O, et al. 2013, Nat Commun, Vol. 4, p. 1908. https://doi.org/10.1038/ncomms2921 PMid:23715267 PMCid:PMC3674280

74. The spindle checkpoint, aneuploidy, and cancer. Bharadwaj R, Yu H. 2004, Oncogene, Vol. 23, pp. 2016-2027. https://doi.org/10.1038/sj.onc.1207374 PMid:15021889

75. Microtubules and actin filaments: dynamic targets for cancer chemotherapy. Jordan MA, Wilson L. 1998, Curr Opin Cell Biol, Vol. 10, pp. 123-130. https://doi.org/10.1016/S0955-0674(98)80095-1

76. Paclitaxel-dependent cell lines reveal a novel drug activity. Ganguly A, Yang H, Cabral F. 2010, Mol Cancer Ther, Vol. 9, pp. 2914-2923. https://doi.org/10.1158/1535-7163.MCT-10-0552 PMid:20978163 PMCid:PMC2978777

77. Pathogenesis of paclitaxel-induced peripheral neuropathy: A current review of in vitro and in vivo findings using rodent and human model systems. Staff NP, Fehrenbacher JC, Caillaud M, Damaj MI, Segal RA, Rieger S. 2019, Exp Neurol.

78. Microtubules do not promote mitotic slippage when the spindle assembly checkpoint cannot be satisfied. Brito DA, Yang Z, Rieder CL. 2008, J Cell Biol, Vol. 182, pp. 623-629. https://doi.org/10.1083/jcb.200805072 PMid:18710927 PMCid:PMC2518701

79. p21 and p27: roles in carcinogenesis and drug resistance. Abukhdeir AM, Park BH. 2008,

Expert Rev Mol Med, Vol. 10, p. e19. https://doi.org/10.1017/S1462399408000744 PMid:18590585 PMCid:PMC2678956

80. Cyclin E2 overexpression is associated with endocrine resistance but not insensitivity to CDK2 inhibition in human breast cancer cells. Caldon CE, Sergio CM, Kang J, Muthukaruppan A, Boersma MN, Stone A, Barraclough J, Lee CS, Black MA, Miller LD, et al. 2012, Mol Cancer Ther, Vol. 11, pp. 1488-1499. https://doi.org/10.1158/1535-7163.MCT-11-0963 PMid:22564725

81. Ectopic expression of cyclin E in estrogen responsive cells abrogates antiestrogen mediated growth arrest. Dhillon NK, Mudryj M. 2002, Oncogene, Vol. 21, pp. 4626-4634. https://doi.org/10.1038/sj.onc.1205576 PMid:12096339

82. Roscovitine confers tumor suppressive effect on therapy-resistant breast tumor cells. Nair BC, Vallabhaneni S, Tekmal RR, Vadlamudi RK. 2011, Breast Cancer Res, Vol. 13, p. R80. https://doi.org/10.1186/bcr2929 PMid:21834972 PMCid:PMC3218960

83. Flavopiridol administered using a pharmacologically derived schedule is associated with marked clinical efficacy in refractory, genetically high-risk chronic lymphocytic leukemia. Byrd JC, Lin TS, Dalton JT, Wu D, Phelps MA, Fischer B, Moran M, Blum KA, Rovin B, Brooker-McEldowney M, et al. 2007, Blood, Vol. 109, pp. 399-404. https://doi.org/10.1182/blood-2006-05-020735 PMid:17003373 PMCid:PMC1785084

84. Roscovitine and other purines as kinase inhibitors. From starfish oocytes to clinical trials. Meijer L, Raymond E. 2003, Acc Chem Res, Vol. 36, pp. 417-425. https://doi.org/10.1021/ar0201198 PMid:12809528

85. The kinase inhibitor O6-cyclohexylmethylguanine (NU2058) potentiates the cytotoxicity of cisplatin by mechanisms that are independent of its effect upon CDK2. Harrison LR, Ottley CJ, Pearson DG, Roche C, Wedge SR, Dolan ME, Newell DR, Tilby MJ. 2009, Biochem PHarmacol, Vol. 77, pp. 1586-1592. https://doi.org/10.1016/j.bcp.2009.02.018 PMid:19426695

86. Cyclin-dependent kinase inhibitors for cancer therapy: a patent review (2009-2014). Malinkova V, Vylicil J, Krystof V. 2015, Expert Opin Ther Pat, pp. 1-18.

87. The history and future of targeting cyclin-dependent kinases in cancer therapy. Asghar U, Witkiewicz AK, Turner NC, Knudsen ES. 2015, Nat Rev Drug Discov, Vol. 14, pp. 130-146. https://doi.org/10.1038/nrd4504 PMid:25633797 PMCid:PMC4480421

88. PD0332991, a selective cyclin D kinase 4/6 inhibitor, preferentially inhibits proliferation of luminal estrogen receptor-positive human breast cancer cell lines in vitro. Finn RS, Dering J, Conklin D, Kalous O, Cohen DJ, Desai AJ, Ginther C, Atefi M, Chen I, Fowst C, et al. 2009, Breast Cancer Res, Vol. 11, p. R77. https://doi.org/10.1186/bcr2419 PMid:19874578 PMCid:PMC2790859

89. MDA-MB-435 cells are derived from M14 melanoma cells - a loss for breast cancer, but a boon for melanoma research. Rae JM, Creighton CJ, Meck JM, Haddad BR, Johnson MD. 2007, Breast Cancer Res Treat, Vol. 104, pp. 13-19. https://doi.org/10.1007/s10549-006-9392-8 PMid:17004106

90. The Rb-related p107 protein can suppress E2F function independently of binding to cyclin A/cdk2. Smith EJ, Nevins JR. 1995, Mol Cell Biol, Vol. 15, pp. 338-344. https://doi.org/10.1128/MCB.15.1.338 PMid:7799940 PMCid:PMC231964

91. Developmental activation of the Rb-E2F pathway and establishment of cell cycle-regulated cyclin-dependent kinase activity during embryonic stem cell differentiation. White J, Stead E, Faast R, Conn S, Cartwright P, Dalton S. 2005, Mol Biol Cell, Vol. 16, pp. 2018-2027. https://doi.org/10.1091/mbc.e04-12-1056 PMid:15703208 PMCid:PMC1073679

92. CDK4/6 inhibition triggers anti-tumour immunity. Goel S, DeCristo MJ, Watt AC, BrinJones H, Sceneay J, Li BB, Khan N, Ubellacker JM, Xie S, Metzger-Filho O, Hoog J, Ellis MJ, Ma CX, Ramm S, Krop IE, Winer EP, Roberts T, Kim HJ, McAllister SS, Zhao JJ. 2017, Nature, Vol. 548, pp. 471-475. https://doi.org/10.1038/nature23465 PMid:28813415 PMCid:PMC5570667

93. Cyclin D3 compensates for the loss of Cyclin D1 during ErbB2-induced mammary tumor initiation and progression. Zhang Q, Sakamoto K, Liu C, Triplett AA, Lin W-C, Rui H, Wagner, K-U. 2011, Cancer Res, Vol. 71, pp. 7513-7524. http://doi.org/10.1158/0008-5472.CAN-11-1783 PMid:22037875 PMCid:PMC324818

94. NeoPalAna: Neoadjuvant Palbociclib, a Cyclin-Dependent Kinase 4/6 Inhibitor, and Anastrozole for Clinical Stage 2 or 3 Estrogen Receptor-Positive Breast Cancer. Ma CX, Gao F, Luo J, Northfelt DW, Goetz M, Forero A, Hoog J, Naughton M, Ademuyiwa F, Suresh R, Anderson KS, Margenthaler J, Aft R, Hobday T, Moynihan T, Gillanders W, Cyr A, Eberlein TJ, Hieken T, Krontiras H, Guo Z, Lee MV, Spies NC, et al. 2017, Clinical Cancer Res, Vol. 23, pp. 4055-4065.

95. The cyclin-dependent kinase 4/6 inhibitor palbociclib in combination with letrozole versus letrozole alone as first-line treatment of oestrogen receptor-positive, HER2-negative, advanced breast cancer (PALOMA-1/TRIO-18): a randomised phase 2 study. Finn RS, Crown JP, Lang I, Boer K, Bondarenko IM, Kulyk SO, Ettl J, Patel R, Pinter T, Schmidt M, et al. 16, 2015, Lancet Oncol, pp. 25-35. https://doi.org/10.1016/S1470-2045(14)71159-3

96. Overcoming Therapeutic Resistance in HER2-Positive Breast Cancers with CDK4/6 Inhibitors. Goel S, Wang Q, Watt AC, Tolaney SM, Dillon DA, Li W, Ramm S, Palmer AC, Yuzugullu H, Varadan V, Tuck D, Harris LN, Wong KK, Liu XS, Sicinski P, Winer EP, Krop IE, Zhao JJ. 2016, Cancer Cell, Vol. 29, pp. 255-269. https://doi.org/10.1016/j.ccell.2016.02.006 PMid:26977878 PMCid:PMC4794996

97. CDK4/6-dependent activation of DUB3 regulates cancer metastasis through SNAIL1. Liu T, Yu J, Deng M, Yin Y, Zhang H, Luo K, Qin B, Li Y, Wu C, Ren T, Han Y, Yin P, Kim JJ, Lee SB, Lin J, Zhang L, Zhang J, Nowsheen S, Wang L, Boughey J, Goetz MP, Yuan J, Lou Z. 2017, Nature Communications, Vol. 8. https://doi.org/10.1038/ncomms13923 PMid:28067227 PMCid:PMC5228031

98. Estrogen-dependent cyclin E-cdk2 activation through p21 redistribution. Planas-Silva MD, Weinberg RA. 1997, Mol Cell Biol, Vol. 17, pp. 4059-4069. https://doi.org/10.1128/MCB.17.7.4059 PMid:9199341 PMCid:PMC232259

99. Down-regulation of p21WAF1/CIP1 or p27Kip1 abrogates antiestrogen-mediated cell cycle arrest in human breast cancer cells. Cariou S, Doovan JC, Flanagan WM, Milic A, Bhattacharya N, Singerland JM. 2000, Proc Natl Acad Sci USA, Vol. 97, pp. 9042-9046. https://doi.org/10.1073/pnas.160016897 PMid:10908655 PMCid:PMC16818

100. Cyclin-dependent kinase inhibitor therapy for hematologic malignancies. Bose P, Simmons GL, Grant S. 2013, Expert Opin Investig Drugs, Vol. 22, pp. 723-738. https://doi.org/10.1517/13543784.2013.789859 PMid:23647051 PMCid:PMC4039040

101. Flavopiridol and Trastuzumab Synergistically Inhibit Proliferation of Breast Cancer Cells: Association with Selective Cooperative Inhibition of Cyclin D1-dependent Kinase and Akt Signaling Pathways 1. Wu K, Wang C, D'Amico M, Lee RJ, Albanese C, Pestell RG, and Mani S. 2002, Molecular Cancer Therapeutics, Vol. 1, pp. 695-706.

102. Flavopiridol Synergizes with Sorafenib to Induce Cytotoxicity and Potentiate Antitumorigenic Activity in EGFR/HER-2 and Mutant RAS/RAF Breast Cancer Model Systems. Nagaria TS, Williams JL, Leduc C, Squire JA, Greer PA, Sangrar W. 2013, Neoplasia, Vol. 15, pp. 939-951.

https://doi.org/10.1593/neo.13804
PMid:23908594 PMCid:PMC3730045

103. Rapid onset of nucleolar disintegration preceding cell cycle arrest in roscovitine-induced apoptosis of human MCF-7 breast cancer cells. Wojciechowski J, Horky M, Gueorguieva M, Wesierska-Gadek J. 2003, Int J Cancer, Vol. 106, pp. 486-495. https://doi.org/10.1002/ijc.11290
PMid:12845642

104. Roscovitine-induced up-regulation of p53AIP1 protein precedes the onset of apoptosis in human MCF-7 breast cancer cells. Wesierska-Gadek J, Gueorguieva M, Horky M. 2005, Mol Cancer Ther, Vol. 4, pp. 113-124.

105. Seliciclib (CY202, R-roscovitine) enhances the antitumor effect of doxorubicin in vivo in breast cancer xenograft model. Appleyard MV, O'Neill MA, Murray KE, Paulin FE, Bray SE, Kernohan NM, Levison DA, Lane DP, Thompson AM. 2009, Int J Cancer, Vol. 124, pp. 465-472. https://doi.org/10.1002/ijc.23938
PMid:19003963

106. Roscovitine targets, protein kinases and pyridoxal kinase. Bach S, Knockaert M, Reinhardt J, Lozach O, Schmitt S, Baratte B, Koken M, Coburn SP, Tang L, Jiang T, et al. 2005, J Biol Chem, Vol. 280, pp. 31208-31219. https://doi.org/10.1074/jbc.M500806200
PMid:15975926

107. Dinaciclib, a novel CDK inhibitor, demonstrates encouraging single-agent activity in patients with relapsed multiple myeloma. Kumar SK, LaPlant B, Chng WJ, Zonder J, Callander N, Fonseca R, Fruth B, Roy V, Erlichman C, Stewart AK. 2015, Blood, Vol. 125, pp. 443-448. https://doi.org/10.1182/blood-2014-05-573741 PMid:25395429 PMCid:PMC4296007

108. Combined depletion of cell cycle and transcriptional cyclin-dependent kinase activities induces apoptosis in cancer cells. Cai D, Latham Jr VM, Zhang X, Shapiro GI. 2006, Cancer Res, Vol. 18, pp. 9270-9280. https://doi.org/10.1158/0008-5472.CAN-06-1758 PMid:16982772

109. A phase 1 study with dose expansion of the CDK inhibitor dinaciclib (SCH 727965) in combination with epirubicin in patients with metastatic triple negative breast cancer. Mitri Z, Karakas C, Wei C, Briones B, Simmons H, Ibrahim N, Alvarez R, Murray JL, Keyomarsi K, Moulder S. 2015, Invest New Drugs, Vol. 33, pp. 890-894.
https://doi.org/10.1007/s10637-015-0244-4
PMid:25947565