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Abemaciclib

Abstract

Hormone receptor-positive (HR+) disease is characteristic of over 70% of metastatic breast cancer (MBC) patients and, therefore, are candidates for endocrine therapy (ET); however, with the onset of resistance, clinical outcome has failed to improve. Evidence suggests a role for CDK4 and CDK6, key regulators of the cell cycle, in ET resistance. Cyclin D, which directly activates CDK4 and 6, is upregulated in more than 50% of breast cancer patients. Following the development and preclinical characterization of abemaciclib, a CDK4/6 inhibitor that shows higher selectivity for CDK4, a key driver of breast tumorigenesis, Eli Lilly explored its therapeutic potential in HR+/HER2- MBC. By targeting CDK4 and 6, abemaciclib presents an adjuvant therapy capable of overcoming ET resistance in the aggressive HR+/HER2- MBC.

CDK4/6-Rb Pathway and Cancer

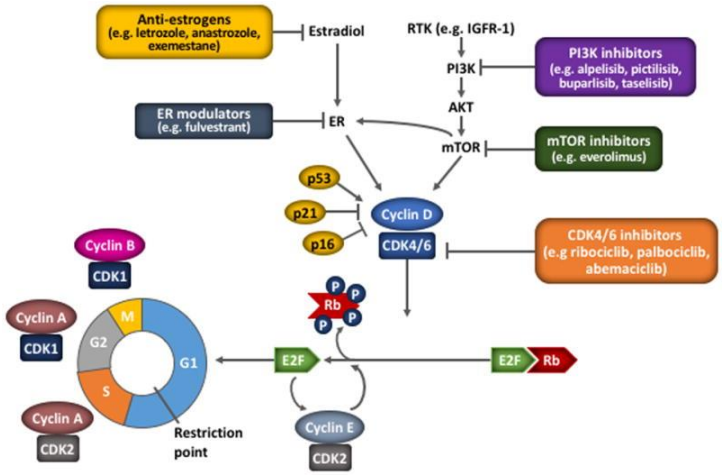

Figure 1. The role of the cyclin D–CDK4/6–p16–Rb pathway in the cell cycle and the existing therapeutic landscape. The different modes of targeting the pathway in cancer provides a rationale for dual inhibition and combination therapy (10).

The cell cycle is a highly regulated process. Because of this regulation, every cell cycle phase except G1 must complete entirely; therefore, they occur after having reached a point of no return and progress independently of mitogens and growth factors. During G1, the cell grows and prepares for DNA replication and mitosis in response to growth factors and mitogens. However, G1 has its own point of no return after which the cell also becomes independent called the restriction point (1).

Growth factors regulate cyclin D1 and, in turn, allow them to form a complex with cyclin D kinases, particularly CDK4 and CDK6. Upon binding cyclin D, the active kinases phosphorylate the retinoblastoma protein (Rb), a repressor, and consequently induce the expression of genes that drive the cell to the restriction point. Another kinase, CDK2, is derepressed, and, therefore amplifies Rb phosphorylation which leads to the expression of many other genes that are necessary for the transition into the S-phase, during which DNA replication occurs. Therefore, Rb acts as a tumor suppressor, maintaining the restriction point and preventing uncontrolled proliferation. In cancer cells, however, markedly increased activation of CDK4 and CDK6 leads to increased inactivation of Rb. As a result, the cell can bypass the restriction point (1).

CDK4/6 activation can occur through gene amplification, epigenetic modifications, or changes in the activity of key regulators. The multiple mechanisms of activation suggest their importance in both cancer progression and aggression. Although CDK4 and CDK6 assume redundant functions in cell-cycle regulation, their relative contribution is highly context-specific and, therefore, another important factor in clinical outcome. For example, CDK4 is important for breast tumorigenesis, while CDK6 drives

hematopoietic stem cell differentiation, or blood cell formation by the bone marrow. However, for these same reasons, CDK4 and CDK6 are prime targets for cancer therapy (7).

HR+/HER2- Breast Cancer

Increased CDK4/6 activity is observed commonly in breast cancer, especially the HR+/HER2- type associated with poor clinical outcome. Endocrine therapy was previously considered the standard of care for adjuvant treatment of postmenopausal women with HR+, HER2- breast cancer. Aromatase inhibitors (AI) prevent the conversion of androgens to estrogen. However, for premenopausal women, inhibition decreases their generally high estrogen levels only to activate the HPA axis and stimulate the ovary to secrete more estrogen. Adjuvant treatment involving AIs, such as the addition of tamoxifen, a competitive antagonist of estrogen, or fulvestrant, a selective estrogen-receptor degrader (SERD), improves clinical outcome in some cases (2).

Fulvestrant, like AIs, are mostly ineffective if not harmful to premenopausal women; however, unlike AIs, it can effectively reduce ER-dependent cell cycle upregulation at high doses such as 750 mg. This is likely a consequence of the mechanism of action which consists of not only accelerating the degradation of ERs but decreasing ER dimerization which may otherwise trigger estrogen-independent signaling cascades (2).

Nevertheless, AIs and SERDs, like CDK4/6 inhibitors, can induce G1 cell cycle arrest by decreasing ER-induced cyclin D1 expression and, therefore, CDK4 and CDK6 activation or by decreasing estrogen which normally promotes the transition from G1 to the S phase. However, with the evolution of resistance driven by the multiple mechanisms of CDK4/6 activation, finding other modes that either replace or complement endocrine therapy becomes necessary. Particularly, cyclin D1 seems to be an essential component of ER-induced proliferation; therefore; cyclin D1, CDK4 and CDK6 may together drive the observed resistance to both forms of endocrine therapy (1, 2, 9).

Preclinical Characterization:

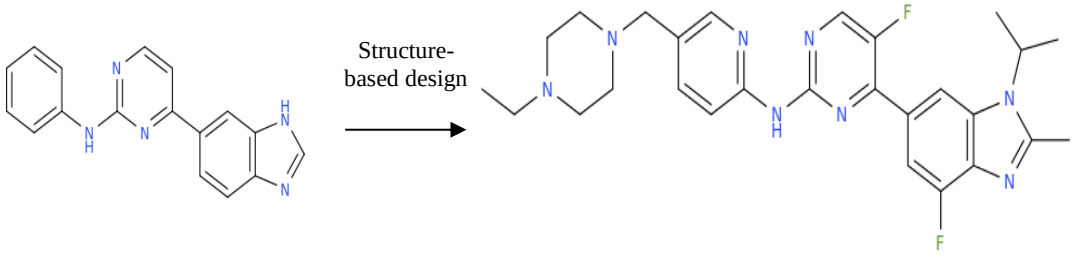

Figure 2. Compound screening revealed that the 2-anilino-2,4-pyrimidine-[5-benzimidazole] scaffold potently inhibited CDK4/6. The scaffold was optimized through structure-based design by biochemical screening to improve potency and CDK4/6 selectivity (4, 5).

Beginning with a potent scaffold, researchers developed abemaciclib, a reversible CDK4/6 inhibitor that binds competitively with ATP. Abemaciclib is highly selective because it is less active against other kinases. For example, it is at least 160-fold less potent against CDK1 (4). Additionally, abemaciclib is a more potent inhibitor of CDK4 with an IC50 of 2 nmol/L compared to CDK6 with an IC50 of 10 nmol/L. Recent analysis of binding site interactions revealed the importance of introducing the fluorine to the benzimidazole as well as the piperazine which happen to be involved in the multiple binding interactions of abemaciclib with GLU_{144}, a crucial residue for CDK4-specific inhibition. Other successful CDK4/6 inhibitors that lack this selectivity, such as Palbociclib and Ribociclib, also lack interactions with this residue (8).

Administration of abemaciclib at doses as low as 12.5 mg/kg to colorectal cancer patient-derived tumor xenografts caused tumor growth rate to decrease. Measuring the level of cell cycle markers such as TopoIIα, pHH3, and the previously described pRb, revealed more than 50% inhibition. TopoIIα is an enzyme that is responsible for resolving any DNA tangling during replication and, is therefore, a marker of progress into the S phase. Therefore, the decreased pRb demonstrates successful inhibition of CDK4 and CDK6 while decreased TopoIIα demonstrates that the tumor cells remain in G1 and that the mechanism of the observed antitumor activity is G1 cell cycle arrest mediated by CDK4 and CDK6 inhibition (4, 6). Both continuous and intermittent administration of 50 mg/kg for a relatively long 56 days maintained significant inhibition of tumor growth for 2 months following the last dose, while also failing to produce any adverse effects in the treated mice. Similar antitumor activity was also seen in patient-derived xenografts for other types of cancer, such as glioblastoma (4).

Efficacy

Based on evidence of the desired inhibitory activity both *in vitro* and *in vivo,* clinical trials evaluated the pharmacokinetic profile, pharmacodynamic effects and antitumor activity of abemaciclib. All patients had advanced cancer; therefore, most had more than 2 metastatic sites and had received other lines of therapy (3, 7, 9).

Pharmacodynamics and Pharmacokinetics

Orally administered abemaciclib was absorbed slowly and reached a maximum plasma concentration at 4 to 6 hours but was well distributed. Its mean terminal elimination half-life ranged from 17 to 38 hours. At steady state, the maximum plasma concentration reached, on average, 249 ng/mL for twice-daily administration of 150 mg abemaciclib and 298 mg/mL for twice-daily administration of 200 mg. More interestingly, the concentration of abemaciclib in cerebrospinal fluid was approximately 2 to 15 nmol/L, which is greater than the dissociation constant (Ki) for the CDK4 and cyclin D1 complex, 0.6 nmol/L. Therefore, abemaciclib effectively penetrates the blood-brain barrier and may be capable of optimal inhibitory activity in the context of glioblastoma and brain metastases (7).

pRB and TopoIIα levels were then measured in a proliferative cell population at twice-daily doses of both 150 mg and 200 mg abemaciclib and found to be reduced significantly ($P < 0.0001$). Reduced expression levels were maintained at steady state; therefore, CDK4/6 inhibition was also sustained. Additionally, both doses induced comparable levels of inhibition, as determined by the expression of pRB and TopoIIα, defining a recommended dose range for consistent *in vivo* CDK4/6 inhibition. By further investigating the relationship between pRb expression levels and response, a decrease in pRb of more than 60% was found to be associated with optimal clinical outcome ($P < 0.0001$), either stable disease or a partial response (7).

Antitumor Activity

During phase I trials, the disease control rate with abemaciclib was higher for HR+ breast cancer than that of HR- breast cancer. 11 of 36 HR+ breast cancer patients achieved partial response with 7 having HER2- disease and 4 having HER2+ disease. 11 HR+ breast cancer patients also achieved stable disease for over 2 years. The overall HR-positive population maintained progression-free survival (PFS) for a median 8.8 months. These results motivated the pursuit of therapy with abemaciclib for HR+/HER2- breast cancer in phase II and III trials. However, abemaciclib also achieved stable disease in 3 of 17 glioblastoma patients. Therefore, abemaciclib not only penetrates the blood-brain barrier but affects CDK4 activity as well. Although further optimization will be required, abemaciclib may also be relevant to the treatment of breast cancer brain metastases (7).

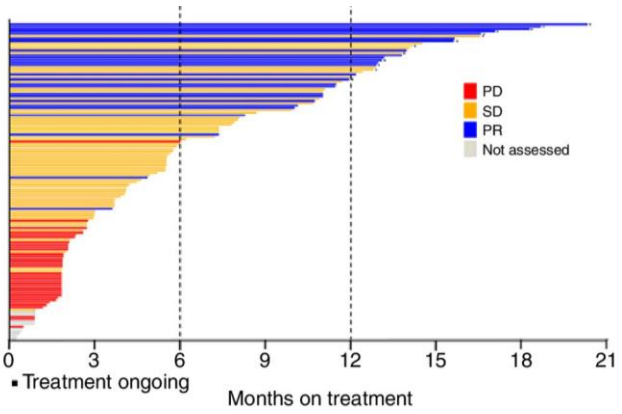

Figure 3. Time on treatment for patients in the phase II trials and their corresponding response status. A large percentage of patients achieved a partial response (PR) or stable disease (SD), while a relatively smaller percentage of patients had progressive disease (PD).

Phase II trials consisted of 132 HR+, HER2- metastatic breast cancer patients with the majority having 3 or more metastatic sites and previously received treatment, including chemotherapy and endocrine therapy. However, patients with brain metastases were excluded from this study. This more focused trial sought to determine the efficacy and safety of abemaciclib monotherapy in this subpopulation associated with poor clinical outcome (3).

At 12 months of follow-up, 26 of 132 patients achieved a partial response resulting in an objective response rate of 19.7% and a clinical benefit rate of 42.4%. 24 of these 26 patients had visceral disease, and 12 had more than 3 metastatic sites. A response occurred within 3.7 months and lasted for 8.6 months. Despite a low median progression free survival, overall survival was high at 17.7 months (3).

Overall, abemaciclib shows promising clinical activity. As mentioned before, the CDK4/6 inhibitors palbociclib and ribociclib both require intermittent dosing schedules. Additionally, the objective response rate for palbociclib monotherapy in a phase II trial was 6% in patients with HR+ metastatic breast cancer, while one of 18 patients with ER+ breast cancer achieved a partial response with ribociclib monotherapy during a phase I trial. Therefore, abemaciclib monotherapy achieves more clinical activity. The objective response rate of abemaciclib was also comparable to approved chemotherapy with the potential for a preferable safety profile (3).

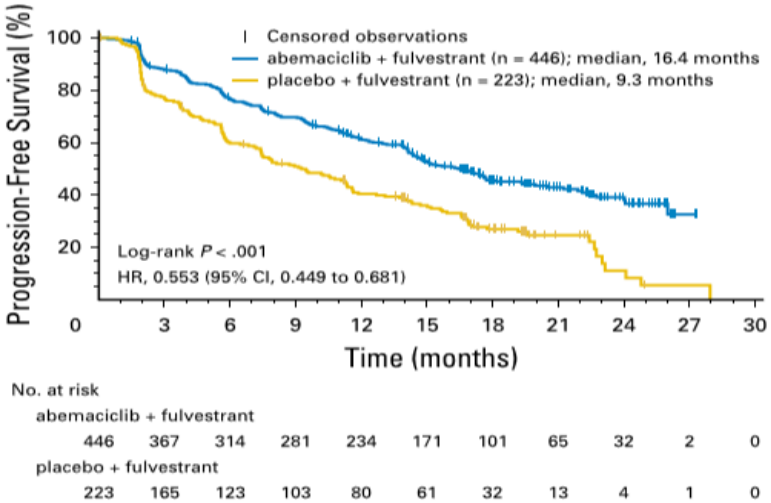

Figure 4. Progression-free survival of patients receiving abemaciclib and patients receiving the placebo. Abemaciclib increases PFS and, therefore, displays strong antitumor activity while also reducing adverse events (9).

Phase III trials consisted of two treatments: abemaciclib and fulvestrant or a placebo and fulvestrant in a 2:1 ratio until progressive disease, death or participant withdrawal. The efficacy of both treatments was compared in women above 18 with endocrine therapy resistant HR+/HER2- advanced breast cancer (ABC) and any menopausal status. Prior therapy excluded the fulvestrant used in this study

and other CDK4 and CDK6 inhibitors. Additionally, participants with any reported brain metastasis were excluded from these trials. In addition to one of the described treatments, pre- and perimenopausal women were also administered a gonadotropin releasing hormone agonist to limit estrogen production because, as mentioned before, a decrease in estrogen level in response to treatment increases estrogen production which may cause more harm to the participant. Survival analysis of both treatment conditions revealed consistently increased survival with abemaciclib and fulvestrant. Risk was assessed based on tumor measurements using CT and MRI as well as hematologic chemistry lab tests. Particularly, participants receiving abemaciclib maintained progression free survival for a median 16.4 months which is greater than that of participants receiving the placebo. Hazard ratios for abemaciclib vs placebo in different participant subgroups, including metastatic site, ET resistance type, menopausal status, were consistently less than 1 suggesting that abemaciclib had higher efficacy (9).

Safety

During phase I trials, dose escalation with a daily administration schedule revealed a lack of dose-limiting toxicity and, therefore, maximum dose tolerance. However, twice-daily administration resulted in grade 3 fatigue for 1 of 7 patients given 200 mg and 2 of 3 patients given 275 mg. This inidicates that the maximum tolerance dose for the twice-daily administration schedule is 200 mg (7).

Besides fatigue, gastrointestinal, renal, and hematopoietic adverse events were also observed during phase I, II, and III trials (3, 7, 9). Notably, continuous dosing increased serum creatinine largely due to direct inhibition of renal efflux pumps in the proximal tubular where creatinine secretion occurs (3, 7, 9). For example, during phase III trials, 25% more patients receiving abemaciclib had increased serum creatinine (9). Neutropenia, or low white blood cell count (WBC), was less of a concern, which offers more flexibility and reduces the need for the intermittent dosing schedules required by other CDK4 and CDK6 inhibitors such as ribociclib and palbociclib. 87.7% of patients had decreased WBC counts; however, only 10 % received hematopoietic growth factor support during phase II trials. This may be a consequence of the drug's selectivity for CDK4 and reduced suppression of white blood cell formation by CDK6 inhibition (3).

Another commonly observed abnormality was diarrhea. During phase II trials, 90.2% of patients receiving abemaciclib experienced grade 2 or 3 diarrhea within 7 days of initiating therapy. Diarrhea was as frequently observed in phase III trials. In most cases, it was treatable with antidiarrheal medication and, therefore, lasted no longer than 1 week and did not merit a change in treatment (3, 7, 9).

Conclusion

Abemaciclib is a CDK4/6 inhibitor that has demonstrated high potency against both kinases but higher selectivity for CDK4. This characteristic partially explains its success in breast cancer. More importantly, abemaciclib shows more efficacy than other CDK4/6 inhibitors for exactly this reason. To complement improved efficacy, abemaciclib has a safety profile marked by the lack of concern of low WBC counts that necessitate intermittent dosing schedules and hematopoietic growth factor support. Finally, abemaciclib, in combination with ET, improves clinical outcome in HR+/HER2- MBC, providing a strategy to overcome ET resistance and may have applications to other types of cancers or, even, brain metastases.

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