Critical Reviews in Oncology / Hematology 196 (2024) 104324

Contents lists available at ScienceDirect

Critical Reviews in Oncology / Hematology

journal homepage: www.elsevier.com/locate/critrevonc

Cyclin-dependent kinase 2 (CDK2) inhibitors and others novel CDK inhibitors (CDKi) in breast cancer: clinical trials, current impact, and future directions

Riccardo Gerosa [a,b], Rita De Sanctis [a,b,*], Flavia Jacobs [a,b], Chiara Benvenuti [a,b], Mariangela Gaudio [a,b], Giuseppe Saltalamacchia [a], Rosalba Torrisi [a], Giovanna Masci [a], Chiara Miggiano [a,b], Francesco Agustoni [c,d], Paolo Pedrazzoli [c,d], Armando Santoro [a,b], Alberto Zambelli [a,b]

[a] Humanitas University, Department of Biomedical Sciences, Humanitas University, Via Rita Levi Montalcini 4, Milan, Pieve Emanuele 20090, Italy
[b] Humanitas Clinical and Research Center-IRCCS, Humanitas Cancer Center, Via Manzoni 56, Milan, Rozzano 20089, Italy
[c] Department of Internal Medicine and Medical Therapy, University of Pavia, Pavia, Italy
[d] Medical Oncology Unit, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy

ARTICLE INFO

Keywords:
Breast cancer (BC)
Cyclin-dependent kinase 2 inhibitor (CDK2i)
Cyclin-dependent
Kinase 4 inhibitor (CDK4i)
Pan-Cyclin-dependent kinase inhibitor (Pan-CDKi)
Cyclindependent
Kinase 2/4/6 inhibitor (CDK2/4/6i)
Metastatic breast cancer (mBC)

ABSTRACT

Aberrant cyclin-dependent kinase 2 (CDK2) activation has been identified as a main resistance mechanism to CDK4/6 inhibition in hormone-receptor positive (HR+) breast cancer. Additionally, consistent preclinical evidence states its crucial role in MYC and CCNE1 overexpressed cancer survival, such as triple-negative breast cancers (TNBC), thus representing an appealing and relatively unexplored target treatment opportunity. Despite emerging initial results of novel CDK2 inhibitors (CDK2i) activity, a comprehensive outcomes collection is currently absent from the scientific literature. We aim to provide an overview of ongoing clinical trials involving CDK2i in the context of metastatic breast cancer (mBC), either as monotherapy or in combination with other agents. The review extends beyond CDK2i to encompass novel emerging CDK4 inhibitors, combined CDK2/4/6 inhibitors, and the well-known pan-CDK inhibitors including those specifically directed at CDK2. Delving into the results, we critically appraise the observed clinical efficacy and offer valuable insights into their potential impact and future applications.

1. Introduction

Aberrant control of the cell cycle, leading to sustained cellular proliferation, is a hallmark of cancer (Watt and Goel, 2022). The progression through the distinct phases of the cell cycle is meticulously regulated by a complex system of cyclin proteins and their partner cyclin-dependent kinases (CDKs), thus making them attractive targets in cancer treatment (Ghafouri-Fard et al., 2022). CDK inhibitors (CDKi) encompass small molecules or antibodies that inhibit the enzymatic activity of CDKs, halting cell cycle progression and inducing cell death (proving effective even in quiescent cancer cells) (Criscitiello et al., 2014). Specifically, cyclin-dependent kinase 4/6 (CDK4/6) and their D-type cyclins control the transition from gap 1 (G1) to synthesis (S) cell cycle phase, playing a crucial role in hormone-receptor positive (HR+) breast cancer (BC) tumorigenesis and endocrine resistance. Hence, blocking this pathway with CDK4/6 inhibitors (CDK4/6i) combined with endocrine therapy (ET), has demonstrated substantial improvements in progression-free survival (PFS) and overall survival (OS) in HR+ metastatic breast cancer (mBC), leading to their clinical approval (Mittal et al., 2023; Cogliati et al., 2022). This pivotal achievement has generated greater interest in targeting other members of the CDK family, including cyclin-dependent kinase 2 (CDK2) (Tadesse et al., 2020).

The traditional cell-cycle progression model posits that mitogens stimulate the mitogen-activated protein kinase (MAPK) pathway, triggering the expression of D-type cyclins and activation of CDK4/6. Subsequently, CDK4/6-cyclin D complexes phosphorylate retinoblastoma tumor suppressor (Rb), releasing the adenoviral early region 2 binding factor (E2F) and promoting transcription of cyclins E1/E2 and A. CDK2

* Correspondence to: Humanitas University - Research Hospital, Via Manzoni 56, Milan, Rozzano 20089, Italy.
E-mail address: rita.de_sanctis@hunimed.eu (R. De Sanctis).

https://doi.org/10.1016/j.critrevonc.2024.104324
Received 1 November 2023; Received in revised form 31 January 2024; Accepted 7 March 2024
Available online 8 March 2024

activation, by cyclins E1/2 and A, leads to hyperphosphorylation of Rb, establishing a positive feedback loop that ensures sustained expression of essential proteins for S phase, committing cells to the complete cell cycle (Arora et al., 2023)

With a few exceptions, CDK2 is generally not upregulated or amplified in cancer, but rather, its activity is altered through its binding partners or by alterations to post-translational modifications. For example, certain oncogenic molecular pathways, including the upregulation of cyclin E1/amplification of the G1/S-specific cyclin-E1 encoding gene (CCNE1) and overexpression of the basic helix–loop–helix transcription factor (MYC), converge on CDK2, thereby modifying its activity as a crucial node in cell cycle control (Fig. 1.) (Tadesse et al., 2020; Panagiotou et al., 2022; Gomatou et al., 2021). Notably, in tumours marked by MYC overexpression, as approximately 70% of triple-negative breast cancer (TNBC), CDK2 activity appears indeed to be vital for preventing senescence and permitting the immortalization of cancer cells (Agostinetto et al., 2021; Freeman-Cook et al., 2021). On the other hand, a broad range of aggressive cancers overexpress cyclin E and/or harbour CCNE1 gene amplifications (such as high-grade serous ovarian cancers), with preclinical evidence suggesting that the addiction to CDK2/cyclin E activity results in high sensitivity to CDK2 inhibition (Patel et al., 2023). Zi-Ming Zhao et al. brilliantly described that overexpression of CCNE1 was a significantly frequent event in TNBC patients (48,7% and 42.1%, for TCGA and METABRIC databases, respectively) and may confer resistance to chemotherapy, as it is associated with poor overall survival (Zhao et al., 2019). Despite the extensive heterogeneity in the mechanisms driving TNBC, encompassing various potentially targetable pathways, the lack of alternative targeted therapies beyond poly ADP ribose polymerase (PARP) inhibitors and the newly emerging antibody-drug conjugates (ADCs), is remarkable and challenging (Zhu et al., 2023) Notably, the use of CDK4/6i as monotherapy in metastatic TNBC (mTNBC) has yielded unsatisfactory results, likely due to the frequent Rb loss event, distinguishing it from the luminal subtype. Despite preclinical indications suggesting potential responsiveness in certain TNBC subtypes (such as Rb-proficient, Luminal Androgen Receptor [LAR] or Mesenchymal Stem-like [MSL]), recent findings reported that abemaciclib monotherapy lacked meaningful clinical activity in Rb or androgene receptor (AR)-positive mTNBC. Future trials exploring CDK4/6i monotherapy in TNBC may not be warranted, and the combined approach with chemotherapy appears controversial and not worth pursuing (Agostinetto et al., 2021; Jovanović 2 et al., 2023). Nonetheless, the emergence of CDK2 aberrant activation as a key oncogenic driver in mTNBC represents a potentially appealing avenue for a novel therapeutic approach.

Broadening the scope of analysis beyond patients with mTNBC, consistent preclinical discoveries have also unveiled CDK2 crucial role in driving endocrine and CDK4/6 resistance in HR+ breast cancer (Ma et al., 2023). Generally, mechanisms of resistance to CDK4/6i (independent of potential primary or secondary resistance to the ET, which may occur simultaneously) can be broadly categorized as aberrations affecting cell cycle progression or activation of other signaling pathways (Fig. 1.). The latter include the activation of the phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR) pathway, neurofibromin 1 (NF1) / mitogen-activated protein kinase (MAPK) cascade, as well as activating mutations or amplifications in other growth factor receptor genes like epidermal growth factor receptor 2 (HER2) and fibroblast growth factor receptor (FGFR) (Ma et al., 2023). Among resistance mechanisms driven by alterations in cell cycle regulators, including Rb loss-of-function mutations, increased expression of cyclin-dependent kinase 6 (CDK6) or 7 (CDK7) and Aurora kinase A, CDK2 aberrant activation hold a pivotal role, primarily steaming from the same CCNE1 and C-MYC alterations mentioned earlier. For instance, the upregulation of cyclin E1/E2, that interact with

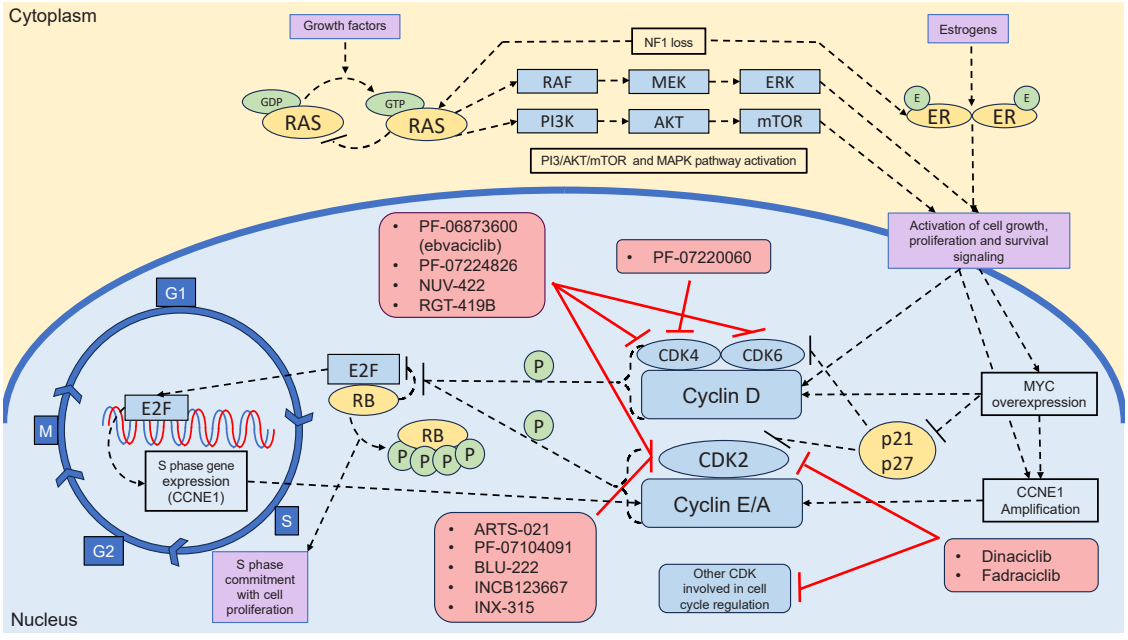

Fig. 1. Oncogenic signaling and resistance pathways converging on CDK2 in mBC (HR+/- HER2neg), with potential therapeutic strategies targeting CDK2 and other partner CDKs. The potential treatment strategies are shown in pink boxes, the oncogenic and resistance crosstalk pathways are indicated by dotted lines with arrows for activation and bars for inhibition. (figure created with Powerpoint) CDK (cyclin-dependent kinase), CCNE1 (G1/S-specific cyclin-E1 encoding gene), PI3K (phosphatidylinositol-3-kinase), AKT (protein kinase B), mTOR (mammalian target of rapamycin), MAPK (mitogen-activated protein kinase), RB (retinoblastoma tumor suppressor), E2F (adenoviral early region 2 binding factor), p21 (cyclin-dependent kinase inhibitor 1), MYC (Proto-Oncogene-BHLH Transcription Factor). G1 (gap 1 cell cycle stage), G2 (gap 2 cell cycle stage), S (synthesis cell cycle stage), M (mitosis cell cycle stage), P (phosphorylation), MYC (basic helix–loop–helix transcription factor), NF1 (neurofibromin 1), p21 (Cdk Interacting Protein 1), p27 (Kinase Inhibitory Protein 1), ER (estrogen receptor), E (estrogen), RAS (Rat sarcoma virus), MEK (Mitogen-activated protein kinase), ERK (extracellular signal-regulated kinases), RAF (Rapidly Accelerated Fibrosarcoma), GTP (Guanosine-5′-triphosphate), GDP (Guanosine-5′-diphosphate).

CDK2 and trigger its activation, has been shown in preclinical CDK4/6i resistant models and thus suggested as a main resistance mechanism. Interestingly, the overexpression of cyclin E may arise from the upregulation of other established signaling pathways associated with resistance to CDK4/6i and ET, including the PI3K/AKT/mTOR pathway (Gomatou et al., 2021). From 302 patients enrolled in the PALOMA-3 trial, palbociclib efficacy was indeed lower in patients with high cyclin E expression (Gomatou et al., 2021). Furthermore, Al-Qasem et al. have observed that high levels of CDK6, p-CDK2, and/or cyclin E1 were associated with adaptation and resistance to endocrine therapy (ET) and CDK4/6i in HR+ mBC. Hence, their combined expression was found to be an independent prognostic factor in these patients (Al-Qasem et al., 2022). Therefore, Freeman-Cook et al. uncovered that in both preclinical and clinical contexts, C-MYC overexpression results in resistance to endocrine therapy and CDK4/6 inhibitors. This resistance occurs by suppressing the cyclin dependent kinase inhibitor 1 A (CDKN1A) gene, which encodes p21, a well-known G1-CDK blockade protein. Consequently, this suppression releases CDK2–cyclin-E complexes, facilitating cell cycle progression (Tadesse et al., 2020).

While CDK2 gained prominence in cancer drug development during the 1990 s, the initial enthusiasm was tempered by the limited specificity and off-target effects of both first and second-generation CDKi. Nonetheless, the emerging preclinical evidence underscoring the pivotal role of abnormal CDK2 activity in breast cancer (as well as in other advanced solid tumors), as just highlighted, has revitalized the interest in developing novel more selective inhibitors and testing their clinical activity (Panagiotou et al., 2022). Therefore, considering emerging preliminary results, this review aims to fill a current gap in the scientific literature by providing an overview of clinical impact and ongoing clinical trials involving novel CDK2 inhibitors (CDK2i) in the context of mBC. On the same trajectory, it is worth mentioning that other selective inhibitors targeting different CDK, such as cyclin-dependent kinase 7 (CDK7) and 9 (CDK9), are currently under evaluation in early-phase clinical trials in the context of breast disease. In both instances, preliminary promising efficacy data along with a favourable toxicity profile, prompt further investigations and research (Patnaik 1 et al., 2023; Clack 1 et al., 2023; Mita 1 et al., 2023).

Moreover, informed by robust preclinical evidence, there has been a pursuit of combination therapeutic strategies to restore cell cycle control effectively, through the simultaneous inhibition of CDKs, particularly those crucially involved in the G1-S phase transition. In preclinical models resistant to ET and/or CDK4/6i, Al-Qasem et al. have indeed demonstrated that co-targeting of CDK2 and CDK4/6 in a triple combination with ET has a synergistic effect. This combination effectively inhibits cellular growth, induces cell cycle arrest, promotes apoptosis, and delays disease progression (Al-Qasem et al., 2022). Moreover, Arora et al. have demonstrated that acute response to selective CDK2 inhibition alone, despite an immediate reduction kinase activity, lead to cells rapid adaptation via a CDK2/4/6-Rb-E2F-dependent mechanism that circumvents CDK2 block and enables cell-cycle completion (Arora et al., 2023). Indeed the maintenance of Rb1 hyperphosphorylation by unblocked CDK4/6, results in active E2F transcription therefore sustaining cyclin A2 production, enabling a final paradoxical CDK2 reactivation that maintain the positive feedback loop with cell-cycle commitment. Interestingly, the novel CDK2i preferentially inhibits CDK2-cyclin E complex over CDK2-cyclin A2 and, despite the rebound on short timescale, long-term CDK2 inhibition is particularly effective in cyclin E-amplified patient-derived mouse cancers xenografts. Hence, at the light of these findings, Arora et al. serendipitously emphasize the usefulness of the current CDK2i for cancers that are heavily reliant on cyclin E. On the other hand, the co-inhibition of both CDK2 and CDK4/6 stops the rebound, undermining cell's CDK2-increasing proliferative trajectory and breaking the positive feedback loop that reinforces Rb1 phosphorylation. Furthermore, on extended timescales of CDK2 inhibition, there is a shift from CDK4/6 to CDK1 reliance for cell proliferation. Given that CDK1 inhibition is expected to be poorly tolerated in people, co-targeting CDK2 and CDK4/6 represents a potential treatment strategy to ablate the early adaptive rebound and even stave off more problematic CDK1-mediated adaptation to CDK2 inhibition (Arora et al., 2023).

Taking into account these preclinical findings, the review expands its scope beyond CDK2i to encompass the new combined CDK2/4/6 inhibitors (CDK2/4/6i) and the novel selective CDK4 inhibitors (CDK4i). Thanks to the sparing of CDK6, CDK4i hold promise for a more favorable toxicity profile and permit higher dosages, making them indeed ideal partners for a combination strategy with CDK2i.

Therefore, encompassing the older broad-spectrum CDK inhibitors (pan-CDKi) ,the review also describes the impact of non-selective CDK2 targeting along with concurrent inhibition of multiple other CDKs. Specifically, it reviews those that include CDK2 in their spectrum of action, that have available clinical data in mBC setting.

Remarkably, other alternative therapeutic strategies target in a different way CDK2 activity, rather than inhibiting it, such as the novel MK-8776 drug, which inhibits checkpoint kinase 1 (CHK1). Ordinarily, CHK1 facilitates cell cycle arrest to aid DNA damage repair, and its inhibition in sensitive cells, particularly those with CDK2 activation, permits the accumulation of DNA breaks, ultimately leading to cytotoxicity (Ma et al., 2023). A comprehensive exploration of such alternative strategies is beyond the scope of this review.

2. CDK2 inhibitors

2.1. ONGOING clinical trial, safety and efficacy results

Currently, ongoing phase 1/2 clinical trials are actively exploring the effectiveness of CDK2 selective inhibitors in treating HR+ mBC after CDK4/6i failure, as well as heavily pre-treated mTNBC patients (Table 1.).

2.1.1. PF-07104091

PF-07104091 (also known as PF-4091 or Tagtociclib), a novel CDK2-selective inhibitor, is currently under investigation in a first-in-human, open-label, multicenter, multiple-dose phase 1/2a study (NCT04553133) (Yap et al., 2023a). At data cut-off, 35 patients with advanced solid tumors were enrolled in PF-07104091 monotherapy dose-escalating cohorts, receiving the treatment twice daily orally in 28-day cycles. Among them, 29 were HR+/ human epidermal growth factor receptor 2 negative (HER2-) mBC and only one mTNBC, who had previously undergone $\geq$ 2 lines of treatment in an advanced or metastatic setting [median of 4 lines (Interquartile range IQR: 2–12)]. Notably, all luminal patients had received CDK4/6i+ ET, 86.2% of them fulvestrant and 72.4% up to two prior lines of cytotoxic chemotherapy, while the only one enrolled mTNBC patient had received $\leq$3 lines of treatment in metastatic setting. Preliminary findings point towards a positive safety and tolerability profile of PF-07104091 in monotherapy. Treatment adverse events (TAEs) were observed in 34 patients (97.1%), of which 20 patients (57.1%) were grade (G) $\geq$ 3. The most frequent TAEs included nausea (77.1%; 14.3% G3), diarrhea (48.6%; 8.6% G3), vomiting (48.6%; 2.9% G3), fatigue (45.7%; 20.0% G3) and anemia (45.7%; 8.6% G3). No G4 TAEs were reported. An encouraging clinical response was observed in 16 patients with evaluable responses. More precisely, six patients (37.5%) had stable disease (SD) and three (18.8%) showed partial responses (PR). Two patients exhibited response durations longer than six months, and one patient was still responsive at the time of data cutoff. Within this cohort of heavily pretreated patients, an impressive disease control rate (DCR) of 61.5% (95% Confidence Interval [CI]: 40.6–79.8) was achieved. Currently, dose escalation and expansions of PF-07104091 in combination with fulvestrant for HR+ mBC are ongoing. Moreover, a dedicated arm is planned to explore the combination of PF-07104091, PF-07220060 and ET (Fulvestrant or Letrozole), as discussed later in this review.

Table 1
Summary of Results from Trials Exploiting CDK2 Selective Inhibitors. BC (breast cancer), pts (patients), CDK (cyclin-dependent kinase), CDK4/6i (cyclin-dependent kinase 4/6 inhibitors), ET (endocrine treatment), HR+/HER2- (hormone-receptor positive/ human epidermal growth factor receptor 2 negative), TNBC (triple-negative breast cancer), mBC (metastatic breast cancer), SOC (standard of care), TAEs (treatment adverse events), SAEs (serious adverse events), G (grade), PR (partial response), SD (stable disease), DCR (disease control rate), CCNE1(G1/S-specific cyclin-E1 encoding gene).

NCT number	Compound	Targeted Pathway	Regimen for Phase/Part	BC population for the enrollment (n)	Toxicities in all pts	Clinical response in BC pts	Status (last access to the trial status)
NCT04553133	PF-07104091	CDK2	**Part 1 A** PF-07104091 Monotherapy **Part 1B-Part 2** PF-07104091 +Fulvestrant PF-07104091+ Palbociclib+ET	**Part 1 A** HR+HER2- mBC (n=29) after CDK4/6i TNBC mBC (n=1) after SOC	**Part 1 A** TAEs 97.1% ≥G3 57.1%, SAEs 20%	**Part 1 A** 18.8% PR 37.5% SD 61.5% DCR	Recruiting (10 / 03 / 2023)
NCT05252416 (VELA trial)	BLU-222	CDK2	**Part 1 A** BLU-222 Monotherapy **Part 1B** BLU-222+Ribociclib +Fulvestrant **Part 2B-D** BLU-222+Fulvestrant +/- Ribociclib	**Part1A** mBC (n=13) after SOC, regardless of CCNE1 status **Part1B** HR+/HER2-mBC after progression on CDK4/6i **Part2B-D** HR+/HER2-mBC after CDK4/6i failure	**Part 1 A** TAEs 63%, ≥G3 19% (Part 1 A)	**Part 1 A** 1 PR, with no other clinical response data	Recruiting (20 / 12 / 2023)
NCT05238922	INCB123667	CDK2	**Part 1A-B** INCB123667 Monotherapy	**Part 1A-B** HR+HER2- and TNBC mBC after SOC Advanced or metastatic CCNE1 amplified or cycline E1 overexpressed solid tumors after SOC	/	/	Recruiting (27 / 12 / 2023)
NCT05735080	INX-315	CDK2	**Part A** INX-315 Monotherapy **Part C** INX-315 +ET+ CDK4/6	**PartA** Advanced or metastatic CCNE1 amplified solid tumors after SOC **Part A-C** HR+/HER2-mBC after CDK4/6i failure	/	/	Recruiting (01 / 09 / 2023)
NCT05867251	ARTS-021	CDK2	**Part 1 A** ARTS-021 Monotherapy **Part2A** ARTS-021 Monotherapy **Part^{1}B–^{2}B** ARTS-021+CDK4/6i +Fulvestrant or Letrozole	**Part 1 A** mBC after SOC **Part 2 A** Advanced or metastatic CCNE1 amplified solid tumors (including TNBC) after SOC **Part ^{1}B–^{2}B** HR+/HER2-mBC after CDK4/6i failure	/	/	Recruiting (20 / 12 / 2023)

2.1.2. BLU-222

BLU-222, a potent and selective CDK2 inhibitor, in combination with ribociclib, showed sustained tumor regression in both CDK4/6-resistant and sensitive models of HR+/HER2- breast cancer in preclinical studies. Efficacy, safety, tolerability, pharmacokinetics, and pharmacodynamics of BLU-222 are being evaluated in the VELA trial, an international, open-label, first-in-human phase 1/2 study (NCT05252416) (Patel et al., 2023). As of January 2023, 27 patients with advanced solid tumors after standard of care (SOC) had been recruited to receive BLU-222 in monotherapy escalating cohorts, administered twice daily in continuous 28-day cycles. Thirteen patients (48%) had mBC, without any specific subtype classification report, regardless of CCNE1 status, although CCNE1 amplification or progression after a CDK4/6 inhibitor were of specific interest. The most common (≥15%) adverse events (AEs) were (all-cause AEs; treatment-related AEs) nausea (33%; 26%), vomiting (22%; 11%), anemia (22%; 19%), diarrhea (22%; 22%), and fatigue (18%; 15%). Additionally, five patients (19%) experienced transient visual AEs, including symptoms like blurred vision, photophobia, and changes in vision, with only one G3 blurred vision/photophobia. In February 2023, the Food and Drug Administration (FDA) placed a partial clinical hold on the VELA trial because of the visual adverse events. Patients already enrolled in the trial continued to receive BLU-222 but no additional patients were allowed to enroll. All patients fully recovered after dose interruption or reduction. Further, no treatment-emergent abnormal findings have been reported in patients given detailed ophthalmologic examinations. Hence, in March 2023, the partial clinical hold was lifted, with trial sites reinitiating patient enrollment (Blueprint Medicines Corp., 2023; Sava, 2023). Translational pharmacodynamic data has shown early evidence of pathway modulation, with one reported partial response in a patient with HR+/HER2− mBC previously treated with 5 lines of therapy. Nevertheless, no additional clinical data regarding antitumoral activity was reported. Future research will be focused on BLU-222 dose escalation in combination with ribociclib and fulvestrant in patients with HR+ mBC after CDK4/6i.

2.1.3. Other CDK2i agents

ARTS-021, INX-315, and INCB123667 are other investigational, potent, orally available CDK2 selective inhibitors, with promising therapeutic potential based on consistent preclinical data. For refractory HR+/HER2- mBC (post CDK4/6i+ ET), both monotherapy and combination dose escalation-expansion strategies (with ET and CDK4/6i) will be employed. Notably, all the trials will evaluate their safety and activity as monotherapy in a dedicated arm with CCNE1 amplified or cyclin E1 overexpressed advanced/metastatic solid tumors after SOC, including mTNBC patients. Currently, while ARTS-021 trial has started recruiting (NCT05867251), there aren't available clinical data from INX-315 and INCB123667 trials (NCT05735080, NCT05238922). To conclude, other CDK2 inhibitors and degrader modalities are still in preclinical development (Arora et al., 2023).

3. Beyond CDK2 inhibitors

3.1. CDK4i AND CDK2/4/6i ongoing clinical trial, safety, and efficacy results

As previously mentioned, the concept of co-targeting CDK2 in a triple combination with conventional CDK4/6i and ET has been proposed, supported by robust preclinical evidence, and will be investigated in the above-mentioned trials. However, moving beyond the already approved CDK4/6i, new combined compounds are being developed to individually target all the relevant G1/S CDKs or selectively CDK4 (Table 2.).

3.1.1. PF-07220060

Over the past decade, the clinical development of CDK4/6 inhibitors has led to practice-changing outcomes in breast cancer treatment. Dual CDK4/6 inhibition has shown impressive antitumor activity with a manageable toxicity profile, although myelotoxicity remains a concern in daily use and can limit the dosage. All three approved CDK4/6i drugs, hinder CDK4 and CDK6 kinase activity. However, palbociclib and abemaciclib have greater potency against CDK4 than CDK6, with the latter that has five-fold more potency for CDK4 than the others and displays less selectivity by inhibiting multiple other kinases in-vivo, including CDK1, CDK2, and CDK9 (George et al., 2021). For this different pharmacodynamic activity on CDK4 and CDK6, they actually display distinct efficacy and toxicity profiles. PF-07220060 is a next-generation highly selective CDK4i with significant sparing of CDK6 (Yap et al., 2023b). To note, CDK4 is only essential for pancreatic lineages and reproductive functions, whereas both CDK4 and CDK6 are required for hematopoietic lineages (Arora et al., 2023). Hence, because of its greater selectivity for CDK4 over CDK6, it leads to less neutropenia in vivo models and, consequently, can be dosed higher to attain tolerated plasma concentrations that exceed those reported for dual CDK4/6i. PF-07220060, either alone or in combination with ET, has been investigated in a multicenter, first-in-human phase 1/2a study (NCT04557449), for

Table 2
Summary of Results from Trials Exploiting CDK4 and CDK2/4/6 Inhibitors. BC (breast cancer), Pts (patients), CDK (cyclin-dependent kinase), CDK4/6i (cyclin-dependent kinase 4/6 inhibitors), ET (endocrine treatment), HR+/HER2- (hormone-receptor positive/ human epidermal growth factor receptor 2 negative), TNBC (triple-negative breast cancer), ABC (advanced breast cancrr), mBC (metastatic breast cancer), SOC (standard of care), TAEs (treatment adverse events), SAEs (serious adverse events), G (grade), CR (complete response), PR (partial response), SD (stable disease), DCR (disease control rate), mPFS (median progression-free surviva)l, wks (weeks), mo (months), Cth (chemotherapy).

NCT number	Compound	Targeted Pathway	Regimen for Phase/ Part	BC population for the enrollment (n)	Toxicities in all pts	Clinical response	Status (last access to the trial status)
NCT04557449	PF-07220060	CDK4	**Part 1 A** PF-07220060 Monotherapy **Part 1B-C** PF-07220060 combination with letrozole or fulvestrant	**Part 1 A** HR+HER2+/- ABC/mBC (n=7) after at least 1 line of SOC (respectively CDK4/6i and anti HER2) **Part 1B-C** HR+HER2neg ABC/mBC (n=26) after at least 1 line of SOC (CDK4/6i)	**Part1A** 100%TEAEs ≥G3 50% SAEs 32.4% **Part1B-C** 96.2%TEAEs ≥G3 34.6% SAEs 15.4%	**Part1A (all pts)** 0% CR/PR 62.1%SD 75.9%DCR mPFS 23,9 wks **Part1B-C** 4.7% CR 23.8%PR 85.7%DCR mPFS 24,7 wks (30.8% still ongoing > 60+ wks)	Recruiting (29 / 12 / 2023)
NCT05262400	PF-07220060 + PF-07104091	CDK4+ CDK2	**Part1** PF-07220060 + PF-07104091 **Part2** PF-07220060 + PF-07104091 + letrozole or Fulvestrant	**Part1** HR+HER2+/-ABC/ mBC **Part2** HR+HER2neg ABC/ mBC	/	/	Recruiting (28 / 12 / 2023)
NCT05304962	RGT-419B	CDK2/4/6	**Part 1A** RGT-419B Monotherapy **Part 1B** RGT-419B combination with ET	**Part1A-B** HR+HER2+/- ABC/mBC (n=12) after CDK4/6i+ET and ≤ 1 of CT in ABC setting	**Part1A** 100%TEAEs ≥G3 25% (1 pt G4 hypertension and hyponatraemia) SAEs 0%	**Part1A (evaluable pts)** 50% PR (1 unconfirmed) 16,6% SD	Recruiting (13 / 10 / 2023)
NCT03519178	PF-06873600 (ebvaciclib)	CDK2/4/6	**Part1A** PF-06873600 monotherapy **Part1B** PF-06873600 + Letrozole or Fulvestrant	**Part1A** HR+/HER2– ABC/mBC (n=50) after CDK4/6i and 1–2 lines of Cht and mTNBC (n=2) up to 2 lines of Cth **Part1B** HR+/HER2– ABC/mBC (n=9) after CDK4/6i and 1–2 lines of Cht	**Part1A** nausea 50% / G3 0% anemia 38% / G3 14% neutropenia 29% / G3 16% **Part1B** nausea 67% / G3 0% anemia 44% / G3 11% neutropenia 22% / G3 11%	**Part1A** 48%DCR 2 PR (1 pt who remained on Treatment for >13 mo) **Part1B** 67%DCR 4 SD (3>13 mo, 1 >28 mo)	Program discontinued for mBC (26 / 12 / 2023)
NCT05905341	PF-07224826	CDK2/4/6	**Part1** PF-07224826 monotherapy in TNBC PF-07224826 +ET in HR+ **Part2** PF-07224826 +Fulvestrant	**Part1** HR+HER2neg or TNBC ABC/mBC **Part2** HR+HER2neg ABC/ mBC after (ArmA) or naïve (ArmB) to CDK4/6i	/	/	Withdrawn (Sponsor decision) (08 / 11 / 2023)
NCT04541225 NCT05191004	NUV-422	CDK2/4/6	NUV-422 monotherapy (NCT04541225) NUV-422+ Fulvestrant (NCT05191004)	HR+HER2neg ABC/mBC after SOC with at least I line of ET+CDK4/6i	/	/	Terminated (Sponsor decision) (14 / 07 / 2023) Withdrawn (Sponsor decision) (11 / 07 / 2023)

advanced solid tumors and HR+/HER2- mBC. At the data cut-off, 26 HR+/HER2- mBC patients (out of 34 with advanced solid tumors) were included. These patients had received escalating doses of PF-07220060 in combination with letrozole or fulvestrant. They were mainly heavily pretreated patients (median number of prior lines: 5 [IQR 1–11]); all had prior CDK4/6i, 19 (73.1%) prior fulvestrant and 20 (76.9%) prior chemotherapy. Most frequent TEAEs with PF-07220060 plus ET were diarrhea (50.0%; 0% G3), neutropenia (50.0%; 15.4% G3), and nausea (38.5%; 3.8% G3), with no G4 TEAEs. A similar safety profile was reported in monotherapy for the other advanced solid tumors. In evaluable mBC cases, the combination of PF-07220060 with ET has demonstrated 6 (28.6%) confirmed responses, including 1 complete response (CR) and 5 partial responses (PR). Clinical benefit response (CR, PR, or ≥24 weeks stable disease) was seen in 11 (52.4%) patients, with a median progression-free survival (PFS) of 24.7 weeks (95% CI: 23.1–47.4). As standalone treatment, 62.1% patients with SD as best response were reported with no partial or complete response and a mPFS of 23,9 wks. At the data cut-off, eight patients (30.8%) continued PF-07220060 + ET without progression for up to 60 plus weeks. To note, a dedicated dose expansion cohort will enroll HR+/HER2- mBC naïve to CDK4/6i. Moreover, the combined approach of novel CDK4i and CDK2i is currently under investigation in another ongoing phase 1b/2 study (NCT05262400). The results of dose escalation involving PF-07220060, PF-07104091 with or without ET, in HR+ mBC patients who are treatment-naïve or have previously received CDK4/6i are eagerly awaited.

3.1.2. RGT-419B . RGT-419B is a novel next-generation CDK inhibitor with anti-cancer activity in preclinical breast cancer models resistant to approved CDK4/6i (Wander 1 et al., 2023). It has shown a high potency against CDK4 with additional activity against CDK2 and selectivity against CDK6 to overcome resistance and reduce hematologic toxicity. Eligible patients for the first-in-human, multicenter trial assessing RGT-419B as a single agent were post-menopausal individuals affected by HR+ HER2- advanced breast cancer. These patients should have received ≥2 lines of treatment and progressed on CDK4/6i and ET. Twelve eligible patients received RGT-419B in 4 escalating cohorts as oral monotherapy twice daily in continuous 28-day cycles. The median age was 64.8 y (range 50–80 y) and all had prior palbociclib + ET (2 pts had abemaciclib or ribociclib after palbociclib); a majority received fulvestrant (67%) and prior chemotherapy (50%). The most observed TEAEs with RGT-419B were nausea, reduced white blood cell counts (neutrophils and/or lymphocytes) and diarrhea. The most frequent ≥G3 AEs were hyperglycaemia (33% all grades;G3 8%), vomiting (25% all grades; G3 8%), fatigue (25% all grades; G3 8%). No ocular toxicity or discontinuation has been observed due to RGT-419B. Among six evaluable patients for efficacy analysis, 3 achieved partial response (1 unconfirmed), while 1 achieved stable disease. In the RGT-419B 150 mg BID cohort, all 3 patients had tumor size reduction. The current data on RGT-419B monotherapy dose-escalation support further evaluation either as a single agent or as in combination with ET (Wander 1 et al., 2023)

3.1.3. PF-06873600 (PF-3600). PF-06873600 (also known as PF-3600 or ebvaciclib) is a first-in-class selective pharmacologic inhibitor targeting all three major G1/S CDKs: CDK2, 4 and 6 (CDK2/4/6 inhibitor) (Yap et al., 2022). It has shown robust pharmacodynamics and tumor growth inhibitory activity in multiple models of cancer, particularly for those with elevated MYC activity (Freeman-Cook et al., 2021). At the 2021 San Antonio Breast Cancer Symposium (SABCS), the findings of dose escalation both in monotherapy and in combination with ET were presented (NCT03519178). Among 67 patients enrolled, 59 were heavily pretreated HR+/HER2- mBC and 2 mTNBC patients (median 4; range [1-8] for monotherapy, range [3-6] for combination). A total of 58 patients received PF-3600 as monotherapy, while 9 patients received the combination of PF-3600 and Fulvestrant. The most frequently reported TAES (all G; G ≥3) for monotherapy and combination were nausea (50%; 0% and 67%; 0%), anemia (38%;14% and 44%;11%), and neutropenia (29%;16% and 22%;11%), respectively. The disease control rate (DCR) for PF-3600 alone was 48% (28 out of 58 patients). Among these patients, two had a partial response, and one patient sustained treatment for over 13 months. In the combination cohort, DCR reached an impressive 67% (6 out of 9 patients), showcasing remarkable stability as three patients maintained stability for more than 13 months, and one patient's stability extended beyond 28 months. Dose expansion in combination with endocrine therapy (ET) was initially planned for patients naïve to or after CDK4/6i , but its clinical development has been discontinued by the sponsor (Dietrich et al., 2023).

3.1.4. Other CDK2/4/6 agents. In the same setting, another innovative oral small molecule co-targeting CDK2/4/6, PF-07224826, has been proposed as a single agent or in combination with Fulvestrant in mBC and other advanced solid tumors (NCT05905341); However, the enrollment for this trial has been withdrawn by the same sponsor of the aforementioned PF-06873600. Apparently, the decision was driven by business considerations rather than specific safety concerns or a request from a regulatory authority (Dietrich et al., 2023). Moreover, two further trials were evaluating NUV-422, another selective CDK2/4/6 inhibitor, combined with fulvestrant or in monotherapy, for solid tumors including HR+ HER2- mBC post CDK4/6i+ET. Unfortunately, the FDA has placed a partial clinical hold on the phase 1/2 NUV-422–02 trial (NCT04541225) due to reported cases of uveitis. Consequently, also the NUV-422–03 trial's recruitment (NCT05191004) had been also halted (Jordyn Sava., 2022)

3.2. PAN-CDKi targeting CDK2: ongoing clinical trial, safety and efficacy results

The efficacy of pan-CDKi compared to highly selective ones is still a matter of debate. Most first-generation pan-CDKi (such as flavopiridol and roscovitine), faced non-approval for clinical application due to their non-specific CDK targeting and significant side effects. However, the second-generation pan-CDKi with higher selectivity for CDK1–2 and reduced toxicity through combination therapies, have thus regained clinical potential.

3.2.1. Dinaciclib. Dinaciclib (also known as MK-7965 and SCH727965) is a notable second-generation pan-CDK inhibitor, primarily targeting CDK1, CDK2, CDK5 and CDK9. Notably, CDK9 complex is involved in transcriptional elongation, and its inhibition resulted in the loss of transcripts with short half-life. Additionally, the survival of cancer cells carrying strong oncogenic signals (such as MYC-driven cancers) is maintained through overexpression of pro-survival driver (such as pro-survival protein Myeloid Cell Leukemia 1, MCL1, member of the BCL2 family). Mechanistically, some tumors depending heavily on transcription of selected driver genes such as MCL1, MYC, MYCN, result in being highly CDK9-dependent, and thus its inhibition induce rapid apoptosis in cancer cells (Frame et al., 2020). Therefore, it is worth emphasizing that, given the complexity and pleiotropic action of pan-CDKi, they predominantly function more as cytotoxic compounds rather than targeted drugs. Despite preclinical evidence of Dinaciclib significant efficacy and its promising clinical results observed in hematological malignacies, recent Phase 2 trials conducted in the context of advanced solid tumors have unfortunately yielded unsatisfying results (Agostinetto et al., 2021) (Table 3.). Similarly, as monotherapy regimen, Dinaciclib regrettably failed to yield any significant responses or disease control in patients with mBC. This outcome held true for both weekly and every-3-week intravenous infusion schedules (NCT00871663, NCT00871910) (Nemunaitis et al., 2013; Mita et al., 2017). In a randomized Phase 2 trial (NCT00732810) (Mita et al., 2014), the duration of time to progression (TTP) for Dinaciclib monotherapy was shorter than Capecitabine (2.73 vs 4.17 months, hazard ratio [HR] 1.67; 95%

Table 3
Summary of Results from Trials Exploiting pan-CDK Inhibitors. BC (breast cancer), pts (patients), CDK (cyclin-dependent kinase, CDK4/6i (Cyclin-dependent kinase 4/6 inhibitors), ET (endocrine treatment), HR+/HER2- (hormone-receptor positive/ human epidermal growth factor receptor 2 negative), mTNBC (metastatic triple-negative breast cancer), mBC (metastatic breast cancer), SOC (standard of care), TAEs (treatment adverse events), SAEs (serious adverse events), G (grade), PR (partial response), SD (stable disease), DCR (disease control rate), AST (aspartate aminotransferase), ALT (alanine aminotransferase), TTP (time to progression), ORR % (objective response rat%), CR (complete response), PR (partial response), SD (stable disease), DCR (disease control rate), mPFS (median progression-free survival), wks (weeks), mo (months), Cth (chemotherapy), IV (intravenous administration), BID (twice a day), d-q (day of cycle scheme).

NCT number	Compound	Targeted Pathway	Regimen for Phase/Part	BC population for the enrollment (n)	Toxicities in all pts	Clinical response	Status (last access to the trial status)
NCT02552953	Fadraciclib	CDK2/9	Fadraciclib monotherapy IV 4-hour infusion	Advanced solid tumors including mBC after SOC (not other specified)	**All patient** Most common mild-moderate TAEs constipation, diarrhea, decreased appetite, dehydration, fatigue, nausea, vomiting, (not other specified)	6 SD ≥ 6 cycles (0 mBC)	Active, not recruiting (25 / 04 / 2022)
NCT04983810	Fadraciclib	CDK2/9	Fadraciclib monotherapy oral administration BID q28	HR+HER2neg or HER2+ TNBC ABC/ mBC after SOC (CDK4/ 6i for HR+)	/	/	Recruiting (04 / 04 / 2022)
NCT00871910	Dinaciclib	CDK 1/2/ 5/9	**Part 1** Dinaciclib monotherapy every 3 week (2-h IV infusions) **Part 2** Dinaciclib monotherapy every 3 week (8- and 24-h IV infusions)	**Part1** mBC after SOC (n=1) **Part2** mBC after SOC (n=4)	**Part1 (G≥3)** leukopenia 17% neutropenia 46% (G4 6%) increased AST 9%, increased ALT 6% hyperuricemia 6% **Part2 (ArmA G≥3)** neutropenia 38% (G4 13%) hypotension 13% **(ArmB G≥3)** fatigue 20	0% ORR (all pts) **Part1** 5 SD (0 mBC) **Part2** 3 SD (0 mBC)	Completed (23 / 10 / 2017)
NCT00871663	Dinaciclib	CDK 1/2/ 5/9	Dinaciclib monotherapy 2 hours IV infusion d1,8,15 q28	mBC after SOC (n=3)	**All pts** AEs G≥3 60% (and ≥10% G3 AEs) Neutropenia 10% Anemia 10% Hyperbilirubinemia 10% Hypophosphatemia 10%	0% ORR 10 SD (O mBC)	Completed (20 / 04 / 2015)
NCT00732810	Dinaciclib	CDK 1/2/ 5/9	**Part1 Randomized (1:1)** Dinaciclib monotherapy (2-hour IV infusion q21 at 50 mg/mq) **vs** Capecitabine monotherapy 1250 mg/m2 bid q21 oral administration **Part2** Cross over to Dinaciclib after progression on Capecitabine	**All pts** mBC 1 up to 2 lines of Cth and 2 anti-HER2 lines if HER2+ **Part1** (n=30: 15 Dinaciclib vs 15 Capecitabine; 4 TNBC, 3 HER2+,15 HR+ HER2-, 1 HR+HER2+, 7 unknown) **Part2** (n=6, 1 TNBC, 4 HR+ HER2-, 1 unknown)	**Part1–2** -All Dinaciclib pts G≥3 neutropenia 47% (11% G4) leukopenia 21%, AST and ALT increase, Cough Diarrhea, vomiting, hypersensitivity, and fatigue 5% each	**Part1** -Dinaciclib 8% ORR (1 PR, another 1 unconfirmed) TTP 2.73 mo -Capecitabine 7% ORR (1 PR) TTP 4.17 mo HR 1.67 (95% CI,0.68–4.15) **Part2** 0% ORR	Completed (05 / 08 / 2015)
NCT01676753	Dinaciclib	CDK 1/2/ 5/9	Dinaciclib d1,8 q21 IV combination with Pembrolizumab IV infusion 200 mg q21	mTNBC ABC/mBC up to 2 lines of Cth (n=32)	**All patients** G≥3 neutropenia 37.5% (G4 12.5%) fatigue 12.5% transaminitis 3.2%, neuromuscular weakness 3.2%	**All pts** CR 3.4%) had a CR, 4 pts (13.8%) had a PR, and 6 pts (20.6%) had SD	Completed (21 / 09 / 2022)
NCT01624441	Dinaciclib	CDK 1/2/ 5/9	Dinaciclib IV infusion d1q21 + Epirubicin IV infusion d2 q21	mTNBC up to 2 lines of Cth (n=9)	**All patients** G≥3 leuko-neutropenia 22% (G4 22%) syncope 22% diarrhea 11% vomiting 11%	**All pts** 0 ORR% median TTP 5.5 wks	Early stopped (30 / 03 / 2018)

CI, 0.68–4.15). This outcome persisted despite an initial reported response observed with Dinaciclib treatment. Moreover, considering the combinational strategy, Dinaciclib was investigated with Epirubicin in a Phase 1 dose-escalation study involving patients with mTNBC, with a prior history of receiving ≤2 lines of chemotherapy (Mitri et al., 2015). Among the patients evaluated, no objective responses were observed. Median TTP was brief, standing at 5.5 weeks (IQR 3–12 weeks). Dose escalation ceased after the second cohort due to both toxicity concerns and the complete lack of response. Consequently, patient enrollment was halted, and lower dosage levels were not explored. In contrast, the combination of Dinaciclib with Pembrolizumab in a Phase 1 trial demonstrated an initial clinical benefit for mTNBC (n=32 patients with a median of 2 L), as supported by the preclinical evidence that MYC-driven TNBC models are associated with an increased programmed cell death 1 (PD-1) expression on tumor-infiltrating lymphocytes (sTILs) (Chien et al., 2020). In this study, five patients (16.7%) had an objective response, including one complete response (3.4%), four partial response (13.8%) and six stable disease (20.6%). Interestingly, an exploratory analysis revealed a noteworthy correlation between MYC expression and treatment response, suggesting that MYC could serve as a predictive biomarker in this context.

3.2.2. Fadraciclib. The anti-cancer efficacy of fadraciclib (CYC065), potent and selective inhibitor of CDK2 and CDK9, has been observed preclinically in breast cancer models, either alone or combined with other antineoplastic regimens, such as trastuzumab or eribulin (Frame et al., 2020). The administration of fadraciclib through 4-hour infusion every 3 weeks was examined in a pioneering Phase 1 study involving patients with advanced cancers (NCT02552953) (Do et al., 2018). A total of 26 patients were enrolled, although it was not explicitly mentioned whether breast cancer patients were included. The severity of the most frequent adverse events that occurred was mild to moderate. Also, six patients (23.1%) had stable disease after 6 or more treatment cycles; however, none of these were specifically breast canncer patients. Utilizing a more convenient oral administration for Fadraciclib in advanced solid tumors, including mBC after SOC, a dedicated phase 1/2 trial (NCT04983810) is actively recruiting participants. As of now, there are no clinical available data.

4. Discussion

Collectively, consistent preclinical evidence establishes that aberrant CDK2 activity, through MYC overexpression and CCNE1 amplification, represents either an oncogenic driver for certain cancer types (such as TNBC) and a main mechanism of endocrine-CDK4/6i resistance in HR+ patients. Hence, targeting CDK2 has emerged as an appealing and relatively unexplored treatment opportunity in the context of mBC, either triple negative or HR+, where targeted therapy options are limited after primary progression, being single-agent chemotherapy the most common choice with limited survival (Modi et al., 2022). In response to compelling preclinical evidence and the unmet clinical need, there has been a push towards the development of novel CDK2 selective inhibitors, resulting in numerous phase 1/2 trials to investigate their clinical activity.

4.1. Patient setting for novel CDKi use

All the novel CDKi have been exclusively investigated in pretreated mBC patients, especially HR+ patients after CDK4/6i failure, representing their primary target population. The emphasis on TNBC was limited, since they were excluded from trials involving CDK4i and CDK2/4/6i, and only one patient was formally mentioned to have been enrolled in CDK2i trial, specifically with PF-07104091.

4.2. Patient selection for novel CDKi use

The identification of reliable biomarkers to predict response to novel target therapy is an urgent need, being indispensable for patient selection and to magnify clinical benefit. Interestingly, in current clinical practice, specific predictive signatures for primary resistance to CDK4/6i, such as Rb loss, aren't considered for first-line treatment selection (Gomatou et al., 2021). Conversely, the identification of secondary resistance biomarkers, is mandatory to guide the best second-line treatment in HR+ mBC (such as Estrogen Receptor 1 [ESR1] mutation, germline breast cancer gene 1 mutation [gBRCA], PI3K/AKT/mTOR pathway alteration). Notably, the enrollment in CDK2i trials, for patients with accessible clinical data, was solely guided by experiencing progression after the standard of care treatment (CDK4/6i and ET for HR+ cases), regardless for example of MYC or CCNE1 status. Considering the latter, given their role as surrogate biomarkers for CDK2 "dependency", they could be therefore used for treatment selection, either for TNBC or HR+ patients. Notably, on this trajectory, certain CDK2i trials have planned expansion/escalation arms specifically designed for advanced solid tumors or particular histology (high-grade serous epithelial ovarian cancer) with CCNE1 amplification or cyclin E1 overexpression, including also mBC (especially TNBC). Hence, they will provide valid information regarding their surrogacy and clinical reliability as predictive biomarker of response to CDK2i.

However, further data and genomic analyses from larger and randomized trials are needed to move beyond the current "one-size-fits-all" approach.

4.3. CDK2i monotherapy treatment, rational and toxicity

Inevitably resistance to CDK4/6i occurs and optimal subsequent therapeutic strategy is still an open question (Mittal et al., 2023; Cogliati et al., 2022; Ma et al., 2023). Despite the conventional chemotherapy regimens, the new emerging ADCs and PARP inhibitors, there are still therapeutic approaches being pursued to target hormone-related pathways and cell cycle control after CDK4/6i failure. Among them, some involve blocking the specific mechanisms driving resistance to CDK4/6i, while others explore the option of continuing the treatment with switched molecules. Therefore,as previously reported, two ongoing phase 1/2 trials have published preliminary clinical results of targeting CDK2 resistance pathway with novel selective inhibitors, as standalone therapy in mBC patients. The first molecule, PF-07104091, in dose escalation exhibited a significant antitumor activity in HR+/HER2- mBC patients after progression on CDK4/6i, with an impressive DCR of 61.5%, without any data available for the only one enrolled TNBC patient. Despite a favourable tolerability profile stated by the authors, more than half of the patients reported G3 adverse events (most of them non-hematological: nausea 14.3%, diarrhea 8.6%, vomiting 2.9% and fatigue 20.0%). Conversely, the second one, BLU-222, has achieved only one partial response without any additional clinical available information, although with a lower incidence of overall grade 3 or higher toxicities compared to PF-07104091. Nevertheless, 19% of the patients encountered transient visual AEs, including one G3 case of blurred vision/photophobia. This resulted in an initial clinical hold by the FDA, then lifted after full toxicity recovery following dose interruption or reduction, with no treatment-emergent abnormal findings at the subsequent ophthalmologic examinations.

4.4. CDK2i combinational approaches, rational and toxicity

Combination approaches with novel CDK2i, currently under investigation or slated for evaluation, concentrate primarily on HR+ patients, being ET and CDK4(/6)i ideal candidates for synergistic partnership. The focus on luminal patients stems from the limited representation of TNBC subgroup in ongoing trials and the lack of CDK2 efficacy and safety data in monotherapy.

Firstly, irrespective of their notable clinical efficacy as standalone treatments, it would be intriguing to observe the outcomes of CDK2 combination with ET. Counteracting upstream resistance pathways by targeted drugs along with endocrine backbone treatment (PI3K/AKT/mTOR with Alpelisib/Capivasertib/Everolimus) resulted in remarkable clinical benefit in several randomised phase 2/3 trial, being a valuable choice after CDK4/6i failure (Turner et al., 2023; André et al., 2019; Baselga et al., 2012; Bidard et al., 2022). Furthermore, the potential for any specific cumulative toxicity from endocrine therapy (ET) is expected to be marginal. Secondly, combining CDK2i with other selective G1/S CDK inhibitors, with or without ET, presents another appealing opportunity. It stems from the notion, as previously discussed, that an alternative strategy following CDK4/6i failure involves maintaining cell cycle control by "only" switching both endocrine and CDK4/6i molecules. This approach capitalizes on drug's different pharmacodynamic impact on CDKs and endocrine pathways, aiming to overcome the acquired resistance. Furthermore, the "MAINTAIN trial" has been the first randomized, placebo-controlled study to reveal a clinical benefit with this strategy. A significant median PFS advantage with Ribociclib compared to placebo with switched ET was registered in patients with HR+/HER2– mBC after a prior CDK4/6i (Kalinsky et al., 2023). Consistent with this concept, switching to a different novel highly selective CDK4i (PF-07220060) with or without ET, after CDK4/6i failure, has shown a notable preliminary clinical benefit. Interestingly, CDK4i+ET combination not only exhibited superior clinical performance, with eight patients (30.8%) without progression for over 60 weeks at the data cut-off, but also demonstrated a seemingly more favourable toxicity profile compared to the standalone approach (34.6% vs 50% $\geq$G3 and 15.4% vs 32.4% SAEs).

Certainly, integrating CDK2i into a triple combination with switched ET and CDK4/6i molecules could offer synergistic advantages after CDK4/6i failure. Although supported by the previously mentioned preclinical evidence (Arora et al., 2023; Freeman-Cook et al., 2021; Al-Qasem et al., 2022), and high clinical responses yielded by novel combined CDK2/4/6i (PF-06873600) with ET, this strategy involves balancing the magnification of benefits at the cost of increased toxicities. Notably, all CDK2/4/6i, except for RGT-419B, have been currently stopped in their clinical development either due to unacceptable toxicity or on sponsor decision. Moreover, Dietrich C. et al. have finely depicted some theoretical disadvantages of combined compounds that inhibit all G1/S CDKs: first, low flexibility with dosing to find an effective and tolerable treatment schedule, rather than balancing separate drugs; second, sole CDK2 inhibition may be sufficient to regain control of tumor proliferation avoiding futile toxicities related with CDK4/6 block (Dietrich et al., 2023). Although the results of planned CDK2i dose

expansion combination with CDK4/6i and ET are still pending, these considerations cast doubts about its feasibility and advancement. On the contrary, this challenging three-pronged strategy could potentially be pursued through the synergistic and less toxic combination of CDK2i, highly selective CDK4i with ET.

4.5. Pan-CDKi treatment and toxicity

Notably, the remarkable clinical activity of the above-mentioned novel CDKi in refractory HR+ mBC contrasts with poor results of the "old" pan-CDKi drug, converging mainly on pre-treated mTNBC. Overall pan-CDKi yielded unsatisfactory outcomes, except for Dinaciclib when combined with immunotherapy. Moreover, combination of the latter with chemotherapy exhibited a concerning toxicity profile. Furthermore, their inconvenient infusion-based administration method further diminishes their clinical relevance in breast cancer treatment. However, it is worth emphasizing that the trials assessing pan-CDKi did not focus on HR+ patients and did not incorporate a combination of endocrine interventions. This omission may have missed a potential area for their application. Likewise, employing highly selective CDK2i in conjunction with chemo/immunotherapy for mTNBC could potentially introduce a new landscape for a population, lacking effective targeted therapies.

5. Conclusion

At light of the current research and preliminary clinical findings a bunch of open questions bends over novel CDK2i. Expected forthcoming data will provide more robust information of their clinical activity and toxicity, offering valuable insights into questions regarding patient selection, potentially predictive molecular markers, the necessity for combinational approaches and the best-partnering drugs in those scenarios. Triple-negative patients characterized by a "CDK2-driven" biology, being frequently MYC overexpressed or CCNE1 amplified, may benefit from a standalone treatment. Otherwise luminal-like cancers, appropriately selected for molecular resistance signature, might deserve, as highlighted by preclinical evidences, a combinational approach to reverse or delay (in naïve patients) CDK4/6i-ET resistance. To note, another key point to be addressed is the best fitting for CDK2i in the rapidly evolving and expanding treatment algorithm, considering the other cutting-edge molecules, emerging with positive results from phase 2/3 clinical trials. These advancements encompass the new revolutionary ADCs, already representing a new standard of care in both pretreated HR+ and mTNBC (Bardia et al., 2021; Robson et al., 2019; Rugo et al., 2023; Bardia et al., 2023). In conclusion, despite their very early clinical development and undefined place in the therapeutic algorithm, these innovative drugs hold the promise of offering a novel target therapy, potentially reshaping the treatment landscape for both HR+ resistant and mTNBC patients.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of Competing Interest

Armando Santoro: Advisory Board: Bristol-Myers Squibb (BMS), Servier, Gilead, Pfizer, Eisai, Bayer, Merck Sharp & Dohme (MSD). Consultancy: Arqule, Sanofi, Incyte. Speaker's Bureau: Takeda, BMS, Roche, Abb-Vie, Amgen, Celgene, Servier, Gilead, Astrazeneca, Pfizer, Arqule, Lilly, Sandoz, Eisai, Novartis, Bayer, MSD (all outside the submitted work); Alberto Zambelli has received personal fees and non-financial support from Novartis, AstraZeneca, Lilly, Pfizer, Daiichi Sankyo, MDS Merck Sharp&Dome, Roche, Seagen, Exact Sciences, Gilaed, Istituto Gentili; Rosalba Torrisi has received funding from Astra Zeneca, Pfizer, Eli Lilly, Exact Sciences and MSD; Rita De Sanctis honoraria for advisory board consultancy from Novartis, Istituto Clinico Gentili, Amgen, EISAI, Lilly and Gilead (all outside the present work). The other authors declare no competing interests.

Acknowledgments

none

Author contributions

All the authors, including: Riccardo Gerosa, Rita De Sanctis, Rosalba Torrisi, Giovanna Masci, Chiara Miggiano, Giuseppe Saltalamacchia, Mariangela Gaudio, Chiara Benvenuti, Flavia Jacobs, Francesco Agustoni, Paolo Pedrazzoli, Armando Santoro, Alberto Zambelli, have made substantial contributions to all of the following: (1) the conception and design of the original research, (2) drafting the article or revising it critically, (3) final approval of the submitted version of the manuscript.

References

Agostinetto, E., Eiger, D., Punie, K., de Azambuja, E., 2021. Emerging therapeutics for patients with triple-negative breast cancer. Curr. Oncol. Rep. 23 (5), 57.

Al-Qasem, A.J., Alves, C.L., Ehmsen, S., Tuttolomondo, M., Terp, M.G., Johansen, L.E., et al., 2022. Co-targeting CDK2 and CDK4/6 overcomes resistance to aromatase and CDK4/6 inhibitors in ER+ breast cancer. NPJ Precis Oncol. 6 (1), 68.

André, F., Ciruelos, E., Rubovszky, G., Campone, M., Loibl, S., Rugo, H.S., et al., 2019. Alpelisib for PIK3CA -mutated, hormone receptor–positive advanced breast cancer. N. Engl. J. Med. 380 (20), 1929–1940.

Arora, M., Moser, J., Hoffman, T.E., Watts, L.P., Min, M., Musteanu, M., et al., 2023. Rapid adaptation to CDK2 inhibition exposes intrinsic cell-cycle plasticity. Cell 186 (12), 2628–2643.e21.

Bardia, A., Hurvitz, S.A., Tolaney, S.M., Loirat, D., Punie, K., Oliveira, M., et al., 2021. Sacituzumab govitecan in metastatic triple-negative breast cancer. N. Engl. J. Med. 384 (16), 1529–1541.

Bardia, A., Jhaveri, K., Kalinsky, K., Pernas, S., Tsurutani, J., Xu, B., et al., 2023. TROPION-Breast01: datopotamab deruxtecan vs chemotherapy in pre-treated inoperable or metastatic HR+/HER2– breast cancer. Future Oncol.

Baselga, J., Campone, M., Piccart, M., Burris, H.A., Rugo, H.S., Sahmoud, T., et al., 2012. Everolimus in postmenopausal hormone-receptor–positive advanced breast cancer. N. Engl. J. Med. 366 (6), 520–529.

Bidard, F.C., Kaklamani, V.G., Neven, P., Streich, G., Montero, A.J., Forget, F., et al., 2022. Elacestrant (oral selective estrogen receptor degrader) versus standard endocrine therapy for estrogen receptor–positive, human epidermal growth factor receptor 2–negative advanced breast cancer: results from the randomized phase III emerald trial. J. Clin. Oncol. 40 (28), 3246–3256.

Blueprint medicines announces lift of partial clinical hold on phase 1/2 vela trial of blu-222 (no date) Blueprint Medicines Corp. Available at: ⟨https://ir.blueprintmedicines.com/news-releases/news-release-details/blueprint-medicines-announces-lift-partial-clinical-hold-phase⟩ (Accessed: 14 October 2023).

Chien, A.J., Gliwa, A.S., Rahmaputri, S., Dittrich, H.F., Majure, M.C., Rugo, H.S., et al., 2020. A phase Ib trial of the cyclin-dependent kinase inhibitor dinaciclib (dina) in combination with pembrolizumab (P) in patients with advanced triple-negative breast cancer (TNBC) and response correlation with MYC-overexpression. J. Clin. Oncol. 38 (15_suppl), 1076–1076.

Glen Clack (1) Sercan Aksoy (2) Meritxell Bellet- Ezquerra (3) Begoña Bermejo (4) Cagatay Arslan (5) Maria Castro-Henriques (6) Irfan Cicin (7) Mahmut Gumus (8) Carmen Hinojo-González (9) Nuri Karadurmus (10), Denisse Lee Cervantes (11) Rebeca Lozano Mejorada (12) Emmanuel de la Mora Jimenez (13) Fernando Moreno Antón (14) Sonia Pernas (15) Timothy Pluard (16) Carlos Zuloaga Fernandez del Valle (17) Ozan yazıcı (18) Stuart McIntosh (1). Session: Poster Session 3 SABCS. 2023. (PO3-04-12) SUMIT-BC: Phase 2 randomized study of fulvestrant with or without the cyclin-dependent kinase 7 inhibitor (CDK7i) samuraciclib in advanced hormone receptor positive (HR+) breast cancer after CDK4/6i.

Cogliati, V., Capici, S., Pepe, F.F., di Mauro, P., Riva, F., Cicchiello, F., et al., 2022. How to treat HR+/HER2- metastatic breast cancer patients after CDK4/6 inhibitors: an unfinished story. Life 12 (3), 378.

Criscitiello, C., Viale, G., Esposito, A., Curigliano, G., 2014. Dinaciclib for the treatment of breast cancer. Expert Opin. Invest. Drugs 23 (9), 1305–1312.

Dietrich, C., Trub, A., Ahn, A., Taylor, M., Ambani, K., Chan, K.T., et al., 2023. INX-315, a selective CDK2 inhibitor, induces cell cycle arrest and senescence in solid tumors. Cancer Discov.

Do, K.T., Chau, N., Wolanski, A., Beardslee, B., Hassinger, F., Bhushan, K., et al., 2018. Abstract CT037: phase I safety, pharmacokinetic and pharmacodynamic study of CYC065, a cyclin dependent kinase inhibitor, in patients with advanced cancers (NCT02552953). Cancer Res. 78 (13_Supplement). CT037–CT037.

Frame, S., Saladino, C., MacKay, C., Atrash, B., Sheldrake, P., McDonald, E., et al., 2020. Fadraciclib (CYC065), a novel CDK inhibitor, targets key pro-survival and oncogenic pathways in cancer. PLoS One 15 (7), e0234103.

Freeman-Cook, K., Hoffman, R.L., Miller, N., Almaden, J., Chionis, J., Zhang, Q., et al., 2021. Expanding control of the tumor cell cycle with a CDK2/4/6 inhibitor. Cancer Cell 39 (10), 1404–1421.e11.

George, M.A., Qureshi, S., Omene, C., Toppmeyer, D.L., Ganesan, S., 2021. Clinical and pharmacologic differences of CDK4/6 inhibitors in breast cancer. Front Oncol. 11.
Ghafouri-Fard, S., Khoshbakht, T., Hussen, B.M., Dong, P., Gassler, N., Taheri, M., et al., 2022. A review on the role of cyclin dependent kinases in cancers. Cancer Cell Int. 22 (1), 325.
Gomatou, G., Trontzas, I., Ioannou, S., Drizou, M., Syrigos, N., Kotteas, E., 2021. Mechanisms of resistance to cyclin-dependent kinase 4/6 inhibitors. Mol. Biol. Rep. 48 (1), 915–925.
Jordyn Sava https://www.targetedonc.com/view/fda-halts-phase-1-2-trial-evaluating-nuv-422-in-solid-tumors. 2022. FDA Halts Phase 1/2 Trial Evaluating NUV-422 in Solid Tumors.
Jovanović (2), Bojana, Chu (3), Xiangying, Hughes (3), Melissa, Mohammed-Abreu (3), Ayesha, Kasparian (2), Julie, Erick (2), Timothy, DiLullo (4), Molly, Wrabel (2), Eileen, Jeselsohn (5), Rinath, Tayob (5), Nabihah, Schnitt (6), Stuart, Lin (7), Nancy, Tolaney (5), Sara, 2023. Session: poster spotlight session SABCS, 2023. (PS12-06) A phase 2 study of abemaciclib monotherapy for patients with retinoblastoma-positive (Rb+), triple-negative metastatic breast. cancer.
Kalinsky, K., Accordino, M.K., Chiuzan, C., Mundi, P.S., Sakach, E., Sathe, C., et al., 2023. Randomized phase II trial of endocrine therapy with or without ribociclib after progression on cyclin-dependent kinase 4/6 inhibition in hormone receptor–positive, human epidermal growth factor receptor 2–negative metastatic breast cancer: maintain trial. J. Clin. Oncol. 41 (24), 4004–4013.
Ma, J., Chan, J.J., Toh, C.H., Yap, Y.S., 2023. Emerging systemic therapy options beyond CDK4/6 inhibitors for hormone receptor-positive HER2-negative advanced breast cancer. NPJ Breast Cancer 9 (1), 74.
Mita, M.M., Joy, A.A., Mita, A., Sankhala, K., Jou, Y.M., Zhang, D., et al., 2014. Randomized phase II trial of the cyclin-dependent kinase inhibitor dinaciclib (MK-7965) versus capecitabine in patients with advanced breast cancer. Clin. Breast Cancer 14 (3), 169–176.
Mita, M.M., Mita, A.C., Moseley, J.L., Poon, J., Small, K.A., Jou, Y.M., et al., 2017. Phase 1 safety, pharmacokinetic and pharmacodynamic study of the cyclin-dependent kinase inhibitor dinaciclib administered every three weeks in patients with advanced malignancies. Br. J. Cancer 117 (9), 1258–1268.
Monica Mita (1) Alain Mita (2) Miguel Villalona-Calero (3) Noah Federman (4) Drew Rasco (5) David Spigel (6), Jia Luo (7) Gregory Cote (8) Richard Cutler (9) Pavan Kumar (9) Crystal MacKenzie (9) Charles Lin (9) Jorge DiMartino (9) Elizabeth Olek (9) Brian Van Tine (10). Poster Session 4, SABCS. 2023. (PO4-18-07) A dose escalation and cohort expansion study of the CDK9 inhibitor KB-0742 in triple negative breast cancer and transcriptionally addicted relapsed or refractory solid tumors.
Mitri, Z., Karakas, C., Wei, C., Briones, B., Simmons, H., Ibrahim, N., et al., 2015. 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. Invest N. Drugs 33 (4), 890–894.
Mittal, A., Molto Valiente, C., Tamimi, F., Schlam, I., Sammons, S., Tolaney, S.M., et al., 2023. Filling the Gap after CDK4/6 Inhibitors: novel endocrine and biologic treatment options for metastatic hormone receptor positive breast cancer. Cancers 15 (7), 2015.
Modi, S., Jacot, W., Yamashita, T., Sohn, J., Vidal, M., Tokunaga, E., et al., 2022. Trastuzumab deruxtecan in previously treated HER2-low advanced breast cancer. N. Engl. J. Med. 387 (1), 9–20.
Nemunaitis, J.J., Small, K.A., Kirschmeier, P., Zhang, D., Zhu, Y., Jou, Y.M., et al., 2013. A first-in-human, phase 1, dose-escalation study of dinaciclib, a novel cyclin-dependent kinase inhibitor, administered weekly in subjects with advanced malignancies. J. Transl. Med. 11 (1), 259.
Panagiotou, E., Gomatou, G., Trontzas, I.P., Syrigos, N., Kotteas, E., 2022. Cyclin-dependent kinase (CDK) inhibitors in solid tumors: a review of clinical trials. Clin. Transl. Oncol. 24 (2), 161–192.
Patel, M.R., Juric, D., Henick, B.S., Duska, L.R., Wu, R., Guo, J., et al., 2023. BLU-222, an oral, potent, and selective CDK2 inhibitor, in patients with advanced solid tumors: phase 1 monotherapy dose escalation. J. Clin. Oncol. 41 (16_suppl), 3095–3095.
Amita Patnaik (1) Silvia Antolin Novoa (2) Meritxell Bellet- Ezquerra (3) Francois-Clement Bidard (4) Valentina Boni (5) Maxime Brunet (6) Mario Campone (7) Carlos Garay (8) Raquel Gómez-Bravo (9) Sacha Howell (10), Rinath Jeselsohn (11) Simon Lord (12) Paolo Mazzei (13) Maria De Miguel (14) Erin Roesch (15) Agostina Stradella (16) Helene Vanacker (17) Cecile Vicier (18) Seth Wander (19) Glen Clack (20) Stuart McIntosh. Poster Session 3 SABCS. 2023. (PO3-04-13) SUMIT-ELA: Phase 1b/2 combination of cyclin-dependent kinase 7 inhibitor (CDK7i) samuraciclib and selective estrogen receptor degrader (SERD) elacestrant in advanced hormone receptor positive (HR+) breast cancer after CDK4/6i.
Robson, M.E., Tung, N., Conte, P., Im, S.A., Senkus, E., Xu, B., et al., 2019. OlympiAD final overall survival and tolerability results: olaparib versus chemotherapy treatment of physician's choice in patients with a germline BRCA mutation and HER2-negative metastatic breast cancer. Ann. Oncol. 30 (4), 558–566.
Rugo, H.S., Bardia, A., Marmé, F., Cortés, J., Schmid, P., Loirat, D., et al., 2023. Overall survival with sacituzumab govitecan in hormone receptor-positive and human epidermal growth factor receptor 2-negative metastatic breast cancer (TROPiCS-02): a randomised, open-label, multicentre, phase 3 trial. Lancet.
Sava, J. (2023) FDA lifts partial hold on phase 1/2 vela trial of blu-222 for solid tumors, Targeted Oncology. Available at: ⟨https://www.targetedonc.com/view/fda-lifts-partial-hold-on-phase-1-2-vela-trial-of-blu-222-for-solid-tumors⟩ (Accessed: 14 October 2023).
Tadesse, S., Anshabo, A.T., Portman, N., Lim, E., Tilley, W., Caldon, C.E., et al., 2020. Targeting CDK2 in cancer: challenges and opportunities for therapy. Drug Discov. Today 25 (2), 406–413.
Turner, N.C., Oliveira, M., Howell, S.J., Dalenc, F., Cortes, J., Gomez Moreno, H.L., et al., 2023. Capivasertib in hormone receptor–positive advanced breast cancer. N. Engl. J. Med. 388 (22), 2058–2070.
Seth A. Wander 1 Dave Valacer, 2 Richard Zuniga, 3 Heather Yeckes-Rodin, 4 Christopher Perkin, 5 Lisa Ge, 5 Lili Yao, 2 Jing Lin, 2 Dawn Begley, 2 Feifei Sun, 2 Andie Zheng, 2 Jing Han, 2 Julie Xie, 2 Aditya Bardia, 1 Kevin Kalinsky 6. San Antonio Breast Cancer Symposium Poster ID: PO3-18-06. 2023. First-in-human Phase 1 A study of RGT-419B, a next generation CDK4 inhibitor, in patients (pts) with HR+/HER2- ABC who progressed on prior CDK4/6 inhibitors.
Watt, A.C., Goel, S., 2022. Cellular mechanisms underlying response and resistance to CDK4/6 inhibitors in the treatment of hormone receptor-positive breast cancer. Breast Cancer Res. 24 (1), 17.
Yap, T.A., Basu, C., Goldman, J.W., Gordon, M., Hamilton, E., Kelly, A., et al., 2022. Abstract P5-16-06: a first-in-human phase 1/2a dose escalation/expansion study of the first-in-class CDK2/4/6 inhibitor PF-06873600 alone or with endocrine therapy in patients with breast or ovarian cancer. Cancer Res. 82 (4_Supplement). P5-16-06-P5-16–06.
Yap, T.A., Elhaddad, A.M., Grisham, R.N., Hamm, J.T., Marks, D.K., Shapiro, G., et al., 2023a. First-in-human phase 1/2a study of a potent and novel CDK2-selective inhibitor PF-07104091 in patients (pts) with advanced solid tumors, enriched for CDK4/6 inhibitor resistant HR+/HER2- breast cancer. J. Clin. Oncol. 41 (16_suppl), 3010–3010.
Yap, T.A., Giordano, A., Hamilton, E.P., LoRusso, P., Bowers, M., Basu, C., et al., 2023b. First-in-human first-in-class phase 1/2a study of the next generation CDK4-selective inhibitor PF-07220060 in patients (pts) with advanced solid tumors, enriched for HR + HER2- mBC who progressed on prior CDK4/6 inhibitors and endocrine therapy. J. Clin. Oncol. 41 (16_suppl), 3009–3009.
Zhao, Z.M., Yost, S.E., Hutchinson, K.E., Li, S.M., Yuan, Y.C., Noorbakhsh, J., et al., 2019. CCNE1 amplification is associated with poor prognosis in patients with triple negative breast cancer. BMC Cancer 19 (1), 96.
Zhu, S., Wu, Y., Song, B., Yi, M., Yan, Y., Mei, Q., et al., 2023. Recent advances in targeted strategies for triple-negative breast cancer. J. Hematol. Oncol. 16 (1), 100.

Riccardo Gerosa, MD, Resident in Clinical Oncology at Humanitas University/Cancer Center, Milano, Italy. His professional interests include clinical and pre-clinical research focused on breast cancer.

Rita De Sanctis, MD, PhD, Oncologist, Assistant Professor of Medical Oncology at Humanitas University, and Consultant at Humanitas Cancer Center, Milano, Italy. Extensive experience in clinical and laboratory projects. Her noteworthy contributions include diverse presentations at conferences and publications in renowned journals. Her primary research focus encompasses pre-clinical, translational, and clinical projects related to breast cancer and quantitative classifiers in medical oncology.

Flavia Jacobs, MD, Oncologist working at Humanitas University/Cancer Center, Milano, Italy. Experience in clinical and laboratory projects and publications in renowned journals. Her professional interests mainly converge on breast cancer.

Chiara Benvenuti, MD, Resident in Clinical Oncology at Humanitas University/Cancer Center, Milano, Italy. Experience in clinical and laboratory projects and publications in renowned journals. Her professional interests mainly converge on breast cancer.

Mariangela Gaudio, MD, Resident in Clinical Oncology at Humanitas University/Cancer Center, Milano, Italy. Experience in clinical and laboratory projects and publications in renowned journals. Her professional interests mainly converge on breast cancer.

Giuseppe Saltalamacchia, MD, Oncologist working at Humanitas University/Cancer Center, Milano, Italy. Experience in clinical and laboratory projects and publications in renowned journals. His professional interests mainly converge on breast cancer.

Rosalba Torrisi, MD, Oncologist working at Humanitas University/Cancer Center, Milano, Italy. Extensive experience in clinical and laboratory projects. Her noteworthy contributions include diverse presentations at conferences and publications in renowned journals. Her primary research focus encompasses pre-clinical, translational, and clinical projects related to breast cancer.

Giovanna Masci, MD, Oncologist working at Humanitas University/Cancer Center, Milano, Italy. Extensive experience in clinical and laboratory projects. Her noteworthy contributions include diverse presentations at conferences and publications in renowned journals. Her primary research focus encompasses pre-clinical, translational, and clinical projects related to breast cancer.

Chiara Miggiano, MD, specialized in Internal Medicine and Medical Oncology working at Humanitas University/Cancer Center, Milano, Italy. Extensive experience in clinical and laboratory projects and publications in renowned journals. His professional interests mainly converge on breast cancer.

Francesco Agustoni, MD, Assistant Professor of Medical Oncology at the Department of Internal Medicine and Medical Therapy, University of Pavia, Italy, and Oncologist working at Department of Oncology, Fondazione IRCCS Policlinico San Matteo. Extensive expertise, encompassing clinical, translational, and clinical aspects, with numerous scientific

contributions in terms of publications and articles in highly indexed journals, as well as participation in national and international conferences.

Paolo Pedrazzoli, MD, Onco-hematologist, Director of the Department of Oncology, Fondazione IRCCS Policlinico San Matteo Pavia, Italy, and Associate Professor of Medical Oncology at the Department of Internal Medicine and Medical Therapy, University of Pavia, Italy. Extensive expertise in the field of onco-hematology, encompassing clinical, translational, and clinical aspects, with numerous scientific contributions in terms of publications and articles in highly indexed journals, as well as participation in national and international conferences.

Armando Santoro, MD, Onco-hematologist, Extraordinary Professor of Medical Oncology at Humanitas University and Director of Humanitas Cancer Center, Milano, Italy. Extensive expertise in the field of onco-hematology, encompassing clinical, translational, and clinical aspects, with numerous scientific contributions in terms of publications and articles in highly indexed journals, as well as participation in national and international conferences.

Alberto Zambelli, MD, Oncologist, Associate Professor of Medical Oncology at Humanitas University, Head of the Breast Unit at Humanitas Cancer Center, Milano, Italy. Extensive expertise in the field of breast-cancer, encompassing clinical, translational, and clinical aspects, with numerous scientific contributions in terms of publications and articles in highly indexed journals, as well as participation in national and international conferences.