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Breast Cancer (EA Comen, Section Editor)

The Role of CDK4/6 Inhibitors in Breast Cancer

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Opinion statement

Oral inhibitors of CDK4/6 have been shown to increase response rates and prolong disease control when combined with endocrine therapy in hormone-responsive (HR+) HER2-negative advanced breast cancer. Palbociclib, ribociclib and abemaciclib are all approved in combination with non-steroidal aromatase inhibitors in first-line therapy for post-menopausal women, with a 40–45% improvement in progression-free survival seen with the addition of any of these CDK4/6 inhibitors. Additional approved indications, including first- and second-line combination therapy for pre-menopausal women, combination with fulvestrant and use as monotherapy, vary with each agent and are reviewed fully in the subsequent texts. These agents also differ in their toxicity profiles and monitoring requirements, and prescribers should be aware of the individual requirements for each agent. Current clinical trials are investigating the expanded use of these agents in other breast cancer subtypes, such as HER2-positive and triple-negative breast cancer, as well as in the adjuvant and neoadjuvant treatments of early breast cancer. Resistance to CDK4/6 inhibition can occur through multiple mechanisms. Rational combinations with other therapies, such as PI3K inhibitors, HER2-directed therapies and immunotherapy, are being explored.

Introduction

One of the hallmarks of cancer cells is to evade extracellular and intracellular signals limiting proliferation [1]. The retinoblastoma (RB)-associated protein is a key negative regulator of cell cycle progression, involved in determining whether a cell will proceed though the cycle of growth and division or enter a quiescent phase. RB's repressive effect on the E2F family of transcription factors is disrupted upon hyperphosphorylation by a complex composing cyclin D in partner with cyclin-dependent kinase 4 or its homolog cyclin-dependent

kinase 6 (CDK4/6) [2]. CDK4 and 6, therefore, are involved in the promotion of cell cycle progression via the release of the negative regulatory effect of RB. Upregulation of the cyclin D–CDK4/6–pRB axis is a common event in breast cancer particularly of the luminal subtypes, with cyclin D1 gene amplification seen in 58% of luminal B and 29% of luminal A cancers and CDK4 gain seen in 25 and 14%, respectively [3]. This axis is also dysregulated in other breast cancer subtypes, with cyclin D1 amplification seen in 38% of the HER2-enriched subtype and RB loss in 20% of basal-like cancers [3].

Pharmacologic treatment

The initial pharmacologic inhibitors of CDK were pan-CDK inhibitors whose further development was limited by issues including pharmacokinetic complexity, unfeasible dosing schedules and toxicity [4, 5]. In contrast, the agents that have more recently been approved by the US Food and Drug Administration (FDA) for use in breast cancer are oral highly selective inhibitors of CDK4/6 and include palbociclib, ribociclib and abemaciclib. The data from the landmark randomised clinical trials supporting FDA approval of these agents in various clinical settings is summarized in Table 1. The warnings, precautions and monitoring requirements included in the FDA prescribing information for these agents are detailed in Table 2.

Palbociclib

Palbociclib (Ibrance®, Pfizer Inc.) is an orally active highly selective inhibitor of CDk4/6 that showed preclinical activity in a panel of molecularly characterised breast cancer cell lines, particularly of the luminal subtypes [6]. Initial human studies established the following dosing schedule that was utilised for subsequent studies and subsequently approved in clinical practice: 125 mg daily orally on a 21-day on, 7-day off schedule [2]. The randomised phase 2 PALOMA-1 study enrolled previously untreated post-menopausal women with advanced HR+, HER2-negative breast cancer and confirmed a prolongation of progression-free survival (PFS) when palbociclib was added to endocrine therapy with the non-steroidal aromatase inhibitor (NSAI) letrozole (10.2 vs 20.2 months, HR 0.4888, 95% CI 0.319–0.748) [7]. Palbociclib also improved overall response rate (ORR) from 39.4 to 55.4%. The phase 3 registration PALOMA-2 study enrolled a similar population and again randomised between letrozole–placebo versus letrozole–palbociclib [8••]. It confirmed an approximately 10-month improvement in median PFS with the addition of palbociclib (14.5 vs 24.8 months, HR 0.58; 95% CI, 0.46 to 0.72; $P < 0.001$).

Palbociclib has also been evaluated in combination with the selective estrogen receptor downregulator (SERD) fulvestrant in in patients who had experienced disease progression after prior endocrine therapy in the phase 3 PALOMA-3 study. Pre-menopausal women were also eligible provided they received ovarian function suppression (OFS) with goserelin. Final PFS analysis confirmed a doubling of PFS from 4.6 to 9.5 months (HR 0.46, 95% CI 0.36–0.59; $P < 0.0001$) [9••]. The final analysis of OS was not significant in the intention to treat population despite an absolute difference between arms of 6.9 months (34.9 vs 28 months, stratified HR 0.81, 95% CI 0.64 to 1.03; $P = 0.09$) [10]. Of note, this study was markedly underpowered to show a significant

Table 1. Randomised controlled trial data supporting endocrine–CDK4/6 inhibitor combinations in advanced HR-positive HER2-negative breast cancer in various clinical settings

			Palbociclib		Ribociclib		Abemaciclib	
Partner	**Line**	**Menopausal status**						
NSAI	1st	Pre			✓	MONALEESA-7		
		Post	✓	PALOMA-2	✓	MONALEESA-2	✓	MONARCH 3
	2nd	Pre						
		Post						
Fulvestrant	1st	Pre						
		Post			✓	MONALEESA-3		
	2nd	Pre	✓	PALOMA-3			✓	MONARCH 2
		Post	✓	PALOMA-3	✓	MONALEESA-3	✓	MONARCH 2
Tamoxifen	1st	Pre			✓[a]	MONALEESA-7		
		Post						
	2nd	Pre						
		Post						
Monotherapy	2+	Pre					✓	MONARCH 1
		Post						

[a]Not approved by the FDA because of QTc prolongation with this combination

difference in OS. Similarly, the OS analyses of the first-line studies with all three currently approved agents (palbociclib, ribociclib and abemaciclib) will be underpowered in the absence of very dramatic prolongation of OS, and a meta-analysis would likely be required to confirm a difference in OS if present [11].

Palbociclib is generally well-tolerated. Pooled safety analysis from the PALOMA studies reveals a peak incidence of adverse events in the first 6 months of treatment, with a subsequent decrease over time [12]. Neutropaenia is exceptionally common (80.6% among palbociclib-treated patients versus 3.6% on patients receiving endocrine therapy alone). Reassuringly, febrile neutropaenia was very rare among palbociclib-treated patients (1.6%) but non-neutropaenic infections were increased (54.7% vs 36.9%, typically low grade). The rate of alopecia is also increased by the addition of palbociclib (25.9 vs 10.2%).

Ribociclib

Ribociclib (Kisqali®, Novartis Pharmaceuticals Corp.) is another oral highly selective CDK4/6 inhibitor that yielded promising preclinical activity in models of endocrine-resistant breast cancer, including activations of PIK3CA and HER2 pathways [13]. A phase 1 study in patients with advanced cancer established a dosing schedule for future studies of 600 mg daily orally on a 21-day on, 7-day off schedule [14]. The phase 3 randomised MONALEESA-2 study enrolled a similar patient population to the PALOMA-2 palbociclib study and investigated also the addition of a CDK4/6 inhibitor to letrozole in first-line therapy [15••]. At an interim analysis performed at a median duration of follow-up of 15.3 months, the median PFS in the letrozole–palbociclib arm was not reached.

Table 2. FDA warnings, precautions and advice re monitoring parameters for patients receiving currently approved CDK4/6 inhibitors

	Palbociclib Ibrance®	Ribociclib Kisqali®	Abemaciclib Verzenio™
Starting dose	125 mg	600 mg	150 mg with AI/fulvestrant 200 mg as monotherapy
Dosing frequency	Once daily	Once daily	Twice daily
Cycle length	28 days	28 days	28 days
Treatment period	21 days	21 days	28 days
Rest period	7 days	7 days	None
CBC monitoring	Every 2 weeks for first 2 cycles, at beginning of each subsequent 4 cycles, and as clinically indicated.	Every 2 weeks for first 2 cycles, at beginning of each subsequent 4 cycles, and as clinically indicated.	Every 2 weeks for first 2 months, monthly for next 2 months, and as clinically indicated.
EKG monitoring	Not required	At baseline, day 14 of cycle 1, day 1 of cycle 2 and as clinically indicated. Monitor electrolytes at beginning of each cycle for 6 cycles and as clinically indicated.	Not required
LFT monitoring	Not required	Every 2 weeks for first 2 cycles, at beginning of each subsequent 4 cycles, and as clinically indicated.	Every 2 weeks for first 2 months, monthly for next 2 months, and as clinically indicated
Embryo–fetal toxicity	Advise patients of potential risk to a fetus and to use effective contraception	Advise patients of potential risk to a fetus and to use effective contraception	Advise patients of potential risk to a fetus and to use effective contraception
Diarrhea advice	Not required	Not required	Advise patients to initiate antidiarrheal therapy at the first sign of loose stools, increase oral fluids and notify their healthcare provider.
VTE advice	Not required	Not required	Monitor patients for signs and symptoms of thrombosis and pulmonary embolism and treat as medically required.
Dose level—1	100 mg	400 mg	100 mg with AI/fulvestrant 150 mg as monotherapy
Dose level—2	75 mg	200 mg	50 mg with AI/fulvestrant 100 mg as monotherapy

Full FDA information for prescribers available at the following: https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/209092s000lbl.pdf; https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/207103s004lbl.pdf; https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/208855s000lbl.pdf

However, the HR for PFS seen in this study of 0.56 was virtually identical to that seen with palbociclib in PALOMA-2 (0.58). Updated analysis confirmed a PFS benefit with ribociclib of 9.3 months (PFS 25.3 vs 16.0 months, HR 0.568; 95% CI 0.457–0.704; log-rank $P = 9.63 \times 10^{-8}$) [16].

Meanwhile, MONALEESA-7 investigated ribociclib with endocrine therapy and OFS in pre-menopausal women with advanced HR+ breast cancer. A total

of 672 pre-menopausal women not previously treated with endocrine therapy in the advanced setting were randomised to ribociclib or placebo in combination with tamoxifen or non-steroidal aromatase inhibitor therapy, all in the context of OFS with goserelin. Ribociclib resulted in a similar proportional improvement in PFS to that seen in other CDK4/6 inhibitor studies (PFS 23.8 months vs 13.0 months, HR 0.55, 95% CI 0.44–0.69; $P < 0.0001$) [17•].

The MONALEESA-3 study tested the addition of ribociclib to fulvestrant therapy [18••]. Its design differed from that of the PALOMA-3 study in that treatment-naïve patients were included (and indeed made up approximately 50% of patients in each arm) and that pre-menopausal patients were not eligible. This study confirmed the utility of the ribociclib–fulvestrant combination in both the first-line and second-line settings with an improvement in PFS for the overall population from 12.8 to 20.5 months (HR 0.593, 95% CI 0.480 to 0.732; $P < 0.001$) [18••]. Subgroup analysis demonstrated a HR for progression of 0.58 in the treatment-naïve subgroup despite an impressive median PFS of 18.3 months in the fulvestrant monotherapy arm; this is the first randomised study to provide data supporting a fulvestrant–CDK4/6 inhibitor combination in the first-line setting.

In general, the toxicity profiles of ribociclib and palbociclib are remarkably similar. The registration studies of each drug in combination with letrozole in the first-line setting demonstrated rates of neutropaenia of around 75–80% with febrile neutropaenia in 1.5–1.8% [8••, 15••]. Alopecia was seen in approximately 1/3 of patients treated with the letrozole–CDK4/6 combination in both studies. Additional toxicities seen with ribociclib included elevated aminotransferases in around 15% of patients (including 1.2% who met the Hy's law definition of drug-induced liver injury) and prolongation of QTcF interval to more than 480 ms in 3.3%. For this reason, the "Warnings and Precautions" section of the FDA prescribing information for ribociclib includes sections on hepatobiliary toxicity and QT interval conduction prolongation as well as the sections on neutropaenia and embryo–fetal toxicity common to both drugs [19]. Additional monitoring of liver function tests and electrocardiograms is required in the initial months of treatment and as clinically indicated with this drug. The FDA has not approved the ribociclib–tamoxifen combination because of concerns regarding QTc prolongation: an increase of > 60 ms from baseline in the QTcF interval was seen in 14/87 (16%) of patients receiving this combination compared with 18/245 (7%) of patients receiving ribociclib with an NSAI.

Abemaciclib

Abemaciclib (Verzenio™, Eli Lilly and Company) is the latest oral CDK4/6 inhibitor to receive FDA approval for use in patients with advanced HR+, HER2-negative breast cancer having being granted breakthrough therapy designation in two indications in September 2017 [20]. It was simultaneously approved in combination with fulvestrant after progression on prior endocrine therapy and as monotherapy in patients with progression after prior endocrine therapy and chemotherapy. A third indication in combination with NSAI in first-line therapy was added in 2018 [21]. The MONARCH 2 study confirmed the benefit of abemaciclib 150 mg twice daily orally continuously added to fulvestrant in women whose advanced breast cancer had progressed on prior endocrine therapy [22••]. Premenopausal women were eligible for this study and received

OFS. Median PFS was prolonged from 9.3 to 16.4 months with the combination (HR 0.55; $P < .001$). The approval as monotherapy was based on the single-arm phase 2 MONARCH 1 study [23•]. A total of 132 patients with at least one line of prior chemotherapy as well as endocrine therapy for HR+, HER2-negative ABC received abemaciclib 200 mg twice daily orally. This resulted in an ORR of 19.7% in this heavily pretreated population. A total of 90% of patients experienced diarrhea of which 19.7% were grade 3. Laboratory abnormalities, including neutropaenia (88%), elevated ALT (30%), hypokalaemia (26%) and hyponatraemia (20%), were also seen.

In the first-line setting, the MONARCH 3 study enrolled a study population very similar to those of the PALOMA-2 and MONALEESA-2 studies and employed an identical randomisation to letrozole with or without the CDK4/6 inhibitor, here given in the combination therapy dose of 150 mg twice daily orally continuously. The improvement in the primary endpoint of PFS was very similar to that seen in those studies with a HR for PFS of 0.54 (HR 0.41 to 0.72; $P = 0.000021$) [24••]. For patients with measurable disease, all three CDK4/6 inhibitors lead to response rates of 50–60% when combined with letrozole in this setting. As a result of the MONARCH 3 results, abemaciclib was approved by the FDA for use in combination with an aromatase inhibitor in first-line therapy in February 2018.

The toxicity profile of first-line abemaciclib in combination with letrozole differs markedly from those of palbociclib and ribociclib, with a much lower rate of neutropaenia (21% vs 75–80%) and a higher rate of diarrhea (81.3% overall, 9.5% grade 3 or 4 despite protocol-mandated intensive supportive measures) [8••, 15••, 24••]. Venous thromboembolic events, including deep vein thrombosis and pulmonary embolism, occurred in 5% of patients treated with abemaciclib plus NSAI in MONARCH3 and in 5% of patients treated with abemaciclib and fulvestrant in MONARCH2 [22••, 24••].

Predictors of response

In the early clinical development of palbociclib, *CCND1* amplification and loss of p16 were investigated as potential biomarkers of response, but an interim analysis of the PALOMA1 study indicated that these factors did not appear to predict response, and they were jettisoned as eligibility criteria for this and subsequent studies [7]. Subsequent gene expression analyses of tumour tissues from patients participating in the PALOMA-2 study showed that expression of genes in the cyclin D–CDK4/6–RB pathway did not correlate with benefit from palbociclib [25]. Recent work has focused on the development of gene expression signatures predicting response to CDK4/6 inhibition. The abundance of the T172-phosphorylated active form of CDK4 varies among breast cancer cell lines and molecular subtypes [26]. A gene expression signature that acts as a surrogate for this active CDK4 has been shown to predict sensitivity of cell lines to palbociclib. A separate gene expression signature of RB loss-of-function (composed of genes correlated with E2F1 and E2F2 expression) is capable of discriminating between palbociclib-sensitive and -resistant breast cancer cell lines [27]. Such signatures may better identify which patients are most likely to benefit from CDK4/6 inhibitor therapy, but, at the current time, they remain investigational, and no clinical tools can identify patients likely to derive benefit apart from the immunohistochemical features of ER+, HER2-negative breast cancer.

Resistance

In a substudy of the PALOMA3 study, Turner and colleagues found mutations in *PIK3CA* and *ESR1* among patients receiving fulvestrant with or without palbociclib while mutations in the *RB1* gene were found exclusively in palbociclib-treated patients, indicating distinct events driving resistance to fulvestrant versus palbociclib [28]. Acquired resistance due to decreased dependence on cyclin D1–CDK4/6 may arise through various mechanisms, including loss of RB protein expression and overexpression of cyclin E1 [29, 30]. In addition, non-canonical RB phosphorylation by CDK2–cyclin D1 complex can occur in the setting of CDK4/6 inhibition. Such resistance may be abrogated by upfront PI3K inhibition in combination with endocrine-CDK4/6 doublet therapy but interestingly could not be rescued by the addition of PI3K inhibition after resistance had developed [30]. The role of the PI3K/AKT/mTOR pathway in mediating sensitivity to CDK4/6 inhibition is further illustrated by the enhanced anti-tumour efficacy seen with the addition of inhibition of 3-phosphoinositide-dependent protein kinase 1 (PDK1) to CDK4/6 inhibition in vivo [31]. Of note, early circulating tumour DNA (ctDNA) dynamics (specifically an increase in *PIK3CA* ctDNA after 15 days of treatment) strongly predicts PFS in patients treated with fulvestrant plus palbociclib, indicating a potential population to consider the addition of upfront PI3K inhibition to endocrine-CDK4/6 doublet therapy [32]. Additional mechanisms of CDK4/6 inhibitor resistance include CDK6 gene amplification and increased CDK6 expression [33]. A further resistance event may be via activation of autophagy. Autophagy is a cellular stress tolerance mechanism characterised by the digestion and recycling of cellular proteins and organelles [34]. It is implicated in resistance to antineoplastic therapies. Autophagy has been implicated as a resistance mechanism in the setting of palbociclib treatment, and the combination of autophagy inhibitors with CDK4/6 inhibition led to increased growth inhibition in vitro and in vivo [35].

CNS penetration

Animal studies investigating brain penetration of palbociclib have confirmed that this agent is a substrate for the efflux proteins p-glycoprotein and breast cancer resistance protein (BCRP), limiting the brain distribution of this drug [36]. Similarly, brain distribution of ribociclib and abemaciclib following systemic dosing is suboptimal although abemaciclib efflux is less marked than palbociclib [37, 38]. Rational development of candidate agents that are not efflux protein clients holds promise for therapies with enhanced activity against brain metastases and primary brain tumours [39].

Combination therapy

The preclinical evidence of potential synergy between inhibitors of CDK4/6 and PI3K has led to the investigation of triplet regimens of

drugs from these classes in combination with endocrine therapy. To date, early-phase studies have demonstrated promising signals of activity without evidence of unexpected toxicity with various iterations of this triplet approach [40, 41].

As outlined earlier, CDK4/6 inhibitors act by preventing hyperphosphorylation of RB and therefore maintaining RB's repressive effect on the E2F family of transcription factors, thereby reducing transcription of pro-proliferative proteins. Another target of the E2F family is DNA methyltransferase 1 (DNMT1), and reduced transcription of this protein leads to enhanced efficiency of tumour cell antigen production as well as suppression of the proliferation of regulatory T cells (Tregs) [42•]. Together, these factors lead to enhanced clearance of tumour cells mediated by cytotoxic T lymphocytes (CTLs). Furthermore, inhibition of CDK6 leads to increased nuclear levels of Nuclear Factor of Activated T cell (NFAT) proteins ultimately enhancing effector T cell activity [43]. Together, these data suggest a potential role for combination therapy with CDK4/6 inhibitors and immunotherapy. In vivo models confirm enhanced tumour regression when immune checkpoint blockade with an anti-PD-L1 antibody is added to CDK4/6 inhibition [42•, 43]. A multicentre phase II study is currently evaluating the combination of letrozole, palbociclib and pembrolizumab in postmenopausal women with HR+ advanced breast cancer [44], and a number of other CDK4/6 inhibitor–immunotherapy combination studies are ongoing in various settings [45].

Triple-negative breast cancer

The androgen receptor (AR) has become an attractive target in triple-negative breast cancers (TNBCs), with AR expression in up to one third of these cancers [46]. Cluster analysis of gene expression profiles in TNBCs identifies distinct molecular subtypes, including the luminal androgen receptor (LAR) cell type, characterised by high expression of AR mRNA and protein [47]. TNBCs of the LAR subtype tend to have a high proportion of cells exiting mitosis into a quiescent phase, from which CDK4/6 activity is required to re-enter the cell cycle, and have been shown to be sensitive to CDK4/6 inhibition [48]. Conversely, basal-like TNBCs have a high proportion of cells exiting mitosis into a proliferative state, bypassing the restriction point and exhibiting resistance to CDK4/6 inhibition. Palbociclib has been shown to enhance the activity of the AR antagonist bicalutamide in vitro in AR+ RB-proficient TNBC cells [49]. An open-label phase 2 study is currently exploring the combination of bicalutamide and ribociclib in patients with advanced AR+ triple-negative breast cancer [50]. Meanwhile, the combination of CDK4/6 and PI3K inhibition that has been explored in ER+ breast cancer also showed synergistic effects in TNBC cell lines (including basal-like, mesenchymal-like and LAR subtypes) and human xenograft mouse models and enhanced tumour immunogenicity [51]. The addition of immune checkpoint blockade to PI3K and CDK4/6 inhibition further enhanced the anti-tumour effect in a syngeneic TNBC mouse model [51].

HER2-positive breast cancer

The early cell line data confirming the activity of palbociclib in luminal breast cancer cell lines also indicated activity in HER2-positive breast cancer cell lines [6]. Sensitivity to CDK4/6 inhibition was evident in preclinical models of HER2-positive breast cancer with acquired resistance to HER2 inhibition through deregulation of cyclin D1 control, with an additive benefit from combined palbociclib and HER2-directed therapies [52]. A synergistic effect of abemaciclib with HER2-directed therapies has also been shown in sensitive and resistant models of HER2-positive breast cancer, with greatest synergy in the resistant models [53]. These data form the basis for the currently accruing studies that are combining HER2-directed and CDK4/6 inhibitory therapies in the clinical setting. Early promising results of this approach are available from the small NA-PHER2 study of neoadjuvant trastuzumab, pertuzumab, fulvestrant and palbociclib in 36 women with ER+ HER2+ early breast cancer [54]. The combination led to a marked reduction in Ki67 after 2 weeks and at surgery and resulted in a rate of pathologic complete response (pCR) in breast and axillary lymph nodes of 27%, impressive for a non-chemotherapy regimen in an ER+ population.

Adjuvant and NACT

Having demonstrated a clear role in increasing endocrine responsiveness and delaying progression in HR+ advanced breast cancer, the logical next step is to investigate the addition of CDK4/6 inhibitors to endocrine therapy in the early breast cancer setting. Several early "window-of-opportunity" neoadjuvant trials have demonstrated promising indications of activity through effects on endpoints such as complete cell cycle arrest, Ki67-positive cell fraction and EndoPredict scores [55, 56, 57]. A relatively brief (14 weeks) period of combination therapy with letrozole and palbociclib in the randomised phase 2 PALLET study led to a greater decrease in Ki67 than letrozole alone but disappointingly did not lead to higher clinical response rates [58]. Meanwhile, the NeoPAL study randomised 106 patients with genomically defined high-risk luminal breast cancer to anthracycline and taxane chemotherapy or letrozole plus palbociclib [59]. The non-chemotherapy combination had a more favourable toxicity profile, while clinical response and breast conserving surgery rates were similar. However, the primary endpoint of residual cancer burden (RCB) scores of 0 or 1 post-treatment was numerically higher in the chemotherapy arm (15.7 vs 7.7%). These studies are characterised by their small sample sizes and short duration of neoadjuvant therapy compared to the prolonged duration typically utilized in neoadjuvant endocrine therapy. In contrast, the large adjuvant studies which are ongoing or recently completed accrual are evaluating prolonged (1–2 years) CDK4/6 inhibition. Two large randomised studies (PENELOPE-B and PALLAS) of adjuvant palbociclib in patients with high-risk HR+ HER2-negative early breast cancer have completed accrual, and the results are eagerly awaited [60, 61].

Compliance with Ethical Standards

Conflict of Interest
Conleth G. Murphy declares that he has no conflict of interest.

Human and Animal Rights and Informed Consent
This article does not contain any studies with human or animal subjects performed by the author.

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