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Editorial Comment

EDITORIAL COMMENT

The open system of FGF-23 at the crossroad between additional P-lowering therapy, anemia and inflammation: how to deal with the intact and the C-terminal assays?

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ABSTRACT

Fibroblast growth factor 23 (FGF-23) has been associated with increased cardiovascular risk and poor survival in dialysis patients. It is well established that FGF-23 synthesis is directly induced by positive phosphate (P) balance. On the other hand, P-lowering treatments such as nutritional P restriction, P binders and dialysis are capable of reducing FGF-23 levels. However, there are many uncertainties regarding the possibility of adopting FGF-23 to guide the clinical decision-making process in the context of chronic kidney disease–mineral bone disorder (CKD-MBD). Furthermore, the best assay to adopt for measurement of FGF-23 levels (namely the intact vs the C-terminal one) remains to be determined, especially in conditions capable of altering the synthesis as well as the cleavage of the intact and biologically active molecule, as occurs in the presence of CKD and its complications. This Editorial discusses the main insights provided by the *post hoc* analysis of the NOPHOS trial, with particular attention given to evidence-based peculiarities of the intact and the C-terminal assays available for measuring FGF-23 levels, especially in patients receiving additive P-lowering therapy in the presence of inflammation, anemia and iron deficiency.

Keywords: anemia, C-term, FGF-23, inflammation, intact, iron deficiency, phosphate binders

Chronic kidney disease–mineral and bone disorder (CKD-MBD) is one of the most common complications of CKD, and is associated with increased risk of cardiovascular disease (CVD), fractures and mortality [1]. Compared with other mineral biomarkers, serum levels of the phosphaturic hormone fibroblast growth factor 23 (FGF-23) start to rise from earlier stages of CKD as a compensatory mechanism to maintain phosphate (P) homeostasis. Therefore, it has been proposed that FGF-23 could be an early indicator of P overload and that screening for it could help to identify which patients might benefit from intervention, independently of serum P levels [2]. Moreover, in dialysis patients, high FGF-23 levels are independently associated with increased mortality, even in normophosphatemic patients [3]. The association between FGF-23 and mortality likely involves a cardiovascular (CV) mechanism, since high FGF-23 levels have been associated with left ventricular hypertrophy [4], impaired vasoreactivity [5] and coronary artery calcification [6], independent of traditional CV risk factors and serum P levels. Thus, it was hypothesized that FGF-23 could be another therapeutic target for CV protection in CKD.

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Although randomized clinical trials primarily investigating the effect of FGF-23 reduction on clinical outcomes are still lacking, therapeutic interventions suggested for treating CKD-MBD and improving prognosis proved capable of indirectly lowering FGF-23 levels in CKD [7]. These mainly include calcimimetics [8–14] and P-lowering therapies [15–26], such as nutritional P restriction, P binders (PB), inhibitors of intestinal P absorption and increased dialysis efficiency. Conversely, the direct inhibition of FGF-23 activity by neutralizing anti-FGF-23 antibodies induced hyperphosphatemia, hypercalcemia, accelerated vascular calcification and mortality in animal models [27, 28]. These findings highlight the importance of identifying therapies capable of jointly lowering both FGF-23 and P levels, or lowering FGF-23 specifically through P reduction. A variable reduction in FGF-23 concentrations (by up to 40%) was achieved by the use of calcium-free PB [15–22, 24, 29], highlighting the importance of achieving recommended P targets to secondarily impact on FGF-23 reduction. However, despite the widespread use of PB, up to 70% of hemodialysis (HD) patients in Europe do not manage to reach normophosphatemia [30]. Thus, additional P-lowering treatments, such as the inhibitors of intestinal P transport, have been advocated especially in patients on dietary P restriction and PB, which are prone to the compensatory increase of intestinal P absorption. Tenapanor is a nonbinder inhibitor of paracellular intestinal P absorption, capable of altering the permeability of the tight junction to P flow by directly inhibiting the sodium–hydrogen exchanger isoform 3. Tenapanor significantly decreased both P and FGF-23 levels in HD patients alone [25], as well as on top of standard PB therapy [26]. In contrast, nicotinamide (NA), an inhibitor of the sodium-dependent phosphate cotransporter 2b (NaPiIIb), first showed an inconsistent effect on P and FGF-23 reduction in CKD stage 3–4 [31, 32] but later was shown to significantly reduced P levels in dialysis patients [33–35].

In this issue of *CKJ*, Egli-Spicht *et al*. present their findings on FGF-23 levels in the NOPHOS cohort, consisting of HD patients treated with NA modified-release (NAMR) or placebo, in addition to PB [36]. In this *post hoc* analysis, the authors aim to assess the relationship between P, FGF-23, inflammation and iron metabolism, providing some encouraging insights into a P-mediated reduction of FGF-23, and some food for thought on some of the open problems in FGF-23 application in clinical practice, including the ideal assay to use, its association with the pathways involved in inflammation and iron deficiency, and its role in elderly patients.

The NOPHOS trial consisted of a multicentric, double-blinded, placebo-controlled trial, conducted in Germany, Poland and Austria, on patients undergoing regular maintenance HD, who had hyperphosphatemia (with serum P concentration between 4.5 and 8.7 mg/dL) despite the use of one or two PB, who were randomized 3:1 to receive NAMR versus placebo on top of ongoing traditional PB. The authors have previously reported that after 12 weeks of treatment, patients in the NAMR arm had significantly lower levels of P and parathormone (PTH) compared with placebo [P: 5.36 ± 1.38 vs 5.88 ± 1.32 mg/dL; PTH: 227 (121–366) vs 252 (141–447) pg/mL] [37]. Moreover, the serum of patients treated with NAMR presented reduced calcification propensity, assessed by T50 test. In the current *post hoc* analysis patients in the active treatment arm also had a significant reduction in the trajectory of intact FGF-23 (iFGF-23) levels, although not yet leading to significant differences in FGF-23 concentrations, at 12-week follow-up. Although modest in size, this effect is consistent with previous findings and somewhat encouraging, considering that it might have been limited by the short follow-up, the reduced sample size, and the high prevalence of patients receiving calcium-containing PB (51%) and active vitamin D (48%), both established inducers of FGF-23 synthesis. The effect on FGF-23 could have been reduced also by the poor compliance to NMAR, represented by the low rate (46%) of patients undergoing NMAR who completed the study, 30% of whom were not compliant to prescribed therapy. The high pill burden (averaging 3.8 and 4.9 capsules prescribed per day at baseline and 12 weeks, respectively) and common side effects (diarrhea, nausea, vomiting, thrombocytopenia and pruritus) might have contributed to the unsatisfactory adherence to NMAR and to the non-significant long-lasting benefit on serum P against placebo at the 52-week follow-up [38].

Despite the limits of NMAR in achieving a prolonged reduction in serum P and FGF-23 levels, this *post hoc* analysis recognized basal FGF-23 levels as the best independent predictor of the response to P-lowering therapy in terms of P reduction. It could be argued that higher FGF-23 levels may represent severe P overload, which could be more difficult to counteract, due to considerable amount of stored P and individual patients' characteristics responsible for positive P balance, such as incomplete dialysis efficiency and poor adherence to nutritional counselling and PB. In searching for the clinical plausibility of introducing FGF-23 in clinical practice, more accurate recognition of patients affected by severe P overload, who may benefit from additional P-lowering interventions independently of serum P levels, may represent a promising application of FGF-23 assessment, especially in younger patients with prolonged life expectancy and those suitable for kidney transplantation.

However, uncertainties about the best way to assess FGF-23 (either the intact or the C-terminal assay) and the susceptibility of FGF-23 metabolism to uremic perturbations, such as inflammation, anemia and iron deficiency, still limit any evidence-based suggestion of orienting clinical decision-making according to FGF-23 levels. Egli-Spicht *et al*. provided a significant contribution to shed light on these unsolved issues.

Two assays are currently available for measuring FGF-23: the C-terminal and the intact one (namely cFGF-23 and iFGF-23, respectively). The first recognizes two epitopes on the C-terminal part of the molecule, thus capturing both biologically active iFGF-23 and its presumed biologically inactive C-terminal fragments. The second recognizes epitopes on either side of the cleavage site, thus identifying only the biologically active iFGF-23. Consensus is still lacking about which is the ideal assay to use for measuring circulating FGF-23 levels in CKD. Probably influenced by the previous experience with second- and third-generation assays for PTH detection, many of us argue that the assay targeted on the biologically active molecule could be the most appropriate for clinical application. However, the evaluation is more complex than expected, and requires a better understanding of the pathophysiological mechanisms involved in the regulation of cFGF-23 and iFGF-23 metabolism (Fig. 1).

iFGF-23 is secreted by osteocytes and osteoblasts as a 32-kDa glycoprotein, composed of a hydrophobic signal sequence, an N-terminal domain homologous with other FGFs and a C-terminal domain, unique to FGF-23 and essential for interaction with the FGF receptor (FGFR)–Klotho complex. Between the N- and C-terminal domains there is a proteolytic cleavage site, where the biologically active iFGF-23 can be processed and inactivated, resulting in two presumably inactive N- and C-terminal fragments [39]. Of note, C-terminal fragments can bind, but not transactivate, the FGFR–Klotho complex, acting as a competitive inhibitor for iFGF-23 [40]. Serum levels of iFGF-23 are regulated by a still partially unknown interplay between systemic and local bone

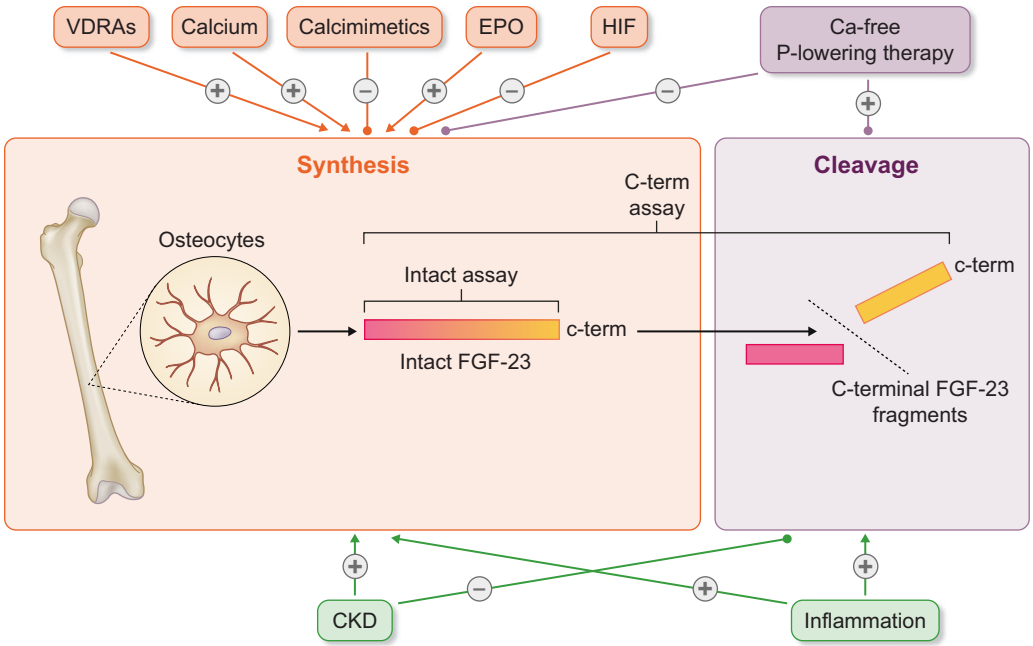

Figure 1: Hypothetical pathways linked to FGF-23 synthesis and cleavage. Ca, calcium; VDRAs, vitamin D receptor activators.

factors. Certainly, iFGF-23 secretion is triggered by dietary P loading and increased levels of active vitamin D and PTH [41]. High P levels increase FGF-23 activity, by inhibiting iFGF-23 cleavage trough overexpression of N-acetylgalactosaminyltransferase 3 (GALNT3), the enzyme responsible for O-glycosylation of the proteolytic cleavage site [42]. Conversely, high levels of calcium and 1,25$(OH)_2$D downregulate GALNT3 expression, leading to decreased FGF-23 activity [42]. However, to date it is established thought that the net clinical effect of calcitriol on FGF-23 system consists of increased FGF-23-mediated pathways, due to a stronger effect of calcitriol on FGF-23 synthesis than on FGF-23 cleavage. Thus, when interpreting the entity of FGF-23-driven effects on mineral and CV homeostasis, not only its synthesis, but also the rate of its cleavage should be carefully considered. This could be relevant especially when the iFGF-23 to cFGF-23 ratio is unbalanced, as it occurs in the presence of CKD, P overload, inflammation, anemia and iron deficiency.

Uremia itself is associated with impaired cleavage of FGF-23. In animal models of CKD, early FGF-23 increments were not associated with its increased production in bone, suggesting that they could result from an alternative tissue source or impaired cleavage [43]. The hypothesis of impaired cleavage was sustained by the observation that as CKD progresses, the ratio of circulating iFGF-23 to cFGF-23 rises, and that circulating FGF-23 in dialysis patients is mostly intact and biologically active [44]. However, in a large cohort of patients with and without CKD, the association between iFGF-23 and heart failure and mortality was completely attenuated after adjustment for kidney function, while cFGF-23 levels remained significantly associated with both outcomes in the same model [45]. Of note, C-terminal FGF-23 fragments have been shown to directly increase the size of adult rat cardiomyocytes and the cFGF-23 circulating levels (and not those of iFGF-23) have been shown to be positively correlated to heart hypertrophy in sickle cell disease [46]. Further research is needed to elucidate whether kidneys differently metabolize iFGF-23 and C-terminal fragments. Furthermore, a hypothetical longer half-life of C-terminal chains, although biologically inactive, might be taken as a proxy or memory of the iFGF-23 synthesis through a longer time frame, which may reflect the FGF-23 biological activity over a longer time than what is expected by the punctual assessment of the single biologically active intact molecule. Unfortunately, no assay is currently available to accurately assess only the C-terminal component of the whole circulating FGF-23 levels. If confirmed, this hypothesis could be theoretically similar to what is already accepted for adopting glycated hemoglobin and 25(OH)D, in respect of glycemia and 1–25$(OH)_2$D, respectively. Notably, the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) Committee on bone metabolism on FGF-23 determination has recently highlighted the importance of developing assays capable of separately detecting intact FGF-23 and its fragments, for better discrimination of the functions of FGF-23 fragments. Despite the challenges related to the very low FGF-23 circulating levels, liquid chromatography with tandem mass spectrometry was suggested as potential solution for standardizing FGF-23 assays [47].

Previous studies analyzed the effect of different medications on FGF-23 levels by only one of the two available assays (Table 1), making the comparison between the clinical utility of the intact and C-terminal assays difficult to interpret. Egli-Spicht *et al.* provided FGF-23 assessment by both the intact and C-terminal assay, making their comparison more achievable than previous investigations. Although iFGF-23 and cFGF-23 were highly correlated (with a Pearson correlation coefficient of 0.8), and equally predicted serum P levels, only the trend for iFGF-23 significantly differed between the NAMR and the placebo arms. A similar effect was previously described in a small group of HD patients, where sustained control of serum P levels (target range <4.5 mg/dL) was associated with lower levels of only iFGF-23, whereas patients with uncontrolled hyperphosphatemia presented increased levels of both iFGF-23 and cFGF-23 [23]. Aforementioned data may hypothetically suggest that reduction in P levels mediates a decrease in FGF-23 mainly

Table 1: Studies on therapeutic strategies to reduce FGF-23.

Author, journal, year	Intervention	Population	cFGF-23	iFGF-23
Phosphate binders				
Koiwa [16], *Ther Apher Dial*, 2005	Sevelamer + CC vs CC only	N = 46 HD High P	n.t.	↓ with sevelamer + CC ↔ with CC only
Gonzalez-Parra [17], *Nephrol Dial Transplant*, 2011	LC	N = 18 CKD 3 Normal P	↓	n.t.
Shigematsu [18], *Nephrol Dial Transplant*, 2012	LC + CC	N = 36 HD High P	n.t.	↓
Toida [19], *Clin Nephrol*, 2012	LC vs CC	N = 42 HD	n.t.	↓ with LC ↔ with CC
Yilmaz [20], *Am J Kidney Dis*, 2012	Sevelamer vs CA	N = 100 CKD 4 High P	n.t.	↓ with sevelamer ↔ with CA
Soriano [21], *Clin Nephrol*, 2013	CC vs LC	N = 32 CKD 4–5ND High P	n.t.	↔ with CC ↓ with LC
Spatz [22], *Nephron Clin Pract*, 2013	Sevelamer	N = 40 CKD 3–5ND High P	↔	n.t.
Chang [29], *Clin Exp Nephrol*, 2017	CC vs LC	N = 25 HD High P	n.t.	↔ with CC ↓ with LC
Rodelo-Haad [23], *PLoS One*, 2018	PB to achieve sustained P control <4.5 mg/dL vs less strict P control	N = 21 HD	↔ with sustained P control ↑ without sustained P control	↓ with sustained P control ↑ without sustained P control
Ketteler [24], *Nephrol Dial Transplant*, 2019	Sevelamer vs SO	N = 549 CKD 5D High P	n.t.	↓ with both treatments
Transport inhibitors				
Ix [31], *J Am Soc Nephrol*, 2019	NA + LC vs NA + placebo vs placebo + LC vs placebo + placebo	N = 205 CKD 3b–4	n.t.	↔ with all treatments
Block [25], *Nephrol Dial Transplant*, 2019	Tenapanor vs placebo (after PB withdrawal)	N = 162 HD High P	n.t.	↓ with tenapanor ↑ with placebo
Pergola [26], *J Am Soc Nephrol*, 2021	Tenapanor + PB vs placebo + PB	N = 235 HD High P	↓ with tenapanor ↔ with placebo	↓ with tenapanor ↔ with placebo
Wetmore [9], *Clin J Am Soc Nephrol*, 2010	Cinacalcet + calcitriol vs calcitriol alone	N = 91 HD	n.t.	↓ with cinacalcet + calcitriol ↔ with calcitriol alone
Koizumi [8], *Nephrol Dial Transplant*, 2012	Cinacalcet	N = 55 HD Parathyroid hyperplasia	n.t.	↓
Moe [10], *Circulation*, 2015	Cinacalcet vs placebo	N = 2602 HD High PTH	n.t.	↓ with cinacalcet ↔ with placebo
Sprague [14], *Clin J Am Soc Nephrol*, 2015	Cinacalcet vs vitamin D	N = 312 HD High PTH	n.t.	↓ with cinacalcet ↑ with vitamin D
Wolf [12], *Clin Kidney J*, 2020	Etelcalcetide vs cincalcet vs placebo	N = 1706 HD High PTH	n.t.	↓↓ with etelcalcetide ↓ with cinacalcet ↔ with placebo
Hashimoto [11], *Nephrology*, 2022	Etelcalcetide vs control	N = 124 HD High PTH	n.t.	↓ with etelcalcetide ↔ in controls

CA, calcium acetate; CC, calcium carbonate; LC, lanthanum carbonate; N, number of participants; NA, nicotinamide; n.t., not tested; SO, sucroferric oxyhydroxide.

by increasing its cleavage through post-translational modifications, resulting in lower iFGF-23 but unchanged cFGF-23 levels, while the synthesis of iFGF-23 might require a longer time to be downregulated.

Animal and human studies have shown that both cFGF-23 and iFGF-23 increase due to iron deficiency [48–50]. Furthermore, an association between inflammation and FGF-23 was found, mediated by either functional iron deficiency or inflammatory cytokines [51, 52]. In particular, acute inflammation stimulates both FGF-23 production and cleavage leading to an increase in cFGF-23 but unchanged iFGF-23, while chronic inflammation seems to increase both cFGF-23 and iFGF-23 levels [51]. This evidence was confirmed by a stronger correlation found *in vivo* between markers of both iron deficiency and inflammation with cFGF-23 compared with iFGF-23 concentrations [45]. Interleukin-6 (IL-6) contributes to high FGF-23 levels in uremic rats, with a positive feedback loop in which increased FGF-23 expression promotes inflammation [52]. Finally, a bi-directional relationship was found between erythropoietin (EPO) and FGF-23, with a similar time-dependent effect. While acute increased EPO concentration resulted in higher cFGF-23 but unchanged iFGF-23 levels, chronically increased EPO led to high iFGF-23 levels, which in turn inhibited EPO synthesis by a negative feedback loop [53]. In contrast, the effect of hypoxia inducible factor (HIF) stabilizers on FGF-23 is still uncertain and probably influenced by residual renal function [53, 54].

The *post hoc* analysis of the NOPHOS trial by Egli-Spicht *et al*. confirmed a significant interplay between the FGF-23 system, anemia and inflammation in the real-world setting [35]. IL-6 and C-reactive protein were positively associated with cFGF-23 levels, but not with iFGF-23, possibly confirming that inflammation acts on both production and cleavage, leading to increased cFGF-23 and unchanged iFGF-23. Among other variables, hemoglobin was positively associated with iFGF-23, but with an effect size that was not clinically relevant, while P and PTH were strongly associated with both iFGF-23 and, to a lesser extent, cFGF-23. Of note, the well-controlled levels of iron stores at baseline might have accounted for the absence of a significant association between ferritin, transferrin and FGF-23, independently of the analytical assay. In line with previous evidence, these findings suggest that in HD patients, despite the strong correlation that exists between iFGF-23 and cFGF-23, the intact should be the assay of choice, as it shows stronger associations with mineral metabolism and it is prone to a lesser interaction with inflammation and uremic anemia. Furthermore, while anticipation of an early debut of FGF-23 in the clinical arena is growing rapidly, the present data increase awareness of how metabolism of FGF-23 is regulated by pathways other than mineral metabolism. To date, inflammation, anemia and iron depletion, and their treatment by EPO, HIF stabilizers and iron supplementation, should be taken as possible confounders or even mediators of the link existing between FGF-23 (especially as cFGF-23) and clinical hard endpoints (Fig. 1).

Although the HD population is ageing rapidly, the knowledge on how to deal with CKD-MBD in the elderly is lacking, especially in frail patients with high pill burden, poor quality of life and short life expectancy [55]. Egli-Spicht *et al*. observed a negative association between age and FGF-23. This could be in line with the expected lower P intake in the elderly, consequent to a general reduction of nutritional intake and hyporexia. It remains difficult to determine whether lower FGF-23 levels may orient toward softer P-lowering treatment in older patients. Certainly, data from Egli-Spicht *et al*. reinforce the need for dedicated studies in elderly cohorts, to better understand the real need for a stringent control of mineral parameters with related pill burden in such frail patients.

In conclusion, the NOPHOS study and its *post hoc* analysis presented in this issue of *CKJ* collectively highlight that an additional P reduction could be beneficial in dialysis patients, as it could lead to lower PTH and iFGF-23 levels and delayed vascular calcifications. While we await the results of the HiLo randomized clinical trial, which will better clarify which serum P target to pursue in these patients [56], it is reasonable to seek new therapies for lowering P towards the normal range as currently suggested by KDIGO guidelines [1], possibly combining different strategies including diet, HD removal, PB and transport inhibitors. Unfortunately, the efficacy of NAMR was not maintained in the long term, possibly due to the high burden of gastrointestinal side effects and high pill burden, leading to a considerable non-compliance rate. Many expectations rely on the potential reduction of FGF-23 elicited by HIF stabilizers. In this scenario, FGF-23 remains a promising biomarker of P overload and a mediator of its CV toxicity. However, the FGF-23 system should be always taken as being open to pathways other than mineral metabolism, like inflammation, anemia and iron deficiency. Further investigations are needed to elucidate which assay is the best to adopt to guide decision-making with reference to FGF-23 levels in the clinical setting.

CONFLICT OF INTEREST STATEMENT

M.C. is the *ad interim* Editor-in-Chief of *CKJ*.

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NephroCan

Rethinking Hemodialysis

NephroCan is a Canadian, fully integrated product and service provider for patients affected by chronic kidney failure and needing hemodialysis (HD) therapy. Our company offers a broad range of HD products including machinery: hemodialysis machine, central and portable reverse osmosis (RO) systems, patient chairs, and disposables: dialyzers, bloodlines, fistula needles, and bicarbonate cartridges and bags.

NephroCan's dialyzers (NephroFilters) are made with high-quality materials and pass rigorous testing to ensure safety, effectiveness, and efficacy. We offer a variety of NephroFilters to assist nephrologists and other healthcare providers in administering personalized care for their patients. NephroFilters are low flux or high-flux permeability and adaptable to different hemodialysis machines, designed for ease of use by healthcare professionals.

Our HD machine (NephroHDM) features technology that enables precise and customized treatment for each patient. Our goal is to improve clinical outcomes and patient safety. The NephroHDM offers various therapeutic options that allow healthcare providers to tailor hemodialysis sessions based on each patient's specific needs. The machine is practical, with an intuitive interface for a fast, easy set up, and safe monitoring of HD treatments.

NephroCan's CE-certified products are trusted by healthcare professionals around the world. Our commitment to quality and safety is reflected in our operations and processes, which ensure our products provide patients with the best hemodialysis treatment throughout their ESRD journey.

Our distribution partners and end users agree on several reasons why NephroCan presents a unique offering:

1. Extensive product portfolio

NephroCan offers a wide range of products and services that cover the "A to Z" of the hemodialysis spectrum. This broad portfolio provides integrated solutions and comprehensive treatments for dialysis patients with various medical needs.

2. Commitment to innovation

NephroCan is committed to innovation and invests heavily in research and development to create new products that can improve patient outcomes. Our focus is to develop products and technologies that will better serve the healthcare industry in the coming years.

3. Global perspective

With an existing presence in the EU, Africa, Asia, and the Middle East, NephroCan's goal is to expand our reach and serve patients in diverse geographical areas. This global vision allows us to share best practices and leverage expertise across regions to improve patient care.

4. Patient and family-centred care approach

NephroCan places a strong emphasis on putting patients and their families first. We tailor our products and services to meet the uniqueness of the communities we serve. This philosophy is reflected in our commitment to quality and safety, ensuring NephroCan is a trusted provider of hemodialysis products.

You can learn more about how our products are driving positive change in the industry and improving patient outcomes worldwide by visiting our website: www.NephroCan.com.

We invite you to see our product portfolio in person at the upcoming ERA 2023 congress:

June 15th - 17th	MiCo - Milano Convention Center	Booth number C.100