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Les calcimimétiques : des mécanismes d'action dans les cellules ostéoclastiques aux nouvelles indications thérapeutiques en pédiatrie

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Julie Bernardor

**Les calcimimétiques : des mécanismes
d'action dans les cellules
ostéoclastiques aux nouvelles
indications thérapeutiques en pédiatrie**

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RESUME

INTRODUCTION :

La maladie rénale chronique (MRC) pédiatrique est une pathologie complexe qui nécessite une prise en charge précoce en raison des complications liées au trouble de la croissance et des conséquences cardiovasculaires. Le traitement des anomalies minérales et osseuses, également appelées « *Chronic Kidney Disease and Mineral Bone Disorders* » (CKD-MBD) est essentiel pour prévenir ces complications et diminuer la morbi-mortalité.

MATÉRIELS ET MÉTHODES :

Notre première étude, RENOCLASTE, a analysé l'effet direct osseux de deux principaux traitements utilisés pour le CKD-MBD : la vitamine D active et un calcimimétique, le KP2326. Nous avons ensuite mené plusieurs études rétrospectives pour évaluer l'efficacité du cinacalcet, calcimimétique de 1^{ère} génération, hors autorisation de mise sur le marché (AMM), notamment en contexte de MRC et en hyperparathyroïdie primaire (HPP) en pédiatrie.

RÉSULTATS :

Le KP2326 et la vitamine D influencent directement la différenciation ostéoclastique par leur effet inhibiteur dans le groupe MRC pédiatrique. Associés à des doses réduites d'analogues de vitamine D, ils peuvent affecter la minéralisation et le renouvellement osseux. Le cinacalcet s'est avéré efficace pour réguler les concentrations d'hormone parathyroïdienne et de calcium dans l'utilisation hors AMM chez l'enfant dialysés de moins de 3 ans, en post-transplantation rénale et en HPP pédiatrique. Une surveillance attentive est indispensable pour anticiper les effets secondaires de l'utilisation du cinacalcet, et en particulier les épisodes d'hypocalcémie.

CONCLUSION :

Les calcimimétiques et leur utilisation hors AMM, en association avec des analogues de la vitamine D, présentent une stratégie thérapeutique potentiellement efficace pour la croissance osseuse et l'équilibre phosphocalcique en MRC et HPP pédiatriques.

SUMMARY

INTRODUCTION:

Pediatric Chronic Kidney Disease (CKD) is a complex condition that demands early intervention due to complications related to growth disorders and cardiovascular diseases. The management of chronic kidney disease and mineral bone disorders (CKD-MBD) is crucial to attenuate these complications and reduce morbidity and mortality.

MATERIALS AND METHODS:

Our preliminary study, RENOCCLASTE, investigated the direct bone effects of two primary treatments used for CKD-MBD: active Vitamin D (1 α ,25(OH) $_2$ D) and a calcimimetic, KP2326. Subsequently, we conducted several retrospective studies to assess the efficacy and safety of off-label use of cinacalcet, especially in CKD and primary hyperparathyroidism.

RESULTS:

Both KP2326 and 1 α ,25(OH) $_2$ D directly influence osteoclast differentiation in children with CKD. When combined with reduced doses of 1 α ,25(OH) $_2$ D, they can impact bone mineralization and turnover. Cinacalcet has proven effective in regulating PTH and calcium levels in off-label use among children under 3 years undergoing dialysis, post-kidney transplantation, and in primary hyperparathyroidism. Close monitoring is essential to preempt side effects from cinacalcet use, particularly episodes of hypocalcemia.

CONCLUSION:

Calcimimetics and their off-label use, in combination with vitamin D analogues, present a potentially effective therapeutic strategy for bone growth and the phosphocalcic balance in pediatric chronic kidney disease and pediatric primary hyperparathyroidism.

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À mes amis, à ma famille,

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ABREVIATIONS ET ACRONYMES

- 1 α ,25(OH) $_2$ D : 1 α ,25-OH vitamine D ou Vitamine D active ou calcitriol
- Akt : *Protein Kinase B*
- AMM : Autorisation de Mise sur le Marché
- [Ca $^{2+}$] : concentration de calcium
- CAKUT: Congenital Abnormalities of Kidney and Urinary Tract)
- CaSR: *Calcium Sensing Receptor*
- CERTAIN: Cooperative European Paediatric Renal TransplAnt Initiative
- CKD-MBD: *Chronic Kidney Disease associated Mineral and Bone Disorders*
- COX-2 : Cyclooxygénase-2
- CV : calcifications vasculaires
- DFG : Débit de Filtration Glomérulaire
- DMO : Densité de Masse Osseuse
- ERK1/2 : *Extracellular signal-regulated kinases*
- ESPN: European Society of Pediatric Nephrology
- FBS : Fœtal Bovine Serum
- FGF23 : Fibroblast Growth Factor 23
- hiPSc :human induced pluripotent stem cells
- HPP : Hyperparathyroïdie Primaire
- KDIGO: Kidney Disease: Improving Global Outcomes
- KO: *Knock-Out*
- MEM : Minimal Essential Medium
- M-CSF : Macophage colony-stimulating factor
- MCV : Maladie Cardiovasculaire
- MRC : maladie rénale chronique
- IRC : Insuffisance rénale chronique
- IRT : Insuffisance Rénale Terminale
- Ob : ostéoblaste
- Oc : ostéoclaste
- PAL : phosphatases alcalines
- PBMC: *Peripheral Blood Mononuclear Cells*
- PGE2 : Prostaglandine E2
- PTH: parathyroid hormone; hormone parathyroïdienne
- RANK-L: Recombinant human soluble Receptor activator of NF κ B ligand,
- sHPT : hyperparathyroïdie secondaire
- TMV : turnover osseux, la minéralisation et le volume osseux
- TRAP: *Tartrate-Resistance acid phosphatase*
- TRPV : Transient Receptor Potential Calcium Channel Subfamily
- Tt combi : traitement combinatoire
- VDR : récepteur de la vitamine D
- VDRE : *Vitamin D Responsive Element*
-

INTRODUCTION

1. Métabolisme phosphocalcique et régulation du calcium

a. Généralités

Le métabolisme phosphocalcique est un processus complexe qui régule les concentrations de calcium et de phosphate dans l'organisme. Ce métabolisme met en jeu trois hormones principales : l'hormone parathyroïdienne (PTH), la vitamine D ($1,25(\text{OH})_2\text{D}$) et le *Fibroblast Growth Factor 23* (FGF23) ; ainsi que trois ions, le calcium, le phosphate et le magnésium. L'intestin, le rein et l'os sont les trois principaux effecteurs.

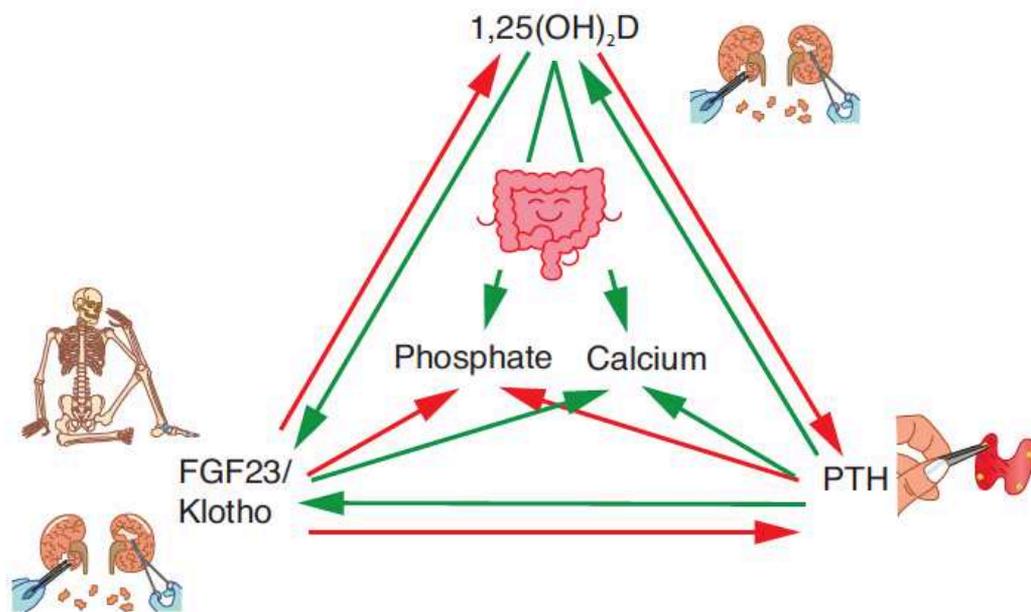


Figure 1. Vision d'ensemble du métabolisme phosphocalcique [1]

Le phosphate et le calcium sont principalement stockés dans les os, mais l'intestin et les reins jouent un rôle clé dans le métabolisme phosphocalcique. Les flèches vertes correspondent à un effet stimulant et les flèches rouges correspondent à un effet inhibiteur.

L'absorption intestinale joue un rôle clé dans l'apport de phosphate et de calcium dans l'organisme. Une fois absorbés, ces minéraux sont transportés dans le sang. En fonction des concentrations de calcémie et de phosphatémie, la PTH est libérée par les glandes parathyroïdes en réponse à une diminution de la calcémie. La PTH favorise également la libération de calcium provenant du lieu de stockage osseux par stimulation de la résorption osseuse et augmente l'absorption intestinale de calcium par un mécanisme actif du transporteur *Transient Receptor Potential Calcium Channel Subfamily TRPV6*. La réabsorption tubulaire du calcium se déroule dans tous les segments du néphron. La PTH agit sur le tube contourné distal et diminue l'excrétion de calcium par un mécanisme actif du TRPV5 (10-15%) [2].

Plusieurs processus sont impliqués pour maintenir une balance phosphocalcique adaptée aux besoins qui varient avec l'âge (**Tableau 1**). Chez l'enfant, la balance calcique doit être positive pour permettre une minéralisation osseuse optimale de ce squelette en croissance. En cas de carence en vitamine D ou d'une hyperparathyroïdie (primaire ou secondaire), des anomalies de minéralisation osseuse peuvent être observées [3].

Age	Calcémie normale (mmol/L)	Calcium ionisé normal (mmol/L)	Apports quotidiens recommandés en calcium (mg)	Phosphatémie normale (mmol/L)	Apports quotidiens recommandés en phosphore (mg)
Naissance-5 mois	2,18-2,83	1,22-1,40	210	1,50-2,40	100
6-12 mois	2,18-2,75	1,20-1,40	270	1,50-2,40	275
1-5 ans	2,35-2,70	1,22-1,32	500	1,50-2,10	460
6-12 ans	2,35-2,58	1,15-1,32	800	1,20-1,90	500 jusqu'à 8 ans puis 1250 après
13-20 ans	2,20-2,55	1,12-1,30	1300	0,70-1,50	1250

Tableau 1 : Valeurs de référence et apports quotidiens recommandés du calcium et du phosphore chez l'enfant et le jeune adulte [4]

b. Le calcium

Le calcium est l'électrolyte le plus abondant dans l'organisme, et 99% de celui-ci est stocké dans les os sous forme de cristaux d'hydroxyapatite. La signalisation calcique est un mécanisme intracellulaire essentiel qui utilise les ions calcium (Ca^{2+}) pour communiquer et réguler divers processus intracellulaires, comme la conduction nerveuse, la contraction musculaire, la coagulation sanguine et joue un rôle crucial dans la différenciation cellulaire ainsi que dans la signalisation intracellulaire. Une fois dans le cytosol, le calcium exerce des effets régulateurs allostériques sur de nombreuses enzymes et protéines, agissant ainsi dans la transduction du signal suite à l'activation de canaux ioniques ou en tant que second messenger dans des voies de transduction du signal indirectes, telles que les récepteurs couplés aux protéines G. La concentration de calcium dans le cytosol est régulée par des mécanismes complexes. Par exemple, la voie de la phospholipase C (PLC) est une voie courante qui augmente la concentration cytoplasmique de calcium. De nombreux récepteurs de surface cellulaire activent l'enzyme PLC, qui utilise l'hydrolyse du phospholipide membranaire PIP₂ pour former l'inositol (1, 4, 5) triphosphate (IP₃) et le diacylglycérol (DAG). L'IP₃ se lie aux récepteurs IP₃ du réticulum endoplasmique (RE), libérant ainsi du calcium dans le RE. De nombreuses fonctions médiées par le calcium se produisent lorsque le calcium libéré se lie à la protéine régulatrice, la calmoduline, qui peut activer les protéines kinases dépendantes du calcium ou agir directement sur d'autres protéines effectrices.

c. Le Récepteur Sensible au Calcium ou Calcium sensing receptor (CaSR)

L'homéostasie du calcium passe par le Récepteur Sensible au Calcium (CaSR), membre de la superfamille des récepteurs couplés aux protéines G, jouant un rôle fondamental dans l'homéostasie du calcium. Une diminution de la calcémie est détectée par le CaSR de la glande parathyroïde, provoquant une augmentation de la sécrétion de PTH. Cet excès de PTH stimule la mobilisation du calcium au niveau osseux par augmentation de la résorption osseuse, la réabsorption tubulaire rénale du calcium et la synthèse rénale de 1 α ,25(OH) $_2$ D. L'expression du CaSR est majoritairement retrouvée au niveau de deux organes : la parathyroïde et le rein. Plus récemment, sa présence a été mise en évidence dans différents tissus, et types cellulaires, comme les ostéoblastes, les ostéocytes mais également les chondrocytes, jouant ainsi un rôle au niveau squelettique.

Dans le tissu osseux, les fonctions et régulations du CaSR sont fondamentales : il régule la formation de la plaque de croissance mais également l'équilibre du remodelage osseux (formation/résorption), comme résumé dans le **Tableau 2** [5].

Modèles <i>in vitro</i>				
Auteur	Année	Ref	Modèles expérimentaux	Phénotypes observés
Dvorak MM.	2004	[6]	CaSR activé en présence de hautes [Ca ²⁺] extracellulaires ; ostéoblastes <i>in vitro</i> : rats Sprague-Dawley au stade fœtal ;	Augmentation des marqueurs de différenciation ostéoblastique après activation du CaSR par de fortes [Ca ²⁺] extracellulaires
Mentaverri R.	2006	[7]	Délétion CaSR exon 5 ; <i>in vitro</i> ostéoclastes souris issu reproduction de souris hétérozygotes (CaSR+/-)	Diminution de la différenciation Oc quand le CaSR est délété
			Activation CaSR en présence de hautes [Ca ²⁺] extracellulaires ; <i>in vitro</i> ostéoclastes souris	Augmentation de l'apoptose quand le CaSR est stimulé
Kaji H.	2008	[8]	Activation du CaSR en présence de fortes [Ca ²⁺] extracellulaires ; <i>in vitro</i> : ostéoblastes souris	Stimulation de la prolifération, différenciation et minéralisation ostéoblastiques quand le CaSR est activé
Modèles <i>in vivo</i>				
Auteur	Année	Ref	Modèles expérimentaux	Phénotypes observés
Chen T-H	2008	[9]	Délétion conditionnelle (exon 7) du CaSR dans les ostéoblastes /souris	Anomalies fœtales et néonatales du développement squelettique : Retard de croissance et défaut de minéralisation en cas de délétion du CaSR
Chen T-H	2008	[9]	Délétion conditionnelle (exon 7) du CaSR dans les chondrocytes/souris	Absence de différenciation des chondrocytes de la plaque de croissance en cas de délétion du CaSR

Tableau 2 : expression du CaSR et effets osseux

Présentation de l'expression du CaSR dans différents tissus constitutifs du squelette ; description des modèles expérimentaux utilisés avec leurs effets en fonction de l'activation ou de la délétion du récepteur.

CaSR: Calcium Sensing Receptor ; [Ca²⁺]: concentration de calcium ; Oc : ostéoclastes ;
KO : Knock-Out

Plusieurs études confirment l'expression du CaSR dans les monocytes, les macrophages [10], mais également dans les ostéoclastes [11]. En 2006, une étude a permis de mettre en évidence l'effet direct du CaSR dans l'ostéoclastogénèse ; en effet, le nombre d'ostéoclastes, différenciés *in vitro*, après biopsie osseuse de souris KO CaSR^{-/-} (issus de souris hétérozygotes (CaSR^{+/-}) pour l'exon 5), était significativement diminué. Il est démontré que l'activation, *in vitro*, du CaSR dans les ostéoclastes matures de lapin, en présence de fortes concentrations de calcium extracellulaire [Ca²⁺] (20mM), stimule de façon aberrante la voie NFκB augmentant ainsi l'apoptose des ostéoclastes matures [7]. On peut donc supposer que l'activation du CaSR dans l'ostéoclaste augmente la différenciation, mais stimule également l'apoptose, et de ce fait diminue la durée de vie et l'activité de résorption ostéoclastique, tout en jouant un rôle primordial dans le remodelage osseux.

Le remodelage osseux est le résultat de l'activité coordonnée des ostéoblastes, des ostéoclastes et des ostéocytes. La présence du CaSR dans les cellules osseuses suggère que cet équilibre peut être régulée directement par le [Ca²⁺] extracellulaire en détectant ses variations au niveau du micro-environnement osseux (0,5mM en phase de minéralisation ; ≥ 2mM au cours de la résorption osseuse ; 40mM au sein de la lacune de résorption) [12, 13]. Étant donné que le recrutement des ostéoblastes est couplé à la résorption osseuse au cours d'un cycle de remodelage osseux, la libération de calcium au sein des sites de résorption osseuse peut activer les ostéoblastes et stopper l'activité ostéoclastique via le CaSR [14]. Des concentrations extracellulaires élevées de [Ca²⁺] ont permis d'augmenter les marqueurs de différenciation ostéoblastique, *in vitro*, dans un modèle animal de rats au stade foetal [6]. Au cours de la résorption osseuse, l'augmentation du [Ca²⁺] fournit un signal d'activation du CaSR au sein des cellules osseuses conduisant au recrutement et à la prolifération de cellules ostéoprogénitrices d'ostéoblastes et à la diminution du nombre d'ostéoclastes [13].

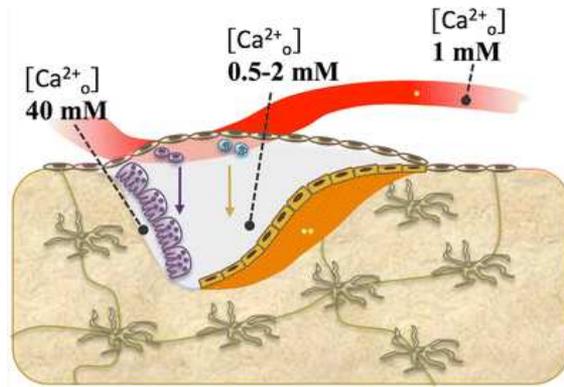


Figure 2 : Rôle du CaSR dans le remodelage osseux [13]

Les concentrations élevées de calcium libérés pendant la résorption osseuse (≥ 2 mM) fournissent un signal d'activation du CaSR dans les cellules osseuses. On observe alors, *in vitro*, une diminution de la différenciation des ostéoclastes, et de façon concomitante une production de cellules ostéoprogénitrices à l'origine de nouveaux ostéoblastes. Cellules violettes : ostéoclastes ; cellules orange : ostéoblastes ; rouge : flux sanguin

Plusieurs voies de signalisation, différentes en fonction du type cellulaire, sont impliquées lors de l'activation du CaSR. Dans les cellules ostéoblastiques, le $[Ca^{2+}]$ extracellulaire active, *in vitro*, la phospholipase C, la voie Akt (Protein Kinase B) mais également la voie des *Extracellular signal-regulated kinases* (ERK1/2), stimulent la prolifération de cellules ostéoprogénitrices et préviennent leur apoptose [15].

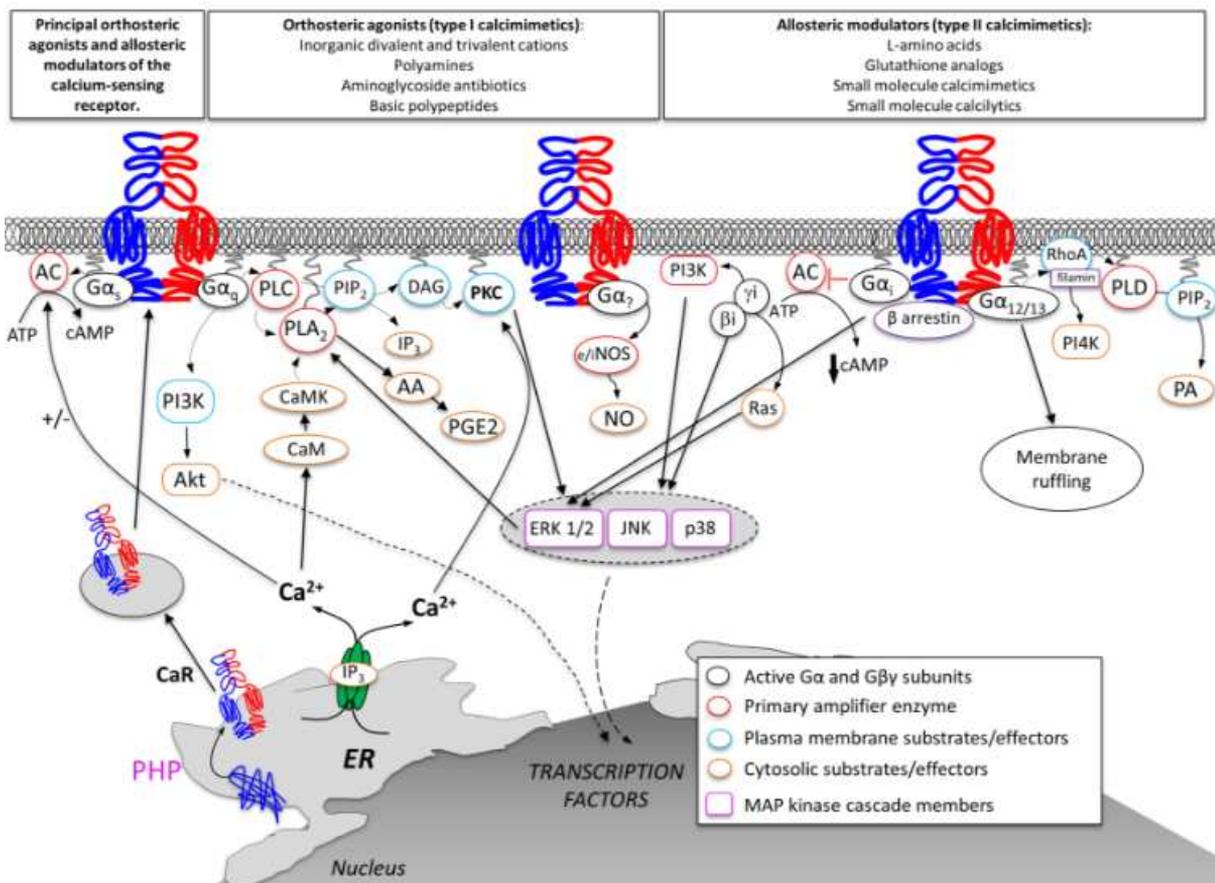


Figure 3. Voies de signalisation du CaSR et ses ligands modulateurs [16]

La liaison de certains traitements au CaSR, comme les calcimimétiques, conduit à des réarrangements structurels. L'agonisme biaisé est défini par la capacité du ligand à stabiliser des sous-ensembles de conformation du récepteur afin de d'activer une voie de signalisation spécifique. La voie activée ERK/pERK (ERK1/2) stimule la synthèse de PGE2 via la phospholipase A2 (PLA2) [17]. On peut noter que la phospholipase C (PLC), impliquée dans l'apoptose des ostéoclastes, permet également la synthèse de PGE2, qui inhibe la différenciation des pré-ostéoclastes.

En aval de l'activation de ces voies, des seconds messagers peuvent intervenir dans l'ostéoblastogénèse et l'ostéoclastogénèse via le CaSR. En effet, la production de prostaglandine E2 (PGE2), après stimulation de la cyclooxygénase-2 (COX-2), *in vitro*, permet la différenciation de nouveaux ostéoblastes [18]. Plus récemment, à partir de monocytes humains, l'action inhibitrice directe du PGE2 sur la différenciation des précurseurs ostéoclastiques a pu être mise en évidence, sans effet sur les ostéoclastes matures [19]. La signalisation du CaSR permet une régulation physiologique à la fois de l'ostéoclastogénèse et de l'ostéoblastogénèse. Ces données ouvrent ainsi une perspective d'applications thérapeutiques ciblées sur le CaSR des cellules osseuses dans un contexte de déséquilibre du remodelage osseux. Dans cet esprit, des drogues à visée calcilytique (inhibition du CaSR) ont ainsi été testées dans le traitement de l'ostéoporose. Ces molécules bloquent le CaSR des cellules parathyroïdiennes et stimulent la sécrétion de PTH. Des augmentations transitoires des concentrations circulantes de PTH permettent alors de stimuler la formation osseuse et par conséquent le remodelage osseux. Ils sont en général associés à des molécules anti-résorptives.

2. Maladie rénale chronique en pédiatrie

La maladie rénale chronique (MRC) se caractérise par une altération de la fonction rénale, définie par un débit de filtration glomérulaire (DFG) inférieur à 60 mL/min pour 1,73 m², avec ou sans protéinurie associée, depuis au moins 3 mois. Les stades de la MRC sont classifiés en fonction des valeurs de DFG, exprimées en mL par minute par 1,73 m² de surface corporelle. Les stades de la MRC comprennent une progression allant du stade 1 (atteinte rénale avec DFG ≥ 90 mL/min/1,73m² avec atteintes rénales depuis plus de 3 mois) au stade 5, correspondant à l'insuffisance rénale terminale nécessitant un traitement de suppléance ou une transplantation (IRT, DFG < 5 mL/min/1,73m²) [20]. La MRC au stade 5 nécessitant un traitement de suppléance (ou IRT) à l'âge pédiatrique est rare par rapport à celle des adultes avec moins de 1% des enfants et des adolescents parmi les patients incidents en 2021 en France [21]. En 2021, 91 enfants et adolescents de moins de 18 ans ont démarré un premier traitement de suppléance, dont 5 enfants avaient moins d'un an en France. L'incidence brute de la MRC stade 5 traitée chez les moins de 18 ans était de 6.2 par million d'enfants en 2021 [22]. Il est important de préciser que les patients, ayant bénéficiés d'une transplantation rénale, présentent toujours une MRC

après celle-ci. L'évaluation du débit de filtration glomérulaire est définie selon les formules de calcul adaptés et identifiés avec la lettre T (exemple : DFG=60mL/min correspond à un stade 2T) [23].

L'hypertension artérielle et le diabète sont les principales causes de MRC chez l'adulte. Les étiologies de MRC pédiatrique sont différentes et correspondent principalement à des malformations congénitales rénales et des voies urinaires, appelées également *CAKUT (Congenital Abnormalities of Kidney and Urinary Tract)*. Selon le rapport REIN 2021 [22], environ 52% des cas de MRC sont attribuables à ces pathologies congénitales, comprenant des maladies monogéniques, des uropathies et/ou des hypodysplasies rénales. D'autre part, environ 20% sont secondaires à des glomérulopathies acquises, avec une prédominance de syndromes néphrotiques idiopathiques de l'enfant présentant des lésions glomérulaires minimales ou des hyalinoses segmentaires et focales [22]. L'âge d'apparition de l'IRT diffère entre ces deux groupes, avec une moyenne d'environ 11 ans pour les pathologies congénitales et 16 ans pour les glomérulopathies acquises [22]. D'autres pathologies peuvent causer une MRC comme certaines pathologies vasculaires ou des néphrites interstitielles acquises mais il est important de noter que chez presque 20% des cas, l'étiologie de l'IRT reste encore indéterminée [22].

3. Retentissement et complications de la MRC

Au cours de la MRC, les reins perdent leur capacité à effectuer une filtration adéquate du sang circulant, entraînant une accumulation de toxines urémiques [24]. Bien que certains signes cliniques, tels que des antécédents familiaux de MRC ou la présence d'une protéinurie, puissent faire évoquer cette pathologie, d'autres manifestations, plus insidieuses, peuvent également être associées à une MRC. Parmi celles-ci, on note le retard de croissance, l'asthénie, les anomalies osseuses et le syndrome polyuro-polydipsique. De plus, la MRC est souvent accompagnée d'autres complications comme une hypertension artérielle, augmentant significativement le risque de développer une maladie cardiovasculaire (MCV) [25]. L'anémie est fréquente chez les patients atteints de MRC, en raison d'une production insuffisante d'érythropoïétine et de perturbations du métabolisme du fer [26]. Des déséquilibres métaboliques, tels que l'hyperkaliémie et l'acidose métabolique, peuvent survenir en raison de l'altération de la régulation rénale des électrolytes. Par ailleurs, l'ostéodystrophie rénale est secondaire à une altération du métabolisme phosphocalcique, conduisant à une fragilité osseuse accrue [27].

a. MRC et atteinte minérale et osseuse (CKD-MBD)

La diminution de la masse néphronique et/ou de leur fonction glomérulaire ont pour conséquence une diminution de l'excrétion urinaire de phosphate, ainsi qu'une diminution de la concentration de $1\alpha,25(\text{OH})_2\text{D}$ induisant une hypocalcémie séquellaire et une hyperparathyroïdie secondaire (sHPT) [2].

Les anomalies du métabolisme osseux et minéral, également dénommées *Chronic Kidney Disease associated Mineral and Bone Disorders* (CKD-MBD), secondaires à la MRC, peuvent se traduire par des conséquences immédiates (hypocalcémie, hyperphosphatémie, sHPT) mais également des conséquences tardives (fractures, ostéodystrophie rénale, calcifications vasculaires) ayant des répercussions sur la morbi-mortalité, la croissance et la qualité de vie de l'enfant atteint de MRC [28] ; au moins 30 à 40% des enfants présentent des atteintes squelettiques lorsque l'IRC progresse, et d'autant plus qu'ils nécessitent un traitement par dialyse [29]. La **Figure 4** résume la physiopathologie des anomalies biologiques observées lors du CKD-MBD.

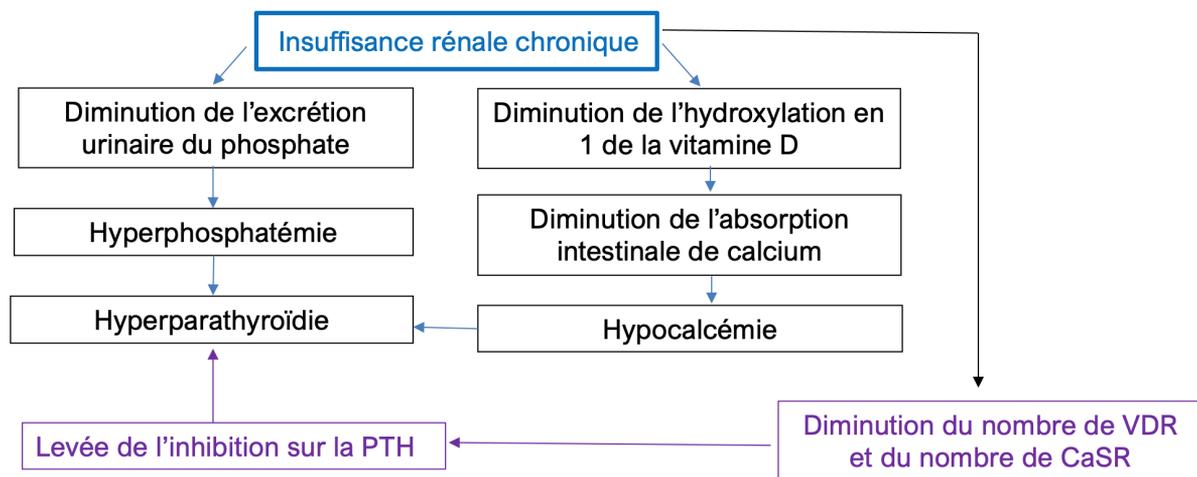


Figure 4. Physiopathologie des anomalies biologiques observées lors du CKD-MBD

L'insuffisance rénale chronique (IRC) ou MRC secondaire à la diminution de la masse néphronique fonctionnelle, diminue l'hydroxylation rénale en 1 de la 1 α ,25(OH) $_2$ D. Il en résulte une diminution de l'absorption intestinale de calcium. L'hypocalcémie séquellaire stimule ainsi la sécrétion d'hormone parathyroïdienne (PTH) des cellules parathyroïdiennes, via le Calcium Sensing Receptor (CaSR). Dans un même temps, la diminution de l'excrétion urinaire du phosphate, secondaire à l'IRC, augmente la phosphatémie et stimule également la sécrétion de PTH. La 1.25-D régule la réabsorption tubulaire distale rénale de calcium et phosphate tout en inhibant la synthèse de PTH via le récepteur de la vitamine D (VDR).

Le terme d'ostéodystrophie rénale réfère spécifiquement aux anomalies osseuses induites par la MRC. L'ostéodystrophie est censée être définie par analyse histomorphométrique après biopsie osseuse, mais cette exploration invasive est exceptionnellement réalisée à l'âge pédiatrique [30]. Le turnover osseux (T), la minéralisation (M) et le volume osseux (V) constituent ainsi les trois caractéristiques pouvant être atteintes au cours de la MRC, ce qui permet de définir les différents types d'ostéodystrophie rénale par la classification TMV des recommandations *Kidney Disease: Improving Global Outcomes* (KDIGO) 2009 [31]. La lésion primitive de l'os en situation d'ostéodystrophie rénale pédiatrique, tout du moins chez les enfants incidents en dialyse sans traitement adapté préalable, est la lésion de turnover élevé de l'hyperparathyroïdie, avec une modification morphologique progressive aboutissant dans le cas le plus extrême à l'ostéite fibreuse, secondaire à l'augmentation chronique des concentrations circulantes d'hormone parathyroïdienne (PTH) et au déficit en 1 α ,25(OH) $_2$ D. A

l'opposé, les lésions de bas turnover (ou os adynamique) peuvent aussi s'observer chez les enfants dialysés, mais elles sont dans ce cas le fait d'un traitement excessif par analogues de la vitamine D et sels calciques ; ces lésions sont délétères sur le long terme, puisqu'elles sont associées à une augmentation du risque de calcifications vasculaires, de fractures et de retard de croissance. Dans le contexte de la MRC pédiatrique, les anomalies de la minéralisation apparaissent dès les stades précoces, alors que les anomalies du turnover sont beaucoup plus tardives [32]. La physiopathologie de l'ostéodystrophie rénale pédiatrique est multifactorielle, incluant des facteurs nutritionnels, l'acidose métabolique, l'anémie, la résistance à l'hormone de croissance ainsi que le métabolisme phosphocalcique ; son traitement reste un défi quotidien pour les néphropédiatres [33].

b. MRC et maladie cardiovasculaire (MCV)

Les enfants et les adolescents atteints de MRC présentent une augmentation de la morbi-mortalité en lien avec le développement d'une maladie cardiovasculaire (MCV). En effet , il a été démontré que l'état vasculaire d'un jeune adulte dialysé de 25 ans est comparable à celui d'une personne âgée de plus de 80 ans [34]. Après l'analyse d'une cohorte de 6473 enfants dialysés, 25% des décès étaient secondaires à la MCV [35]. Différents facteurs de risque, dits « traditionnels », sont impliqués dans la MCV, tels que la dyslipidémie, l'obésité ; mais également des facteurs de risque en lien avec la MRC comme l'inflammation, le stress oxydatif et le déséquilibre phosphocalcique avec l'hyperphosphatémie [36].

L'hypertrophie ventriculaire gauche est un marqueur précoce de la MCV et est retrouvée chez un tiers des enfants dès le stade de MRC légère à modérée [37]. Ce phénotype peut être complété par la présence d'une vasculopathie détectée par l'augmentation de l'épaisseur de l'intima-média des artères carotidiennes, de la rigidité de la paroi artérielle carotidienne et de la calcification de l'artère coronaire [38].

Les calcifications vasculaires sont une complication fréquente et grave de la MRC chez l'adulte. Ces calcifications peuvent se manifester sous deux formes distinctes : les calcifications de la couche intima, en lieu avec un dépôt lipidique ; et celles de la couche média des vaisseaux secondaire à une transdifférentiation des cellules musculaires vasculaires lisses en cellules ostéoblastes-like [39]. Plusieurs publications ont établi une corrélation entre la phosphatémie, et le produit calcium/phosphate avec le risque de mortalité chez les patients pris en charge en hémodialyse [40]. Par ailleurs, deux études ont mis en évidence la présence de calcifications coronaires chez de jeunes adultes suivis pour MRC depuis la jeune enfance et traités par dialyse, démontrant ainsi l'impact précoce de ces calcifications sur la MCV et donc de la morbi-mortalité [41, 42].

Il a été récemment rapporté une corrélation inverse entre la densité minérale osseuse (DMO) et l'apparition des calcifications vasculaires chez des enfants et de jeunes adultes avec MRC, aux stades 4-5 ou en dialyse. En effet, une augmentation de la DMO trabéculaire était associée à un risque six fois plus élevé d'augmentation de l'épaisseur intima-média de la carotide et de la progression du processus de calcification vasculaire. Il a été également rapporté que la croissance osseuse atténuée la progression de la MCV, suggérant un rôle protecteur du squelette en croissance [43].

La physiopathologie des mécanismes impliquées dans la calcification vasculaire et dans la balance de l'axe os/vaisseaux restent méconnues chez l'enfant et une meilleure compréhension de leur évolution naturelle en MRC pédiatrique ou après transplantation rénale permettrait l'optimisation et de la prise en charge du CKD-MBD et de diminuer la morbi-mortalité chez ces adultes en devenir [44].

4. Prise en charge du CKD-MBD

Afin d'obtenir une croissance optimale et diminuer la morbi-mortalité, la prise en charge actuelle du CKD-MBD, décrite dans la **figure 5**, se base sur le traitement de l'hyperphosphatémie, de l'hypovitaminose D native et de sHPT grâce à plusieurs classes thérapeutiques et à des règles hygiéno-diététiques [45, 46]. Elle reste un défi quotidien pour le néphrologue et le pédiatre [47]. Avec la progression de la MRC, on observe une diminution de l'expression des récepteurs CaSR, VDR et PTH/PTHrP [48–51]. La diminution du nombre de ces récepteurs permet de lever l'inhibition de la sécrétion de PTH, et a donc des conséquences néfastes sur la formation et la minéralisation osseuses. Ces dernières années, plusieurs recommandations européennes ont été publiées concernant l'utilisation de la vitamine D et du cinacalcet, les apports phosphocalciques, et l'évaluation du CKD-MBD chez l'enfant insuffisant rénal [45, 52, 30, 53].

Prise en charge du CKD-MBD en 2023

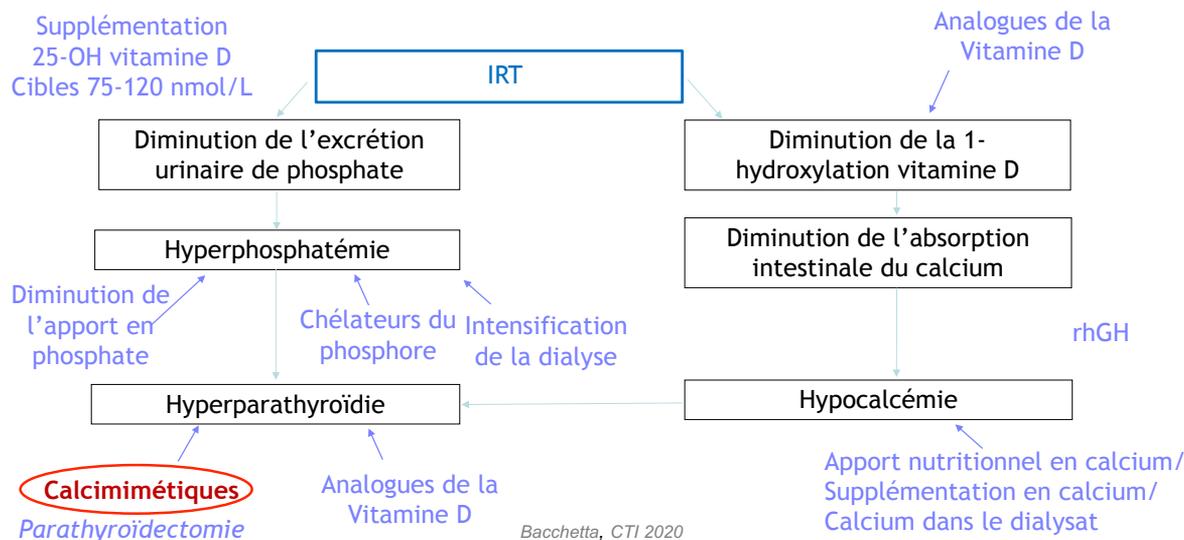


Figure 5 : prise en charge actuelle du CKD-MBD

Pour pallier l'hypovitaminose D, le traitement par $1\alpha,25(\text{OH})_2\text{D}$ et ses analogues est débuté lorsque l'IRC progresse, en l'absence d'hypocalcémie. Ce traitement est recommandé en une prise orale quotidienne chez l'insuffisant rénal chronique présentant une hyperparathyroïdie, permettant ainsi de contrôler la PTH en maintenant une normo-calcémie. L'hyperphosphatémie est prise en charge par la limitation des apports alimentaires en phosphore, la mise en place de chélateurs du phosphore et l'intensification de la dialyse. Les calcimimétiques (cinacalcet, etelcalcétide) sont utilisés chez les patients insuffisants rénaux chroniques pour corriger la sécrétion de PTH en modulant l'activation du CaSR.

a. Rôle de la $1\alpha,25\text{-OH}$ vitamine D dans la prise en charge du CKD-MBD

La vitamine D ($1\alpha,25(\text{OH})_2\text{D}$) est une hormone synthétisée dans l'organisme et elle est peu retrouvée dans l'alimentation hormis dans les poissons gras. Lors de l'exposition cutanée aux rayons ultraviolets du soleil, la vitamine D3 est synthétisée par une isomérisation spontanée de la pré-vitamine D3. Cette dernière est elle-même dérivée de la photo-isomérisation du 7-déhydrocholestérol présent dans l'épiderme. Une fois formée, la vitamine D3 est transportée dans la circulation sanguine en se liant à la protéine de liaison de la vitamine D (*VitD binding protein*). Contrairement à d'autres vitamines, la vitamine D3 n'est pas stockée de manière significative dans le foie. Dans le foie, la vitamine D subit une première hydroxylation au niveau du carbone 25, catalysée par l'enzyme CYP2R1. Cette étape est suivie d'une seconde hydroxylation au niveau du carbone 1 dans le rein par l'enzyme CYP27B1, produisant ainsi la forme bioactive de la vitamine D, la $1\alpha,25(\text{OH})_2\text{D}$. Cette enzyme est active dans de nombreux types cellulaires avec une action locale importante et est majoritairement retrouvée dans le rein. La régulation de ces enzymes hydroxylases est orchestrée par la PTH en corrélation avec la calcémie. En présence de concentrations faibles de calcium, la sécrétion augmentée de PTH va à son tour stimuler l'expression des enzymes CYP2R1 et CYP27B1. De plus, le $1\alpha,25(\text{OH})_2\text{D}$ stimule

l'expression de CYP24A1, une enzyme responsable de sa dégradation en acide calcitroïque, une molécule inerte éliminée par voie urinaire [54].

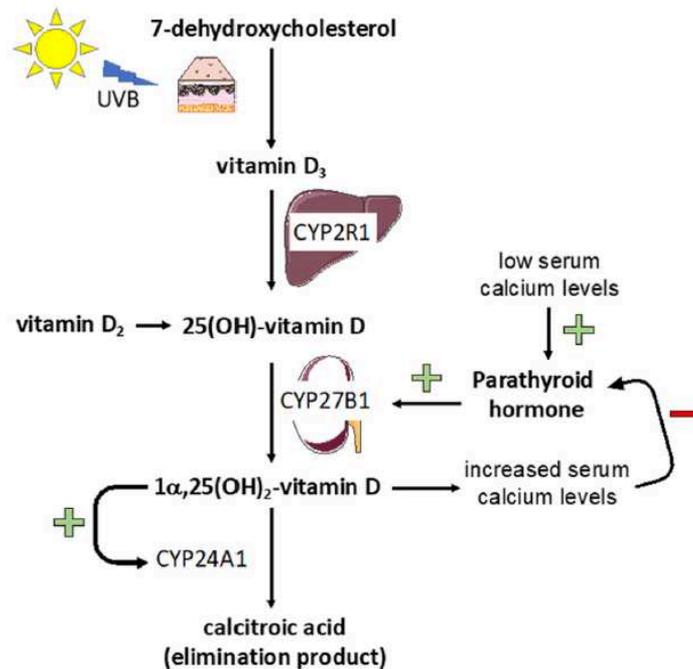


Figure 6 : Synthèse de la vitamine D active [54]

Pour pallier la diminution de la 1-hydroxylase vitamine D au cours de la MRC, enzyme permettant de transformer la 25-OH vitamine D en $1\alpha,25(\text{OH})_2\text{D}$, le calcitriol et les analogues de la vitamine D ($1\alpha,25(\text{OH})_2\text{D}$), restent le traitement de référence du CKD-MBD. Après liaison cytoplasmique au VDR (superfamille des récepteurs nucléaires), puis translocation dans le noyau, la fixation sur la *Vitamin D Responsive Element* (VDRE) permet l'expression des gènes cibles de la $1\alpha,25(\text{OH})_2\text{D}$, comme celui du VDR. Elle régule l'absorption intestinale de calcium, la réabsorption tubulaire distale rénale de calcium et de phosphate, et inhibe la synthèse d'hormone parathyroïdienne (PTH) [55].

La $1\alpha,25(\text{OH})_2\text{D}$ a des effets physiologiques reconnus sur la croissance et régule le métabolisme du phosphate, du calcium et de la PTH [54]. En 2015, Hahn *et al.* ont rapporté l'effets de la $1\alpha,25(\text{OH})_2\text{D}$ sur le métabolisme osseux, la croissance et le métabolisme phospho-calcique, chez 576 enfants insuffisants rénaux, stades 2 à 5D, inclus dans 18 essais contrôlés randomisés [56]. L'administration de $1\alpha,25(\text{OH})_2\text{D}$, que ce soit par voie orale ou intraveineuse, a réduit efficacement les concentrations de PTH par rapport au placebo ou à l'absence de traitement spécifique chez l'enfant avec MRC [56]. Cependant les résultats varient selon la voie d'administration du calcitriol. Si certaines études ont rapporté une réduction plus importante des concentrations de PTH avec le calcitriol intrapéritonéal qu'avec l'administration orale [57], d'autres études n'ont pas trouvé de différences significatives entre les deux voies d'administration [58]. De plus, la fréquence d'administration orale

du calcitriol (quotidienne ou intermittente) n'a pas entraîné de variations significatives des concentrations de PTH [59–61].

Une hypercalcémie iatrogène peut survenir suite au traitement par $1\alpha,25(\text{OH})_2\text{D}$, liée à une augmentation de l'absorption du calcium. Bien que des études aient comparé les voies d'administration intrapéritonéale et orale, ainsi que la fréquence d'administration, aucune différence n'a été observée concernant le nombre d'enfants présentant une hypercalcémie ou la fréquence de ces épisodes [57–61]. Alors que le calcitriol intraveineux semble augmenter le risque d'hypercalcémie [62], d'autres stéroïdes de la vitamine D, tels que la 1 α -hydroxyvitamine D et le paricalcitol, apparaissent des alternatives plus sûres [63], quoique non forcément disponibles en France. Il est à noter que ces épisodes d'hypercalcémie pourraient être associés à l'apparition de calcifications vasculaires [64, 65].

Malgré l'instauration de ce traitement à dose optimale, on observe la persistance d'un défaut de minéralisation chez les patients insuffisants rénaux pédiatriques, décrit dans une cohorte de 60 enfants dialysés, après analyse histomorphométrique [66]. En 2015, au cours d'un travail visant à évaluer l'effet du FGF23 sur la différenciation et la résorption ostéoclastiques, les effets de la $1\alpha,25(\text{OH})_2\text{D}$ sur l'ostéoclaste humain ont déjà été étudiés et rapportés par notre équipe chez des témoins sains. En effet, à la dose de 5 nM, la $1\alpha,25(\text{OH})_2\text{D}$ inhibe de manière significative et importante la différenciation ostéoclastique (d'environ 90%), sans avoir d'effet sur l'activité de résorption [67]. Plus récemment, l'équipe d'I. Salusky (UCLA, USA) a rapporté dans un abstract présenté au congrès de l'*American Society of Nephrology*, en 2015, une résistance progressive à la $1\alpha,25(\text{OH})_2\text{D}$, *in vitro*, dans des ostéoblastes issus de patients à différents stades de l'IRC. Chez des témoins sains, cette même équipe a montré que la $1\alpha,25(\text{OH})_2\text{D}$ diminue la prolifération ostéoblastique tout en augmentant la minéralisation [68].

b. Rôle des calcimimétiques dans la prise en charge du CKD-MBD

Comme décrit précédemment, l'objectif de la prise en charge du CKD-MBD est de normaliser la phosphatémie et d'obtenir des concentrations de PTH dans les cibles en fonction du degré de MRC [45]. L'utilisation des calcimimétiques, tels que le cinacalcet ou l'etelcalcétide, est la seconde pierre angulaire dans le traitement du sHPT du CKD-MBD. Le cinacalcet est un calcimimétique de « première génération » administré quotidiennement par voie orale. Il agit sur le CaSR tel un modulateur allostérique positif par fixation sur un site transmembranaire. Il potentialise la liaison et/ou l'efficacité d'un ligand orthostérique. Ce phénomène est nommé « coopérativité positive ». Le ligand, ici le calcium, doit être présent et l'ampleur de la coopérativité dépend de celui-ci. Le cinacalcet a été approuvé en 2004 aux États-Unis et en 2005 en Europe ; une diminution significative des concentrations de PTH sans augmentation du calcium et du phosphate sériques a été rapporté [69].

Le cinacalcet peut prévenir l'hypertrophie de la glande parathyroïde et l'hyperparathyroïdie résistante au traitement standard du CKD-MBD [70–72]. Il affecte également le remodelage osseux, réduisant le turnover osseux sans affecter la minéralisation osseuse [73].

L'etelcalcetide, est un agoniste peptidique allostérique activateur positif du CaSR, après liaison à un domaine extracellulaire, agissant par un mécanisme distinct de celui du cinacalcet. A l'inverse du cinacalcet qui nécessite une liaison du calcium sur le CaSR pour être actif, ce calcimimétique de « seconde génération » peut activer le CaSR indépendamment du calcium extracellulaire par sa liaison à une cystéine à la position 482 pour créer un pont disulfure, essentielle pour l'activation du récepteur [74]. Ce traitement est indiqué en cas de sHPT chez le patient dialysé. Il est administré par voie intraveineuse à la fréquence de trois injections par semaine, améliorant l'observance du patient. L'etelcalcetide, approuvé en 2013, présente une efficacité similaire à celle du cinacalcet dans la freination de la synthèse de la PTH, mais offre l'avantage d'une demi-vie plus longue, ce qui permet une administration trois fois par semaine [75–78]. Il a également des effets sur le remodelage osseux, améliorant la structure de l'os cortical chez les rats atteints de hyperparathyroïdie secondaire (sHPT) [79].

Les deux médicaments ont montré des résultats prometteurs dans la réduction de la calcification vasculaire, et certaines études suggèrent un bénéfice potentiel dans la réduction des événements cardiovasculaires [80]. Ils abaissent les concentrations de FGF23, ayant un effet protecteur sur le système cardiovasculaire [81, 82].

Chez les enfants dialysés, l'utilisation du cinacalcet a été recommandée en cas d'hyperparathyroïdie secondaire à la MRC, sévère et persistante malgré un traitement optimal du CKD-MBD, mais les données concernant ses effets sur l'os et les vaisseaux sont limitées [83]. Warady *et al.* ont rapporté des essais cliniques contrôlés randomisés menés par la société Amgen®, 7,4 à 57,1 % des sujets ayant reçu du cinacalcet ont atteint des concentrations de PTH dans les cibles recommandées, et 22,2 à 70,6 % ont obtenu une réduction ≥ 30 % de la PTH. Des réductions significatives de la PTH ont également été retrouvées dans toutes les études non soutenues par Amgen® [84]. Une étude évaluant l'effet de l'etelcalcetide sur le CKD-MBD chez des enfants dialysés est en cours.

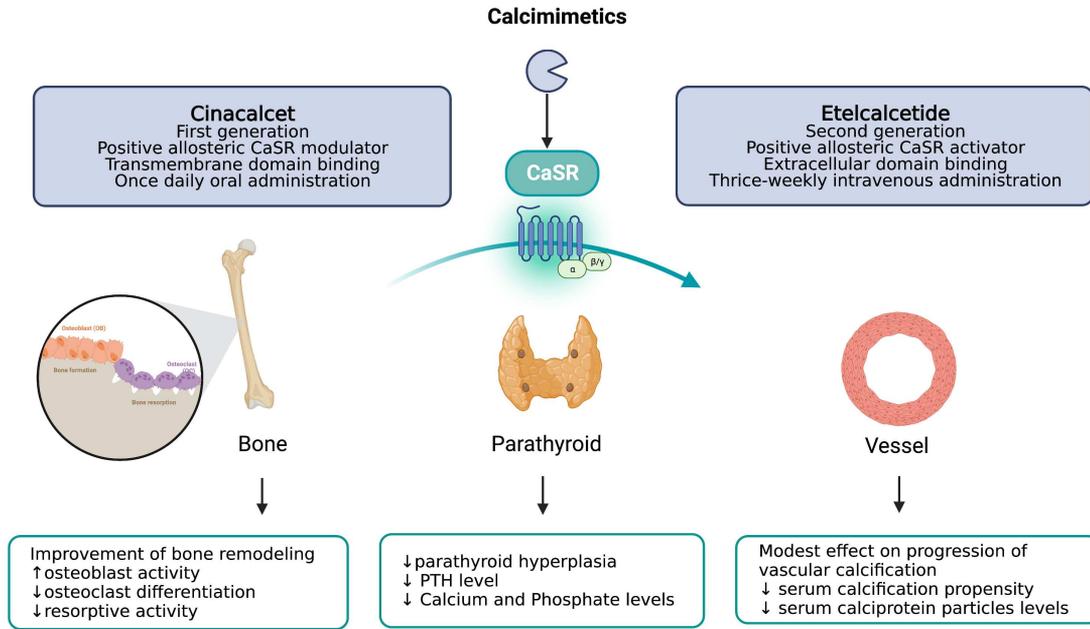


Figure 7 : Effets des calcimimétiques sur les cellules osseuses, parathyroïdiennes et vasculaires [85]



Impact of Cinacalcet and Etelcalcetide on Bone Mineral and Cardiovascular Disease in Dialysis Patients

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Abstract

Purposes of Review With chronic kidney disease (CKD) progression, secondary hyperparathyroidism (sHPT) and mineral and bone metabolism disease (MBD) almost inevitably develop and result in renal osteodystrophy and cardiovascular disease (CVD). Together with active vitamin D, calcimimetics are the main therapy for sHPT in CKD. This review provides an overview of the therapeutic effects of oral cinacalcet and intravenous etelcalcetide on CKD-MBD and vascular disease, with a focus on pediatric dialysis patients.

Recent Findings Randomized controlled trials in adults and children demonstrate efficient lowering of parathyroid hormone (PTH) by the calcimimetics together with a reduction in serum calcium and phosphate when combined with low-dose active vitamin D, while therapy with active vitamin D analogs alone increases serum calcium and phosphate. Cinacalcet and etelcalcetide both improve bone formation and correct adynamic bone, i.e., have a direct bone anabolic effect. They decrease serum calciprotein particles, which are involved in endothelial dysfunction, atherogenesis, and vascular calcification. Clinical trials in adults suggest a modest slowing of the progression of cardiovascular calcification with cinacalcet.

Summary Calcimimetic agents represent a major pharmacological tool for improved control of CKD-MBD, by efficiently counteracting sHPT and allowing for better control of calcium/phosphate and bone homeostasis. Albeit definite evidence is lacking, the beneficial effects of calcimimetics on CVD are promising. Routine use of cinacalcet has been suggested in children.

Keywords Calcimimetic · Cinacalcet · Etelcalcetide · CKD · PTH · Bone · Vascular calcification · Children

Introduction

With chronic kidney disease (CKD) progression, the regulation of mineral and bone metabolism is disturbed and involves calciotropic hormones with high parathormone (PTH) levels, low active vitamin D (25-OHD), and an

increase of fibroblast growth factor 23 (FGF23) [1]. CKD-mineral bone disorder (CKD-MBD) results in a higher morbi-mortality with a decreased bone mass, increased risk of fragility bone fractures and bone pain, and of vascular calcifications [2, 3].

The optimal management of CKD-MBD is a daily challenge for physicians and consists mainly of correcting native and active vitamin D deficiency, maintaining nutritional

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calcium intake in the recommended range, lowering serum phosphate concentrations by dietary means and oral phosphate binders, and to keep PTH levels within the target range [1, 4]. According to the Kidney Disease: Improving Global Outcomes (KDIGO) recommendations, active vitamin D analogs and calcimimetics, such as cinacalcet and etelcalcetide, should both be used to manage secondary hyperparathyroidism (sHPT) [1, 5].

The class C G-protein-coupled calcium-sensing receptor (CaSR) is ubiquitously expressed in the human body, notably in parathyroid and renal tubular cells but also in bone and vascular tissues [6]. This receptor plays a crucial role in calcium homeostasis by sensing variations of extracellular ionized calcium concentration. Cinacalcet is a “first generation” calcimimetic acting on the CaSR as a positive allosteric modulator by binding to a transmembrane site of the receptor, increasing its calcium sensitivity and thereby decreasing parathyroid PTH secretion [7]. Cinacalcet is administered once daily and induces marked circadian PTH fluctuations with a nadir after 4–8 h after oral administration. Etelcalcetide is a peptide that binds to the extracellular domain and is a positive CaSR allosteric activator, by a mechanism distinct from that of cinacalcet [8]. This “second generation” calcimimetic does not require extracellular calcium binding to CaSR to activate the CaSR.

This review reports the therapeutic effects of cinacalcet and etelcalcetide (a) on sHPT and mineral metabolism, and more specifically (b) on bone, and (c) vascular disease in dialysis patients, (d) with a special focus on pediatric patients.

Effects on sHPT and Mineral Metabolism

For more than one decade, cinacalcet was the only approved calcimimetic used in CKD dialysis patients to treat sHPT, since 2004 in the USA and since 2005 in Europe, based on a series of prospective clinical trials. In 2002, Goodman et al. reported lower PTH and serum calcium concentrations in 52 adult hemodialysis patients treated with cinacalcet during 8 days with a nadir 4 h after dosing [9]. Randomized double-blind controlled studies demonstrated significant suppression of PTH levels without increments of serum calcium and phosphate in 78 hemodialysis patients daily treated during 18 weeks [10]. Similar findings have emerged from subsequent larger studies with longer follow-up ([10, 11, 12•, 13–18, 19••, 20–25, 26•, 27, 28]; Table 1). Block et al. described in two-thirds of 741 patients treated with cinacalcet for 26 weeks a 30% reduction in PTH from baseline values, compared with 11% of patients receiving standard care [12•]. Serum calcium and phosphate were unchanged with standard care but decreased both in the treatment arm by about 15% [12•]. A randomized controlled

trial (RCT) reported lower FGF23 levels with cinacalcet and low-dose calcitriol analogs compared to the regimen using vitamin D analogs alone [29]. FGF23 induces cardiac hypertrophy, modifies cardiomyocyte contractility, and has been associated with an increased cardiovascular risk, including the risk of arrhythmic events [30]. Goodman et al. demonstrated that the CaSR activation by calcimimetics is independent of the serum phosphate concentrations, even in the case of hyperphosphatemia, which is increasingly prevalent with progression of CKD [31].

Etelcalcetide was approved in 2013 as an activator of CaSR to treat sHPT in adults, following in vitro and in vivo characterization of the activity and of the clinical utility [32]. In a prospective crossover study design, Martin et al. described significant dose-dependent reductions in PTH and FGF23 levels in a hemodialysis population and attenuation in the interdialytic rise in serum phosphate [21]. The noninferiority of etelcalcetide compared to cinacalcet was reported in 343 patients with more than a 30% reduction in mean predialysis PTH concentrations from baseline during weeks 20–27; 178 patients of the 343 patients had a more than 50% PTH reduction [26•]. A major advantage of etelcalcetide is the pharmacokinetic profile with an effective half-life of 3–5 days that allows thrice-weekly dosing [8, 32–34].

Calcimimetics may slow or even prevent the development of parathyroid gland hypertrophy and tertiary, therapy-resistant HPT. In in vitro models, rats and humans, the development of parathyroid hyperplasia was attenuated by cinacalcet [35–37]. An observational study with repeated ultrasounds measurement suggests regression of parathyroid gland hypertrophy with extended cinacalcet use also in dialysis patients [38] which may explain the reduced rate of parathyroidectomy with cinacalcet therapy demonstrated in a large RCT [19••].

In parathyroidectomized and thyroidectomized rats, respectively, an additional calcitonin secretion stimulating effect of the calcimimetic NPS R-568 was demonstrated independent of the inhibition of PTH secretion; calcitonin inhibits osteoclast activity [39]. Of interest for clinicians is the interpatient variability of the parathyroid gland response to cinacalcet depending on CaSR gene variants. Heterozygous and homozygous carriers of the *CASR* A990G variant have a 4 times greater, baseline serum phosphate, calcitriol dose, and age-adjusted odds to achieve a 30% reduction in serum PTH after 12 weeks of cinacalcet treatment than respective noncarriers [40]. This genetic variant increases CaSR function with increased post receptor signaling activity in response to calcium binding [41, 42]. Cinacalcet is also effective and approved for patients with primary hyperparathyroidism and hypercalcemia in adults. A recent observational study in 18 children with primary hyperparathyroidism and hypercalcemia, of which 13 had

Table 1 summary of the randomized controlled trials reporting the use of cinacalcet or etelcalcetide in hemodialysis patients

Author, journal, year	Calcimimetic, dose	patient	Time of follow-up (weeks)	Effect of calcimimetic on mineral homeostasis	Effect of calcimimetic on bone turnover	Effect of calcimimetic on vascular calcifications	Calcimimetic-related adverse events
[10] Lindberg JS., <i>Kidney Int.</i> , 2003	Cinacalcet vs. placebo, 20 mg daily, up-titration to max. 50 mg/day	78 adult HD patients	18 weeks (12-week dose titration phase)	- PTH decreased by 26% (versus PTH increased by 22% with placebo), independent of baseline vitamin D usage - 11.9% decrease in calcium × phosphorus product	NA	NA	Asymptomatic hypocalcemia: 3/38 subjects
[11] Quarles LD., <i>J Am Soc Nephrol.</i> , 2003	Cinacalcet vs. placebo, up-titration to max. 100 mg/day	71 adult HD patients	18 weeks (12-week dose titration phase)	- Decrease in PTH ≥ 30% in 53% of patients (23% with placebo) - 7.9% decrease in calcium × phosphorus product	NA	NA	- Vomiting more frequent
[12•] Block GA., <i>N Engl J Med.</i> , 2004	Cinacalcet vs. placebo, up-titration to max. 180 mg/day	741 adult HD patients	26 weeks (12-week dose titration phase)	- 43% reached PTH levels ≤ 250 ng/L (PTH increased by 9% with placebo) - 15% decrease in calcium × phosphorus product	NA	NA	- Nausea reported in 32% of patients up - Upper respiratory tract infection (13% in cinacalcet vs. 7% in controls)
[13] Lindberg JS., <i>J Am Soc Nephrol.</i> , 2005	Cinacalcet vs. placebo, up-titration to max. 180 mg/day	395 adult HD and PD patients	26 weeks (16-week dose titration phase)	- 46% reached PTH levels ≤ 300 ng/L (versus 9% in controls) - Significant reduction in serum calcium and phosphorus	NA	NA	- Nausea in 30% of patients (vs. 22% in controls)
[14] Messa P., <i>Clin J Am Soc Nephrol.</i> , 2008	Cinacalcet vs. CT (vitamin D analog phosphate binders) Cinacalcet up-titration to max. 180 mg/day	552 adult HD patients 2:1 ratio	26 weeks (16-week dose titration phase)	- 71% reached PTH levels ≤ 300 ng/L (vs. 22% with CT) - Significant reduction in serum calcium and phosphate	NA	NA	- Nausea in 32% of patients (vs. 3% in CT) - Hypocalcemia in 5% of patients (vs. 1% in controls)

Table 1 (continued)

Author, journal, year	Calcimimetic, dose	patient	Time of follow-up (weeks)	Effect of calcimimetic on mineral homeostasis	Effect of calcimimetic on bone turnover	Effect of calcimimetic on vascular calcifications	Calcimimetic-related adverse events
[15] Akiba T., Ther Apher Dial., 2008	Cinacalcet vs. placebo, 20 mg daily, up-titration to max. 50 mg/day	120 adult Japanese HD patients	5 weeks	- Dose-dependent decline in PTH-significant reduction in serum calcium and phosphate - Significant decline in PTH	- Osteocalcin and tartrate-resistant acid phosphatase decreased significantly - Diminished activation frequency, bone formation rate/bone surface, and fibrosis surface/bone surface - Bone mineralization parameters remained normal	NA	- 2 patients with symptomatic hypocalcemia NA
[16] Malluche HH, Clin Nephrol., 2008	Cinacalcet vs. placebo with CTtherapy	32 adult HD patients	NA	- No difference between the two groups for serum PTH, calcium, and phosphate - Significant reduction in serum PTH, calcium, and phosphate	NA	NA	- Gastrointestinal adverse events lower when cinacalcet was administered in the evening NA
[17] Schaefer RM, Clin Nephrol., 2008	Cinacalcet daily with the post-dialysis meal vs. with food during the dialysis session	673 adult HD patients	21 weeks	- Significant reduction in serum PTH, calcium, and phosphate	NA	NA	- Hypocalcemia risk seven-fold higher as in the placebo group - Nausea and vomiting twice as common
[18] Raggi P., Nephrol Dial Transplant., 2011	Cinacalcet plus low-dose vitamin D sterols o vs. flexible doses of vitamin D sterols alone	360 adult HD patients	52 weeks (20-week dose titration phase)	- Dose-dependent significant decreases in serum iPTH at 30 min, FGF23 at 24 h, and iCa at 12 h post dose	NA	NA	NA
[19•••] EVOLVE Trial Investigators, N Engl J Med., 2012	Cinacalcet vs. placebo, up-titration to max. 180 mg/day	3883 adult HD patients	64 months (20-week dose titration phase)	- Dose-dependent significant decreases in serum iPTH at 30 min, FGF23 at 24 h, and iCa at 12 h post dose	- Reduction in fracture risk by 16–29%	- No significant reduction in the risk of death or major cardiovascular event	- Hypocalcemia risk seven-fold higher as in the placebo group - Nausea and vomiting twice as common
[20] Martin KJ., Nephrol Dial Transplant., 2014	Etelcalcetide vs. placebo, up-titration to max. 10 mg/day intravenously	32 healthy male volunteers	24 h and 48 h post dose	- Dose-dependent significant decreases in serum iPTH at 30 min, FGF23 at 24 h, and iCa at 12 h post dose	NA	NA	NA

Table 1 (continued)

Author, journal, year	Calcimimetic, dose	patient	Time of follow-up (weeks)	Effect of calcimimetic on mineral homeostasis	Effect of calcimimetic on bone turnover	Effect of calcimimetic on vascular calcifications	Calcimimetic-related adverse events
[21] Martin KJ., <i>Kidney Int.</i> , 2014	Etelcalcetide vs. placebo (max 60 mg/day i.v.); two-period crossover design	28 adult HD patients	24 h and 48 h post dose	- Dose-dependent reduction in PTH, serum calcium, and FGF-23 - Attenuated rise in serum phosphate during interdialytic period	NA	NA	- Hypocalcemia in 25% of patients (vs. 0% in placebo) - Paresthesia in 7% of patients (vs. 3% in placebo)
[22] Sprague SM., <i>Clin J Am Soc Nephrol.</i> , 2015	Cinacalcet or vitamin D analog	312 adult HD patients	52 weeks	- Decreased serum FGF-23 - Changes in FGF-23 were correlated with changes in Ca x P product in both arms	NA	NA	- Safe and well tolerated, no adverse effects reported
[23] Floege J., <i>Clin J Am Soc Nephrol.</i> , 2015	Cinacalcet vs. placebo, up-titration to max. 180 mg/day	24 HD patients with calcific uremic arteriolopathy (moderate to severe sHPT)	64 months (20-week dose titration phase)	NA	NA	Significant reduced incidence of arterial calcification	NA
[24] Wetmore JB., <i>Clin J Am Soc Nephrol.</i> , 2015	Cinacalcet vs. vitamin D analogs	312 adult HD patients	12 months	- No difference achieving plasma PTH < 300 ng/L or a ≥ 30% decrease in PTH - Calcium and phosphorus levels decreased in the cinacalcet arm, whereas a modest increase in calcium was noted in the vitamin D analog arm	NA	NA	- Hypocalcemia was more common - Hypercalcemia and hyperphosphatemia less frequent

Table 1 (continued)

Author, journal, year	Calcimimetic, dose	patient	Time of follow-up (weeks)	Effect of calcimimetic on mineral homeostasis	Effect of calcimimetic on bone turnover	Effect of calcimimetic on vascular calcifications	Calcimimetic-related adverse events
[25] Fukagawa M, Nephrol Dial Transplant, 2017	Etelcalcetide vs. placebo, up-titration to max. 15 mg/day intravenously	155 adult Japanese HD patients	12 weeks	- PTH target range (60–240 ng/L) was reached in 59% vs. 1.3% - Serum albumin—corrected calcium, phosphorus, and FGF-23 levels were decreased in the etelcalcetide group	NA	NA	- Asymptomatic blood calcium decreased (6.4% with etelcalcetide; 0% with placebo) - Vomiting (3.8% with etelcalcetide; 0% with placebo), nausea (1.3% with etelcalcetide; 0% with placebo) - Symptomatic hypocalcemia (1.3% with etelcalcetide; 0% with placebo) - Hypocalcemia in 68.9% of patients with etelcalcetide (vs. 59.8% with cinacalcet) - Vomiting in 32% of patients (24% in controls), hypocalcemia in 23% (vs. 19% in controls), nausea in 18% (vs. 14%), hypertension in (14% (vs. 24% in controls)
[26] Block GA, JAMA, 2017	Etelcalcetide vs. placebo and cinacalcet vs. placebo	683 adult HD patients	26 weeks	Etelcalcetide was not inferior to cinacalcet in reducing serum PTH concentrations	NA	NA	- Hypocalcemia in 68.9% of patients with etelcalcetide (vs. 59.8% with cinacalcet) - Vomiting in 32% of patients (24% in controls), hypocalcemia in 23% (vs. 19% in controls), nausea in 18% (vs. 14%), hypertension in (14% (vs. 24% in controls)
[27] Warady BA, Pediatr Nephrol, 2019	Cinacalcet vs. placebo	43 pediatric HD patients	30-week controlled phase and 30-week open label use	- Cinacalcet decreased serum intact PTH levels ($\geq 30\%$ decrease) - Significant reduction in serum calcium and phosphorus	NA	NA	- Vomiting in 32% of patients (24% in controls), hypocalcemia in 23% (vs. 19% in controls), nausea in 18% (vs. 14%), hypertension in (14% (vs. 24% in controls)
[28] Eddington H, BMC Nephrol, 2021	Cinacalcet vs. standard therapy alone	36 adult HD patients	52 weeks (12-week dose titration phase)	- Cinacalcet decreased serum intact PTH and serum phosphate levels	- No difference, bone mineral density slightly changed in both arms	- No difference in the progression of calcification	NA

Abbreviations: N^o, number; PTH, parathyroid hormone; NA, not available; sHPT, secondary hyperparathyroidism; PD, peritoneal dialysis; HD, hemodialysis; CT, conventional treatment; sHPT, secondary hyperparathyroidism

inactivating CaSR mutations, reconfirmed the PTH and Ca-lowering effect in pediatric patients, but side effects were not reported [43].

Impact of Cinacalcet and Etelcalcetide on CKD-MBD

Bone remodeling is the result of the coordinated activity of osteoblasts, osteoclasts, and osteocytes. The presence of CaSR on osteoblast, osteoclasts, and osteocytes suggests that this bone remodeling balance can be regulated directly by extracellular calcium concentrations and sensing its variations in the bone microenvironment (0.5 mM during mineralization; ≥ 2 mM during bone resorption; 40 mM within the resorption gap) [44, 45]. These findings also argue in favor of a direct impact of calcimimetics on the bone mineralization process. By means of osteoclasts differentiated from CaSR^{-/-} KO mice and transfected with the CaSR, Mentaverri et al. demonstrated that stimulation of the CaSR may play a pivotal role in the control of both osteoclast differentiation and apoptosis [46]. In mouse osteoblastic cells, mineralization increased upon exposure to the calcimimetic NPS-R467, an effect abolished after transfection with CaSR antisense and the inactivating R185Q variant of the CaSR. Abolition of CaSR function reduced alkaline phosphatase activity, osteocalcin expression, and mineralization in the osteoblastic cells. In mouse models of rickets and sHPT due to 1 α -hydroxylase deficiency, cinacalcet administered without concomitant active vitamin D reduced PTH and normalized the increased osteoblast activity but increased the number and activity of osteoclasts and did not normalize bone mineralization deficit [47]. In human monocytes from healthy donors, cinacalcet exposure inhibited osteoclastic differentiation, reduced TRAP activity, and inhibited resorptive activity [48]. In 2022, Chiu et al. revealed the molecular pathways in osteoclasts regulated by cinacalcet. Cinacalcet inhibits the receptor activator of NF- κ B ligand (RANKL)/receptor activator of NF- κ B (RANK)-induced osteoclast differentiation in a concentration-dependent manner through a close interaction between activation of caspase-related apoptosis, reversal of osteoclast autophagy, and attenuation of the osteoclast endoplasmic reticulum stress [49].

In 2008, 32 hemodialysis patients were randomly assigned 2:1 to receive cinacalcet or placebo with concurrent vitamin D and/or phosphate binder therapy and underwent bone biopsies performed before and after 1 year of treatment [16]. Cinacalcet treatment decreased serum PTH, bone formation rate, and fibrosis per bone surface. Adynamic bone was observed in three out of 19 patients receiving cinacalcet; in two of these, PTH levels were persistently below 100 ng/L. The changes in histomorphometric bone parameters corresponded to serum PTH-, bone-specific alkaline phosphatase- and N-telopeptide reductions. Bone mineralization parameters remained normal [16]. The EVOLVE trial

randomizing 3883 hemodialysis patients to cinacalcet versus placebo demonstrated a clinical fracture risk of 16–29%, after adjustment for baseline characteristics, multiple fractures, and/or cinacalcet discontinuation [19••]. The effect was more pronounced in patients above 64 years of age. Since bone imaging or bone biopsies were not performed, the bone-specific effects of cinacalcet remained elusive but should be on cortical bone, preferentially altered in CKD [19••, 50, 51]. Etelcalcetide should also act on the bone. In nephrectomized rats with sHPT, etelcalcetide reduced bone remodeling and mineralization defect by improving cortical bone structure [52].

Effects on Vascular Disease

Vascular disease is already prevalent in young children with CKD and on dialysis [53], the arterial calcium content markedly increased [54]. Arterial calcifications are related to hypertrophy of the left ventricle and to high pulse wave velocity [55]. Two types of vascular calcifications (VC) can be distinguished, intimal calcification related to lipid deposition, associated with infiltration of inflammatory cells, and medial calcification as a consequence of vascular smooth muscle cell transformation into an osteoblast-like cell type. Initiation and progression of VC are similar to the bone formation process [56] and are promoted by several CKD-related risk factors. In vitro, the osteogenic differentiation of vascular smooth muscle cells (VSMC) is promoted by DNA damage and cellular senescence [57]. Dysregulated mineral metabolism promotes oxidative DNA damage and vascular inflammation [58]. High phosphate concentrations particularly promote the VC process and have therefore been named “silent killer” [55, 59]. However, comprehensive analyses of molecular mechanisms underlying CKD-MBD-induced arterial remodeling, e.g., by means of vascular transcriptomics or proteomics, have not been accomplished yet. In a uremic rat model, cinacalcet inhibited aorta and heart calcifications, reduced serum calcium, phosphate, and PTH, and the expression of osteoblast-specific markers [60]. Joki et al. confirmed these results and reported delayed progression of atherosclerosis with cinacalcet [61]. Cinacalcet reduces oxidative DNA damage, endothelial dysfunction, and carotid artery intima-media thickness, due to the suppression of endothelial-to-mesenchymal transition [62, 63]. Odenwald et al. reported an initial BP increase in sham-operated and uremic rats in response to the calcimimetic R-568, which in uremic rats was followed by a marked and sustained antihypertensive effect, suggesting another protective mechanism of calcimimetics in CKD [64].

Based on these promising experimental findings, several RCTs were accomplished. In the ADVANCE trial, 360 hemodialysis patients were randomized to cinacalcet or placebo and underwent baseline and follow-up computed

tomography scans to assess coronary artery and aortic calcification [18]. While the primary outcome, the Agatston score of coronary artery calcification, did not reach statistical significance ($p=0.073$), aortic and mitral valve calcification scores were significantly lower in the cinacalcet group, and the progression of coronary artery calcification scores was slightly slower [18]. In the EVOLVE trial described above, cinacalcet did not significantly reduce the risk of death or major cardiovascular events [19••]. The results of this RCT suggested a modest but clinically meaningful benefit in cardiovascular endpoints, particularly cardiovascular death, sudden death, and heart failure, presumably related to the benefits of cinacalcet on nonatherosclerotic processes, such as myocardial fibrosis and arterial calcification [19••, 65]. Of interest, cinacalcet treatment significantly lowered serum FGF23, and these reductions were associated with lower rates of cardiovascular death and major cardiovascular events [65, 66].

Several RCTs with etelcalcetide have meanwhile been accomplished. Analyses of the 26-week randomized trials that compared the effects on PTH of etelcalcetide ($n=509$) versus placebo ($n=514$) and etelcalcetide ($n=340$) versus cinacalcet ($n=343$) in adults with sHPT receiving hemodialysis convincingly demonstrate potent lowering of serum FGF23 together with reductions in PTH, calcium, and phosphate [67]. A recent RCT including 425 hemodialysis patients demonstrated a more efficient decrease in serum

calcification propensity with etelcalcetide than with vitamin D analogs by means of an in vitro assay, which quantifies the transformation time from primary to secondary calciprotein particles [68]. Hashimoto et al. confirmed the decrease in serum calciprotein particle levels during 12 weeks of etelcalcetide treatment [69]. Altogether, these RCTs are consistent with experimental studies and suggest the major beneficial effects of cinacalcet and etelcalcetide on CVD in patients on dialysis, but definitive evidence on improved CV outcome in patients is still lacking (Fig. 1).

Calcimimetics and CKD-MBD Control in Children

In 2020, a European consensus statement from the European Society of Paediatric Nephrology (ESPN) suggests routine use of cinacalcet in pediatric patients on dialysis above 3 years of age with persistent and severe hyperparathyroidism in the presence of high or high-normal calcium levels, and despite optimized conventional management, including active vitamin D. The recommendations were based on two pediatric randomized controlled trials, nine uncontrolled interventional or observational studies in children, and case reports that describe the PTH-lowering efficacy of cinacalcet, which was similar to findings in adults [70]. Warady et al. summarized the cinacalcet pediatric clinical development program, which consisted of two phase 3 randomized studies, one

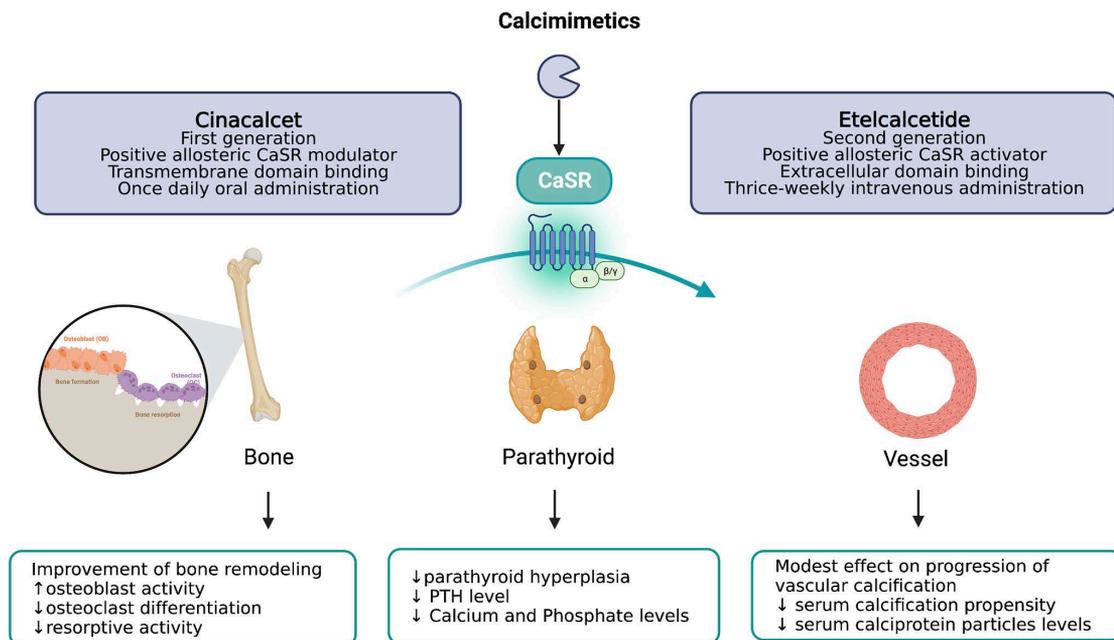


Fig. 1 Beneficial effects of calcimimetics on bone, parathyroid, and vessel cells (created with BioRender.com)

phase 3 single arm extension study, one open-label phase 2 study, and two open-label phase 1 studies. A total of 7.4 to 57.1% of subjects who received cinacalcet in a clinical trial by Amgen attained PTH levels within recommended target ranges and 22.2 to 70.6% a $\geq 30\%$ reduction in PTH. Significant reductions in PTH were also demonstrated in all non-Amgen-supported studies [71••]. None of the pediatric trials reported clinical endpoints related to bone or vascular disease. In children below 3 years of age, for whom cinacalcet has not been licensed, observational evidence suggests similar PTH-lowering efficacy, but with a higher incidence of hypocalcemic episodes, presumably due to the particular high calcium needs in these rapidly growing young children [72]. Three cases of precocious puberty were reported in children, of which one had renal hypodysplasia with a de novo mutation in the *TCF2/HNF1 β* [72, 73].

To gain further insights into the effects of etelcalcetide in children, our team analyzed the in vitro action of etelcalcetide and active vitamin D on mature human primary osteoclasts derived from peripheral blood mononuclear cells from 19 pediatric CKD patients and 6 healthy donors. Etelcalcetide inhibited osteoclastic differentiation of the cells derived from pediatric controls and the children with CKD, albeit then active vitamin D on CKD. The inhibitory effect was independent of the stage of CKD. Combined treatment with etelcalcetide and active vitamin D analog did not result in synergistic effects. Etelcalcetide significantly inhibited osteoclast-mediated bone resorption [74].

In view of the experimental and clinical findings together with the large body of evidence in adults, including the inhibitory effects on FGF23, that has deleterious systemic effects in CKD patients and the fact that active vitamin D analogs stimulate FGF 23 production [75], lower doses of active vitamin D should be used together with calcimimetics in order to control sHPT in growing children. Adult recommendations published in 2017 did not prioritize any PTH-lowering treatment (calcimimetics, calcitriol, or vitamin D analogs); all have been classified as acceptable first-line options (KDIGO) [1]; combining cinacalcet and low doses active vitamin D at well-tolerated concentrations is justified [76].

Conclusion

CKD-MBD management is a daily challenge in dialysis patients, but the calcimimetics cinacalcet and etelcalcetide are novel potent tools to more efficiently control sHPT, calcium, and phosphate balance and to improve bone and presumably cardiovascular health. Experimental and clinical studies suggest the combined use of low-dose active vitamin D analogs together with calcimimetics, especially in children requiring a positive calcium balance for the growing bones.

Compliance with Ethical Standards

Conflict of Interest JBa has been and is an investigator for the Amgen-sponsored trials on the use of cinacalcet and etelcalcetide in pediatric dialysis. JBa is the coordinator of the Amgen-sponsored international registry on the use of cinacalcet in pediatric dialysis.

JBa received research grants from Amgen for the Renoclaste study (effects of etelcalcetide on human osteoclasts obtained from children with chronic kidney disease).

CPS has been and is the principal investigator for the Amgen-sponsored trials on the use of cinacalcet and etelcalcetide in pediatric dialysis and of the Amgen-sponsored international registry on the use of cinacalcet in pediatric dialysis.

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

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c. Effet combinatoire de la 1,25-D et de l'etelcalcetide sur la biologie ostéoclastique chez le témoin sain et le patient pédiatrique atteint de MRC

Comme décrit précédemment, la prise en charge du CKD-MBD est souvent complexe, et certains patients reçoivent à la fois plusieurs classes thérapeutiques, comme la 1,25(OH)₂D et des calcimimétiques, selon les recommandations internationales les plus récentes [31].

Le traitement combinatoire utilisant à la fois des calcimimétiques et des analogues de la vitamine D active est devenu une approche de plus en plus pertinente pour la prise en charge du CKD-MBD. Les recommandations du KDIGO suggèrent que les calcimimétiques et les analogues de la vitamine D active devraient être utilisés en combinaison pour traiter sHPT chez les patients adultes atteints de MRC et en dialyse [86, 87]. La diminution des doses de 1,25(OH)₂D permet de réduire le risque d'hypercalcémie et d'hyperphosphatémie, tout en diminuant les concentrations de PTH, et d'améliorer l'état osseux du patient en MRC.

L'efficacité de ce traitement combinatoire a été rapportée dans deux essais cliniques. L'étude américaine CONTROL est un essai clinique de 16 semaines qui étudiait l'efficacité de la combinaison du cinacalcet avec des doses réduites de 1,25(OH)₂D chez 72 patients adultes hémodialysés avec une sHPT et dont le produit calcium-phosphate (Ca×P) n'était pas contrôlé. Les patients inclus présentaient des concentrations de PTH contrôlés (80-160 pg/mL). Lors de la première phase de l'étude, tous les patients recevaient des doses modérées à élevées de stéroïdes de vitamine D et de chélateurs de phosphate par voie intraveineuse. Les patients ont ensuite intégré la phase de titration de la dose de cinacalcet et celle de 1,25(OH)₂D afin d'atteindre les objectifs du traitement. L'efficacité était définie par l'obtention d'une PTH ≤160 pg/mL et d'un produit Ca × P ≤55 mg²/dL². L'étude a démontré que l'association de cinacalcet et de 1,25(OH)₂D à dose réduite permettait de maintenir le contrôle de la PTH tout en améliorant de manière significative le contrôle du produit Ca × P. Simultanément, les concentrations de calcium et de phosphate sériques ont été réduits, et davantage de patients ont atteint les objectifs de PTH et de Ca × P [88].

L'étude européenne multicentrique randomisée OPTIMA a inclus 552 patients adulte dialysés et suivis pendant 23 semaines, et a évalué l'efficacité du traitement combinatoire (cinacalcet+1,25(OH)₂D) versus traitement standard (1,25(OH)₂D et chélateurs du phosphate) dans le contrôle de la PTH, ainsi que la réduction du calcium et du phosphate sériques. Le schéma thérapeutique a été conçu pour optimiser la combinaison du cinacalcet, des stéroïdes de la vitamine D et des chélateurs du phosphate afin d'atteindre les concentrations cibles de l'PTH. Le critère d'évaluation principal était la proportion

de patients parvenant à contrôler la concentration d'PTH (≤ 300 pg/mL) au cours des semaines 17 à 23. L'étude a rapporté que le traitement combinatoire a permis à une plus grande proportion de patients d'atteindre le critère d'évaluation primaire par rapport au traitement standard. Le cinacalcet a réduit efficacement les concentrations d'PTH indépendamment de la concentration initiale de PTH ; la réduction la plus importante ayant été observée chez les patients atteints d'une sHPT plus sévère [89].

Sur le plan osseux, une analyse secondaire de l'étude EVOLVE, un essai clinique randomisé (cinacalcet *versus* placebo) chez 3883 patients hémodialysés, traités ou non par $1\alpha,25(\text{OH})_2\text{D}$, a permis de montrer, après ajustement, une diminution du risque fracturaire de 16 à 29% chez les patients traités par cinacalcet sans que les mécanismes physiopathologiques sous-jacents ne soient élucidés [90].

En plus de son effet freinateur sur la PTH, un essai randomisé contrôlé a rapporté des concentrations de FGF23 plus faibles avec le cinacalcet associés aux analogues du calcitriol à faible dose que dans le groupe traité uniquement par des analogues de la vitamine D [91]. Le FGF23 induit une hypertrophie cardiaque, modifie la contractilité des cardiomyocytes et a été associé à un risque cardiovasculaire accru, y compris le risque d'événements arythmiques [92].

Le traitement combinatoire (cinacalcet+ $1\alpha,25(\text{OH})_2\text{D}$) ciblent différentes voies de régulation du métabolisme minéral et osseux, et pourrait entraîner des effets synergiques pour améliorer la santé osseuse et cardiovasculaire des patients atteints de CKD-MBD. Des recherches supplémentaires seront nécessaires pour mieux comprendre les mécanismes d'action impliqués et leur impact sur le CKD-MBD, et en particulier en population MRC pédiatrique.

II- OBJECTIFS DE LA THÈSE

La thèse est structurée autour de trois axes de recherche :

L'objectif du premier axe de travail est d'évaluer l'impact direct sur l'ostéoclaste d'un calcimimétique, l'etelcalcetide (et plus précisément sa pro-drogue le KP2326), chez des patients pédiatriques avec MRC à différents stades, après avoir évalué leur propre effet chez des témoins sains. L'hypothèse de travail est que l'etelcalcetide inhibe la différenciation ostéoclastique et l'activité de résorption. Cet effet biologique est moindre lorsque l'IRC progresse et le DFG diminue.

Le deuxième axe de la thèse s'intéresse à l'utilisation du cinacalcet en dehors de son autorisation de mise sur le marché (AMM), en MRC pédiatrique, chez les enfants de moins de 3 ans et après transplantation rénale pédiatrique. Deux études rétrospectives européennes rassemblant des données cliniques et biologiques d'enfants ayant reçu du cinacalcet, dans ces deux indications, ont été rapportées. Nous supposons que le cinacalcet a un effet freinateur sur la synthèse de la PTH et normalise la calcémie chez les enfants avec MRC. Ces éléments permettent d'évaluer l'utilisation en vie réelle du cinacalcet et de recueillir des informations sur son efficacité et sa tolérance.

Les indications retenues pour l'AMM du cinacalcet en France, sont l'hyperparathyroïdie de l'adulte, qu'elle soit primaire ou secondaire à une maladie rénale chronique, et les carcinomes parathyroïdiens. En pédiatrie, les données concernant son utilisation sont restreintes en raison de son AMM récente et limitée aux patients en dialyse de plus de 3 ans présentant une hyperparathyroïdie secondaire. Néanmoins, certains patients présentant des pathologies phosphocalciques sans insuffisance rénale, pouvant être liés avec des mutations du CaSR, doivent être traités hors AMM par cinacalcet devant des hyperparathyroïdies sévères, sans aucun autre traitement chronique disponible à ce jour.

L'objectif du troisième et dernier axe de la thèse est d'évaluer l'utilisation en France du cinacalcet dans des pathologies phosphocalciques pédiatriques hors AMM, afin d'obtenir des données d'efficacité et de sécurité sur ce traitement utilisé pour la prise en charge de ces maladies orphelines. Nous analysons de façon rétrospective et multicentrique, l'utilisation en France du cinacalcet dans des pathologies phosphocalciques sans insuffisance rénale, afin d'obtenir des données d'efficacité et de sécurité afin d'améliorer nos connaissances sur la prise en charge de ces maladies rares. Nous supposons que le cinacalcet a un effet freinateur sur la synthèse de la PTH et normalise la calcémie chez les enfants atteints d'hyperparathyroïdie primaire en France.

Au total, cette thèse a pour objectif principal de contribuer à une meilleure compréhension des mécanismes d'action des calcimimétiques dans le métabolisme osseux, ainsi qu'à définir leurs indications, leur efficacité et leur sécurité dans le traitement des pathologies pédiatriques rares. Ces recherches permettent ainsi de renforcer les connaissances scientifiques dans ce domaine et de promouvoir une prise en charge médicale optimale pour les enfants atteints de MRC et de pathologies phosphocalciques, au sein des Centres de Référence des Maladies Rares du Calcium et du Phosphore, et au sein des Centres de Référence des Maladies Rénales Rares, en France et en Europe.

III- MATERIELS ET MÉTHODES

1. *Ostéoclastogénèse humaine dans l'étude RENOCLASTE*

L'étude RENOCLASTE porte sur l'ostéoclastogénèse humaine, processus de formation des ostéoclastes, à partir de monocytes circulants isolés provenant de poches de sang non thérapeutiques, obtenues dans le cadre d'une convention avec l'Établissement Français du Sang. Cette méthode de différenciation ostéoclastique à partir de monocytes de sang total a été développée par notre laboratoire et est décrite dans la **cf. Revue de la littérature annexe N°2 : Utilisation des PBMC en MRC**. Les monocytes sont purifiés par gradient de densité, d'abord avec du Ficoll pour isoler les globules blancs, puis avec du Percoll pour séparer les monocytes des lymphocytes. Les monocytes sont ensuite mis en culture dans un milieu spécifique, α MEM (Minimal Essential Medium) Alpha Medium [-] Ribonucleosides, [-] Deoxyribonucleosides, et du sérum de veau foetal (Foetal Bovine Serum, FBS, PAN Biotech). Deux cytokines stimulant l'ostéoclastogénèse *in vivo* ont été utilisées au cours des expériences : le M-CSF humain (Recombinant human Macophage colony-stimulating factor, PeproTech) et le RANKL humain (Recombinant human soluble Receptor activator of NF κ B ligand, PeproTech). La différenciation ostéoclastique est étudiée en présence de différents traitements utilisés dans la prise en charge du CKD-MBD, dont la 1 α ,25(OH) $_2$ D et l'Ételcalcétide, appelé ici le KP2326, et le traitement combinatoire (1 α ,25(OH) $_2$ D et KP2326) avec des contrôles éthanol et PBS1x. L'exposition aux différents réactifs utilisés était constante au cours de la différenciation ostéoclastique (J0 à J6 maximum). Des techniques de coloration TRAP et DAPI sont employées pour identifier les ostéoclastes matures et les noyaux, respectivement. Une analyse par Western Blot a permis d'analyser la voie ERK/p-ERK des ostéoclastes après induction. Nous avons également analysé l'effet du KP2326 sur la résorption osseuse d'ostéoclastes issus de témoins sains. Après analyse des effets directs osseux de la 1 α ,25(OH) $_2$ D et du KP2326 sur les ostéoclastes issus de témoins sains, nous avons étudié les ostéoclastes issus de 19 patients pédiatriques atteints de MRC inclus dans RENOCLASTE. Les critères d'inclusion sont les suivants : enfants âgés de plus de 2 ans et de moins de 18 ans ; enfant de plus de 10kg devant avoir un prélèvement sanguin dans le cadre du soin ; patient atteint de MRC suivi dans le service de néphrologie pédiatrique de l'Hôpital Femme Mère Enfant de Bron ; enfant de parent/titulaire de l'autorité parentale ayant été informé de l'étude et ne s'opposant pas à y participer. Les critères de non inclusion sont les suivants : atteinte osseuse en lien avec une maladie rénale génétique pouvant induire une atteinte osseuse spécifique (oxalose, cystinose, syndrome de Pierson, mutation des paracellines, polykystose dominante) ; traitement en cours pouvant avoir un impact spécifique sur l'os (hormone de croissance, biphosphonates, teriparatide). Un comptage semi-automatique de comptage d'ostéoclastes a été réalisée à l'aide d'un logiciel développé en collaboration avec un ingénieur informaticien, du laboratoire INSERM 1033, avec interface graphique par superposition des images en lumière blanche et en lumière à fluorescence.

Différenciation ostéoclastique

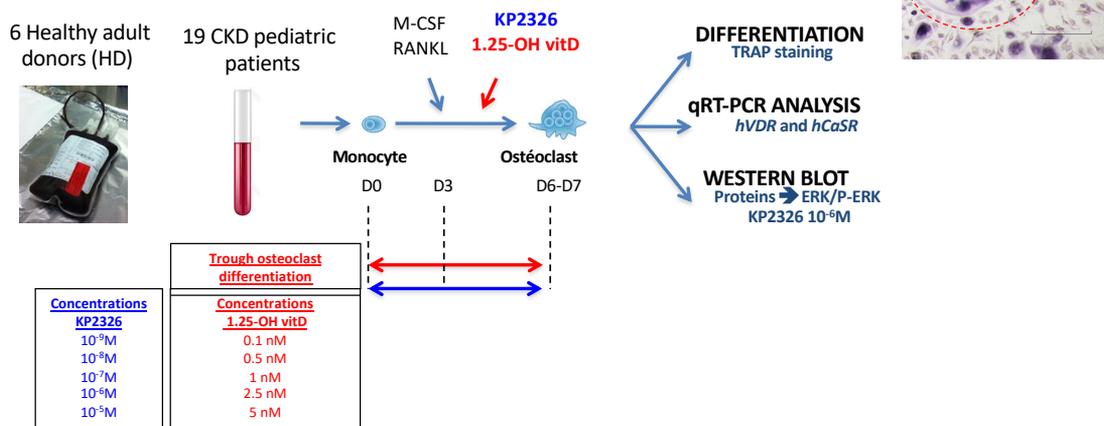


Figure 8 : Plan expérimental de la différenciation ostéoclastique dans l'étude RENOCLASTE

Du sang total a été prélevé chez 6 donneurs sains, puis chez 19 patients pédiatriques atteints de MRC. Nous avons étudié la différenciation des ostéoclastes à partir de monocytes circulants, en présence de deux cytokines (M-CSF et RANK-L), et de 1-25 Vitamine D et/ou KP2326. La vitamine D et le KP2326 ont été utilisés à différentes concentrations, dans le cadre de la différenciation des ostéoclastes. A la fin du processus de différenciation, nous avons le nombre de cellules TRAP positives. Nous avons également effectué une analyse par qRT-PCR pour évaluer les marqueurs d'intérêt des ostéoclastes tels que RANK ; VDR ; CaR et un Western blot après l'induction par le KP2326.



Peripheral Blood Mononuclear Cells (PBMCs) to Dissect the Underlying Mechanisms of Bone Disease in Chronic Kidney Disease and Rare Renal Diseases

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Abstract

Purpose of Review To describe the methods that can be used to obtain functional and mature osteoclasts from peripheral blood mononuclear cells (PBMCs) and report the data obtained with this model in two peculiar diseases, namely pediatric chronic kidney disease-associated mineral and bone disorders (CKD-MBD) and nephropathic cystinosis. To discuss future research possibilities in the field.

Recent Findings Bone tissue undergoes continuous remodeling throughout life to maintain bone architecture; it involves two processes: bone formation and bone resorption with the coordinated activity of osteoblasts, osteoclasts, and osteocytes. Animal models fail to fully explain human bone pathophysiology during chronic kidney disease, mainly due to interspecies differences. The development of in vitro models has permitted to mimic human bone-related diseases as an alternative to in vivo models. Since 1997, osteoclasts have been generated in cell cultures, notably when culturing PBMCs with specific growth factors and cytokines (i.e., M-CSF and RANK-L), without the need for osteoblasts or stromal cells.

Summary These models may improve the global understanding of bone pathophysiology. They can be used not only to evaluate the direct effects of cytokines, hormones, cells, or drugs on bone remodeling during CKD-MBD, but also in peculiar genetic renal diseases inducing specific bone impairment.

Keywords PBMCs · Osteoclasts · Osteoblasts · Bone remodeling · CKD · Cystinosis

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Machuca-Gayet and Justine Bacchetta are equally contributed to the manuscript

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Introduction

Bone tissue undergoes continuous remodeling throughout life to maintain bone architecture and calcium/phosphate homeostasis. Bone remodeling involves two interconnected processes, i.e., bone formation and bone resorption with the coordinated activity of osteoblasts, osteoclasts, and osteocytes [1, 2]. Four consecutive phases compose bone remodeling: recruitment of mononuclear progenitor cells to differentiate into mature multi-nuclear osteoclasts, resorption of bone matrix by osteoclasts, preparation of the resorbed surface, formation of new osteoid, and further mineralization by osteoblasts [3].

Osteoblasts, derived from mesenchymal stem cells (MSCs), appear on bone surface and synthesize an organic matrix with collagen type I and non-collagenous proteins. Mineralized bone tissue results from the secondary deposition of calcium-phosphate crystals on osteoid bone [4]. At the end of bone formation, osteoblasts can be transformed into osteocytes (i.e., mechano-sensing cells embedded in the

mineralized matrix with a crucial role in the regulation of both bone turnover and phosphate/calcium metabolism through the secretion of the anti-anabolic sclerostin and the phosphaturic FGF23, respectively) or into lining cells but also undergo apoptosis [5]. On the other hand, osteoclasts differentiate from the monocytic cell lineage from hematopoietic stem cells (HSCs). This differentiation requires the presence of two cytokines, namely the cytokine receptor activator of NF- κ B ligand (RANKL) and macrophage colony-stimulating factor (M-CSF) which are secreted by osteoblasts and osteocytes [6]. Important to note, MSCs and HSCs are bone marrow cells and can be mobilized from the peripheral blood system [7, 8].

Bone resorption and bone remodeling are tightly coupled, and an imbalance of these two processes leads to bone diseases such as osteoporosis and osteopetrosis [9]. Pathophysiology of mineral and bone disorders associated with chronic kidney disease (CKD-MBD) is complex and multifactorial [10] but is partly explained by impaired bone remodeling [11]. Animal models have provided some insights into the pathophysiology of CKD-MBD [12, 13], but their impact on our understanding of the cellular mechanisms remains to be defined. The development of *in vitro* models has permitted to mimic human bone-related diseases as an alternative to *in vivo* models [14, 15]. Taken together, animal models and studies using PBMCs can offer complementary insights to improve the understanding of CKD pathophysiology.

Since 1997, osteoclasts have been generated in cell cultures, notably when culturing peripheral blood mononuclear cells (PBMCs) with M-CSF and RANK-L, without the need for osteoblasts or stromal cells [16]. These models may improve the global understanding of bone pathophysiology. They can be used not only to evaluate the direct effects of cytokines, hormones, cells, or drugs on bone remodeling during CKD-MBD, but also in peculiar genetic renal diseases inducing specific bone impairment. Using human PBMCs from healthy controls and patients, a functional impairment of osteoclasts has indeed been reported, notably in children with CKD-MBD and in patients with nephropathic cystinosis [15, 17].

The aims of this review are the following: (a) to describe the methods that can be used to obtain functional and mature osteoclasts from PBMCs obtained directly in pediatric and adult patients, (b) to summarize the data obtained with this model in two peculiar diseases, and (c) to highlight future research possibilities in the field.

Mature and Functional Osteoclasts Derived from PBMCs Obtained Directly in Patients

Before recombinant RANKL, osteoclast generation derived from PBMCs was obtained with co-culture of osteoblastic

cells [18], or with human osteoclasts isolated from human osteoclastomas, also known as benign bone giant cell tumors [19]. Since RANKL identification as the osteoclast differentiation factor in 1996 [20, 21], a non-invasive technique was developed to differentiate isolated PBMCs into mature and functional osteoclasts from blood samples [22, 23].

After collection, blood is diluted with an equal volume of phosphate buffered saline (PBS) buffer and loaded on a lymphocyte separation medium density gradient to purify mononuclear cells. Mononuclear cells are isolated by density gradient centrifugation using Ficoll and then centrifugated through a 50 % Percoll gradient. The light density fraction from the pellet is recovered and incubated for 10 min at room temperature in 3 % human serum-PBS. After isolation, flow cytometry and immunohistochemistry are used with antibodies directed against markers to isolated PBMCs and obtain MSCs and HSCs [24]. By the depletion of CD19 (J3-119), CD3 (UCHT1), CD56 (C218), and CD235a (11E4B-7-6), using specific mononuclear antibodies, mononuclear cells are obtained for osteoclast precursors and CD3, CD19, CD38, and CD66b for osteoblast precursors [25, 26]. However, these techniques used a significant amount of total blood (i.e., 200 to 250 mL of total fresh blood) to obtain osteoclasts, thus precluding their use in patients [26, 27].

Therefore, we have developed in the lab a simplified technique allowing us to obtain osteoclasts using only 20 to 25 mL of total blood using Ficoll without Percoll [15, 17]. Monocytes can be purified from fresh samples of peripheral total blood put into a tube with anti-coagulant (e.g., EDTA or citrate). This specific point is particularly relevant for clinical studies in patients, since ethical committees, all over the world, ask researchers to respect a maximal volume for sampling depending on body weight as well as a maximum volume of blood sampling per period of 30 days (including both daily clinical care and research), for example, in France 20 mL and 40 mL, respectively, in a child weighing 10 kg, or 50 and 100 mL, respectively, in a child weighing 25 kg. In an adult of 60 kg, the maximal volume per sample is 120 mL [28]. This PBMC model is easier to obtain, using blood instead of collecting bone marrow. Osteoclasts may also come from the bone marrow, but bone biopsies are not routinely performed in pediatric clinical practice. Furthermore, functional osteoclasts whether derived from bone marrow or peripheral blood do not present differences in terms of resorption activity [29]. This method is less constraining than animal models. For example, 5/6th subtotal nephrectomy rat models, that is an approach widely used to mimic human CKD, need an addition of high phosphorus and lactose diet for 10 weeks to obtain CKD-MBD animal models [30]. PBMCs models permit to avoid interspecies differences such as the differential regulation of VDR expression between humans and mice [31]. Table 1 summarizes the pros and cons of different CKD-MBD models.

Table 1 Pros and cons of different animal models of CKD-MBD [30]

Animal models	Pros	Cons
5/6th nephrectomy rats combining with high phosphate and lactose diet	Robust functional readouts such as mineralization defects Reliable induction in rodents	Difficult surgery Long time course (10 weeks)
Electrocautery mouse models	Variable severity of CKD High turnover renal osteodystrophy	Two-step and difficult procedure
Adenine-containing diet	Nephrolithiasis and renal failure Vascular calcifications	4-week treatment
Gene knockout mice	Several diseases with CKD-MBD phenotype and natural evolution (polycystic kidney disease, KO of 1- α -hydroxylase, etc.)	Long time course Interspecies differences
Mouse with unilateral ureteral obstruction	Model of tubulointerstitial fibrosis with chronic renal failure Skeletal renin-angiotensin system exploration	Difficult procedure Long time course

Despite humans and mice share many similarities in their genomes, anatomy, and physiology, one should be aware that there are also differences. It seems that the regulation of VDR expression is one of these differences.

Monocytes are cultured at 37°C in 5% CO₂ using a minimum essential medium supplemented with 2 mM L-glutamine, 100 U/mL penicillin, 100 Ig/mL streptomycin, and 10 % fetal bovine serum. Monocytes are seeded in the presence of M-CSF and RANKL from day 1 to the end of the differentiation in order to differentiate them into functioning osteoclasts. Medium and cytokines are changed every 3 days. At the end of the osteoclastic differentiation process (6 to 7 days in culture), the cells are fixed with 4% of paraformaldehyde (PFA) and stained for tartrate-resistant acid phosphatase (TRAP) using a leukocyte acid phosphatase assay according to the manufacturer's instructions and observed in white light microscope. Then, DAPI staining (4',6-diamidino-2-phenylindole) could be perform to color osteoclast nuclei and revealed by blue fluorescence microscope using UltraViolet 800ms filter. Stained-positive cells (>3nuclei) are then counted and classified by nuclei per osteoclast (3–5 nuclei; 5–10 nuclei; 10 nuclei). Our lab uses a semi-automatic cells counting software using superimposing images between white and fluorescence lights.

Of note, several growth factor cocktails can be used to differentiate PBMCs into functional osteoclasts. Tumor necrosis factor- α (TNF α) plays an important role in osteoclastic differentiation without RANKL binding and could be used for human PBMC models in presence of M-CSF [32]. The use of dexamethasone and transforming growth factor beta (TGF- β) increases the number of nuclei with an higher osteoclastic potential from PBMCs [29].

After the osteoclast differentiation process, the obtained osteoclasts can also be detached from plastic plates. Then, they are replated on mineralized plates and cultured at 37°C in 5% CO₂ with the same medium used for differentiation in the presence of M-CSF and RANKL. After 2 to 3 days of additional culture, cells on their mineralized substrates are

lysed and plated in a 5% silver nitrate solution to quantify resorption. In parallel a TRAP staining is performed to quantify multinucleated osteoclasts, allowing the measurement of the resorbed area per osteoclast. Results of resorption can be expressed as resorbed area per well and as resorbed area per osteoclast. Figure 1 illustrates the main experiments that can be performed from this model.

Impact of Inflammatory Context in CKD on Osteoclast Formation

Changes in mineral metabolism during CKD, and notably hypocalcemia, hyperphosphatemia, and decreased 1-25(OH)₂ vitamin D (1.25-D) levels, induce secondary hyperparathyroidism (SHPT), characterized by parathyroid gland hyperplasia and subsequent high serum parathyroid hormone (PTH) levels [11]. SHPT contributes to the complex CKD-MBD, including ectopic calcifications and renal osteodystrophy [11]. SHPT has also been associated with impaired longitudinal growth, fractures, anemia, left ventricular hypertrophy, and increased mortality [33]. The primary bone lesion in CKD, at least in patients reaching end-stage renal disease (ESRD) without any previous management, is the high-turnover osteodystrophy (*osteitis fibrosa*), because of SHPT with low 1.25-D levels [11]. Non-mineral factors also play a pivotal role in CKD-MBD pathogenesis and notably iron deficiency, erythropoietin, and inflammation that all contribute to increase fibroblast growth factor 23 (FGF23) levels [34, 35], with an indirect effect on mineralization and osteoclastic activity through altered circulating phosphate and 1.25-D concentrations [36, 37].

During CKD progression, metabolic changes are observed with enhanced oxidative stress, increased pro-inflammatory cytokines, and impaired immunity [38, 39]. Similarly to what is observed in inflammatory diseases such as rheumatoid arthritis, inflammatory cytokines are produced by CD14⁺CD16⁺ monocytes in CKD, regulating osteoclastic

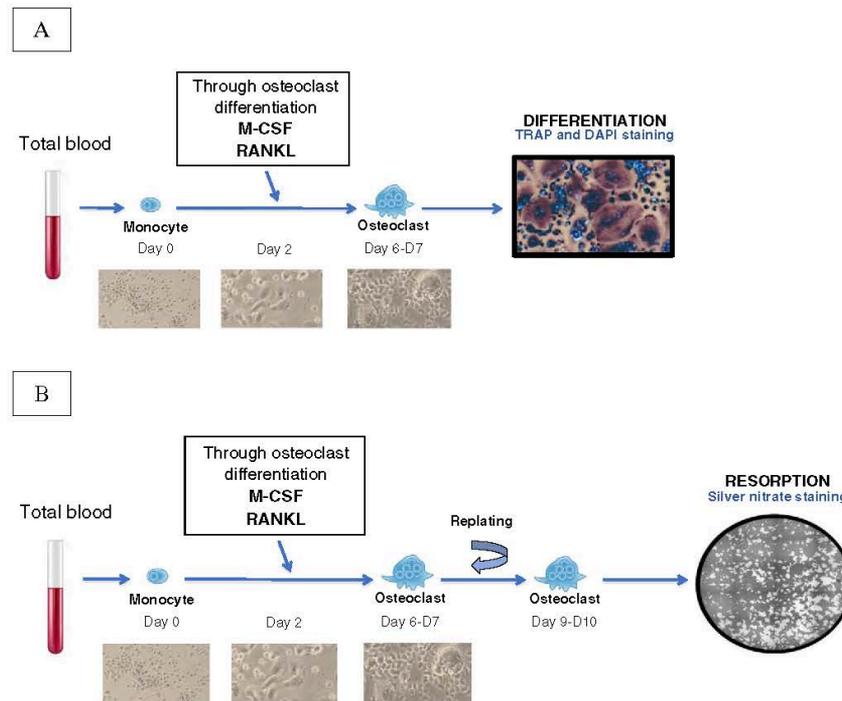


Fig. 1 How to obtain functional osteoclasts from circulating PBMCs in humans? Monocytes are purified from 15 to 20mL of peripheral blood and differentiated into osteoclasts in the presence of M-CSF and RANK-L in 6 to 7 days. At the end of the differentiation process, mature osteoclasts are counted after TRAP and DAPI staining (a). To evaluate osteoclastic activity after normal osteoclastic differentiation, mature

osteoclasts are replated on mineralized plates in the presence of M-CSF and RANK-L for 7 additional days. To quantify resorption, cells are lysed, and plates are stained in a 5% silver nitrate solution to evaluate resorbed areas. In parallel, TRAP staining is performed to quantify multinucleated osteoclasts, allowing the expression of resorption activity as “resorbed area per osteoclast” (b)

differentiation and activity [40, 41]. In 2018, Cafiero et al. observed spontaneous osteoclastogenesis with a significant increase in osteoclastic formation and bone resorbing activity in PBMCs obtained from late-stage CKD and dialysis adult patients ($N=20$ and 18, respectively) when compared with early-stage CKD patients ($N=12$) and healthy donors ($N=10$), likely due to an increased expression of the pro-osteoclastogenic inflammatory RANKL and LIGHT (also known as tumor necrosis factor superfamily member 14, TNFSF14) [42]. These findings underline the association of CKD mineral and bone disorders in CKD-MBD.

Osteoclastogenesis Is Impaired by CKD-Related Toxic Factors

Uremic toxins, among them indoxyl sulfate, accumulate in CKD and contribute to skeletal resistance to PTH [43]. In a murine *in vitro* model, Mozar et al. observed that indoxyl sulfate is an inhibitor of both osteoclastic differentiation and bone-resorbing activity, in a dose-dependent manner; this effect was enhanced in the presence of inorganic phosphate [44,

45], thus suggesting a direct role of uremic toxins in the pathophysiology of bone disease and impaired bone remodeling in CKD. Furthermore, Liu et al. showed that indoxyl sulfate regulates osteoclast differentiation via binding to AhR (aryl hydrocarbon receptor), thereby influencing NFATc1 in a dose- and time-dependent manner [46]. This indoxyl sulfate/AhR/NFATc1 pathway could prevent CKD-MBD, and AhR antagonists may be used as novel drugs for renal osteodystrophy.

Recently, the interest of miRNAs, small non-coding RNAs that negatively regulate gene expression, has dramatically increased in medicine [47]. PBMCs were used to study the role of miRNAs during osteoclastogenesis: in a murine *in vitro* model, M'Baya-Moutoula et al. reported that high levels of inorganic phosphate induced upregulation of miR-223 and consequently could increase osteoclastogenesis as evidenced by osteoclast number and TRAP activity [48]. Conversely, the transfection of an anti-miR-223 agent decreased osteoclast differentiation. In CKD-MBD, these results may be particularly relevant, since miR-223 may also promote vascular calcification through smooth muscle cell trans-differentiation into osteoblast-like cells [49]. It is noteworthy that premature

vascular senescence is now recognized as a hallmark of CKD since childhood [49] and may contribute to explain the dramatic cardiovascular mortality of CKD patient.

The optimal management of CKD-MBD is a daily challenge for physicians, and PTH control is crucial in this setting, the aim being to maintain PTH levels within the target range [10]. Active vitamin D analogs and calcimimetics (e.g., cinacalcet or etelcalcetide) are cornerstones of SHPT management [50–52]. Vitamin D analogs are used as first-line therapy, at least in pediatrics [51]. However, there are pros and cons to their use: even though they are non-expensive, well-known, and available (almost) everywhere, with beneficial global effects notably on the cardiovascular system [53], they may also induce hyperphosphatemia, low bone turnover, vascular calcifications, growth retardation, and last but not least increased FGF23 levels, that have been extensively shown in CKD as a risk factor for cardiovascular morbidity, infections, and global mortality, among other deleterious effects [53]. Specifically for bone outcomes, an histomorphometric study performed in a cohort of 60 pediatric patients undergoing maintenance dialysis receiving vitamin D analogs showed the persistence of mineralization and bone defects, despite suppressed PTH levels, thus questioning the overall positive effects of active vitamin D analogs on the bone [54]. On the other hand, 1.25-D decreases, *in vitro*, osteoblast proliferation and promotes osteoblast differentiation through collagen type I synthesis and osteocalcin secretion [55]. It also increases alkaline phosphatase activity and mineralization of bone matrix synthesized by human osteoblasts [56, 57]. We previously demonstrated a significant and major inhibitory effect of 1.25-D on osteoclastic differentiation with no effect on resorption activity in osteoclasts derived from PBMCs obtained in healthy volunteers [26]. We also showed biphasic effects of FGF23 on osteoclast physiology, inhibiting osteoclast formation while stimulating slightly osteoclast activity [26], that may be relevant to explain part of the underlying CKD-MBD pathophysiology.

We recently evaluated the direct effects of vitamin D analogs and/or calcimimetics on osteoclastic differentiation and resorption activity in PBMCs obtained from pediatric patients at different CKD stages and healthy controls [15]. We confirmed that 1.25-D is a powerful inhibitor of osteoclastogenesis in a dose-dependent manner in healthy controls, but we also demonstrated a resistance to 1.25-D when renal function worsened, with a significant resistance to 1.25-D in advanced CKD and a trendily decrease in VDR expression in patient's osteoclasts [15]. In this RENOCLAST study (ID-RCB 2017-A03241-52), there was a significant albeit less important inhibitory effect of calcimimetics (namely KP2326, a peptide analog of etelcalcetide) on osteoclastic differentiation, observed in both healthy controls and patients, which was not modified by CKD stage. No synergistic effect was observed when co-treating cells with both 1.25-D and KP2326. Last,

unlike 1.25-D [26], KP2326 also inhibited human osteoclast-mediated bone resorption [15]. On the other hand, Diaz-Tocados et al. reported the effects of calcimimetics in osteoblasts and showed that calcimimetics have a direct anabolic effect on bone, through the stimulation of osteogenic differentiation and mineralization in human MSCs *in vitro* [58]. They also found that calcimimetics increased osteoblast number, osteoid surface, and bone formation [58]. Thus, taken together, all these experimental data point towards the use of combined low doses of 1.25-D and calcimimetics in SHPT in dialysis to prevent low bone turnover. Moreover, clinical data showed that this association may slow the progression of coronary artery calcification as compared to high doses of active vitamin D in the ADVANCE trial [59]. This hypothesis obviously requires confirmatory clinical studies, but all these data obtained in PBMCs and MSCs clearly improve our understanding of CKD-MBD.

Bone Cell Models to Understand Bone Disorders in Nephropathic Cystinosis

In addition to CKD-MBD, several genetic renal diseases may induce direct specific bone lesions, among them primary hyperoxaluria type 1 [60, 61], nephropathic cystinosis [62], autosomal dominant polycystic kidney disease [63, 64], Pierson syndrome [65], and ciliopathies associated with skeletal disorders [66]. The underlying pathophysiology obviously differs depending on the disease, from oxalate crystals deposits in the bone marrow vicinity to abnormal primary cilium in the osteocyte [67].

We will discuss here further bone impairment in nephropathic cystinosis (NC), since the use of our simplified PBMCs/osteoclast models directly in patients have allowed a better understanding of what is now called cystinosis metabolic bone disease (CMBD) [62]. Indeed, NC is a rare autosomal recessive lysosomal storage disease due to a deficiency of the cystine lysosomal transport protein cystinosin (CTNS), inducing a systemic accumulation of cystine crystals and further tissue damage and organ dysfunction. Extra-renal complications mainly include hypothyroidism, hypogonadism, myopathy, pancreatic exocrine dysfunction, ocular symptoms (e.g., corneal crystals and photophobia), and bone impairment [68].

The initial description of bone impairment in this disease consisted of a small series of bone biopsies in NC reporting unusual areas of bone resorption in these patients [69], and the publication of two pilot studies confirming a high prevalence of bone symptoms (i.e., pain, deformations, scoliosis, and fractures) with cortical impairment in patients with NC [70, 71], as well as impaired mineralization in most patients [71]. Following these reports and because of the significant deleterious impact on patients' quality of life of this complication, the 2019 international guidelines proposed clinical practice

points for both the diagnosis and management of CMBD [62]. The underlying pathophysiology of CMBD is complex and multifactorial but remains to be fully defined [62].

In addition to “conventional” CKD-MBD before and after transplantation, CMBD can be explained by a combination of factors: (1) renal Fanconi syndrome and bone sequelae of hypophosphatemic rickets of the infancy, one of the main drivers of mineralization defects; (2) deficiency in nutrition and micronutrition, among them copper deficiency; (3) hormonal disturbances such as hypothyroidism, hypogonadism, hypoparathyroidism, and resistance to growth hormone; (4) myopathy; (5) cysteamine toxicity; and (6) intrinsic osteoblastic and osteoclastic defects directly due to *CTNS* mutation [17, 71].

In 2015, Conforti et al. reported that MSCs extracted from bone marrow and of patients with NC display a reduced ability to differentiate into osteoblasts, this later effect being reverted with cysteamine treatment [72]. We further confirmed in a model of murine MSC-derived osteoblasts that in vitro low doses of cysteamine also stimulate osteoblastic differentiation and mineralization, however, with an inhibitory effect at higher doses, likely explaining, at least partly, the bone toxicity observed in patients receiving high doses of cysteamine [17]. Study of human osteoclasts differentiated from PBMCs obtained from healthy donors and patients with NC showed that *CTNS* is required for proper osteoclastic differentiation and resorption activity, with a progressive increase of *CTNS* gene expression during osteoclastogenesis and with a peak observed at day 6 on mature osteoclasts following the same pattern that cathepsin K is a late marker of osteoclastic differentiation crucial for further bone resorption [17]. In culture conditions, an increased number of smaller osteoclasts are observed in cells derived from NC patients (as opposed to healthy controls), despite the fact that they are generated with the same number of monocyte progenitors, suggesting that cystinosis might be a physiological negative modulator of osteoclast formation [17, 70]. Related to this finding, previous studies revealed that cystinosis could be a part of the regulatory complex of mTORC1, interestingly also known to play a role in skeletal development, and that has to be downregulated in the course of osteoclast formation [73, 74]. We evaluated the resorption capacity of these cells after a normal differentiation process, showing that resorption activity is decreased in osteoclasts issued from patients (as compared to healthy controls), with decreased cathepsin K transcript levels [17]. Thus, in vitro, cystinosis deficiency increases osteoclastogenesis (namely increased number of smaller multinucleated cells) but impairs bone resorption activity of osteoclasts derived from human PBMCs. Cysteamine has no effect on osteoclastic differentiation whatever the doses tested [17], but a beneficial anti-resorptive effect was observed in healthy controls, with a 50% inhibition of the resorption activity with 50- μ M cysteamine being observed. A similar effect was observed in cells derived from NC patients, however, significant only at higher doses (i.e., 200 μ M) [17]. Taken together, even

though the content in cystine cells was not measured, these results suggest that neither osteoclast differentiation nor resorption activity is dependent on cystine efflux from lysosomes but may instead rely on cystinosis itself or its interaction with other cellular pathways [75].

How PBMCs-Derived Cells Could Be Involved in Other Rare Renal Diseases Inducing a Peculiar Bone Phenotype?

Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common inherited kidney diseases characterized by development of fluid cysts into kidneys leading to CKD [76]. ADPKD results from a mutation in either *PKD1* (encoding polycystin 1) or *PKD2* (encoding polycystin 2) [66, 76]. Polycystins are expressed both in osteoblasts and osteocytes on the primary cilium [77, 78]; the loss of PKD1 in mice is associated with abnormal bone development and osteopenia because of impaired osteoblastic differentiation through deregulated Runx2-related transcription factor 2 (Runx2) [78, 79]. Evenepoel et al. reported that patients with end-stage renal disease due to ADPKD display suppressed bone turnover, with a better preserved cortical bone mineral density as well as high sclerostin and FGF23 levels [63]. In ADPKD pediatric patients at early CKD stages, hypophosphatemia and suppressed bone formation were observed as compared to healthy controls [80]. Recently, Gitomer et al. confirmed a peculiar bone impairment at early CKD stages in a cohort of 472 ADPKD patients from the HALT-PKD study and 472 non-ADPKD control patients from the CRIC study, with decreased alkaline phosphatase and increased FGF23 levels in ADPKD [64]. In a sub-study comparing 20 bone biopsies from ADPKD patients at a mean glomerular filtration rate of 97 mL/min per 1.73 m² to 17 healthy controls, a significantly lower osteoid volume/bone volume and bone formation rate/bone surface was observed [64]. Immunohistochemical staining confirmed increased expression of FGF23 in ADPKD bone samples compared to controls which supports possible dysregulation of osteocytes in ADPKD [64]. All these clinical data suggest rather osteocytic dysfunction in ADPKD, but the question whether PC1 and PC2 mutations may directly affect osteoclastic biology remains open. Of note, an abnormal orthodontic tooth movement was demonstrated in a PC1/Wnt1-cre murine model, with the absence of osteoclasts on the bone surface; the authors discussed a difference in tooth movement and osteoclast activity between PC1 mutant mice and controls in response to orthodontic force [81]. However, studies using PBMCs to evaluate whether osteoclast impairment and aberrant osteoclastic signaling pathways in ADPKD can be observed would probably not be relevant, since the presence of the primary cilium has never been evidenced in osteoclasts on one hand and since polycystin 1 and polycystin 2 are not expressed in osteoclasts (personal unpublished data and [82]) on the other hand.

Another disease that may benefit from PBMCs experimental studies can be X-linked hypophosphatemia, a proximal tubulopathy inducing renal phosphate loss because of PHEX mutations further increasing FGF23 levels [83]. Indeed, because of the association between FGF23 and inflammation on one hand [83], and because of the key role of osteoclasts in osteoimmunology on the other hand [83], it may be interesting to better characterize these cells from XLH patients.

Conclusion

Over the past decade, alternative *in vivo* models using PBMCs have allowed to better understand the underlying pathophysiology in CKD and rare genetic renal diseases displaying a specific bone impairment. However, several confounding variables, such as inflammation, anemia, hormonal status, and treatment regimens, could limit the interest of PBMCs model, through a “priming” of the cells even *in vitro*. The description of the role of miRNAs in osteoclastogenesis may open perspectives for future therapeutic development [47]. *In vitro* 3D bone models could provide a personalized approach with minimal invasiveness for patients with bone diseases [84]. The global understanding of CKD-MBD becomes more and more complex: indeed, it includes biological abnormalities (such as hormonal resistance and modifications of pathways crucial for bone and vessels), therapies affecting differently FGF23 levels, the uremic milieu, the Framingham cardiovascular risk factors, nutrition, intrinsic factors (e.g., anemia, iron deficiency, acidosis, oxidative stress, and inflammation, among others), and the genetic renal disease *per se*. Taking into account all these components is a real challenge in daily practice and may be the first step for a personalized medicine, even in the field of “CKD-MBD.” In this setting, PBMCs are a promising non-invasive tool to evaluate the osteoclastic component of the cascade.

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Declarations

Conflict of Interest JBa has been and is an investigator for the Amgen-sponsored trials on the use of cinacalcet and etelcalcetide in pediatric dialysis. JBa is the coordinator of the Amgen-sponsored international registry on the use of cinacalcet in pediatric dialysis.

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

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2. Patients et méthodes : étude de l'utilisation hors AMM en MRC

Une première étude rétrospective a été réalisée sur une cohorte de 26 enfants dialysés de moins de 3 ans, traités par cinacalcet pour une hypercalcémie et/ou une sHPT. Ces patients étaient issus de 35 centres de dialyse pédiatrique, affiliés à l'European Society of Pediatric Nephrology et au Réseau européen de référence pour les maladies rénales rares (ERKNet).

La deuxième étude s'est intéressée à l'effet du cinacalcet dans le traitement de sHPT persistante chez des enfants ayant reçu une greffe rénale. Une analyse rétrospective a été réalisée à partir de l'ensemble des données du registre *Cooperative European Paediatric Renal TransplAnt Initiative* (CERTAIN), incluant 20 patients provenant de divers centres européens.

Pour ces deux études, dès l'instauration du traitement par cinacalcet et sur une période minimum d'un an, les données cliniques et biologiques ont été rapportés à différents temps de suivi (Initiation ;1mois ; 3 mois 6mois ; 9 mois ; 12 mois et dernier suivi), comprenant des mesures du calcium total, du phosphate, de la PTH, des phosphatases alcalines (PAL), et des concentrations de vitamine D. Ces études ont respecté les directives de la Déclaration d'Helsinki et les bonnes pratiques cliniques européennes.

3. Patients et méthodes : étude de l'utilisation hors AMM en hyperparathyroïdie primaire (HPP) pédiatrique

Une analyse rétrospective multicentrique a été réalisée dans sept centres de références des maladies rares du métabolisme du calcium et du phosphate en France. Une cohorte de 18 enfants et adolescents atteints d'HPP a été étudiée. Des analyses biochimiques de routine portant sur le calcium, le phosphate, le magnésium, les PAL, la PTH, la créatinine, la 25-OH vitamine D et le calcium urinaire ont été réalisés à différents temps de suivi (Initiation ;1mois ; 3 mois 6mois ; 12 mois et dernier suivi).

4. Analyses statistiques

Pour l'étude Renoclaste, des tests non-paramétriques, en fonction du nombre de groupes à comparer, type Mann-Whitney ou Kruskal-Wallis suivis du test post-hoc de Dunn, ont été utilisés pour la comparaison des paramètres biologiques aux différents temps de suivi. Une analyse bivariée de Spearman a permis de rechercher une corrélation entre le pourcentage d'inhibition induit par la vitamine D et le DFG de chaque patient. La significativité des résultats était obtenue lorsque le p était inférieur à 0.05 pour tous les tests réalisés. Les valeurs des paramètres biochimiques de routine ont été exprimées soit comme médiane avec intervalle interquartile (1^{er} et 3^{ème} interquartile), soit comme médiane avec valeurs minimales et maximales.

En ce qui concerne les études rétrospectives de l'utilisation du cinacalcet (MRC, post-transplantation rénale et hyperparathyroïdie pédiatrique, des tests de Kruskal-Wallis, des modèles mixtes linéaires ANOVA et des tests de Mann-Whitney ont été réalisés, grâce à l'utilisation du logiciel GraphPad afin d'obtenir des informations complètes sur l'impact du traitement au cinacalcet par comparaison des paramètres biologiques aux différents temps de suivi. La significativité des résultats était obtenue lorsque le p était inférieur à 0.05 pour tous les tests réalisés. Les résultats étaient présentés comme médiane avec intervalle d'interquartile (1^{er} et 3^{ème} interquartile).

IV- RÉSULTATS

1. Effets directs du KP2326 sur les ostéoclastes en MRC : publication scientifique N°1 : étude RENOCLASTE

Contexte /objectif

Dans un modèle murin d'hyperparathyroïdie secondaire sur rachitisme par anomalie de la 1 α -hydroxylase, le cinacalcet, donné par voie systémique, corrige la PTH, augmente le nombre d'ostéoclastes, mais ne normalise pas les atteintes de la minéralisation osseuse [93]. Une première étude, en 2003, a étudié l'effet du cinacalcet chez des témoins sains humains, sans retrouver d'effet significatif ni sur les ostéoblastes ni sur les ostéoclastes ; dans ce travail, les auteurs ne retrouvaient en effet dans leurs modèles cellulaires pas d'expression du CaSR [94]. Plus récemment, il a été démontré que l'exposition précoce au cinacalcet inhibe la différenciation ostéoclastique et diminue le nombre d'ostéoclastes issus de monocytes humains sains, tout en inhibant leur activité de résorption [95]. Le **tableau 2**, résume ci-dessous, les effets du cinacalcet *in vitro* et *in vivo*, dans différents modèles expérimentaux.

Auteur, ref	Année	Modèles expérimentaux	Effets du cinacalcet
Goltzmann D, [93]	2010	Modèle murin d'hyperparathyroïdie secondaire ; perte de fonction de la 1 α hydroxylase ; <i>in vivo</i>	Administration par voie systémique ; correction de la PTH ; augmentation du nombre d'Oc ; persistance d'anomalies de la minéralisation osseuse
Padagas J, [94]	2003	Modèle humain de témoins sains ; <i>in vitro</i>	Absence d'effets sur les Oc et les Ob ; absence d'expression du CaSR dans ces cellules
Keller AN, [95]	2018	Modèle humain de témoins sains ; <i>in vitro</i>	Après exposition précoce au cinacalcet ; inhibition de la différenciation ostéoclastique et de l'activité de résorption

Tableau 2. Effets du cinacalcet

Présentation des effets du cinacalcet sur l'ostéoclastes ; description des modèles expérimentaux *in vitro* et *in vivo* utilisés. Oc : ostéoclaste ; Ob : ostéoblaste

Tout comme le cinacalcet, l'etelcalcetide diminue les concentrations de PTH dans un modèle murin d'hyperparathyroïdie secondaire [78]. En 2017, un essai clinique randomisé, réalisé en double placebo et double aveugle, sur 683 patients dialysés a confirmé la non-infériorité de l'etelcalcetide par rapport au cinacalcet, avec une réduction de plus de 30% des concentrations moyennes de PTH [96]. Enfin, il a été démontré que ce traitement réduit le remodelage osseux et le défaut de minéralisation en améliorant la structure de l'os cortical chez le rat néphrectomisé avec une hyperparathyroïdie

secondaire [79]. Cependant, aucune étude, à notre connaissance, n'a évalué le potentiel rôle direct de l'etelcalcetide sur l'ostéoclaste.

L'objectif global de cette publication est d'évaluer l'impact direct sur l'ostéoclaste de la 1a,25(OH)₂D et le calcimimétique KP2326, deux traitements les plus fréquemment utilisés dans la prise en charge du CKD-MBD chez des patients pédiatriques avec MRC à différents stades, après avoir évalué leur effet propre chez des témoins sains. Ces résultats ont permis ainsi de mieux comprendre la physiopathologie du CKD-MBD et d'améliorer la qualité de vie des patients et de diminuer la morbi-mortalité.

Sur la base des observations décrites en introduction concernant les effets de la 1a,25(OH)₂D, notre première hypothèse de travail était la suivante : lorsque la MRC progresse et que le DFG diminue, la 1a,25(OH)₂D inhibe la différenciation ostéoclastique mais de manière moins importante que lorsque la fonction rénale est normale.

Pour évaluer cette hypothèse, le plan expérimental choisi a été le suivant : dans un premier temps, nous avons évalué l'impact de la 1a,25(OH)₂D sur la différenciation ostéoclastique à partir de monocytes issus de témoins sains, en ayant pour objectif de trouver le seuil de 1a,25(OH)₂D suffisant pour ralentir la différenciation sans pour autant l'inhiber totalement (étude dose-réponse). Notre équipe de recherche a en effet développé et validé une technique de différenciation des monocytes circulants en ostéoclastes matures et fonctionnels à partir de sang périphérique de patients (15 à 20 mL) [67, 97]; cependant, ce faible volume ne permet pas d'étudier de nombreuses conditions. Le pré-requis était donc de définir la dose optimale de 1a,25(OH)₂D à évaluer dans les cellules issues de patients avec IRC. Dans un deuxième temps, nous avons réalisé l'étude de la différenciation ostéoclastique chez les patients pédiatriques atteints d'IRC, avec la dose retenue de 1nM.

La deuxième hypothèse de travail était la suivante : l'etelcalcetide inhibe la différenciation ostéoclastique et l'activité de résorption. Cet effet biologique est moindre lorsque l'IRC progresse et le DFG diminue.

Pour évaluer l'impact du KP2326 sur la différenciation ostéoclastique et la résorption osseuse, nous avons d'abord étudié son effet sur des monocytes de témoins sains, aux doses allant de [10⁻⁹M] à [10⁻⁴M]. Nous avons ensuite analysé la différenciation ostéoclastique de monocytes issus de patients pédiatriques atteints de MRC à des doses de 10⁻⁷M et 10⁻⁶M. Enfin, nous avons exploré l'effet combiné

du KP2326, à différentes concentrations, et de la 1 α ,25(OH) $_2$ D (1nM) sur des témoins sains, avant d'examiner leur potentiel effet synergique chez les patients IRC pédiatriques.

Résultats

Au total, 6 donneurs sains et 19 patients pédiatriques atteints de maladie rénale chronique (MRC) ont été inclus dans l'étude RENOCLASTE. L'effet inhibiteur de la 1 α ,25(OH) $_2$ D a été confirmé et la concentration à 1nM a été choisie pour l'analyse de l'effet sur les monocytes issus de patients avec MRC pédiatrique. Cette concentration permet d'inhiber la différenciation ostéoclastique sans la supprimer complètement. Le KP2326 inhibe de manière significative la différenciation ostéoclastique chez les témoins sains et les patients sans effet-dose (**Figure 9**). Cependant, 1 α ,25(OH) $_2$ D s'est avérée être un plus puissant inhibiteur de l'ostéoclastogénèse que le KP2326. Les analyses ont également montré que le KP2326 active la voie Erk des ostéoclastes. Une résistance à la 1 α ,25(OH) $_2$ D a été observée chez les enfants atteints de MRC, avec une diminution de l'inhibition de l'ostéoclastogénèse lorsque la MRC progresse. Enfin, bien que la combinaison de 1 α ,25(OH) $_2$ D et KP2326 ait inhibé la différenciation ostéoclastique chez les enfants atteints de MRC, aucun effet synergique n'a été observé après traitement combinatoire (1 α ,25(OH) $_2$ D +KP2326).

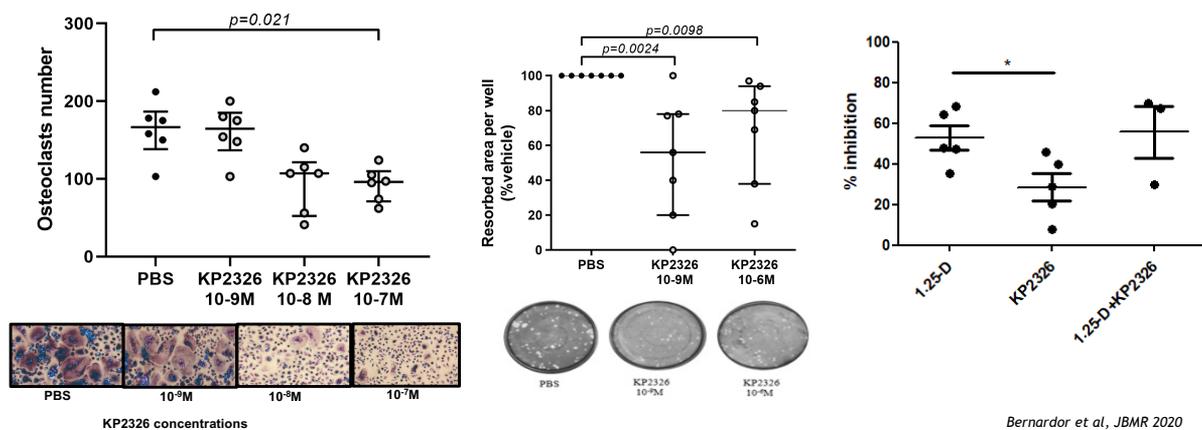


Figure 9 : Effets directs du KP2326 sur l'ostéoclastogénèse et la résorption osseuse chez le sujet contrôlé

Conclusions

L'étude RENOCLASTE a démontré que le 1 α ,25(OH) $_2$ D est plus efficace que le KP2326 dans l'inhibition de la différenciation ostéoclastique. Bien que le KP2326 ait montré une capacité à inhiber cette différenciation, son efficacité varie selon les individus et de la dose utilisée. De plus, une résistance à 1 α ,25(OH) $_2$ D a été identifiée chez les enfants atteints de MRC, en particulier ceux aux stades avancés de la maladie. La combinaison de 1 α ,25(OH) $_2$ D et KP2326 n'a pas montré d'effet synergique, suggérant

que leurs mécanismes d'action pourraient être indépendants l'un de l'autre. Ces découvertes soulignent l'importance de personnaliser les traitements pour les patients atteints de MRC, en tenant compte de la sévérité de leur maladie, de leur réponse individuelle aux médicaments et appuie l'utilisation de la vitamine D à faibles doses associée au calcimimétique.

Inhibition of Osteoclast Differentiation by 1.25-D and the Calcimimetic KP2326 Reveals 1.25-D Resistance in Advanced CKD

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ABSTRACT

Active vitamin D analogs and calcimimetics are the main therapies used for treating secondary hyperparathyroidism (SHPT) in patients with chronic kidney disease (CKD). Peripheral blood mononuclear cells of 19 pediatric patients with CKD1-5D and 6 healthy donors (HD) were differentiated into mature osteoclasts with receptor activator of NF- κ B ligand (RANKL) and macrophage colony-stimulating factor (M-CSF). The effects of single or combined treatment with active vitamin D (1.25-D) and/or calcimimetic KP2326 were evaluated on osteoclastic differentiation and osteoclast-mediated bone resorption. Although 1.25-D inhibited osteoclastic differentiation, a significant resistance to 1.25-D was observed when glomerular filtration rate decreased. A significant albeit less important inhibitory effect of KP2326 on osteoclastic differentiation was also found both in cells derived from HD and CKD patients, through a putative activation of the Erk pathway. This inhibitory effect was not modified by CKD stage. Combinatorial treatment with 1.25-D and KP2326 did not result in synergistic effects. Last, KP2326 significantly inhibited osteoclast-mediated bone resorption. Both 1.25-D and KP2326 inhibit osteoclastic differentiation, however, to a different extent. There is a progressive resistance to 1.25-D in advanced CKD that is not found with KP2326. KP2326 also inhibits bone resorption. Given that 1.25-D has no effect on osteoclastic resorption activity and that calcimimetics also have direct anabolic effects on osteoblasts, there is an experimental rationale that could favor the use of decreased doses of 1.25-D with low doses of calcimimetics in SHPT in dialysis to improve the underlying osteodystrophy. However, this last point deserves confirmatory clinical studies. © 2020 American Society for Bone and Mineral Research.

KEY WORDS: CHRONIC KIDNEY DISEASE; SECONDARY HYPERPARATHYROIDISM; OSTEOCLAST; ETELCACTETIDE; VITAMIN D

Introduction

Changes in mineral metabolism during chronic kidney disease (CKD) induce secondary hyperparathyroidism (SHPT), characterized by parathyroid gland hyperplasia and subsequent high serum parathyroid hormone (PTH) levels. SHPT has also been associated with impaired longitudinal growth, fractures, anemia, left ventricular hypertrophy, and increased mortality.⁽¹⁾ The primary bone lesion in pediatric CKD, at least in pediatric patients reaching end-stage renal disease (ESRD) without any previous management, is the high-turnover osteodystrophy (osteitis fibrosa) because of SHPT with low 1-25 OH₂ vitamin D (1.25-D) levels.⁽²⁾ SHPT results from global disturbances in

mineral metabolism, and notably hypocalcemia, hyperphosphatemia, and decreased 1.25-D levels, contributing to the complex CKD-associated mineral and bone disorders (CKD-MBD), including ectopic calcifications and renal osteodystrophy.⁽²⁾

The optimal management of pediatric CKD-MBD is a daily challenge, consisting mainly of correcting native vitamin D deficiency, keeping nutritional calcium within the age-recommended daily intake, decreasing phosphate levels (using nutritional management and phosphate binders), and keeping PTH levels within the target range.⁽³⁾ Active vitamin D analogs and calcimimetics (such as cinacalcet or etelcalcetide) are cornerstones of SHPT management, and their use has been recently delineated in the European pediatric guidelines.^(4,5) Conversely,

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low turnover osteodystrophy (adynamic bone) can be observed in children undergoing maintenance dialysis who receive too much calcium and/or vitamin D analogs,⁽⁶⁾ therefore requiring a close follow-up with regular therapeutic adjustments.⁽⁷⁾

The direct effects on bone of SHPT remain nevertheless to be determined, especially the effects of the two main therapies used in daily practice, namely vitamin D analogs and calcimimetics. We have already reported the effects of 1.25-D on osteoclastic differentiation and bone resorption in healthy volunteers, demonstrating a significant and major inhibitory effect on differentiation with no effect on resorption activity.⁽⁸⁾ In addition, a progressive resistance to 1.25-D in osteoblasts derived from patients with CKD has been reported when CKD worsens: In this context, 1.25-D decreases proliferation but increases mineralization.⁽⁹⁾

The calcium-sensing receptor (CaSR) plays a crucial role in calcium homeostasis; it is mainly expressed in parathyroid and tubular renal cells but also in bone cells such as chondrocytes, osteoblasts, and osteoclasts.⁽¹⁰⁾ In bone, the CaSR regulates growth plate formation and bone remodeling.⁽¹¹⁾ In animal models, mature osteoclasts undergo apoptosis in the presence of high levels of calcium,⁽¹²⁾ a dominant-negative CaSR construct abrogates this effect.⁽¹³⁾ In young animal models, the activation of CaSR in osteoclasts inhibits bone resorption, thus increasing bone anabolism.⁽¹¹⁾ In contrast, in older animal models, the activation of Ca-SR activates osteoblasts and bone formation but also receptor activator of NF- κ B ligand (RANKL) expression and therefore bone resorption by osteoclasts.⁽¹¹⁾

In a murine model of SHPT due to 1- α hydroxylase deficiency, the calcimimetic cinacalcet corrects PTH levels, normalizes osteoblastic activity, and increases osteoclastic number and activity.⁽¹⁴⁾ It was shown recently that cinacalcet has no effect on osteoclastic differentiation; however, it inhibits resorption activity in osteoclasts derived from healthy human peripheral blood mononuclear cells (PBMCs).⁽¹⁵⁾

We recently developed and validated a noninvasive technique to differentiate PBMCs into mature and functional osteoclasts from blood samples using only 15 mL of total blood allowing us to design patient-derived experimental studies.⁽¹⁶⁾ The objectives of this study were therefore to evaluate the direct effect in vitro of single or combined treatment with 1.25-D and etelcalcetide (and more precisely its peptide analog KP2326⁽¹⁷⁾) on human osteoclasts derived from PBMCs in both healthy controls and pediatric patients with CKD. We hypothesized that when the glomerular filtration rate (GFR) worsens (corresponding to an increase of CKD stages from 1 to 5), bone cells acquire a defective response or resistance to 1.25-D and etelcalcetide treatments that could explain the osteodystrophy observed in some patients.

Materials and Methods

Patients

The inclusion criteria were the following: pediatric patients between 2 and 18 years of age followed in our tertiary center for CKD stages 1 to 5D, with a body weight above 10 kg. The exclusion criteria were the following: renal disease known to induce a specific bone impairment (namely primary hyperoxaluria, cystinosis, Pierson syndrome, and paracellin mutations), and ongoing therapy with growth hormone, bisphosphonates, or teriparatide. The following clinical data were recorded: age, height, body

weight, therapies (and notably active vitamin D analogs or cinacalcet). The following biological data were recorded: creatinine allowing the calculation of the estimated glomerular filtration rate (eGFR) using the bedside 2009 Schwartz formula, calcium, phosphate (expressed as absolute value but also SDS for age), alkaline phosphatase (ALP), PTH (electrochemiluminescence immunoassay, Roche, Mannheim, Germany; local reference values 15 to 65 ng/L) and 25OH vitamin D (chemiluminescence immunoassay, IDS-Ilys-Multi-Discipline Automated System, Immunodiagnostic Systems, Boldon, UK; 75 to 120 nmol/L). The RENOCALST study (ID-RCB 2017-A03241-52) was approved by the IRB (Comité de Protection des Personnes Nord-Ouest II, session December 14, 2017, reference 2017/84). All parents and patients were informed and oral consent was obtained. Total blood from healthy controls was also obtained, with no record of clinical and biological parameters.

Primary osteoclast culture

Monocytes were purified from 15 to 20 mL of peripheral blood. Osteoclasts were obtained as previously reported.⁽¹⁶⁾ Osteoclasts were obtained by incubating monocytes with 20 ng/mL M-CSF (PeproTech, Rocky Hill, NJ, USA) and 35 ng/mL of RANKL (PeproTech) from day 1 to the end of differentiation. All PBMCs were treated with a combination of RANKL and M-CSF to differentiate them into functioning osteoclasts. From the first day on, osteoclastic precursors were treated with 1.25-D (Enzo Life Sciences, Farmingdale, NY, USA) (0.1 nM; 0.5 nM; 1 nM; 2.5 nM; 5 nM) and/or KP2326 (Amgen, Thousand Oaks, CA, USA) (10^{-9} M; 10^{-8} M; 10^{-7} M; 10^{-6} M; 10^{-5} M; 10^{-4} M) during the differentiation process. KP-2326 (Ac-c(C)rrrar-NH2) is a peptide analog of the calcimimetic etelcalcetide (Ac-c(C)arrar-NH2), which are related but clearly distinct branched peptides; the lowercase letters refer to D-amino acids and the uppercase letters refer to the natural L-amino acids (C, Cysteine; A, Alanine; R, Arginine).^(17,18) In fact, both of them are pro-drugs in the sense that the disulfide between D-Cysteine and L-Cysteine at the branch-point must be cleaved before the main chain D-Cysteine residue (c) can bind to the CaSR Venus Flytrap (VFT) residue Cys-482.⁽¹⁹⁾

Osteoclast functional assays

At the end of the osteoclastic differentiation protocol, the cells were harvested for real-time PCR analysis or were fixed with 4% of PFA and stained with leukocyte acid phosphatase (TRAP) staining kit according to the manufacturer's instructions (Sigma-Aldrich, St. Louis, MO, USA). Stained positive cells (>3 nuclei) were then counted. Osteoclast activity was assessed as previously described.⁽⁸⁾ Increasing of KP2326 (10^{-9} M; 10^{-6} M) were added at day 2 and maintained until the end. At day 7, to measure the surface of the resorbed matrix, osteoclasts were washed off with distilled water, and then the matrix was stained with a 5% (wt/vol) silver nitrate solution; the pits produced by osteoclasts appeared as white spots under a light microscope. Their surfaces were quantified by ImageJ software (NIH, Bethesda MD, USA).

Reverse transcription and real-time quantitative PCR (qRT-PCR)

RNA isolation and purification were obtained as previously described.⁽¹⁶⁾ Systematic controls were generated without

iScript and amplified; qPCR was carried out in triplicate or duplicate of triplicate for each data point. Primers for the amplification used in human osteoclasts were *hCaSR* (calcium sensing receptor), *hVDR* (vitamin D receptor). Expression of these genes was quantified and normalized to corresponding RNA L32 values and expressed as relative expression using the $2^{-\Delta\Delta Ct}$ method.

Western blot analyses

After osteoclast differentiation, pre-osteoclastic cells were serum starved in fetal bovine serum 0.1% during 2 hours at 37°C. Then, they were treated with KP2326 [10^{-6} M] at different times: T0 (control), T10, and T30. Cells were lysed and different steps used of Western blot as previously reported.⁽⁶⁾ Membranes were incubated, respectively, with ERK antibody (ref 4695, 1/1000, Cell Signaling Technology, Danvers, MA, USA) and P-ERK antibody (1/1000, ref 4695, 1/1000, Cell Signaling Technology) overnight at 4°C. The membrane was then washed 3 times with TBS-T and incubated with anti-rabbit Cayman (1/20,000, Sigma) secondary antibody for 1 hour at room temperature. After washing the membrane three times with TBST-5% milk, the images were revealed by radiography with the chemiluminescence revealing kit (ECL Amersham and ECL Prime Western Blotting detection reagent, GE Healthcare, Chicago, IL, USA). For each Western blot, the loading was controlled using a monoclonal anti- β tubulin antibody (clone TUB 2.1, Sigma). Western blot analyses were quantified using ImageJ software.

Statistical analyses

Statistical analyses for experimental data were performed using Prism 5.03 (GraphPad, La Jolla, CA, USA). Mann-Whitney tests were used to analyze data from experiments that comprised two groups. Kruskal-Wallis tests (p is given in the figure legends) followed by Dunn's post test (p result for columns comparison is given on the figures) were applied for multiple comparisons to analyze data from experiments containing more than two groups. Bivariate analyses were performed using Spearman correlations. In all cases, $p < 0.05$ was considered significant. Values were expressed as median with interquartile or median (min-max) for biochemical routine parameters.

Results

Nineteen pediatric patients with CKD1-5D and 6 healthy donors (HD) were included in this study: 5 patients were CKD 1-2, 4 were CKD 3, 5 were CKD 4-5, and 5 were undergoing maintenance dialysis. There were no differences for age among the different subgroups. Among CKD 4-5 and 5D patients, no patients received calcimimetics, but 5 and 3 patients received active vitamin D analogs, respectively. Supplemental Table S1 summarizes the main clinical and biological data obtained in patients that are relevant for the analysis of the *in vitro* studies presented below.

Optimal 1.25-D concentration to combine with calcimimetics for dual treatment of bone cells

The dual effect of 1.25-D and calcimimetics has not been tested directly on blood-derived monocytes. We have previously shown that 1.25-D had an inhibitory effect on osteoclast differentiation. Our aim here was to find an optimal 1.25-D concentration generating a significant inhibition of differentiation, without

suppressing it completely: a 1.25-D dose response on osteoclastogenesis was performed, as illustrated in Fig. 1A. Treatment at 1 nM of 1.25-D led to an average 50% decrease of osteoclastogenesis; consequently, this moderate concentration was chosen for further co-treatments. Fig. 1B illustrates the progressive decrease in VDR expression with osteoclastic differentiation, indicating that osteoclast progenitors are potentially more responsive than mature osteoclasts to 1.25-D treatment.

KP2326 inhibits osteoclastic differentiation and bone resorption and activates Erk1/2

To evaluate to what extent the KP2326 calcimimetic directly impacts osteoclastogenesis, a dose-response experiment has been conducted. Blood mononuclear cells from healthy donors were differentiated in presence of M-CSF and RANKL with increasing concentration of KP2326 (Fig. 2A). KP2326 significantly inhibited osteoclastic differentiation; however, the concentration responsible for the maximal inhibition differed greatly among 5 controls, from 10^{-9} M to 10^{-4} M, with a maximal inhibition of osteoclastogenesis varying from 8% with KP2326 10^{-5} M to 46% with KP2326 10^{-4} M. Globally, 1.25D was more effective to inhibit osteoclastic differentiation than KP2326 (Fig. 2B). Based on previous report showing an activation of Erk1/2 by the CaSR in osteoblasts,⁽²⁰⁾ we hypothesized that in osteoclasts derived from PBMCs, the CaSR activation by KP2326 could induce Erk activation. To this end, Western blot analyses were performed to detect Erk phosphorylation after KP2326 treatment in osteoclasts. Fig. 2C shows an activation of the Erk pathway at 10 and 30 minutes after treatment with KP2326.

Last, to explore the effect of KP2326 on osteoclastic activity, a resorption assay was performed. Mature osteoclasts generated under standard conditions were lifted and the same number of osteoclasts were seeded on mineralized plates with or without KP2326. Both low concentration (10^{-9} M) and high concentration (10^{-6} M) of KP2326 impaired resorption. Thus, unlike 1.25-D, KP2326 impaired both osteoclastic differentiation and resorption (Fig. 2D). As illustrated in Fig. 2E, CaSR is regulated throughout osteoclastic differentiation, reaching a significant peak of expression at the latter stage in healthy controls.

There is a progressive resistance to 1.25-D on osteoclastic differentiation when GFR decreases

Osteoclasts derived from PBMCs of CKD children were differentiated in the presence of 1.25-D. The percentage of osteoclastogenesis inhibition obtained for each CKD stage was calculated using the control ethanol (OH; 100%) treatment. As illustrated in Fig. 3A, a significant decreased inhibition in CKD stage 4-5 children compared with CKD stage 1-2 was observed ($p = 0.045$). For dialysis patients, there was no difference compared with CKD 1 and 2. This resistance to 1.25-D is further supported by Spearman bivariate analyses, showing a significant positive association between GFR and the percent of inhibition of differentiation induced by 1 nM 1.25-D ($R = 0.53$, $p = 0.02$) (Fig. 3B). To further investigate the 1.25-D resistance, the expression of 1.25-D receptor (VDR) in osteoclasts was quantified when there was enough material (Fig. 3C): VDR expression tended to decrease when CKD stage increased, as a possible explanation for 1.25-D resistance in osteoclasts.

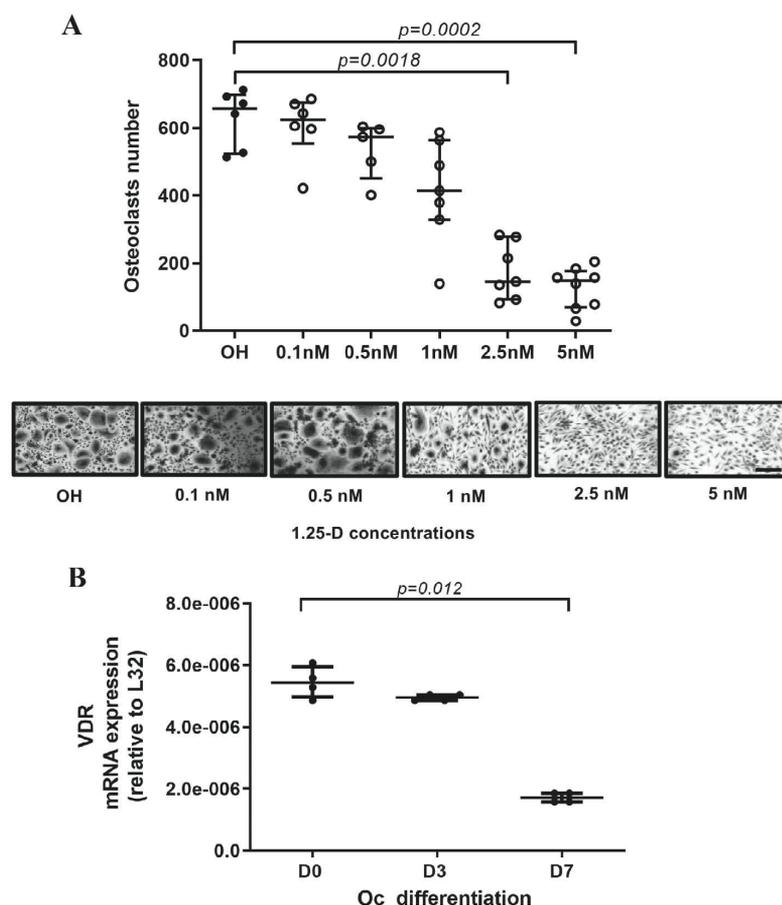


Fig 1. 1.25-D inhibits osteoclast differentiation in healthy controls. (A) The monocytes derived from adult healthy controls were cultured in α -MEM medium and 10% FBS with M-CSF (20 ng/mL) and RANKL (35 ng/mL). Medium and cytokines were changed every 72 hours. Ethanol (OH) represents control vehicle of 1.25-D. Cells were treated with OH or 1.25-D (0.1 nM, 0.5 nM, 1 nM, 2.5 nM, 5 nM) throughout osteoclastogenesis. Osteoclast differentiation was stopped at day 7. 1.25-D significantly inhibits osteoclast differentiation in a dose-dependent manner. Box plots represent median with interquartile range of total TRAP-positive cells relative to OH control. For each condition, 5 to 8 wells were analyzed by Kruskal-Wallis ($p < 0.0001$) followed by multiple comparison Dunn's post-test (p on the figure). The results are presented for one healthy control (osteoclastic inhibition with 1 nM: 38.3% compared with vehicle); similar results were obtained with four other healthy controls. Representative TRAP images at the end of the differentiation process are presented for the different conditions below the graph. The black line scale = 500 μ m. (B) Human osteoclasts were obtained after 7 days of differentiation under standard conditions with M-CSF (20 ng/mL) and RANKL (35 ng/mL) from two healthy controls. Systematic controls were generated without iScript and amplified; qPCR was carried out in duplicate for each data point. VDR expression was quantified and normalized to corresponding RNA L32 values and expressed as a relative expression using the $2^{-\Delta\Delta Ct}$ method. VDR expression was evaluated along the differentiation process at day 0, day 3, and day 7, showing a significant decreased expression in healthy donors at day 7. Statistical analysis was performed using nonparametric Kruskal-Wallis tests ($p = 0.0012$) followed by Dunn's post-test (p on figure for columns comparison).

KP2326 inhibits osteoclastic differentiation to a lesser extent than 1.25-D and independently of the degree of renal impairment

As shown in Fig. 4A, there was a significant inhibition of osteoclastic differentiation in CKD children, independently of the degree of renal impairment. The expression of CaSR in osteoclasts was quantified when there was enough material. As illustrated in Fig. 4B, the expression of CaSR was strikingly dropped off in CKD osteoclasts compared with controls, even though it is significantly increased

as renal function worsened. There was also a trend toward a positive association between PTH and CaSR expression ($R = 0.41, p = 0.43$), however, on a small number of subjects ($n = 6$) (data not shown).

In CKD children, the combination of 1.25-D And KP2326 inhibits osteoclastic differentiation, without synergistic effect

As shown in Fig. 5, the adjunction of KP2326 to 1.25-D in culture did not significantly modify the response in terms of osteoclastic

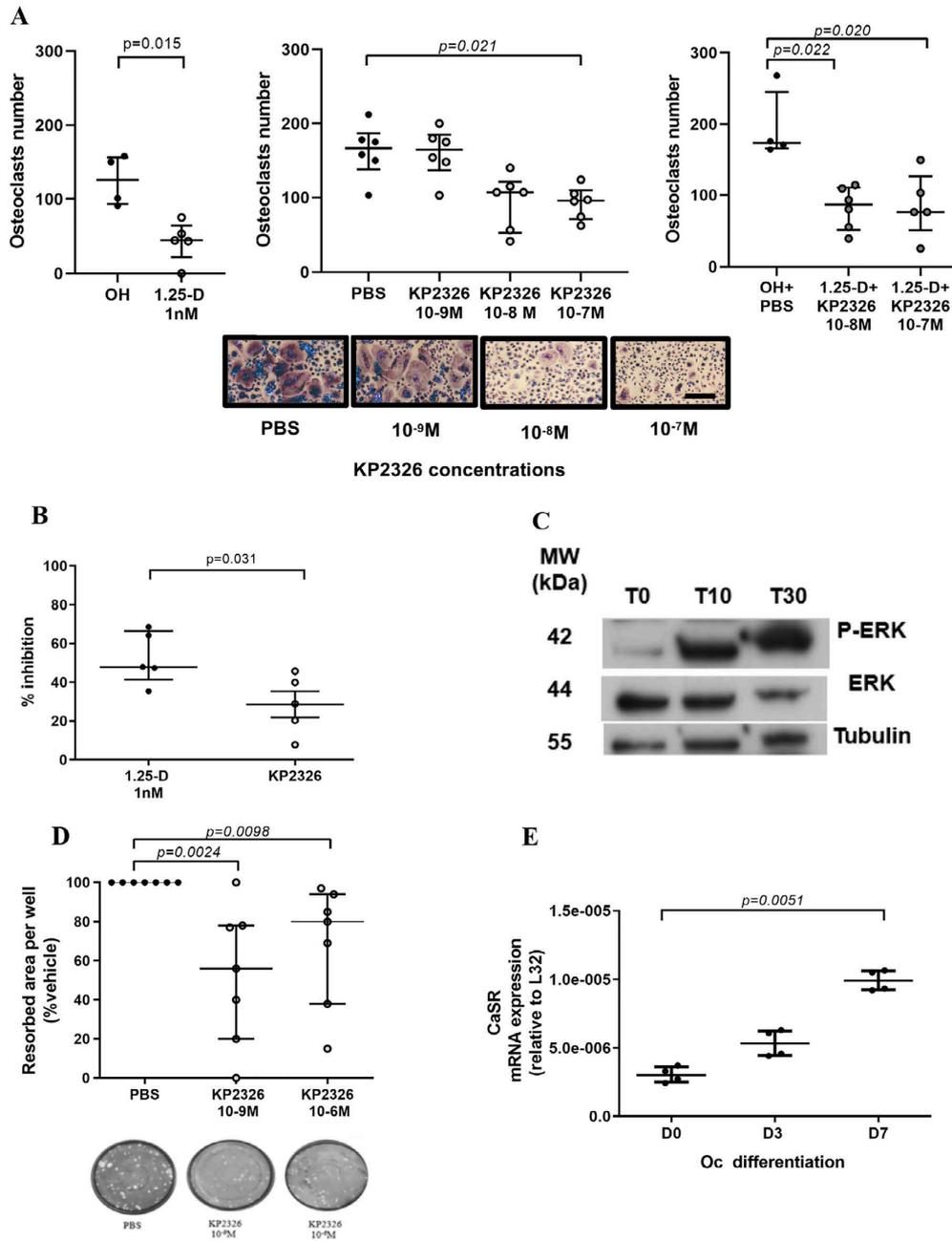


Fig 2. Legend on next page.

differentiation. 1.25-D was more effective to inhibit osteoclastic differentiation than KP2326 in CKD 1–2 and CKD 3. In CKD patients, the combinatorial treatment was not different from 1.25-D or KP2326 alone.

Discussion

This is the first in vitro study evaluating the direct impact on human PBMCs differentiated into functioning osteoclasts of the

two most frequently used treatments in SHPT, namely 1.25-D and/or calcimimetics, both in healthy controls and pediatric patients at different CKD stages. The main results are the following: there is a resistance to 1.25-D when GFR decreases, with a significant decreased inhibitory effect of 1.25-D on osteoclastic differentiation in advanced CKD. There is a significant albeit less important inhibitory effect of KP2326 on osteoclastic differentiation through a putative activation of the Erk pathway, observed for both healthy controls and patients, which is not modified by CKD stage. There is no synergistic effect when co-treating cells with both 1.25-D and KP2326. Last, KP2326 also inhibits human osteoclast-mediated bone resorption unlike 1.25D.

Here, as previously described,⁽⁸⁾ we confirm that 1.25-D is a powerful inhibitor of osteoclastogenesis in a dose-dependent manner in healthy controls. There is a progressive resistance to 1.25-D that appears when GFR decreases. Despite optimal therapy using vitamin D analogs, mineralization and bone defects persisted in a cohort of 60 pediatric patients undergoing maintenance dialysis despite a suppressive effect on PTH levels, thus questioning the overall positive effects of active vitamin D analogs on bone.⁽⁶⁾ The resistance to 1.25-D in osteoclasts observed here with physiological concentrations of active vitamin D is in line with what has been described by Pereira and colleagues in osteoblasts⁽⁹⁾ and is logically associated with a decreased expression of VDR in osteoclasts when GFR decreases, even though we have to acknowledge that only qRT-PCR was used as a quantitative technique. Indeed, we obtained rare samples from pediatric patients with CKD, without the confounding factors that would be associated in adults with CKD. However, the limited amount of material at our disposal did not allow protein quantification of VDR.

This "imprinting" theory, or epigenetic changes secondary to CKD,⁽²¹⁾ is nevertheless not fully explained to date, and it is

surprising that there are no significant differences between CKD stage 4–5 and dialysis patients in terms of inhibition of osteoclastic differentiation. We could even discuss whether cells derived from patients undergoing chronic dialysis improve their response to 1.25-D, but this is not statistically significant. We first hypothesized that this observation could be explained by the proportion of patients receiving alfacalcidol, but patients in dialysis as well as those in stage 4–5 received this drug. However, it is noteworthy that our patients undergoing chronic hemodialysis are well controlled, with PTH levels at the lower limit of the "recommended" target range, depending on which guidelines are taken into account.⁽⁵⁾ This good control of SHPT in dialysis may have blunted the results, and it would be interesting to evaluate the responses in cells from dialysis patients with uncontrolled SHPT. However, the effects on the skeleton may differ between the different compounds used as vitamin D analogs. Indeed, it has been shown that doxercalciferol led to a greater improvement of eroded surface when compared with calcitriol in pediatric dialysis patients with skeletal lesions of SHPT,⁽⁶⁾ and the authors hypothesized a direct effect of doxercalciferol on osteoclastogenesis. It would be interesting to assess such compounds with the current *in vitro* system. In contrast, our CKD stage 4–5 are less well controlled, if we consider that at this CKD stage PTH levels should be at the upper limit of the local normal range (15 to 65 ng/L) or just above the upper normal limit (K-DIGO proposed target range for PTH in CKD stage 4: 70 to 110 ng/L), and this latter fact may explain the observed resistance to 1.25-D observed in pre-dialysis. Last, we also observed a progressive decrease of VDR expression during osteoclastic differentiation in healthy controls; this point confirms that at the early stages of differentiation, cells may be very responsive to 1.25-D, whereas in mature osteoclasts, 1.25-D does not affect bone resorption.⁽⁸⁾

FIGURE 2 In healthy controls, the calcimimetic KP2326 inhibits both osteoclastic differentiation and bone resorption and activates Erk pathway. (A) The monocytes derived from a healthy control were cultured in α -MEM medium and 10% FBS with M-CSF (20 ng/mL) and RANKL (35 ng/mL). Medium and cytokines were changed every 72 hours. Ethanol (OH), PBS, and OH + PBS represent respective control vehicles of 1.25-D, KP2326, and 1.25-D + KP2326. Cells were treated with vehicle, 1.25-D (1 nM) (left graph), KP2326 (10^{-9} M, 10^{-7} M, 10^{-6} M) (middle graph), or combinatorial treatment of 1.25-D and 10^{-9} / 10^{-7} M of KP2326 (right graph) throughout osteoclastogenesis. Osteoclast differentiation was stopped at day 7. Box plots represent median with interquartile range of TRAP-positive cells. For each condition, 5 to 8 wells were analyzed by Mann–Whitney ($p = 0.015$) for left graph, Kruskal–Wallis (middle graph, $p = 0.0068$; right graph, $p = 0.0071$) followed by Dunn's tests (p on figures for columns comparison). The results are presented for one healthy control (the percentage of osteoclastic inhibition with KP2326 10^{-7} M was 40%). A representative photo merge of DAPI and TRAP images at the end of the differentiation process is represented for the different conditions below the graph. The black line scale stands for 500 μ m. (B) Each dot on the graph represents the individual result for each condition expressed as the percent of inhibition of osteoclastic differentiation relative to vehicle treatment that was considered arbitrarily to be at 0%. Ethanol (OH) and PBS represent respective control vehicles of 1.25-D (1 nM) and KP2326. 1.25-D is significantly more effective than KP2326 to inhibit osteoclastogenesis ($p = 0.031$ between 1.25-D and KP2326, Mann–Whitney test). (C) After 5 days of differentiation, osteoclasts were treated with KP2326 10^{-6} M at 0, 10, and 30 minutes. KP2326 at a concentration of 10^{-6} M activates the ERK/p-ERK pathway at 10 and 30 minutes. T0 = 0 minute; T10 = 10 minutes; T30 = 30 minutes; MW = molecular weight. (D) Human osteoclasts obtained after 6 days of differentiation under standard conditions with M-CSF (20 ng/mL) and RANKL (35 ng/mL) were replated on mineralized plates and treated with PBS (vehicle) and KP2326 (10^{-9} M and 10^{-6} M) for 3 days. After osmotic lysis, slices were stained in silver nitrate to quantify the total resorbed areas. KP2326 significantly inhibits bone resorption. Relative results are expressed as the percentage of the resorbed area per well compared with the control PBS (resorbed area per well with KP2326 10^{-6} M: 57.8%, $p = 0.0098$; osteoclast inhibition with KP2326 10^{-9} M: 60.4%, $p = 0.0024$). Micrographs of silver nitrate matrix staining are representative for the three conditions. Statistical analyses were performed with nonparametric paired tests (Kruskal–Wallis [$p = 0.0005$] followed by Dunn's tests [p on the figure for columns comparison]). Experiments were performed in batches from three different donors; each condition for each donor was done in quadruplicate in culture. We present here the representative results obtained in one healthy control. The graphs represent median with interquartile of the percentage of resorbed area per well compared with vehicles that were considered arbitrarily to be at 100%. (E) Human osteoclasts were obtained after 7 days of differentiation under standard conditions with M-CSF (20 ng/mL) and RANKL (35 ng/mL) from two healthy controls. Systematic controls were generated without iScript and amplified; qPCR was carried out in duplicate for each data point. CaSR expression was quantified and normalized to corresponding RNA L32 values and expressed as a relative expression using the $2^{-\Delta\Delta Ct}$ method. CaSR expression was evaluated along the differentiation process at day 0, day 3, and day 7, showing a significant increased expression in healthy donors at day 7. Statistical analyses were performed with nonparametric paired tests (Kruskal–Wallis [$p = 0.0002$] followed by Dunn's tests [p on the figure for columns comparison]).

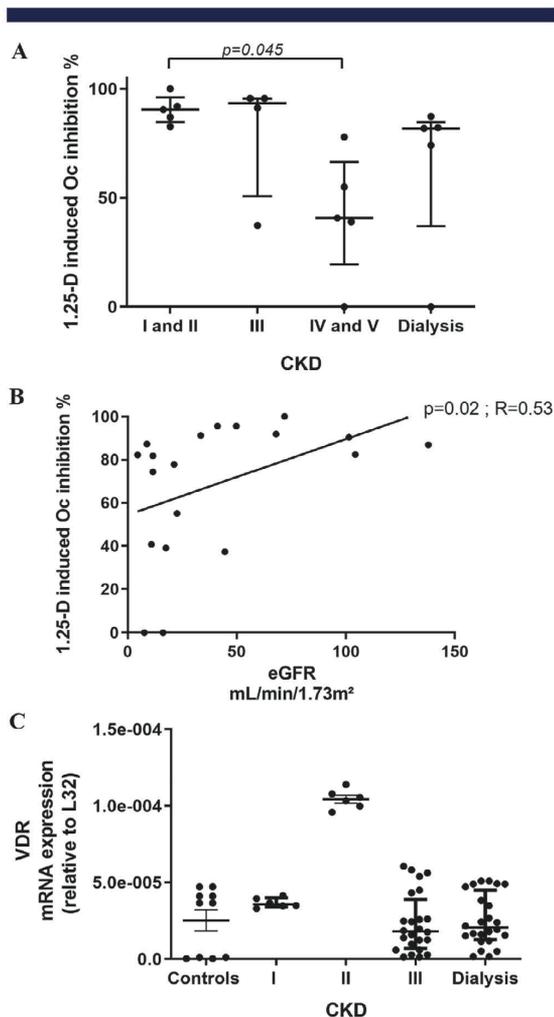


Fig 3. In CKD children, there is a progressive resistance to 1.25-D effects on osteoclastic differentiation in advanced CKD. (A) The graph represents the individual result of CKD patients, with median with interquartile range per CKD group (1–2, 3, 4–5, and dialysis): There is a progressive resistance to 1 nM of 1.25-D when GFR decreases, with a significant decreased inhibition of osteoclastogenesis in CKD 4–5 children compared with CKD stage 1–2 induced by 1 nM of 1.25-D ($p = 0.045$). In dialysis, there is no significant decreased inhibition compared with stage 1–2 with nonparametric paired tests (Kruskal-Wallis [$p = 0.028$] followed by Dunn's tests [p on the figure for columns comparison]). (B) The figure represents eGFR (mL/min/1.73 m²) on the x-axis, and the percent of inhibition with 1 nM of 1.25-D on the y-axis. The statistical bivariate analysis by Spearman correlation confirms the progressive resistance to 1.25-D when CKD worsens ($R = 0.53$; $p = 0.02$). (C) VDR expression in mature osteoclasts. The analysis was possible for 11 CKD patients and 2 controls. For this reason, no data were available for CKD stage 4 and 5. RNA was obtained after osteoclastogenesis on day 7; qPCR was carried out in duplicate or triplicate for each patient and control. VDR expression was quantified and normalized to corresponding RNA L32 values and expressed as a relative expression using the $2^{-\Delta\Delta Ct}$ method. The expression of VDR trendly decreases when GFR decreases (no statistical analysis).

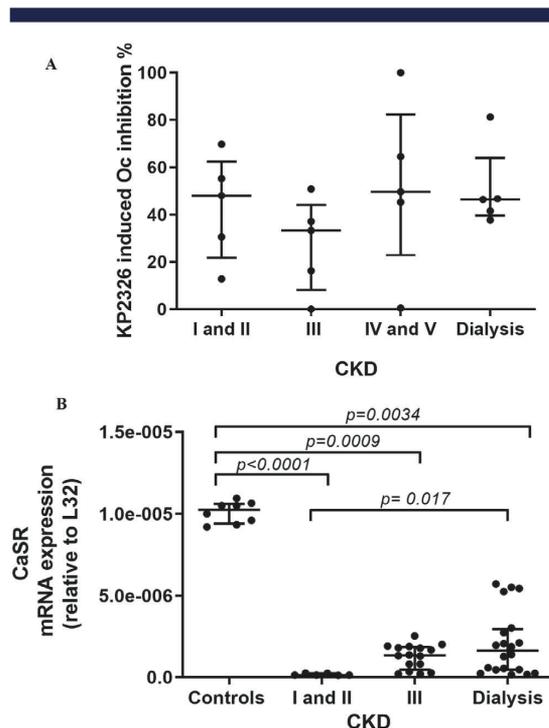


Fig 4. In CKD children, KP2326 inhibits osteoclastic differentiation, however to a lesser extent than 1.25-D, with no effect of the degree of renal impairment. (A) Each dot on the graph represents the individual result of one patient, with median interquartile range per CKD group (1–2, 3, 4–5, and dialysis). Although there is a significant inhibition of osteoclastogenesis with KP2326 in patients with CKD (compared with the control vehicle PBS), there is no difference between groups when CKD worsens. For each patient, 100% reference corresponds to TRAP-positive cells count in PBS condition, as a control vehicle of KP2326. Statistical analyses were performed with nonparametric paired tests (Kruskal-Wallis [$p = 0.38$] followed by Dunn's tests). (B) Analysis of CaSR expression in mature osteoclasts on day 7 (9 pediatric patients and 2 adult controls were represented). qPCR was carried out in duplicate or triplicate for each data point of CKD patients. *hCaSR* expression was quantified and normalized to corresponding RNA L32 values and expressed as a relative expression using the $2^{-\Delta\Delta Ct}$ method. The expression of CaSR significantly increases when renal function worsens. Statistical analyses were performed with nonparametric paired tests (Kruskal-Wallis [$p < 0.0001$] followed by Dunn's tests [p on figure for columns comparison]).

The second cornerstone of SHPT management in dialysis is the use of calcimimetics. Etelcalcetide is a CaSR-positive allosteric peptide agonist, binding to an extracellular domain (CaSR VFT) and acting through a different mechanism compared with the first-generation calcimimetic cinacalcet, binding CaSR transmembrane domain (TMD). Indeed, it does not require the binding of calcium on CaSR to be active and to inhibit PTH synthesis. Thus, it activates the CaSR independently of extracellular calcium, but its activity is, however, less important when extracellular calcium is low or absent.⁽¹⁹⁾ A pharmacokinetic and pharmacodynamic study confirmed that KP2326, the

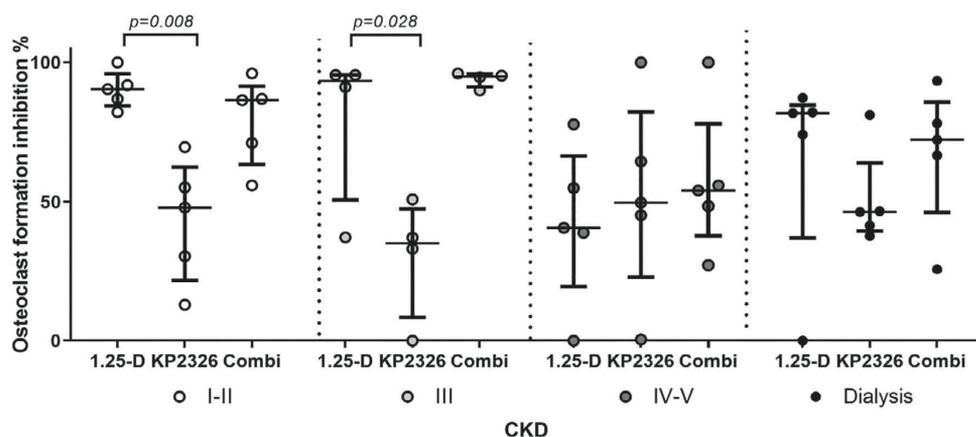


Fig 5. Combinatorial treatment inhibits osteoclastogenesis without synergistic effect. This figure represents the median with interquartile range of percentage of inhibition of osteoclastogenesis compared with the respective controls of 1.25-D, KP2326, and 1.25-D + KP2326, namely ethanol (OH), PBS, and OH + PBS, in the different CKD groups. The results of 19 different patients have been pooled in this analysis. 1.25-D was more effective to inhibit osteoclastic differentiation than KP2326 in CKD 1–2 and 3. In CKD patients, the combinatorial treatment is not different from 1.25-D or KP2326 alone. Statistical analyses were performed with nonparametric paired tests Kruskal-Wallis for group 1–2 ($p = 0.0018$); for group 3 ($p = 0.0071$); for group 4–5 ($p = 0.7$), and dialysis group ($p = 0.38$) followed by Dunn's tests (p on the figure for columns comparison).

peptide analog of etelcalcetide for experimental studies, was an allosteric activator of CaSR.⁽²²⁾ Based on the different distribution volumes of etelcalcetide (blood 50 L, superficial tissue 120 L, deep tissues 625 L), the expected C_0 concentrations in each compartment after an intravenous infusion of 5 mg of etelcalcetide (ie, the dose commonly prescribed in clinical practice in adults undergoing hemodialysis) would rise to 9.5×10^{-8} M, 4×10^{-8} M, and 7.7×10^{-9} M, respectively.^(22,23) These pharmacological estimations led us to test different concentrations of KP2326 in vitro, namely in a range of 10^{-9} M and 10^{-5} M, with heterogeneous results not in terms of biological effect but in terms of the inter-individual concentration required to observe such biological effects. Calcimimetics binding to CaSR leads to structural rearrangements. It was also described a biased agonism, defined as the ability of the ligand to stabilize receptor conformation subsets, coupled to specific signaling pathway.⁽²⁴⁾ This phenomenon might contribute to interindividual variability according to the relative levels of different CaSR activators in different patients.

We demonstrate here an inhibitory effect of KP2326 on osteoclastic differentiation that is significant both in healthy controls and in CKD patients, without any resistance to KP2326 in advanced CKD: As a likely compensatory mechanism, the expression of CaSR significantly increases only in advanced CKD and in dialysis. However, the mechanisms behind this compensation remain unclear. Several signaling pathways are involved during the activation of CaSR in different cells. In osteoblasts, extracellular calcium activates both the phospholipase C_2 ,⁽²⁵⁾ the Akt,⁽²⁰⁾ and the ERK1/2⁽²⁶⁾ pathways, the latter stimulating the proliferation of osteoprogenitor cells and preventing apoptosis. Recently, the direct inhibitory effect of prostaglandin E2 (PGE2) on the differentiation of osteoclastic precursors has been shown, albeit with no effect on mature osteoclasts.⁽²⁷⁾ After activation of CaSR, the ERK/pERK pathway also activates phospholipase A2, thus stimulating the formation of arachidonic acid and PGE2.⁽²⁸⁾

Therefore, one may hypothesize that the decrease in osteoclasts number is related to increased PGE2 levels via the ERK-pERK pathways secondary to the activation of CaSR by KP2326, but this remains to be investigated.

We also demonstrate an inhibiting effect of KP2326 on bone resorption. Thus, in vitro, KP2326 inhibits both osteoclastic differentiation and resorption activity; this provides a strong rationale for a global positive effect of KP2326 on bone remodeling. Even though we provide some mechanistic insights to explain the direct effects of KP2326 on osteoclasts, notably by showing Erk phosphorylation under KP2326 treatment, we still need to delineate why the concentrations required to obtain the maximal effect vary so widely between individuals, as illustrated in Supplemental Table S1. Further experiments adding a CaSR antagonist in the same setting would be valuable to demonstrate that Erk phosphorylation under KP2326 treatment is directly downstream CaSR activation by KP2326 in osteoclasts. Several explanations can be hypothesized to explain this modulation of CaSR at different concentrations. First, calcium concentrations can modify receptor cooperativity within the bone microenvironment and balance bone remodeling,⁽²⁹⁾ but culture conditions were standard and similar for all experiments. Second, CaSR is also able to respond to other extracellular stimuli, such as inorganic cations (strontium, aluminium), polyamines, antibiotics (aminoglycosides), and also magnesium,⁽²⁸⁾ but, again, culture conditions were standard and similar for all experiments. Third, an interindividual variability for CaSR genotypes and expression may exist. Fourth, the expression of CaSR varies at the beginning and during the differentiation process, with a progressive increased expression of CaSR along differentiation. Last, in healthy controls, we did not have biological data such as calcium and 25OH vitamin D levels, which may influence CaSR expression.

We here present data on the effects of KP2326 on osteoclasts, but bone remodeling corresponds to an exquisite balance between bone formation and bone resorption, arising in part

from osteoblast/osteoclast cross-talk. In this study, we present only osteoclastic data, and it was ethically difficult to propose a bone biopsy or an adipose tissue sample (so as to transdifferentiate adipocytes into osteoblasts) because the RENO-CLAST study was totally noninvasive. A recent article evaluated the effect of AMG-641, through CaSR activation, after binding CaSR TMD, on osteoblasts in a very elegant study in normal and uremic rats undergoing a PTH clamp.⁽³⁰⁾ Indeed, Diaz-Tocados and colleagues showed that this calcimimetic, an analog of cinacalcet, has a direct anabolic effect on bone, through the promotion of an osteogenic differentiation and mineralization in human mesenchymal stem cells *in vitro*. Moreover, they also found that the administration of AMG641 increased osteoblast number and osteoid number in normal rats under a PTH clamp; additionally, in uremic rats exposed to PTH at sixfold the usual replacement dose, the administration of the calcimimetic increased osteoblast number, osteoid surface, and bone formation.⁽³⁰⁾ Another recent article also showed that etelcalcetide reduced bone remodeling and mineralization defects by improving cortical bone structure in an animal model of renal osteodystrophy, namely the 5/6th nephrectomized rat with SHPT.⁽³¹⁾ After 6 weeks, this treatment significantly decreased bone turnover, attenuated cortical bone deterioration, mineralization defect, and marrow fibrosis, and preserved bone strength. Taken together, these results on osteoblasts and osteoclasts show a beneficial effect of calcimimetics on bone, clearly favoring bone formation. These collective results can be of utmost importance in clinical practice, especially in dialysis adults who are also prone to osteoporosis.⁽³²⁾

It is also interesting to note that there is no synergy when co-treating cells with 1.25-D and KP2326. The 2017 KDIGO guidelines recommend to use either vitamin D analogs or calcimimetics or both in case of SHPT in dialysis, without any additional details.⁽⁷⁾ In the recent study from Li and colleagues in which the beneficial effect of etelcalcetide on cortical bone structure was described, they showed that etelcalcetide significantly decreased not only PTH level but also fibroblast growth factor-23 (FGF23) levels.⁽³¹⁾ In the setting of global systematic deleterious effects of high FGF23 levels in CKD patients on one hand,⁽³³⁾ and keeping in mind that active vitamin D analogs stimulate FGF 23 production on the other hand,⁽³³⁾ all these results could therefore favor the use of lower doses of 1.25-D with low doses of calcimimetics in dialysis in order to control SHPT without inducing a sharp inhibition of osteoclastic differentiation, and therefore decreasing the risk of worsening the underlying osteodystrophy and further vascular calcifications. The clinical application of these experimental data remains to be evaluated, but this strategy could also decrease (or at least stabilize) FGF23 levels, and therefore be less deleterious on the long term. This point can be of utmost importance, especially because KP2326 significantly inhibits bone resorption at all CKD stages, whereas 1.25-D does not have any effect on bone resorption.⁽⁸⁾ These hypotheses need confirmatory clinical trials with more mechanism experiments to explore osteoclastic regulation and osteoblast/osteoclast cross-talk.

In conclusion, 1.25-vitamin D and KP2326 inhibit osteoclastic differentiation in healthy controls and in CKD patients, however to a different extent. There is a progressive resistance to 1.25-D when CKD worsens that is not found with KP2326.

Disclosures

Justine Bacchetta has been investigator for the Amgen-sponsored trials on the use of cinacalcet and etelcalcetide in pediatric dialysis. Justine Bacchetta is the coordinator of the

Amgen-sponsored international registry on the use of cinacalcet in pediatric dialysis. All other authors state that they have no conflicts of interest.

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Author contributions: Julie Bernardor: Conceptualization; data curation; formal analysis; funding acquisition; investigation; writing-original draft. Sacha Flammier: Conceptualization; formal analysis; investigation; resources; writing-review and editing. Bruno Ranchin: Methodology; writing-original draft; writing-review and editing. Segolene Gaillard: Methodology; writing-review and editing. Diane Platel: Writing-review and editing. Olivier Peyruchaud: Conceptualization; investigation; methodology; writing-original draft; writing-review and editing. Irma Machuca-Gayet: Conceptualization; formal analysis; funding acquisition; investigation; methodology; resources; supervision; writing-original draft; writing-review and editing. Justine Bacchetta: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; resources; supervision; writing-original draft; writing-review and editing.

Peer Review

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2. Utilisation hors AMM du cinacalcet en MRC pédiatrique

En 2017, les directives KDIGO ont recommandé l'utilisation des calcimimétiques en monothérapie ou en association avec des analogues de la 1 α ,25(OH) $_2$ D comme traitement de première intention chez les adultes pris en charge en dialyse [87]. En pédiatrie, des réductions significatives des concentrations de PTH ont été rapportées dans des études observationnelles portant sur 92 enfants [98–104]. Plusieurs essais cliniques incluant deux études de phase 1, une étude de phase 2, deux études randomisées de phase 3, ainsi qu'une étude à bras unique de phase 3, ont été réalisés sur un total de 103 enfants dialysés. Ces essais ont démontré que de 7 à 57 % des enfants traités par cinacalcet ont atteint les concentrations cibles recommandées de PTH, et que 22 à 71 % ont présenté une réduction d'au moins 30 % de la PTH [84]. Sur la base de ces résultats, le cinacalcet a été approuvé, et en 2020, une recommandation européenne publiée par l'European Society of Pediatric Nephrology (ESPN) a délimité l'utilisation du cinacalcet chez les enfants de plus de trois ans pris en charge en dialyse, pour traiter une sHPT persistante et sévère, malgré une prise en charge conventionnelle optimisée [52].

a. Chez l'enfant dialysé de moins de 3 ans : en cours de soumission

Contexte /objectif

La MRC est presque inévitablement associée au CKD-MBD, caractérisé par un ensemble complexe d'anomalies du remodelage osseux et de calcifications vasculaires et extravasculaires [105]. Cette pathophysiologie complexe implique l'hyperphosphatémie, la carence en vitamine D active et/ou native, et l'hypocalcémie subséquente, induisant une sHPT et une évolution vers une maladie osseuse progressive [30]. Bien que de nombreuses publications et des recommandations abordent le diagnostic et la prise en charge du CKD-MBD chez l'enfant, les patients de moins de trois ans restent peu étudiés dans ce contexte avec un nombre faible de cas rapportés [30, 45, 52, 53, 106]. Ce manque de données est particulièrement préoccupant, car l'enfance est la période de croissance la plus rapide, avec un doublement de la taille longitudinale jusqu'à l'âge de trois ans chez les enfants en bonne santé [107]. La croissance osseuse hautement dynamique durant cette phase complique encore davantage la prise en charge du CKD-MBD soulignant la nécessité de thérapeutiques efficaces.

Entre 2009 et 2021, nous avons constitué une cohorte de 26 enfants âgés de moins de trois ans en dialyse chronique, offrant des données significatives sur l'efficacité et la tolérance en vie réelle du traitement hors AMM du cinacalcet.

Résultats

Sur 194 enfants de cette tranche d'âge en dialyse, 26 (13%) ont reçu ce traitement. Le cinacalcet a été prescrit principalement pour traiter l'hyperparathyroïdie secondaire, avec une dose initiale supérieure à la dose recommandée par la recommandation européenne [83]. Au cours du suivi, la PTH a diminué

significativement dès 6 mois de traitement, avec plus de 50% des patients atteignant des concentrations cibles de PTH après 9 mois. Dix épisodes d'hypocalcémie ont été rapportés chez 7 patients. De plus, trois filles ont développé une puberté précoce après 27, 13 et 6 mois de traitement au cinacalcet. Dans les trois cas, l'échographie a révélé la présence de nombreux follicules ovariens, alors que la taille de l'utérus et les résultats sur les surrénales étaient normaux pour l'âge, tout comme les concentrations sériques d'hormone FSH, d'hormone lutéinisante et d'œstradiol. Il est difficile de confirmer le lien entre puberté précoce/cinacalcet ; une surveillance clinique et un suivi endocrinologique sont préconisés lors du traitement par cinacalcet.

Conclusion

L'utilisation hors AMM du cinacalcet chez les patients pédiatriques en dialyse de moins de trois ans permet un contrôle efficace de l'hyperparathyroïdie secondaire sévère. Cependant, le traitement nécessite une surveillance étroite de la calcémie et une adaptation des apports en calcium et en phosphate en fonction de l'âge chez ces enfants présentant une croissance rapide et les formes les plus sévères de CKD-MBD.

Safety and efficacy of cinacalcet in children below 3 years of age on maintenance dialysis

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Abstract

Background

Secondary hyperparathyroidism (sHPT) is common in children on dialysis, and particularly severe in rapidly growing infants. While cinacalcet has proven effective and has been licensed for children on dialysis above 3 years of age; efficacy and safety below 3 years is unknown.

Methods

We identified 26 children on dialysis below three years (nine girls) treated with cinacalcet between 2009 and 2021 in 8 out of 35 European pediatric centers.

Results

Median (IQR) age at start of cinacalcet was 18[IQR 11–27] months, serum parathyroid hormone (PTH) was 792[411–1397] pg/mL; corresponding to 11.6[5.9–19.8] times the upper limit of normal (ULN). Serum calcium was 2.56[2.43–2.75] mmol/L, serum phosphate standard deviation score (SDS) for age -1.7[-3.2 – -0.6]). Serum 25-OHD was 70[60–89] nmol/L, three children were vitamin D deficient (<50nmol/L). The initial cinacalcet dose was 0.4[0.2–0.8] mg/kg/day and the maximum dose was 1.1[0.6–1.2] mg/kg/day after 1.2[0.7–2.0] years. PTH decreased to 4.3[2.2–7.8] times the ULN after 6 months, to 2.0[1.0–5.3] times ULN after 12 months and to 1.6 [0.5–3.4] times thereafter ($p=0.017/0.003/<0.0001$, log-transformed PTH). Seven of the 26 infants developed 10 hypocalcemic episodes <2.10 mmol/L. The daily dose of cinacalcet was almost two-fold higher at time of hypocalcemia than the corresponding dose in the 19 children without hypocalcemia and requiring cinacalcet discontinuation in four. One non-adhering child developed hypocalcemic convulsion. Oral calcium intake was 84[66-117] % of recommended nutrient intake at start, 100[64-142] % at 3 months and declined to 78[65-102] % at 12 months of therapy, dialytic calcium balance was positive. Three children developed clinical signs of precocious puberty.

Conclusion

Cinacalcet efficiently controlled severe sHPT in children below 3 years of age and was associated with hypocalcemic episodes and precious puberty, mandating meticulous control of calcium balance and endocrine changes.

1. Introduction

Chronic kidney disease (CKD) is almost inevitably associated with Mineral and Bone Disorder (CKD-MBD), which is characterized by a combination of bone remodeling abnormalities and cardiovascular disease (CVD) (1). The combination of hyperphosphatemia, deficiency in active and/or native vitamin D, and subsequent hypocalcemia induces secondary hyperparathyroidism (sHPT), resulting in progressive bone disease and parathyroid gland hyperplasia (2). Several consensus papers and guidelines were published to steer both diagnosis and management of pediatric CKD-MBD (2–5). Standard of care includes dietary measures, calcium supplementation, phosphate binders, vitamin D sterols, optimization of dialysate calcium and calcimimetics in children above three years. Evidence is scarce in the youngest children, as discussed recently in the recent European consensus on CKD-MBD in infants (6). This lack of knowledge is particularly concerning, since infancy is the period of fastest growth, with doubling of body length until the age of three years in healthy children and substantial improved growth in CKD children receiving growth hormone therapy (rhGH) from the age of 6 months onwards (7). Growth-associated highly dynamic bone development explains challenges in CKD-MBD management in this peculiar age group. High nutritional supply of calcium is of utmost importance (8), however, is often difficult to achieve in routine clinical practice, especially in children on a phosphate restricted diet (9–11). Even with optimized treatment insufficient control of MBD is prevalent, with significant obstacle to bone strength and CVD worsening (12–17). Conversely, the number of small children requiring dialysis is increasing globally (13,18), as kidney replacement therapy has been widely accepted in otherwise stable children from the first days of life onwards.

The calcimimetic cinacalcet potently inhibits parathyroid hormone (PTH) synthesis and secretion through the sensitization of the parathyroid Calcium-Sensing Receptor (19,20), decreasing calcium and phosphate levels, thus potentially reducing the need for parathyroidectomy (21), decreasing fracture rates (22), and possibly reducing CVD in adults on dialysis (23,24). In 2017, KDIGO recommended calcimimetics as first-line therapy or in combination with vitamin D analogs, in dialysis adults (25). Two open-label phase 1 studies, one open-label phase 2 study, two phase 3 randomized studies and one

phase 3 single arm extension study have been performed in a total of 103 pediatric subjects. Seven to 57% of the pediatric subjects who received cinacalcet attained PTH levels within recommended target ranges, and 22 to 71% a \geq 30% reduction in PTH (26). Significant reduction of PTH levels has been reported in observational studies in 92 children (27–33). Thus, cinacalcet was licensed in children above 3 years and the 2020 European consensus statement suggested routine cinacalcet use in pediatric dialysis patients above three years of age to treat persistent and severe SHPT, despite optimized conventional management (5).

The role of cinacalcet treatment in younger children, with the highest dynamic in bone development and most difficulties to control sHPT, however, remains uncertain. Our aim is to report the European experience of off-label use of cinacalcet in dialysis children below 3 years of age.

2. Patients and methods

2.1 Patients

We performed a retrospective analysis on cinacalcet use in children on maintenance dialysis below three years in 35 pediatric dialysis centers between 2009 and 2021. Centers are members of the European Society of Pediatric Nephrology (ESPN) and/or the European Rare Kidney Disease Reference Network (ERKNet). Patients were identified and analyzed retrospectively at cinacalcet initiation and three-monthly thereafter for one year (when most children have reached the age of 3 years) and at last follow-up in 16 patients treated with cinacalcet for more than one year. Oral calcium intake from diet and medication was expressed relative to the Reference Nutrient Intake (% RNI) for age (4), based on the patients' actual dry body weight. Between 6 months and 3 years, and between 4 and 5 years, recommended calcium intake are 50 and 44 mg/kg/day, respectively (8). Phosphate recommended intakes are 420 and 500 mg, respectively, between 6-12 months, and between 1-3 years (4). Net dialytic calcium uptake during PD was estimated from the total volume of dialysis fluid applied and the delta between dialysate calcium concentration (0.5 mmol/L) and serum calcium concentration, assuming about 55 % cation equilibration after 1 hour of dwell time and 80% after 4 hours (34).

Ultrafiltration associated calcium losses were estimated to amount to 1.1 mmol/L, with a median dwell time of 75 min [IQR 65–90]. Five patients were on hemodialysis at 8 time points. Due to missing data, dialytic Ca gain from hemodialysis could not be estimated appropriately, and the eight time points were excluded from calcium supply studies. Precocious puberty was defined as the onset of puberty before eight years with physical changes, such as breast development and pubic hair apparition, earlier than expected in girls.

2.2 Laboratory analyses

As part of routine follow-up, total calcium, phosphate, total alkaline phosphatase (ALP), PTH and 25-OH vitamin D (25-OHD) were regularly assessed locally by standard methods. Because of the physiological evolution of plasma phosphate and ALP during childhood, these were expressed as standard deviation score (SDS) for age (35,36). Laboratory parameters were collected at seven different time points: initiation of cinacalcet therapy (baseline), and at 1, 3, 6, 9 and 12 months, and at last follow-up beyond 12 months in 16 patients. Normal ranges of PTH assays were reported by each center, thus allowing to standardize PTH data relative to the upper limit of normal (ULN). SDS of body weight and SDS body length were assessed at cinacalcet initiation, 12 months and the last follow-up using WHO growth standards (37). Vitamin D deficiency was defined as serum 25-OHD less than 50 nmol/L and insufficiency as serum 25-OHD less than 75 nmol/L (3).

2.3 Ethics

The study was approved by the local ethical committee in Lyon (*Comité d’Ethique des Hospices Civils de Lyon*, session 14/10/2021, approval 21_634), and declared to the Information Technology and Liberty Commission (CNIL n°21_5634). It respected European relevant regulations. Three children below 3 years of age had been included in an Amgen-sponsored trial before, but data were not published; approval was obtained from Amgen to include the patients’ local results in the present study.

2.4 Statistical analysis

PTH and PTH-ULN had a non-Gaussian distribution and were log-transformed. Linear mixed model (REML) followed by Dunnett's multiple paired comparisons tests compared to baseline were used for biochemical data. Non-parametric Mann-Whitney tests were used to identify risk factors and to compare patients with hypocalcemic episodes to other patients. Results were described as median [interquartile range], *p*-values below 0.05 were considered statistically significant. Kaplan-Meier survival analysis was used to investigate the time-to-attainment of PTH levels three times the ULN, and to estimate the cumulative proportion of patients achieving it. GraphPad Prism software 8.0 (GraphPad, La Jolla, CA, USA) was used.

3. Results

Eight out of 35 pediatric dialysis centers who participated in the survey reported treating children below 3 years with cinacalcet between 2009 and 2021. Of 729 children on dialysis in these centres, 194 (27%) were below 3 years, and 26 (13%) had received cinacalcet. These infants were followed in Heidelberg (n=14), Gdansk (n=3), Lyon (n=2), Birmingham (n=2), Nijmegen (n=2), Genoa (n=1), Rotterdam (n=1) and Rome (n=1).

3.1 Patients

Demographic, clinical and biochemical features at the time of cinacalcet initiation are summarized in Table 1. At 18[11–27] months, 25 children on PD and one on hemodiafiltration started cinacalcet for sHPT, eight (31%) also displaying hypercalcemia; 23 received enteral tube feeding. At cinacalcet initiation, daily calcium intake from diet and medication was 43[33–63]mg/kg/day, providing 84[66–117] % of RNI; dietary phosphate intake was 230[183–256] mg/day, i.e. 47[40–52] % of RNI for age. Estimated dialytic calcium uptake was 2.4[1.7–3.1] mg/kg/day. Two patients received both calcium-based and calcium-free phosphate binders whilst seven patients only received calcium-free binders. All children received vitamin D analogs.

3.2 Cinacalcet dose

In all centers, a suspension was prepared by local pharmacists and provided in capsules. The initial cinacalcet dose was 0.4[0.2–0.8] mg/kg/day, i.e., two-times above the recommended starting dose (5). Follow-up period was 1.2[0.7–2.0] years. Maximal cinacalcet dose and last follow-up doses were 1.1[0.6–1.2] and 1.0[0.4–1.2] mg/kg/day, respectively. Following cinacalcet initiation, intervals for the first biochemical control varied substantially between centers, ranging from 2-3 days (n=14), 7 days (7), 10 days (1), two weeks (1) to one month (1).

3.3 Evolution of CKD-MBD biomarkers

At cinacalcet initiation, PTH was 792[411–1397] pg/mL, corresponding to 11.6[5.9–19.8] times the ULN. Serum calcium concentration was 2.56[2.43–2.75] mmol/L, whilst 8 patients displayed calcium levels above the age specific ULN (38). Serum phosphate was 1.47[1.16–1.71] mmol/L, with a SDS for age of -1.7[-3.2 – -0.6]. Total serum ALP and ALP-SDS were 660[492-905] IU/L and 0.3[-0.5 -2.0], respectively; 25-OHD was 70[60–89] nmol/L, three children were vitamin D deficient and twelve were vitamin D insufficient (Table 1).

Biochemical evolution is displayed in Table 2, and Figures 1/2/3. During cinacalcet treatment PTH levels steadily declined to 4.3[2.2–7.8], 2.0[1.0–5.3] and 1.6 [0.5–3.4] times ULN after 6 months, 12 months and last follow-up, respectively ($p=0.017/0.003/<0.0001$, log-transformed PTH) (Figure 1, Table 2). The probability of achieving serum PTH in the target range (i.e., <3 times ULN) increased over time and was above 50% after 9 months (Figure 2).

Albumin-corrected calcium levels remained in the normal range during the follow-up in the majority of patients (Figure 3A). Patient 26 had persistently increased serum calcium levels despite cinacalcet therapy for 14 months and a low calcium supply of 21% of RNI: PTH declined by 84% but remained above target range. Serum phosphate (absolute and SDS) were low normal and remained stable (Figure 3B/C). Total ALP and 25-OHD concentrations were mostly within the normal range (Figures 3D/E/F). At last follow-up, ALP SDS was significantly lower compared to baseline ($p=0.004$).

X-ray analyses were available for 23 patients: 13 patients presented with radiological signs of MBD including rickets, bone deformities and osteomalacia before cinacalcet initiation. Two patients suffered from low-trauma fractures of long bones during follow-up. One child suffered from an episode of osteomyelitis of the femur without known infectious etiology.

3.4 Safety of cinacalcet and growth in infants

Infants on dialysis frequently suffer from nausea and vomiting, but no additional gastrointestinal symptoms were reported. During the cumulative observation period of 35.5 patient-years, seven of the 26 children developed 10 hypocalcemic episodes (9 asymptomatic) after 8[2-14] months and a cinacalcet dose of 1.5[0.9–3.2] mg/kg/day (Table 3). Compliance to calcium supplements was not good in patient 22, leading to one hypocalcemic convulsion. Patient 19 developed 4 hypocalcemic episodes. At time of hypocalcemic episodes, daily calcium intake was 104[86–127] % of RNI, PTH was 12.3[5.4–19.6] ULN. Albumin-corrected calcium was 1.79[1.76–1.97] mmol/L, phosphate SDS -2.7[-3.0 – -0.8]. The dose of cinacalcet (mg/kg/day) was almost two-fold higher at the time of hypocalcemia than the corresponding dose in the 19 children without hypocalcemia (table 3 and supplemental table 1). Cinacalcet was discontinued in four children.

Three girls, (patients 7, 17 and 26) developed precocious puberty, i.e. thelarche, after 27, 13 and 6 months of cinacalcet therapy. In all three, ultrasounds demonstrated numerous ovarian follicles, while the uterus size and adrenal findings were normal for age as were serum follicle-stimulating hormone, luteinizing hormone and estradiol concentrations. In two girls cinacalcet was stopped at the time of kidney transplantation; 4 and 21 months after diagnosis of precocious puberty. Symptoms persisted after transplantation in both girls, one was treated with gonadotropin releasing hormone agonist. The third girl still had cinacalcet at last follow up, 8 months after diagnosis of precocious puberty, without progression of thelarche.

Body length SDS was largely stable with cinacalcet, and -1.7[-2.5 – -0.8] at start, -1.2[-2.0– -0.6] after 12 months, and -1.5[-1.8 – -1.2] at last follow up. Seven patients concomitantly received rhGH. Body

weight SDS also remained stable and was -0.5 [-0.9– -0.2] at baseline, -0.7 [-1.0– -0.1] at 12 months and -0.5 [-0.9– -0.2] at last follow up.

4. Discussion

The present comprehensive study on the off-label use of cinacalcet in pediatric dialysis patients below three years demonstrates an efficient control of sHPT but also a risk of hypocalcemia in these rapidly growing patients, and a potential risk of precocious puberty.

At start of cinacalcet serum PTH levels were 12-fold above the ULN. PTH levels steadily declined with cinacalcet, with more than 50% of patients reaching PTH levels in the target range after 9 months. The slow but efficient control of sHPT is similar to the one observed in older dialysis children (26), and in children with primary hyperparathyroidism (39). In patients receiving cinacalcet for 12 months, PTH was reduced by around 80%. The cinacalcet starting dose was two-times higher than the initial doses administered in the industry-sponsored trials and the starting dose of cinacalcet of 0.2 mg/kg/day recommended by the 2020 European guidelines, but cinacalcet was started between 2009 and 2021, i.e. mostly prior to publication of the guidelines (43). These doses are not justified by pharmacokinetic data (40–42). In contrast, the maximal dose used was in the range of the phase 2 and 3 trials and the European CPR (42), except for one patient.

Hypocalcemia was observed in 27% of patients, of which 6 children had single, asymptomatic episodes, while one child, not adhering to the prescribed medication, displayed symptomatic hypocalcemia. In the pediatric phase 2 and 3 RCT hypocalcemia was observed in 23% of patients in the cinacalcet group versus 19% in the placebo group (26). A fatal event occurred during the double-blind period in a teenage girl with prolonged QT interval, hypocalcemia and medication interactions. Overall, the hypocalcemia incidence relative to treatment time in infants was not higher as compared to older children.

RNI of calcium should be at least 100% in children with CKD2-5 of RNI in healthy children, with an upper safety threshold of 200% (4). Recent observational studies, however, yielded lower dietary calcium

intake in the majority of children with CKD 3 to 5D and an inverse correlation with serum PTH (10,11). However, when considering calcium from medications, 41% of the children exceeded 200% of the recommended intake (11). 20-40% of formula Ca is absorbed, depending on the native and active vitamin D supply, but may be much lower with high oral calcium intake, since intestinal Ca absorption follows a saturation curve (43). Extrapolating the total Ca intake of the infants to an adult of 75 kg body weight, baseline median calcium intake from diet and medication was 3255 [2477–4691] mg/day, i.e. higher than in most adult dialysis patients, and the active vitamin dose was 14.5 [8.7–20.7] µg thrice weekly, which is close to the highest doses considered in Ca uptake modelling in adults (43). Fitting our findings in the infants to the calculations in adults, and ignoring differences in diets, intestinal uptake rate in the young children should be around 10% only, i.e. about 325mg/day. Accordingly, the relative contribution of dialytic calcium uptake in the children on PD studied here and extrapolated to the adult model, was 181 [127-139] mg/day, i.e. increased net total calcium gain by about 55%. Thus, the relative low average oral Ca intake relative to the 100-200% RNI recommended (4) may have been compensated by a relative high dialytic calcium uptake. In line with this, 8 of the 26 children (31%) had serum calcium levels above the ULN for age at baseline, 2 of the 14 children (14%) still after one year of cinacalcet therapy. Hypercalcemia carries a significant risk of adynamic bone disease and vascular calcification and requires stepwise reduction in calcium supply and vitamin D analogs (4). Of note, 77 % of children exhibited hypophosphatemia at time of cinacalcet initiation and remained stable (79%) during the one year follow-up, which is an additional risk factor for mineralisation defects (44). These findings demonstrate the challenges and the critical importance of the mineral balance in infants and children on dialysis. No systematic studies have been performed, which leaves the pediatric nephrologists with an individual approach, closely monitoring serum calcium, phosphate and PTH and considering trends over time. Cinacalcet may be started in children below 3 years in case of persistent sHPT despite optimized conventional management and in the presence of high or high-normal calcium levels, i.e. in patients with the most severe form of CKD-MBD, as suggested by recently published European guidelines (6) provided informed consent and adequate therapy adherence of the families.

In view of the more than 80% reduction in serum PTH and the associated major influx of calcium into bone increasing oral and dialytic calcium supply should regularly be reconsidered, especially in cases of declining serum calcium.

Different from previous small studies, height SDS did not significantly improve, despite major reduction of exceedingly high PTH values (27,32). This suggests that control of PTH has no major effect on growth, which is in line with observations from the IPPN registry with large patient numbers (45).

Three of the young children on cinacalcet, however, developed precocious puberty, while pubertal development is usually delayed in children on dialysis (46). They had congenital abnormalities of the kidney and urinary tract. Two of them underwent genetic testing, which demonstrated disease causing *HNF1B* variants. No abnormalities in pubertal development or dysgonadism have been reported with such variants so far. In 2009, precocious puberty was reported in a 5-year old-boy with CKD5 due to *HNF1B* variants on cinacalcet (47). The mechanistic link between precocious puberty and cinacalcet therapy is unknown, still these findings indicate the need of close monitoring for endocrine changes in children receiving cinacalcet, especially in patients with *HNF1B* variants.

In conclusion, while limitations inherent to the small number of patients identified in 35 pediatric dialysis centers and inherent to the retrospective design, our findings provide evidence for efficient control of severe sHPT in children below 3 years of age by cinacalcet. This therapy requires close monitoring of calcium and phosphate balance and adaptation of the increasing calcium requirements in these rapidly growing children with the most severe forms of CKD-MBD. The link between precocious puberty and cinacalcet is intriguing and deserves further study and a close endocrine follow-up.

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6. Disclosures of interest

JBa received consulting fees from Amgen and Bayer, and research fees from Amgen. JB is an investigator of the cinacalcet and etelcalcetide Amgen-sponsored trials.

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RS received research grants from Fresenius Medical Care and consultancy fees and / or speaker honoraria from Amgen, Fresenius Medical Care, Astra Zeneca, Vitaflo and Humacyte.

IZ received lecturing honoraria from Baxter and Vitaflo

ADL, SF, JBe, IG, LKK, EV, ED nothing to declare

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Tables

Table 1: Individual patient characteristics, nutrition, medication, baseline serum PTH and cinacalcet dose, and follow up time

ID	Cause of CKD	Age at start of cina (months)	Dialysis mode	Body weight (kg)	Intake (mg/d, % RNI)		P-binder	Active vitamin D (µg/day)	PTH (pg/mL)	PTH, (times above ULN)	Ca _a (mmol/L)	P (mmol/L, SDS)	25-D (nmol/L)	Cina Initial dose (mg/kg/d)	Cina Max. dose (mg/kg/d)	f/u (years)
					Ca	P										
1	CNS	7	PD	5.8	290, 100	155, 37	/	Alfacalcidol 1	810	11.3	2.57	1.01, -4.0	69	0.5	1.6	2.4
2	CNS	8	PD	7.6	352, 92	209, 50	/	Alfacalcidol 1	1245	17.4	2.36	1.55, -1.3	50	0.8	1.0	2.4
3	CAKUT	9	PD	7.8	693, 178	261, 62	/	Alfacalcidol 0.6	631	8.8	2.21	1.17, -3.2	NA	0.5	0.7	1.0
4	CAKUT	11	PD	10.4	396, 76	NA	/	Alfacalcidol 1	563	7.9	2.56	0.95, -4.3	NA	0.3	1.1	1.5
5	CAKUT	11	PD	6.2	355, 114	167, 40	/	Alfacalcidol 1	852	19.6	2.36	1.63, -0.9	79	0.8	1.2	2.2
6	Nephronoph-tis	11	PD	8.6	306, 71	144, 34	/	Alfacalcidol 0.5	410	5.7	2.59	1.13, -3.4	60	0.3	0.4	1.2
7	CAKUT	12	PD	7.5	3653, 975	254, 51	CaCO ₃ Sevelamer	Alfacalcidol 1.5	957	14.7	2.55	1.72, -0.5	34	0.1	0.9	2.6
8	Ischemia	13	PD	8.1	569, 145	233, 47	/	Alfacalcidol 0.6	773	10.8	2.26	1.23, -2.9	NA	1.2	1.2	0.1
9	Glycogenosis	14	PD	8.5	533, 132	344, 69	/	Alfacalcidol 0.2	370	5.2	2.65	1.55, -1.3	65	1.2	1.2	1.7
10	CAKUT	15	PD	12.0	NA	173, 35	Sevelamer	Alfacalcidol 0.8	235	3.5	2.51	1.13, -3.4	97	0.3	0.6	2.5
11	CAKUT	17	PD	9.0	329, 73	235, 47	Sevelamer	Calcitriol 0.75	525	18.1	2.95	2.18, 1.9	95	0.6	0.6	2.0
12	CAKUT	18	PD	11.2	422, 75	NA	Sevelamer	Alfacalcidol 1.2	415	6.4	2.43	1.23, -2.9	NA	0.8	0.8	1.6
13	C-HUS	18	PD	9.5	600, 126%	NA	/	Alfacalcidol 0.4	200	2.8	2.53	1.28, -2.7	72	1.1	1.1	1.8
14	ARPKD	8	PD	5.5	267, 91%	235, 47%	/	Calcitriol 0.5	585	8.2	2.32	1.63, -0.9	68	0.4	1.1	0.9

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15	ADPKD	20	PD	11.4	906, 159%	246, 49%	/	Alfacalcidol 1.5	170	2.3	2.87	1.66, -0.8	133	0.2	0.3	0.3
16	ARPKD	28	HDF	10.3	3525, 684%	444, 89%	CaCO ₃ Sevelamer	Alfacalcidol 1.2	1164	25.6	2.49	2.01, 1.0	80	0.2	0.2	0.1
17	CAKUT	21	PD	8.5	209, 49%	107, 21%	/	Calcitriol 1.5	2320	37.4	2.75	1.16, -3.7	75	0.3	2.1	3.6
18	CAKUT	23	PD	10.9	NA	NA	/	Calcitriol 0.25	1397	21.5	2.60	1.16, -3.2	129	0.2	0.2	0.3
19	ARPKD	25	PD	8.6	176, 41%	112, 22	Sevelamer	Alfacalcidol 0.2	236	3.3	3.38	1.38, -2.0	50	0.9	4.0	0.9
20	ARPKD	27	PD	10.5	374, 71%	232, 46%	Sevelamer	Alfacalcidol 0.5	1829	29.5	2.88	2.81, 5.4	35	0.2	1.7	0.6
21	Pierson syndrome	28	PD	12.6	623, 99%	311, 62%	/	Alfacalcidol 0.5	852	11.9	2.35	1.61, -0.9	65	1.2	1.2	1.3
22	CNS	28	PD	11.1	590, 106%	228, 46%	/	Alfacalcidol 1	1395	19.5	2.09	1.33, -2.2	70	1.4	1.3	1.9
23	CAKUT	29	PD	10.9	323, 59%	235, 47%	/	Alfacalcidol 0.75	945	32.6	2.75	2.25, 2.5	110	0.3	0.6	0.6
24	ARPKD	31	PD	13.0	397, 59%	297, 59%	/	Calcitriol 2.4	91	1.5	2.77	1.16, -3.1	89	0.1	0.3	0.9
25	BBS	32	PD	9.1	181, 40%	182, 36%	Sevelamer	Alfacalcidol 0.2	1422	19.9	2.47	1.95, 0.9	NA	1.1	1.6	0.2
26	CAKUT	35	PD	14.4	547, 76%	270, 54%	Sevelamer	Alfacalcidol 2.5	1757	28.3	2.80	2.45, 3.5	38	0.2	1.1	1.2

Abbreviations: N^o, number; CKD, Chronic Kidney Disease; PD, peritoneal dialysis; HDF, hemodiafiltration; Cina, Cinacalcet; Ca_a, albumin corrected calcium; RNI, Reference Nutrient Intake; P, phosphate; Suppl, supplementation; ULN, upper limit of normal; SDS, Standard Deviation Score for age; f/u, follow-up; CNS, Congenital Nephrotic Syndrome; CAKUT, Congenital Abnormalities of Kidney and Urinary Tract; C-HUS, Congenital Hemolytic Uremic Syndrome; BBS=Bardet-Biedel-Syndrome, ARPKD, Autosomal Recessive Polycystic Kidney Disease; ADPKD, Autosomal Dominant Polycystic Kidney Disease; NA, not available; CaCO₃: calcium carbonate. Calcium intake from diet, medication and PD fluid was expressed relative to the Reference Nutrient Intake (% RNI) for age (8).

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Table 2: Cinacalcet dose, calcium and phosphate intake and biochemical findings at baseline and during follow-up

	Baseline n=26	1 month n=25	3 months n=22	6 months n=20	9 months n=19	12 months n=14	>12 months n=16
Cinacalcet dose (mg/kg/d)	0.4 [0.2-0.8]	0.5 [0.3-1.0]	0.8 [0.3-1.1]	0.9 [0.5-1.1]	0.9 [0.4-1.0]	0.6 [0.4-0.9]	1.0 [0.4-1.2]
Oral Ca intake (% RNI)	84 [66-117]	100 [63-127]	100 [64-142]	93 [78-135]	85 [59-101]	78 [65-103]	76 [55-104]
P intake (% RNI)	47 [40-52]	48 [41-56]	40 [35-51]	48 [45-58]	48 [45-61]	47 [41-51]	62* [57-85]
PTH (pg/mL)	792 [411-1397]	509 [307-916]	490 [207-1056]	323 [165-566]	262 [70-443]	138 [71-361]	90**** [35-239]
Log-PTH (pg/mL)	2.9 [2.6-3.1]	2.7 [2.5-3.0]	2.7 [2.3-3.1]	2.5* [2.1-2.8]	2.4** [1.8-2.7]	2.1** [1.7-2.6]	1.9**** [1.5-2.4]
PTH (times ULN)	11.6 [5.9-19.8]	8.7 [4.7-15.8]	8.0 [3.3-14.7]	4.3* [2.2-7.8]	3.7 [1.0-7.2]	2.0 [1.0-5.3]	1.6**** [0.5-3.4]
Log-PTH (times ULN)	1.1 [0.7-1.3]	0.9 [0.6-1.2]	1.0 [0.5-1.3]	0.7* [0.3-1.0]	0.5** [0.0-0.9]	0.3** [-0.1-0.8]	0.2**** [-0.4-0.6]
Calcium (mmol/L)	2.56 [2.43-2.75]	2.47 [2.38-2.60]	2.51 [2.45-2.58]	2.54 [2.45-2.63]	2.52 [2.44-2.61]	2.48 [2.39-2.53]	2.42 [2.33-2.64]
P (mmol/L)	1.47 [1.16-1.71]	1.28 [1.16-1.62]	1.37 [1.21-1.83]	1.51 [1.08-1.98]	1.34 [1.26-1.90]	1.34 [1.13-1.74]	1.44 [1.23-1.63]
P (SDS)	-1.7 [-3.2--0.6]	-2.5 [-3.2--0.5]	-2.1 [-3.1--0.3]	-1.2 [-3.2-0.9]	-2.1 [-2.6-0.5]	-2.5 [-3.3--0.8]	-1.7 [-2.8--0.6]
25-D (nmol/L)	70 [60-89]	NA	65 [49-82]	70 [62-92]	72 [53-75]	66 [60-72]	65 [54-82]
ALP (IU/L)	660 [492-905]	789 [579-939]	728 [492-857]	613 [540-805]	548 [421-744]	570 [430-861]	438 [270-526]
ALP (SDS)	0.3 [-0.5-2.0]	1.1 [0.1-2.7]	1.1 [-0.3-2.9]	0.5 [-0.2-1.0]	-0.2 [-0.8-1.2]	0.2 [-0.7-1.4]	-0.8** [-2.1--0.2]

Abbreviations: SDS, standard deviation score; Ca, calcium; RNI, Reference Nutrient Intake; P, phosphate ULN, upper limit of normal; 25-D, 25-OH vitamin D; ALP, total Alkaline Phosphatase; SDS, Standard Deviation NA, not available
Linear mixed model (REML) followed by Dunnett's multiple comparisons tests compared to baseline were used for biochemical data. *p<0.05, **p<0.01, and ***p<0.001

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Table 3: Comparison of patients with and without hypocalcemic episodes

	Without hypocalcemic episodes (n = 12)	With hypocalcemic episodes (n = 7)	P
Patient characteristics at initiation			
Age (months)	16 [11-21]	21 [13-23]	0.73
Height SDS	-1.6 [-2.3 -0.7]	-1.7 [-2.4 -1.3]	0.94
Body weight SDS	-1.2 [-2.2 -0.1]	-1.3 [-2.2 -0.2]	0.85
Total Ca supply (%RNI)	84 [68-96]	85 [51-119]	0.74
Cinacalcet dose	0.4 [0.2-0.8]	0.5 [0.3-1.0]	0.44
Maximum dose	1.0 [0.6-1.1]	1.2 [0.9-1.7]	0.31
Serum biochemistry at initiation			
Ca (mmol/L)	2.53 [2.36-2.64]	2.61 [2.51-2.73]	>0.99
Phosphate SDS	-1.1 [-3.2 -0.8]	-2.2 [-3.5 -1.7]	0.31
PTH (ng/L)	698 [414- 1029]	631 [467-1530]	0.65
PTH (times ULN)	11.6 [6.2-17.6]	8.8 [6.5-22.5]	0.84
ALP SDS	1.1 [-0.3-3.2]	1.1 [0-1.9]	0.65
25-D (nmol/L)	68 [55-84]	70 [65-75]	0.89
Patient characteristics at hypocalcemic episodes			
Follow-up (months)	9	8 [2-14]	-
Age (months)	25 [20-30]	32 [28-35]	0.34
Total calcium supply (%RNI)	85 [59-98]	104 [86-127]	0.16
Oral Ca supply	n=5 (45%)	n=4 (57%)	0.69
Cinacalcet dose	0.8 [0.4-0.9]	1.5 [0.9-3.2]	0.03
Serum biochemistry at hypocalcemic episodes			
Ca (mmol/L)	2.52 [2.41-2.58]	1.79 [1.76-1.97]	<0.0001
Phosphate SDS	-2.1 [-2.4 -0.6]	-2.7 [-3.0 -0.8]	0.31
PTH (ng/L)	340 [101-497]	884 [390- 1407]	0.16
PTH (times ULN)	5.1 [1.5-9.1]	12.3 [5.4-19.6]	0.22
ALP SDS	-0.2 [-1.2-1.1]	1.4 [0.7-1.9]	0.08
25-D (nmol/L)	72 [54-74]	82 [70-82]	0.36

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Abbreviations: n, number; SDS, standard deviation score; RNI, Reference Nutrient Intake; ULN, upper limit of normal; PTH, parathyroid hormone; Ca, albumin corrected calcium; ALP, total alkaline phosphatase; 25-D, 25-OH vitamin D. Non-parametric Mann-Whitney test was used for group comparison.

Figures

Figure 1

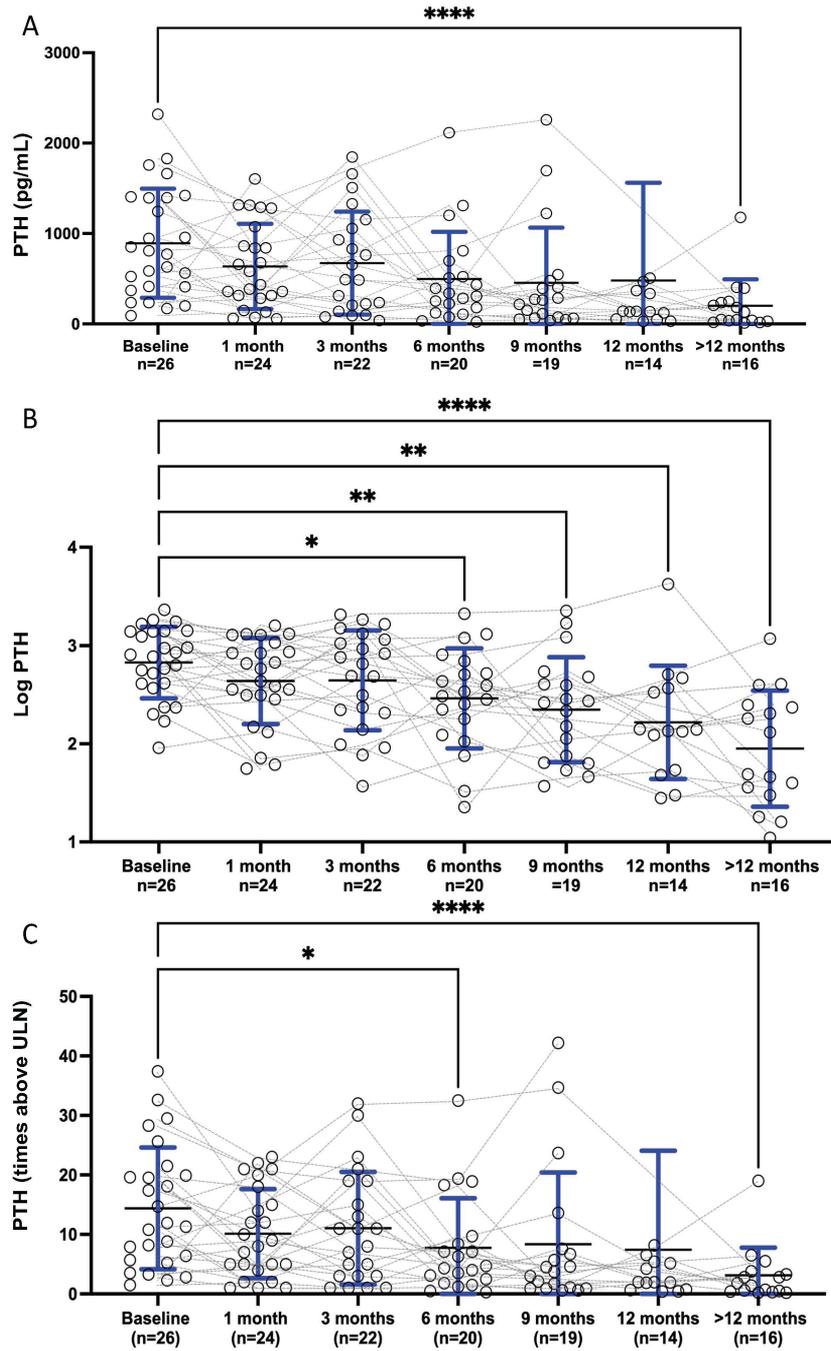


Figure 2

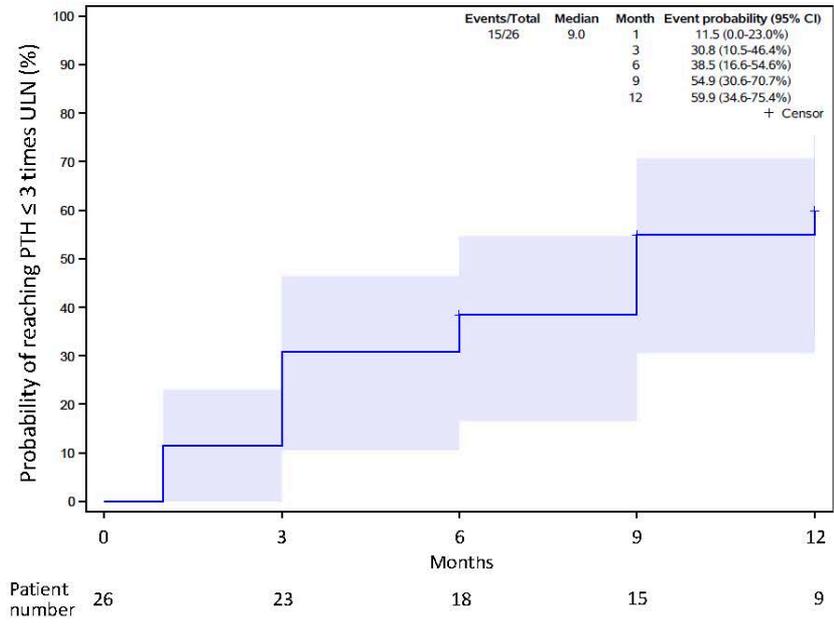
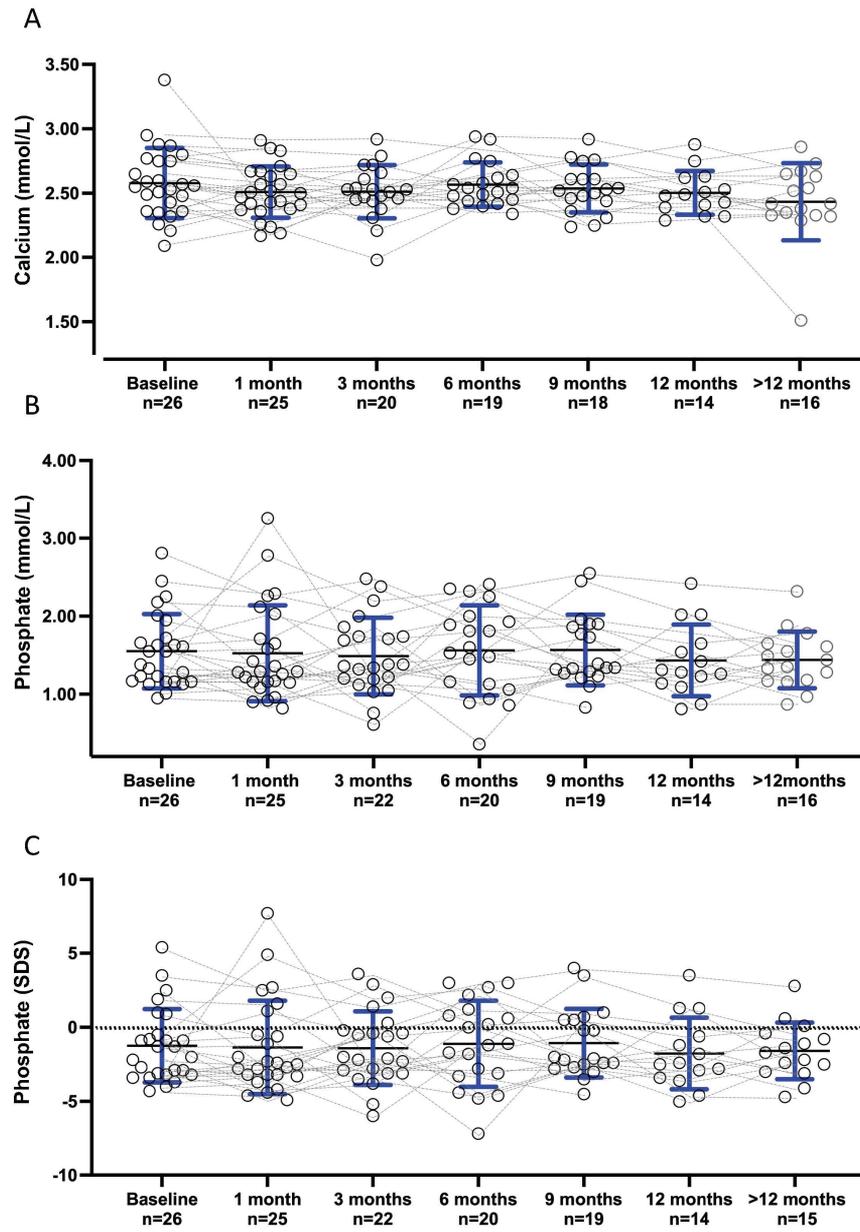


Figure 3



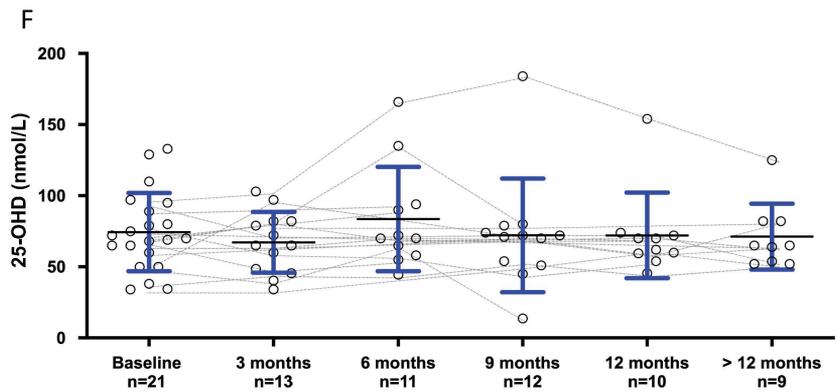
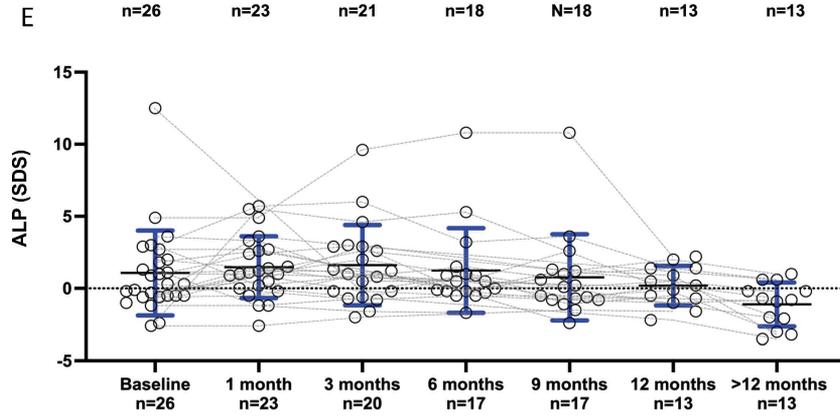
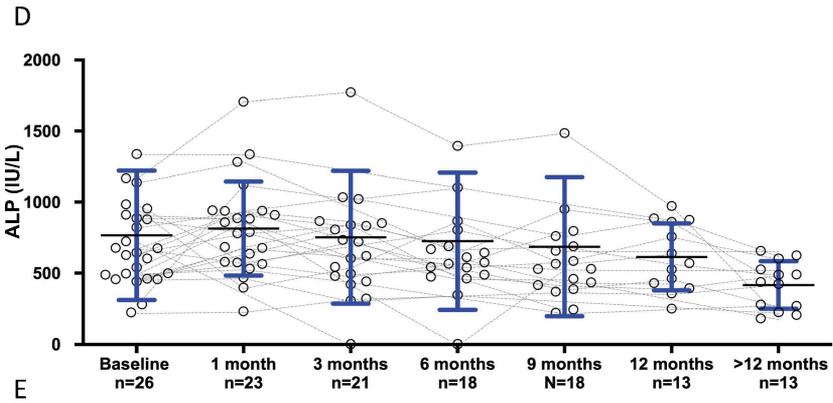


Figure legends

Figure 1: Serum PTH (A), log-PTH (B) and PTH times above upper limit of normal (C) at cinacalcet initiation and during follow-up

Individual values (circles) and median and quartiles are for each time-point. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$ (linear mixed model (REML) followed by Dunnett's multiple comparisons tests compared to baseline)

Figure 2: Probability of achieving a serum PTH below 3 times the upper limit of normal

Kaplan-Meier survival analysis demonstrating the time to achieve parathyroid hormone (PTH) levels below three times the upper limit of normal (ULN), i.e. the target range according to European guidelines(6). The X-axis gives cinacalcet treatment times, the Y-axis the probability, the blue shaded area the 95% confidence interval.

Figure 3: Comparison of corrected calcium levels (A), phosphate (B), phosphate levels as standard deviation score (SDS) for age (C), ALP (D), ALP levels as SDS for age (E) and 25-OH vitamin D (F) levels data at cinacalcet initiation and during follow-up

Individual values (circles) and median and quartiles are for each time-point. $p =$ not statistically significant (NS) and ** $p < 0.01$. (linear mixed model (REML) followed by Dunnett's multiple comparisons tests compared to baseline)

b. Chez l'enfant après transplantation rénale : publication scientifique N°2

Contexte/objectif

Après une transplantation rénale, la sHPT secondaire à la MRC, peut persister et être responsable d'une hypercalcémie et d'une hypophosphatémie avec un rein dont la fonction rénale est généralement proche de la normale. Dans ce contexte, l'utilisation d'analogues de la 1 α ,25(OH) $_2$ D est difficile en raison du risque d'hypercalcémie, et les néphrologues ne s'accordent pas sur la nécessité d'instaurer une supplémentation en phosphate. La prise en charge de cette sHPT post-transplantation est un défi quotidien pour les néphrologues, nécessitant parfois une parathyroïdectomie.

Au moment de la publication, en 2020, 41 articles ont rapporté l'utilisation du cinacalcet après transplantation rénale chez les adultes : certains sont des publications de cas cliniques, plusieurs sont des cohortes rétrospectives [108–120], et des études prospectives [121–141], mais la plupart de ces études étaient de petites séries. Deux essais contrôlés randomisés menés chez des patients atteints de sHPT post-transplantation ont démontré les effets bénéfiques du traitement par le cinacalcet, avec une diminution significative des concentrations de calcium et de PTH [135, 137]. L'utilisation du cinacalcet après transplantation rénale peut permettre d'éviter une parathyroïdectomie. Cependant, certaines études ont rapporté un risque potentiel de néphrocalcinose sur le greffon, mais ce critère n'était pas un critère principal dans les essais contrôlés randomisés. En pédiatrie, seul un cas clinique d'un enfant de 7 ans a rapporté l'efficacité du cinacalcet après la transplantation rénale [142].

Par conséquent, notre étude rétrospective a pour but d'évaluer l'utilisation du cinacalcet, en tant que traitement hors AMM, après la transplantation rénale chez 20 patients pédiatriques, inclus dans le registre européen Cooperative European Paediatric Renal TransplAnt Initiative (CERTAIN), et de considérer ses indications, son efficacité et son innocuité.

Résultats

Sur les 20 patients pédiatriques greffés, 13 avaient des antécédents de sHPT avant la transplantation. L'initiation au cinacalcet variait parmi les patients, certains commençant très précocement après la transplantation et d'autres plus tard en raison d'une dysfonction du greffon. Il n'y avait pas de différence significative concernant la dose quotidienne maximale de cinacalcet ou les concentrations d'hormone parathyroïdienne (PTH) entre celles qui ont commencé le médicament tôt (<1.5 ans après transplantation) et ceux plus tardivement (>1.5 ans). La durée médiane du traitement par cinacalcet était de 3 ans. La PTH diminuait significativement d'environ 50% au cours du suivi. Certains patients présentant une hypercalcémie ont vu une normalisation de leurs concentrations de leur calcémie après traitement par cinacalcet. Les concentrations de phosphate plasmatique sont restés inchangés, et la fonction du greffon est restée stable tout au long de la période de traitement. Un seul épisode

d'hypocalcémie a été rapporté. Trois patients ont arrêté le médicament secondaire à une indication de parathyroïdectomie pour traiter une sHPT sévère et résistante ; une hypocalcémie sévère et une non-observance.

Conclusion

Le cinacalcet semble être efficace pour contrôler le sHPT après une transplantation rénale chez les patients pédiatriques. Cependant, il est essentiel de surveiller attentivement le risque d'hypocalcémie et les effets secondaires du traitement.



The use of cinacalcet after pediatric renal transplantation: an international CERTAIN Registry analysis

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Abstract

Background Secondary hyperparathyroidism (SHPT) may persist after renal transplantation (RTx), inducing hypophosphatemia and hypercalcemia that precludes the use of vitamin D analogs. The calcimimetic cinacalcet improved plasma calcium and parathyroid hormone (PTH) levels in randomized controlled trials in adults after RTx, but pediatric data are scarce.

Methods In this retrospective study, we analyzed 20 pediatric patients from the Cooperative European Paediatric Renal Transplant Initiative (CERTAIN) Registry who received cinacalcet after RTx. The results are presented as median and interquartile range (25th–75th percentile).

Results At 13.7 (11.0–16.5) years of age, 20 pediatric patients received a renal allograft. Cinacalcet was introduced at 0.4 (0.3–2.7) years post-transplant at an estimated glomerular filtration rate (eGFR) of 50 (34–66) mL/min/1.73 m², plasma calcium of 2.58 (2.39–2.71) mmol/L, age-standardized (z score) phosphate of -1.7 (-2.7–0.4), and PTH of 136 (95–236) ng/L. The starting dose of cinacalcet was 0.5 (0.3–0.8) mg/kg per day, with a maximum dose of 1.1 (0.5–1.3) mg/kg per day. With a follow-up of 3.0 (1.5–3.6) years on cinacalcet therapy, eGFR remained stable; PTH levels decreased to 66 (56–124) ng/L at the last follow-up ($p=0.015$). One patient displayed hypocalcemia (1.8 mmol/L). Cinacalcet was withdrawn in three patients (hypocalcemia, parathyroidectomy, incompliance). Nephrocalcinosis of the graft was not reported.

Conclusions This pilot study suggests that cinacalcet as off-label therapy for SHPT after pediatric RTx is efficacious in controlling post-transplant SHPT with acceptable tolerability. Continuing cinacalcet even with normal PTH can lead to dangerous life-threatening hypocalcemia. Therefore, at each subsequent visit, the need to continue cinacalcet must be assessed.

Keywords Secondary hyperparathyroidism · Calcimimetics · Renal transplantation · Children

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Introduction

Secondary hyperparathyroidism (SHPT) is an important and frequent complication of advanced chronic kidney disease (CKD) [1], resulting from global disturbances in mineral metabolism notably hypocalcemia, hyperphosphatemia, and decreased 1,25 [OH]₂ vitamin D (1,25-D) levels. It contributes to the complex CKD-associated mineral and bone disorders (CKD-MBD) including renal osteodystrophy, ectopic, and vascular calcifications, and leading to increased overall morbidity and mortality [2].

Calcimimetic agents, such as cinacalcet or the new-generation etelcalcetide, inhibit parathyroid hormone (PTH) synthesis through sensitization of the parathyroid calcium-sensing receptor by enhancing signal transduction. Calcimimetics are widely used against SHPT in adults receiving dialysis, as a first-line therapy or in combination with vitamin D analogs, as recommended in the 2017 K-DIGO guidelines. Even though modest increases in PTH levels may represent an appropriate adaptive response to declining kidney function, specific therapies aiming at decreasing PTH levels should be introduced when PTH levels progressively rise and remain persistently above the upper target [3]. Cinacalcet was recently licensed in Europe in children for SHPT in children receiving dialysis, with an initial daily dose of 0.2 mg/kg [4]; a European consensus statement from the European Society of Paediatric Nephrology has just been published, delineating the use of this compound in pediatric dialysis [5].

After renal transplantation (RTx), SHPT may persist and is responsible for hypercalcemia and hypophosphatemia on a kidney with usually near-normal renal function. In this setting, the use of vitamin D analogs is difficult because of the prevalent hypercalcemia, and it remains debated among nephrologists whether phosphate supplementation should be initiated. The management of such post-transplant SHPT is a daily challenge for nephrologists, sometimes requiring parathyroidectomy. To date, 41 papers reported the use of cinacalcet after RTx in adults: some are case reports, several retrospective [6–18], and prospective studies [19–39], but most of them were small series. Two randomized controlled trials (RCTs) in patients with post-transplant SHPT demonstrated beneficial effects of cinacalcet therapy, at least on calcium and PTH levels [33, 35]. The use of cinacalcet after RTx may avoid parathyroidectomy. However, some reports highlighted a potential risk of nephrocalcinosis on the renal graft, but this criterion was not a primary outcome in RCTs. In pediatrics, only one case report of a 7-year-old child reported the efficacy of cinacalcet after RTx [40].

Therefore, the main objectives of the current study were to evaluate the use of cinacalcet, as off-label therapy, after RTx in pediatric patients included in the CERTAIN Registry, and to evaluate its indications, efficacy, and safety.

Patients and methods

Patients and study design

The CERTAIN Registry aims to provide pertinent information on the short- and long-term outcomes of pediatric renal allograft recipients for clinical research and quality assurance in order to improve patient care (www.certain-registry.eu) and has already been detailed in previous papers [41]. To avoid redundant data input, CERTAIN has set up data exchange interfaces with Eurotransplant, Collaborative Transplant Study (CTS), and the registry of the European Society of Pediatric Nephrology (ESPN), and European Renal Association-European Dialysis and Transplant Association (ERA-EDTA) (ESPN/ERA-EDTA registry) [42]. For research purposes, an analysis of the multinational dataset requires approval of the predefined study protocol by the CERTAIN Registry Steering Committee. Written informed consent was obtained from all parents/guardians to participate in the registry, with assent from patients as appropriate for their age. The CERTAIN Registry has been approved by the ethics committee of each contributing center and is kept in full accordance with the principles of the Declaration of Helsinki and Good Clinical Practice guidelines. This retrospective study was performed by data extraction of the CERTAIN database. Inclusion criteria were pediatric patients below the age of 21 years with persistent SHPT, having received cinacalcet after RTx, and exclusion criteria were subtotal or total parathyroidectomy before RTx. Twenty patients were identified from five different countries and nine different centers, as follow: Heidelberg (*n* = 10), Hamburg (*n* = 2), Lyon (*n* = 2), Jena (*n* = 1), Essen (*n* = 1), Samsun (*n* = 1), Roma (*n* = 1), Zurich (*n* = 1), and Tübingen (*n* = 1).

Laboratory analyses

Data for plasma total calcium, phosphate, PTH, creatinine, and 25-OH vitamin D levels were collected. Due to the physiological evolution of plasma phosphate during childhood, phosphate levels were normalized and expressed as *z* score for age. Glomerular filtration rate was estimated using the 2009 Schwartz formula [43]. Calcium (Ca), phosphate (Ph), creatinine, and 25-OH vitamin D levels were locally measured by standard methods. Since plasma PTH assessment was a crucial key parameter in this study, we double-checked with each center all the PTH data, asking the physicians to validate both the level of PTH at the different time points and the local assay used for routine assessment of PTH. As summarized in Table 1, different kits were used in the centers with different normal ranges. However, the upper limit of the normal range was not that different among centers. We therefore have not corrected PTH levels and present “raw data” of PTH levels along the manuscript. When available, 24-h urinary excretion

Table 1 Assays used for routine assessment of plasma PTH levels in the contributing CERTAIN centers

Commercial kit	Center	Assay	Normal range
Roche on a Cobas analyzer	Essen, Jena, Lyon, Roma, Samsun, Zürich,	Electro-CLIA	15–65 ng/L
PTH Siemens Analyzer Centaur®	Heidelberg, Tübingen	Two-side CLIA	12.3–71.7 ng/L
NA	Hamburg	Two-side CLIA	17–84 ng/L

NA not available, CLIA chemi-luminescence-immuno assay

rates of calcium were recorded and expressed as millimoles per kilogram per day, the upper normal limit being 0.1 mmol/kg body weight per day in children, teenagers, and adults [44]. These laboratory parameters were analyzed at seven different time points during follow-up, namely at the time of transplant surgery, 4 weeks post-transplant, before cinacalcet initiation, at cinacalcet initiation, at the first documented visit under cinacalcet, at the visit under the maximum cinacalcet dose, and at the last visit under cinacalcet.

Statistical analysis

The main hypothesis was that PTH level declines over time after patients received cinacalcet therapy. There were two main predictions for PTH levels in the sampled patients: PTH levels should be lower at 4 weeks post-transplant than at the time of RTx; PTH decreases as a function of time thereafter. For the secondary objectives, we describe our hypothesis about associated temporal changes with a decrease of calcium levels, an increase of phosphate levels expressed as z score for age, and a stable renal function under cinacalcet therapy.

The 20-patient dataset was incomplete and unbalanced, for all PTH, Ca, eGFR, and phosphate response variables. Kruskal-Wallis test followed by Dunn's test was used to test predictions to analyze times before treatment initiation until the last visit under cinacalcet therapy. To test for decline in PTH level over time, a linear mixed model (LMM) was fitted to the data using the REML (restricted maximum likelihood) approach, in which the random effect was the individual "patient" and the fixed effect was elapsed time post-transplant (Tx, in years), a continuous variable. This approach made full use of the available data, accounting for the repeated measures design, and the fact that error was correlated within subjects while accounting for the variation among them. Since the response of PTH to cinacalcet was of a priori interest, this LMM was fitted to patients' data obtained just before treatment initiation until the last visit under cinacalcet therapy. Likewise, separate LMMs were also fitted for Ca, eGFR, and phosphate to evaluate different predictions. All four LMMs were fitted in GenStat 16th edition (VSN International, Hemel Hempstead, UK). The fits were successful, but in some cases required log transformations (notably for PTH and eGFR). Diagnostic checks showed they had

approximately normal error distributions and homoscedasticity. Importantly, all LMMs used Wald-type F statistics for which the denominator (residual) degrees of freedom were derived using Kenward-Roger computations (recommended for small sample sizes). Mann-Whitney tests were performed to evaluate cinacalcet introduction and eGFR after RTx, and p values below 0.05 were considered statistically significant using GraphPad software. Results are presented as median and interquartile range (25th–75th percentile).

Results

Baseline characteristics

Relevant demographic and clinical and biochemical features of the patients at the time of RTx are presented in Table 2. Only 20 out of a total of 2272 children monitored in the CERTAIN Registry received cinacalcet. At a median age of 13.0 (11.0–16.5) years, a total of 20 patients (15 males) were included (fifteen after a first RTx and five after a second RTx). Ten patients had congenital anomalies of the kidney and urinary tract (CAKUT). The median time spent on dialysis was 1.9 (0.9–3.2) years. With cinacalcet therapy, 11 patients received phosphate supplementation, ten received native vitamin D supplementation, and three received active vitamin D analogs. Two patients received growth hormone therapy.

Cinacalcet post-transplant

Thirteen out of 20 patients (65%) had a history of SHPT pre-transplant, data were not available for 7 patients. Three patients received cinacalcet pre-transplant at a median dose of 0.4 (0.3–0.8) mg/kg per day. Cinacalcet was introduced 0.4 (0.3–2.8) years post-transplant. The starting daily dose was 0.5 (0.5–1.0) mg/kg per day. According to therapeutic responses, the maximum dose was 1.1 (0.5–1.3) mg/kg per day, 1.5 (0.5–3.2) years post-transplant.

Because of the distribution of eGFR among this series at the time of cinacalcet introduction (i.e., $N=2, 3, 8, 2,$ and 3 with eGFR $>90, 60-90, 45-60, 30-45,$ and $15-30$ mL/min per 1.73 m², respectively), it was not possible to analyze the sub-groups of patients requiring cinacalcet because of

Table 2 Patient characteristics at time of renal transplantation

No	Sex	Age (years)	Graft number	Donor	Time spent on dialysis (years)*	Primary renal disease	PTH (ng/L)	Calcium (mmol/L)	Phosphate (z score)	Time between RTx and cinacalcet initiation (years)
1	M	4.3	2	Deceased	0.9	CNS	354	2.53	NA	0.3
2	M	5.2	1	Deceased	3.0	CAKUT	61	2.17	-0.2	5.5
3	M	6.3	1	Living related	Pre-emptive Tx	CAKUT	120	2.18	-1.0	1.9
4	M	6.6	1	Deceased	0.8	Tubulo-interstitial nephritis	NA	NA	NA	6.3
5	M	10.7	2	Deceased	1.5	CAKUT	NA	2.24	4.3	0.3
6	M	11.1	1	Deceased	2.8	CNS	NA	2.38	8.2	0.2
7	F	12.3	1	Deceased	2.5	CAKUT	173	2.59	1.2	2.5
8	F	12.3	1	Living related	0.3	CAKUT	284	2.50	-1.1	1.4
9	M	12.7	2	Living related	Pre-emptive Tx	CAKUT	NA	2.64	-2.4	0.1
10	M	12.9	1	Deceased	10.6	HUS	84	2.42	1.2	2.0
11	M	13.1	1	Deceased	0.9	CAKUT	NA	2.74	-3.4	0.2
12	F	14.2	1	Deceased	4.4	Glomerulonephritis	530	1.77	-1.1	0.1
13	M	15.0	2	Deceased	1.9	CAKUT	451	NA	NA	6.4
14	F	15.5	1	Deceased	0.6	CAKUT	NA	2.45	9.6	0.4
15	M	16.5	1	Deceased	12.8	CNS	327	NA	NA	4.3
16	M	16.8	1	Living related	1.1	IgA nephropathy	245	2.41	6.0	0.0
17	M	17.9	1	Living related	0.5	Watson syndrome	NA	2.23	-0.9	0.1
18	M	18.8	1	Living related	Pre-emptive Tx	CAKUT	126	2.70	2.7	3.0
19	F	19.6	1	Deceased	5.5	HUS	264	2.68	0.8	0.4
20	M	20.9	2	Deceased	3.2	Uncertain etiology	NA	2.44	-0.7	0.4

No number, M male, F female, Tx transplantation, eGFR estimated glomerular filtration rate, CAKUT congenital anomalies of the kidney and urinary tract, CNS congenital nephrotic syndrome, HUS hemolytic uremic syndrome, NA not available

*In patients with the 2nd transplant, the time spent on dialysis was calculated only for the 2nd transplant

advanced CKD. As such, we rather performed a sub-group analysis based on the time of cinacalcet initiation after RTx, with a cutoff of 1.5 years. As such, eGFR was decreased when cinacalcet was introduced more than 1.5 years after RTx ($p = 0.03$), reflecting the likely two indications of cinacalcet therapy in this cohort, either early post-transplant in patients with near-normal renal function and severe pre-dialysis CKD-MBD, or late post-transplant in patients with graft dysfunction (Fig. 1a). However, between these two sub-groups, there was no significant difference neither for the maximal daily dose of cinacalcet (Fig. 1b) nor for PTH levels (Fig. 1c).

The median follow-up under cinacalcet therapy was 3.0 (1.5–3.6) years. At the last visit under treatment, the dose of cinacalcet was 0.6 (0.5–1.2) mg/kg/day. For 55% of patients, follow-up with cinacalcet therapy was above 3 years. Three patients stopped cinacalcet during the post-transplant period of observation (due to parathyroidectomy; severe hypocalcemia; non-compliance).

Evolution of PTH in response to cinacalcet initiation

The evolution of the main biochemical parameters at different time points under cinacalcet therapy is given in Table 3. We observed a nearly a 50% decrease in median PTH levels between before cinacalcet initiation and the last visit ($p = 0.015$), as shown in Fig. 2a. For both predictions, a boot-strapped

paired t test yielded similar results (P values > 0.100 , not shown). Using REML analyses, PTH levels declined significantly linearly over time, before treatment initiation until the last visit under cinacalcet therapy ($R = -0.05$, $p < 0.001$). Figure 2b illustrated the trends for PTH evolution in the two sub-groups (introduction of cinacalcet before or after 1.5 years after RTx).

Evolution of calcium, phosphate, and eGFR in response to cinacalcet

The decrease in calcium levels reached a maximum of 0.18 mmol/L ($p = \text{NS}$) when the daily dose of cinacalcet was maximal, after a period of 1.5 (0.3–6.5) years under cinacalcet therapy (Fig. 3). Using REML analyses, the calcium level declined significantly linearly over time, before treatment initiation until the last visit under cinacalcet therapy ($R = -0.02$, $p = 0.03$). At the time of cinacalcet initiation, 10 patients were hypercalcemic; six normalized their calcium levels after cinacalcet therapy.

As illustrated in Fig. 4, there was no significant changes in plasma phosphate before and after initiation of cinacalcet therapy throughout the observation period. However, as expected, a significant decrease of phosphate was observed between RTx and 4 weeks post-transplant. Using REML analyses, phosphate levels increased significantly linearly over time,

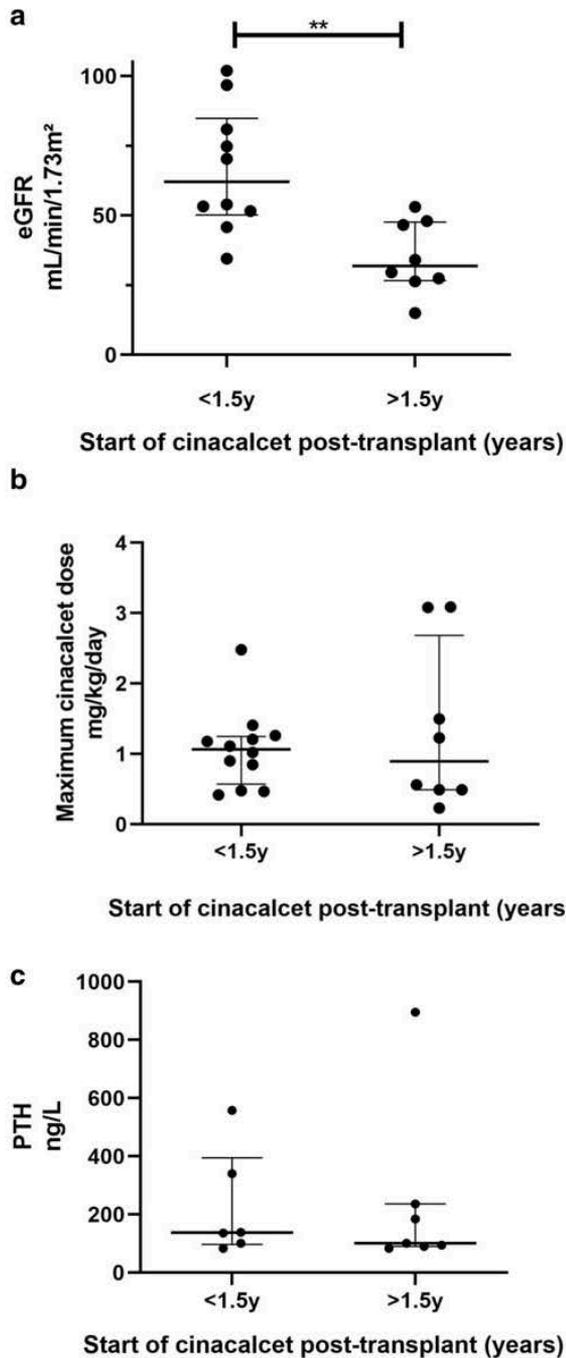


Fig. 1 a Comparison of eGFR data at start of cinacalcet before or after 1.5 years post-transplant. Each dot on the graph represents different follow-up eGFR results, with median and interquartile range, when cinacalcet was started before or after 1.5 years post-transplant. Statistical analyses were performed with non-parametric tests: * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ (Mann-Whitney test). **b** Comparison of the maximum cinacalcet doses at time of cinacalcet initiation (before or after 1.5 years post-transplant). Each dot on the graph represents different follow-up maximum cinacalcet doses results, with median and interquartile range, when cinacalcet was started before or after 1.5 years post-transplant. Statistical analyses were performed with non-parametric paired tests: $p = \text{NS}$ (Mann-Whitney test). **c** Comparison of PTH levels prior to start of cinacalcet (before or after 1.5 years post-transplant). Each dot on the graph represents different follow-up PTH results, with median and interquartile range, when cinacalcet was started before or after 1.5 years post-transplant. Statistical analyses were performed with non-parametric paired tests: $p = \text{NS}$ (Mann-Whitney test)

for calcium and phosphate (as SDS) evolution in the two subgroups (introduction of cinacalcet before or after 1.5 years after RTx).

Adverse effects

During the entire follow-up, no side effects of cinacalcet were reported by the different centers. Tolerance was reported to be good in all patients. However, one patient presented an episode of acute and severe hypocalcemia (1.80 mmol/L) in a context of suppressed PTH of 7 ng/L, with no clinical symptoms, at an eGFR of 17 mL/min/1.73 m², 6.5 years post-transplant, and 3.5 years after cinacalcet initiation, at a daily dose of 1.9 mg/kg per day, thus leading to cinacalcet withdrawal. The previous serum calcium before hypocalcemia was 2.0 mmol/L, 6 years post-transplant (PTH levels 230 ng/L). Retrospectively, as recommended in the 2019 European guidelines [5], cinacalcet should have been withdrawn (or at least decreased) before the onset of clinical symptoms. For one patient, SHPT persisted with parathyroid adenoma: cinacalcet was stopped and subtotal parathyroidectomy was performed. Eventually, for the third patient, cinacalcet was stopped because therapeutic adherence was poor. It is noteworthy that 24-h urine calcium excretion was measured along the study in only two patients. All patients underwent regular follow-up by ultrasound of their graft (usually on a yearly basis), and no nephrocalcinosis was reported during different follow-ups.

Discussion

This is the first case series on the use of cinacalcet in pediatric renal transplant recipients. Our data show that off-label cinacalcet use after pediatric RTx is effective in controlling post-transplant mineral abnormalities, including SHPT with acceptable tolerability.

before treatment initiation until the last visit under cinacalcet therapy ($R = 0.31$, $P < 0.001$). Graft function remained stable with no statistically significant difference between cinacalcet initiation and the last visit under treatment ($p = \text{NS}$), as illustrated in Fig. 5, as confirmed by REML analyses ($R = -0.02$, $p = 0.185$). Figures 3b and 4b illustrated the respective trends

Table 3 Summary of the biochemical data available in patients at the different time points

	eGFR (mL/min/ 1.73 m ²)	PTH (ng/L)	Calcium (mmol/L)	Vitamin D (nmol/L)	Phosphate (mmol/L)	Phosphate (z score)	ALP (U/L)	24-h urine Ca (mmol/kg/day)
Prior to RTX	9 (7–13) n=20	255 (124–334) n=12	2.44 (2.24–2.59) n=17	60 (53–62) n=3	1.54 (1.32–2.30) n=16	0.3 (–1.0–3.1) n=16	154 (91–240) n=10	0.15 (0.09–0.16) n=3
4 weeks post-RTx	65 (57–81) n=20	152 (113–238) n=11	2.57 (2.50–2.64) n=17	39 (36–49) n=3	0.81 (0.68–1.03) n=17	–3.5 (–3.8–2.4) n=17	170 (81–194) n=9	0.12 (0.06–0.13) n=5
First visit before start of cinacalcet	41 (29–61) n=20	136 (91–312) n=15	2.63 (2.41–2.69) n=19	72 n=1	1.11 (0.94–1.31) n=18	–1.5 (–3.1–1.0) n=18	240 (160–317) n=5	NA
At cinacalcet initiation	50 (34–66) n=18	136 (94–236) n=13	2.58 (2.39–2.71) n=18	89 (79–101) n=6	1.17 (0.94–1.33) n=18	–1.7 (–2.7–0.4) n=18	272 (205–394) n=5	0.03 (0.02–0.04) n=2
First visit under cinacalcet	55 (34–72) n=19	107 (78–113) n=14	2.54 (2.35–2.67) n=18	78 (52–90) n=6	1.00 (0.91–1.40) n=17	–2.3 (–2.8–0.7) n=17	219 (119–282) n=6	0.05 n=1
Visit at maximal dose of cinacalcet	53 (33–75) n=19	112 (107–156) n=19	2.43 (2.37–2.60) n=18	74 (54–90) n=6	1.13 (0.99–1.32) n=17	–1.2 (–2.2–0.6) n=17	237 (123–306) n=6	NA
Last visit under cinacalcet	46 (33–76) n=20	66 (56–124) n=14	2.47 (2.36–2.61) n=18	106 (100–118) n=4	1.25 (0.87–1.36) n=17	–1.4 (–2.3–0.6) n=17	222 (101–332) n=5	0.04 n=1

RTx renal transplantation, eGFR estimated glomerular filtration rate, ALP alkaline phosphatase, Ca calcium, NA not available

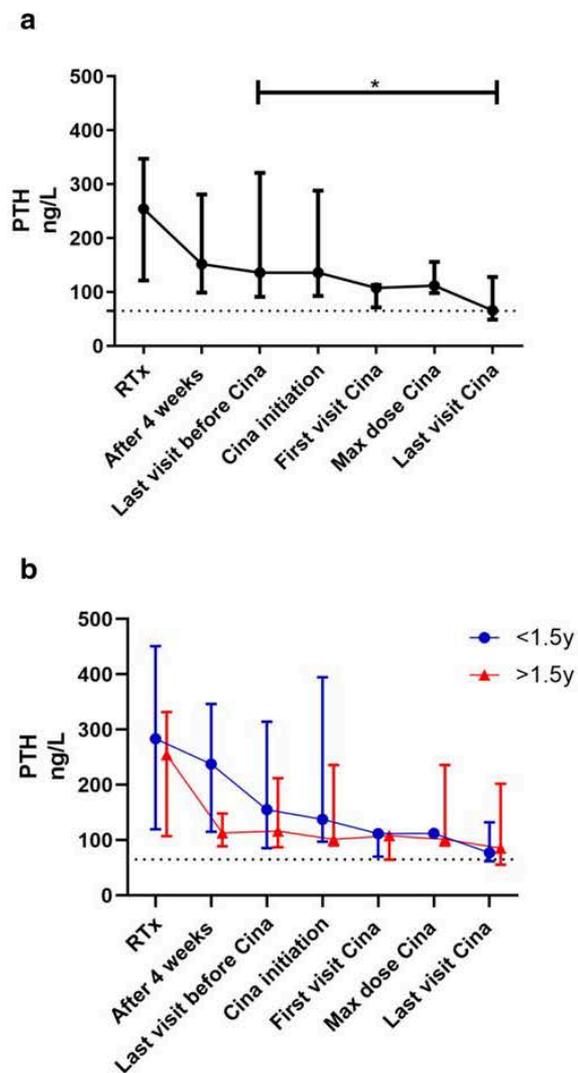


Fig. 2 **a** Evolution of PTH levels before and during cinacalcet therapy. Each dot on the graph represents median with interquartile range of PTH at the different time points. Statistical analyses were performed with Kruskal-Wallis test: * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. Abbreviations: Cina, Cinacalcet. The horizontal line corresponds to the upper normal value for serum PTH (65 ng/L). **b** Comparison of PTH data at start of cinacalcet before or after 1.5 years post-transplant. Each dot on the graph represents different follow-up PTH results, with median and interquartile range, when cinacalcet was started before or after 1.5 years post-transplant. Statistical analyses were performed with non-parametric tests: * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ (Mann-Whitney test). The horizontal line corresponds to the upper normal value for serum PTH (65 ng/L)

In adults, the incidence of post-transplant SHPT varies from 10 to 75%, and usually appears during the first year post-transplant [14]. In a cohort of 100 adults undergoing RTx, 31% of them had SHPT with subsequent hypercalcemia within 1 year after transplantation [45]. Cinacalcet administration pre-transplant and the plasma calcium level at the time of RTx were

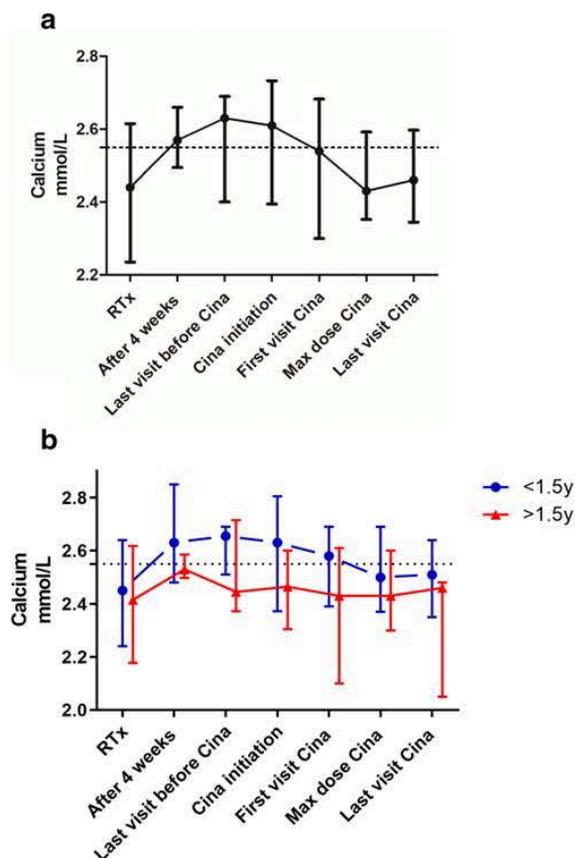


Fig. 3 **a** Evolution of plasma calcium levels before and during cinacalcet therapy. Each dot on the graph represents median with interquartile range of calcium at the different time points. Statistical analyses were performed with Kruskal-Wallis test: $p = NS$ (Kruskal-Wallis test). Abbreviations: Cina, Cinacalcet. The horizontal line corresponds to the upper normal limit for serum calcium (2.55 mmol/L), at 14.7 years of age (i.e., the median age at cinacalcet initiation in this series). **b** Comparison of plasma calcium data at start of cinacalcet before or after 1.5 years post-transplant. Each dot on the graph represents different follow-up plasma calcium results, with median with interquartile range, when cinacalcet was started before or after 1.5 years post-transplant. Statistical analyses were performed with non-parametric tests: * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ (Mann-Whitney test). The horizontal line corresponds to the upper normal limit for serum calcium (2.55 mmol/L), at 14.7 years of age (i.e., the median age at cinacalcet initiation in this series).

independent risk factors [45]. Only four studies have reported long-term follow-up (i.e., more than 3 years) under this treatment [12, 13, 34, 36]. The main manifestation of post-transplant SHPT is hypercalcemia and its incidence varies widely from 9 to 65%. In 2014, a RCT comparing cinacalcet to placebo in 114 adult patients with persistent SHPT post-transplant concluded to a significant beneficial effect of cinacalcet on hypercalcemia and hypophosphatemia, and the absence of safety signals [12]. Nephrocalcinosis was not reported and good cinacalcet tolerance was observed [32]. However, a prospective observational cohort

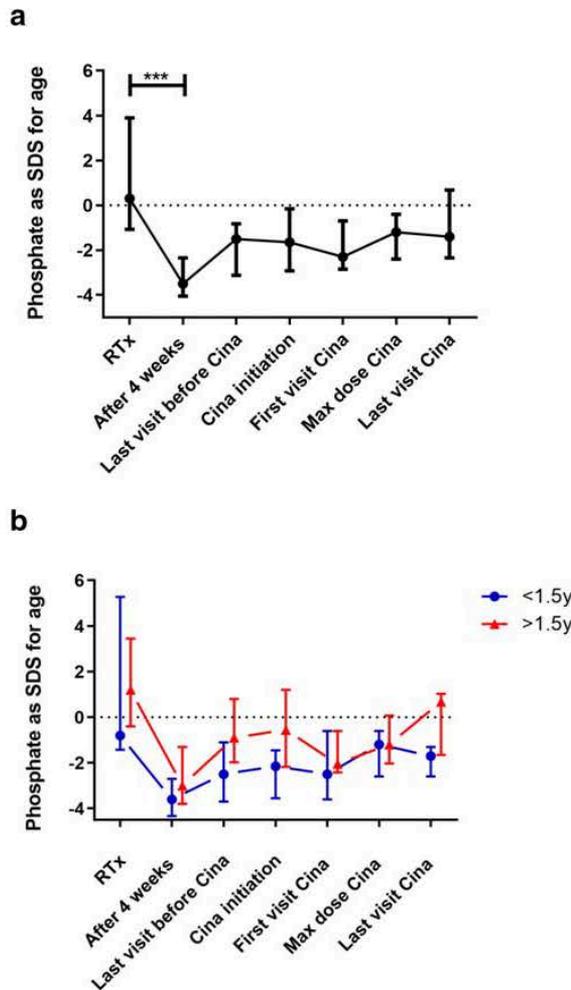


Fig. 4 **a** Evolution of plasma phosphate levels as z score for age before and during cinacalcet therapy. Each dot on the graph represents median with interquartile range of SDS phosphate at the different time points. Statistical analyses were performed with Kruskal-Wallis test: $p = \text{NS}$ (Kruskal-Wallis test). Abbreviations: Cina, Cinacalcet. The horizontal line corresponds to the normal reference range for phosphate as z score (0). **b** Comparison of plasma phosphate levels as z score for age data at start of cinacalcet before or after 1.5 years post-transplant. Each dot on the graph represents different follow-up plasma phosphate levels as z score for age results, with median and interquartile range, when cinacalcet was started before or after 1.5 years post-transplant. Statistical analyses were performed with non-parametric tests: $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$ (Mann-Whitney test). The horizontal line corresponds to the normal reference range for phosphate as z score (0)

study including 303 incident renal transplant recipients with or without cinacalcet at the time of RTx reported an increased incidence of both nephrocalcinosis (i.e., 45% at month 3 versus 33% in controls, $p = \text{NS}$) and parathyroidectomy (i.e., 29% versus 7.2% in controls, $p = 0.02$), with no differences in renal function [31]. In a retrospective study, cinacalcet treatment was not associated with a decline in eGFR or renal graft calcium deposition

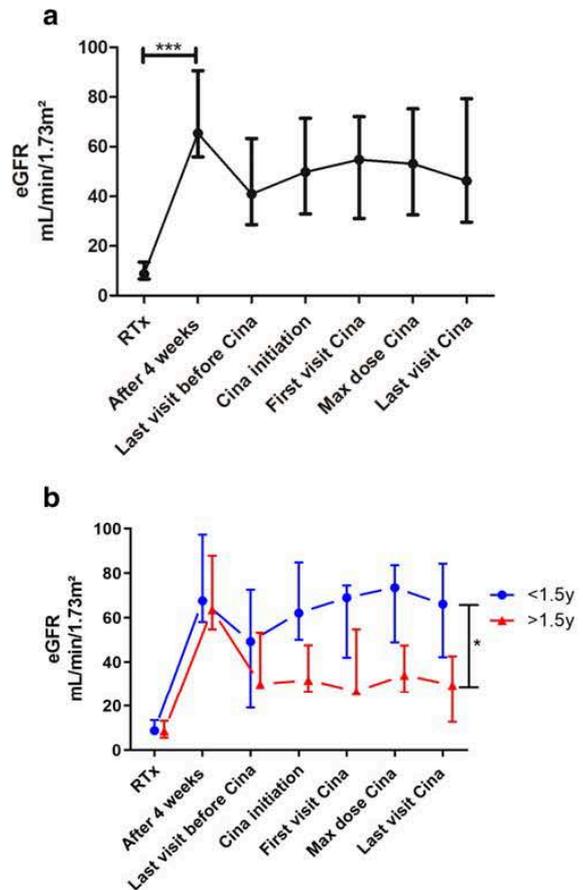


Fig. 5 **a** Evolution of eGFR before and during cinacalcet therapy. Each dot on the graph represents median with interquartile range of eGFR at the different time points. Statistical analyses were performed with Kruskal-Wallis test: $p = \text{NS}$. Abbreviations: Cina, Cinacalcet. **b** Comparison of eGFR data at start of cinacalcet before or after 1.5 years post-transplant. Each dot on the graph represents different follow-up eGFR results, with median, when cinacalcet was started before or after 1.5 years post-transplant. Statistical analyses were performed with non-parametric tests: $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$ (Mann-Whitney test)

on renal biopsies in the first year following RTx, despite sustained and marked hypercalciuria induced by cinacalcet [11].

In children, CKD-MBD may persist after RTx and worsen allograft prognosis [46]. CKD-MBD has also been associated with impaired longitudinal growth, fractures, anemia, left ventricular hypertrophy, and increased mortality [1]; these comorbidities may progress post-transplant. Cinacalcet is only licensed for patients on dialysis [4]. Our study adds real-life data about off-used label cinacalcet that could be used to control SHPT post-transplant. Only one case of cinacalcet use in children after RTx has been reported to date. In this 7-year-old child, cinacalcet was introduced 4 months after RTx, decreased PTH levels from 529 ng/L to 294 ng/L after 3 months treatment [40], but renal

function was not reported. Of note, cinacalcet decreased calcium levels and renal tissue calcium deposits [40].

The present study is the first pediatric case series comprising 20 patients treated with cinacalcet post-transplant. Despite the high prevalence of SHPT after RTx of 26–41% [46, 47] and the proven efficacy of cinacalcet in dialysis patients, pediatric nephrologists are reluctant with off-label cinacalcet use post-transplant. Only 20 out of a total of 2272 children monitored in the CERTAIN Registry received cinacalcet. Routine management of SHPT after pediatric RTx can combine dietary optimization of calcium and phosphate intake, active vitamin D analogs, provided there is no overt hypercalcemia, as well as phosphate supplementation (so as to avoid deficient bone mineralization as a consequence of chronic hypophosphatemia), even though the benefit of the latter is debated. In this cohort, despite the use of cinacalcet and phosphate supplementation in 10 patients, hypophosphatemia was not completely corrected.

Following RTx, mineral metabolism remains compromised for an extended period of time: in addition to SHPT, FGF23 levels remain elevated for weeks to several months after RTx, both resulting in significant phosphaturia and subsequent hypophosphatemia in the post-transplant period [48]. FGF23 levels were not available in the CERTAIN Registry, thus its relative contribution as compared with SHPT remains to be evaluated. In 2018, increased PTH levels before RTx were defined as risk factors for persistent SHPT in children post-transplant [48]. Data on SHPT before RTx were available in 13 out of 20 patients, and all these children had a history of SHPT during the dialysis period. In adults, pre-transplant cinacalcet use predicts the use of cinacalcet post-transplant [15].

PTH levels at which cinacalcet therapy were introduced in this series may appear quite low. However, even though it is difficult to justify the threshold of PTH that was used to introduce cinacalcet on an individual basis in this retrospective analysis of the CERTAIN Registry, recommended target PTH levels are lower in non-dialysis CKD patients. K-DIGO proposed PTH target levels between 35 and 70 pg/mL in CKD stages II and III, between 70 and 110 pg/mL in CKD stage IV, and between 150 and 300 pg/mL in CKD stage V [3]. For the first time, our data obtained in 20 children demonstrates that treatment of children with cinacalcet post-transplant significantly reduces serum PTH and calcium. PTH declined into the target range in 10 of the patients. At the last visit under cinacalcet, PTH had significantly decreased as compared with the pretreatment levels. Since these patients all displayed some degree of CKD and in view of the K-DOQI PTH targets in pre-dialysis patients [3], the PTH targets levels achieved with cinacalcet therapy are adequate. Moreover, 6/10 patients were hypercalcemic at the time of cinacalcet initiation and normalized their calcium levels with cinacalcet therapy. Also, as illustrated in Fig. 3b, patients with near-normal renal function and early introduction of cinacalcet were the ones who benefited most of cinacalcet therapy, at least in terms of calcium levels.

The CERTAIN Registry was not designed to assess cinacalcet therapy post-transplant. We therefore contacted all contributing centers regarding potential adverse effects of cinacalcet. Beyond one episode of hypocalcemia, no other adverse effect was reported. An increased occurrence of nephrocalcinosis of the graft was reported in one study in adults [31]. Limited urinary calcium excretion data were available in this patient cohort, but yearly renal ultrasound findings were available in all patients, with no signs of nephrocalcinosis or nephrolithiasis during a follow-up on cinacalcet therapy of more than 3 years in 55% of patients. Altogether, our observational data suggest that cinacalcet therapy in pediatric renal transplant recipients may be a useful tool to correct SHPT and hypercalcemia with acceptable tolerability.

One episode of severe hypocalcemia occurred in one out of 20 patients who was receiving cinacalcet therapy for a total of 3.5 years. This rate of hypocalcemia (5%) is less than the one observed in the pediatric RCTs in dialysis patients (23% in cinacalcet group versus 5% in placebo group) [49]. The risk of hypocalcemia should be lower in patients with SHPT post-transplant and normal or near-normal renal function, due to the resulting calcium levels within the upper normal range or even above. On the other hand, physicians applied relatively high doses of cinacalcet, a strategy which theoretically increases the risk of hypocalcemia: indeed, the median starting dose of cinacalcet was 0.5 mg/kg per day and the median maximum dose was 3.1 mg/kg per day, whereas the European consensus recommends ≤ 0.2 mg/kg per day for the starting dose and 2 mg/kg per day for the maximal dose in dialysis patients [5]. It also recommends introducing cinacalcet only if calcium levels are above 2.40 mmol/L. Even though this is less likely to be the case after RTx, we believe that this threshold should also be respected. Also, the general contra-indications of cinacalcet, such as the concomitant use of drugs increasing the QT interval, as well as an history of seizures, cardiac arrhythmia, significant liver disease, or poor adherence to medications should be respected after RTx, too [5].

This retrospective uncontrolled single-arm descriptive pilot study is the first step to delineate the use of cinacalcet for treatment of persistent SHPT after pediatric renal transplantation. The strengths of this study are based on the CERTAIN database, which allowed identification of 20 pediatric patients treated with cinacalcet in a large patient cohort. On the other hand, the registry was not specifically designed to evaluate the use of cinacalcet post-transplant. However, we were able to double-check the results with all centers and to obtain additional information. Limitations are the retrospective study design and the lack of a control group. As such, statistical analyses should be seen only as an indicator for potential future studies. A randomized controlled trial would provide the highest evidence on the use of cinacalcet post-transplant, but it requires major efforts and financial support. With this study design, it is impossible to justify the individual decision making of introducing cinacalcet in the different centers,

at a time when guidelines on the use of cinacalcet in pediatric dialysis had not been published, and when cinacalcet was not even approved in pediatrics. Therefore, the next step could be a close, prospective monitoring of all pediatric patients treated with cinacalcet post-transplant to specifically assess therapeutic efficacy but also monitor potential adverse effects including clinical symptoms, transplant function, urinary electrolyte excretion, biomarkers of bone metabolism, and potential interactions with other drugs.

In summary, this pilot study provides the first pediatric data suggesting that the use of cinacalcet, as off-label therapy, for SHPT post-transplant is efficacious in controlling post-transplant SHPT with acceptable tolerability. Continuing cinacalcet after pediatric RTx even with normal PTH can lead to dangerous life-threatening hypocalcemia. Therefore, at each subsequent visit, the need to continue cinacalcet must be assessed. The potential risk of hypercalciuria and nephrocalcinosis previously suggested in adult RTx patients under cinacalcet treatment requires a close follow-up.

Conflict of interest Justine Bacchetta has been an investigator for the Amgen-sponsored trials on the use of cinacalcet in pediatric dialysis. Justine Bacchetta is the coordinator of the Amgen-sponsored international registry on the use of cinacalcet in pediatric dialysis, and the national principal investigator for the Amgen-sponsored trial on the use of etelcalcetide in pediatric dialysis. She received a research grant for experimental studies by Amgen.

Claus Peter Schmitt received financial support from Amgen for investigator-initiated experimental research, a travel grant, and is national principal investigator for the Amgen-sponsored trial on the use of etelcalcetide in pediatric dialysis.

No other relevant conflicts of interest are reported.

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The non-profit pediatric association of *CHU de Nice* (ADHPUN) paid for statistical analyses, performed by SARL Green Grow Scientific.

Compliance with ethical standards For research purposes, an analysis of the multinational dataset requires approval of the predefined study protocol by the CERTAIN Registry Steering Committee. Written informed consent was obtained from all parents/guardians to participate in the registry, with assent from patients as appropriate for their age. The CERTAIN Registry has been approved by the ethics committee of each contributing center and is kept in full accordance with the principles of the Declaration of Helsinki and Good Clinical Practice guidelines.

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3. Utilisation hors AMM du cinacalcet en hyperparathyroïdie primaire (HPP) en pédiatrie : publication scientifique N°3

Contexte/objectif

L'hyperparathyroïdie primaire pédiatrique (HPP) est un trouble endocrinien peu fréquent, secondaire à une sécrétion accrue ou inappropriée de PTH. Cette pathologie a une incidence de 2 à 5 pour 100 000 enfants et est responsable d'une hypercalcémie et d'une hypophosphatémie [143]. L'hypercalcémie chronique se traduit par des différents symptômes tels qu'une polyurie, des nausées, une constipation, des douleurs abdominales ou un retard de croissance [144]. Le diagnostic d'HPP est généralement posé devant une hypercalcémie avec des concentrations de PTH élevés ou "normaux" non adaptés. Un dépistage génétique est recommandé, notamment en raison du risque d'adénocarcinome de la parathyroïde secondaire à des mutations spécifiques, comme par exemple, dans le gène CDC73 ou MEN 1, codant pour une protéine suppresseur de tumeur.

Chez les enfants, l'adénome parathyroïdien bénin unique peut être responsable d'une HPP, survenant à l'adolescence, et nécessite une résection de la parathyroïde [143, 145]. L'hyperparathyroïdie peut également s'intégrer dans différents syndromes endocriniens génétiques, notamment 1/ Néoplasies endocriniennes multiples de type I (hyperparathyroïdie, tumeurs neuroendocrines duodéno-pancréatiques et/ou adénomes hypophysaires, mutation du gène MEN1) [146], ou de type II (hyperparathyroïdie, carcinome médullaire de la thyroïde et/ou phéochromocytome, mutation du proto-oncogène RET) [147]; 2/ Syndrome d'hyperparathyroïdie héréditaire-tumeur mandibulaire (HPT-JT) secondaire à une mutation du CDC73 (également connu sous le nom de HRPT2) [145] ; et 3/ une inactivation du CaSR secondaire à des mutations génétiques (mutations AP2S1, GNA11, CASR) ou à des anticorps anti-CaSR [148, 149].

D'après les recommandations publiées en 2014, la parathyroïdectomie est indiquée chez les adultes âgés de moins 50 ans, présentant une HPP asymptomatique avec hyperplasie parathyroïdienne [150, 151]. Les enfants atteints de HPP sans étiologie déterminée, de MEN1 symptomatique et de HPT-JT, devraient être traités chirurgicalement à la condition qu'ils ne présentent pas de contre-indications [151, 152]. Pour les autres cas ou avant la chirurgie, une prise en charge médicale peut être proposée, et vise à diminuer la calcémie, à obtenir des concentrations de phosphate normaux, à compléter en vitamine D native et à éviter l'hypercalciurie qui peut induire une néphrolithiase et/ou une néphrocalcinose [150]. Chez les adultes, le traitement conventionnel fait appel aux bisphosphates, de préférence à l'alendronate. En cas d'hypercalcémie sévère symptomatique, les calcimimétiques peuvent être ajoutés (8). Il est important de noter qu'il n'est pas recommandé de restreindre l'apport

en calcium en deçà des recommandations établies pour tous les individus afin d'éviter les atteintes osseuses et l'altération du pic de masse osseuse chez l'enfant [153].

De récentes recommandations internationales suggèrent l'utilisation de calcimimétiques chez les adultes atteints de HPP ne pouvant subir une parathyroïdectomie chirurgicale (8). Une méta-analyse récente de 28 études, incluant quatre essais cliniques randomisés, a rapporté que les concentrations sériques de calcium et de PTH étaient significativement réduits, et que les concentrations de phosphate augmentaient significativement avec l'utilisation du cinacalcet chez les patients souffrant d'une HPP [154].

Comme décrit dans la section introduction, une recommandation européenne a défini l'utilisation du cinacalcet pour les enfants dialysés de plus de 3 ans souffrant de sHPT due à une MRC [52]. Par ailleurs, l'utilisation du cinacalcet, hors AMM dans l'HPP, n'a été rapportée que dans quelques cas pédiatriques [155, 156].

L'étude CALCI-CINA rapporte rétrospectivement l'expérience française de 18 enfants traités par cinacalcet dans l'HPP pédiatrique, dans sept centres de références des maladies rares du métabolisme du calcium et du phosphate. Nous avons émis l'hypothèse que le cinacalcet améliore la calcémie sans avoir d'effets indésirables majeurs.

Résultats

Les patients rapportés avaient un âge médian de 10,8 ans et un DFG médian de 120 mL/min/1,73 m² lors de l'initiation du traitement. Après le début du traitement par cinacalcet, une diminution significative des concentrations de calcium a été observée après un mois de traitement, qui s'est maintenue tout au long de la période de suivi. Le DFG et l'excrétion urinaire de calcium sont restés stables, indépendamment de la cause sous-jacente de la HPT (Table 2). Aucun effet secondaire grave associé au cinacalcet, ni cas d'hypocalcémie n'ont été signalés. Trois patients ont arrêté le cinacalcet : pour certains une normalisation de la calcémie ; pour d'autres une mauvaise observance au traitement.

Conclusion

Le cinacalcet s'est avéré être un traitement efficace et sûr pour les patients pédiatriques atteints de HPT. Il permet le contrôle de l'hypercalcémie et de l'hyperparathyroïdie sans effets secondaires notables associés. Cette étude renforce l'importance du cinacalcet comme option thérapeutique pour

les enfants atteints de PHPT, en dépit du manque de données dans la littérature sur son utilisation dans cette population.



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Off-label use of cinacalcet in pediatric primary hyperparathyroidism: A French multicenter experience

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Background: Cinacalcet is a calcimimetic approved in adults with primary hyperparathyroidism (PHPT). Few cases reports described its use in pediatric HPT, with challenges related to the risk of hypocalcemia, increased QT interval and drug interactions. In this study, we report the French experience in this setting.

Methods: We retrospectively analyzed data from 18 pediatric patients from 7 tertiary centers who received cinacalcet for PHPT. The results are presented as median (interquartile range).

Results: At a median age of 10.8 (2.0–14.4) years, 18 patients received cinacalcet for primary HPT ($N = 13$ inactive *CASR* mutation, $N = 1$ *CDC73* mutation, $N = 1$ multiple endocrine neoplasia type 1, $N = 3$ unknown etiology). Cinacalcet was introduced at an estimated glomerular filtration rate (eGFR) of 120 (111–130) mL/min/1.73 m², plasma calcium of 3.04 (2.96–3.14) mmol/L, plasma phosphate of 1.1 (1.0–1.3) mmol/L, age-standardized (z score) phosphate of –3.0 (–3.5;–1.9), total ALP of 212 (164–245) U/L, 25-OHD of 37 (20–46) ng/L, age-standardized (z score) ALP of –2.4 (–3.7;–1.4), PTH of 75 (59–123) ng/L corresponding to 1.2 (1.0–2.3)-time the upper limit for

normal (ULN). The starting daily dose of cinacalcet was 0.7 (0.6–1.0) mg/kg, with a maximum dose of 1.0 (0.9–1.4) mg/kg per day. With a follow-up of 2.2 (1.3–4.3) years on cinacalcet therapy, PTH and calcium significantly decreased to 37 (34–54) ng/L, corresponding to 0.8 (0.5–0.8) ULN ($p = 0.01$), and 2.66 (2.55–2.90) mmol/L ($p = 0.002$), respectively. In contrast, eGFR, 25-OHD, ALP and phosphate and urinary calcium levels remained stable. Nephrocalcinosis was not reported but one patient displayed nephrolithiasis. Cinacalcet was progressively withdrawn in three patients; no side effects were reported.

Conclusions: Cinacalcet in pediatric HPT can control hypercalcemia and PTH without significant side effects.

KEYWORDS

children, primary hyperparathyroidism, cinacalcet, hypercalcemia, Calcium-sensing Receptor (CaSR)

Introduction

Pediatric primary hyperparathyroidism (PHPT) is an uncommon endocrine disorder secondary to an increased secretion of parathyroid hormone (PTH), with an incidence of 2–5 per 100 000 children, responsible for hypercalcemia and hypophosphatemia (1). Heterogenous clinical features such as polyuria, nausea, constipation, abdominal pain and failure to thrive, may be the presenting symptoms of chronic hypercalcemia (2). The diagnosis is usually made in children with hypercalcemia with elevated or non-adapted “normal” PTH levels; genetic screening is recommended, especially because of the risk of adenocarcinoma of the parathyroid with specific mutations as in the *CDC73* gene.

In children, single benign parathyroid adenoma may cause PHPT occurring during adolescence and requires parathyroid resection for definitive treatment (1, 3). PHPT may also be part of genetic endocrine syndromes including 1/ Multiple endocrine neoplasia (MEN) syndromes, type I (PHPT, duodenopancreatic neuroendocrine tumors and/or pituitary adenomas, *MEN1* gene mutation) (4), or type II (PHPT, medullary thyroid carcinoma and/or pheochromocytoma, *RET* proto-oncogene mutation) (5); 2/ Hereditary hyperparathyroidism-jaw tumor (HPT-JT) syndrome secondary to *CDC73* mutation (also known as *HRPT2*) (3); and 3/ Inactive Calcium-sensing Receptor (CaSR) secondary to genetic disorders (*AP2S1*, *GNA11*, *CASR* mutations) or anti-CaSR antibodies (6, 7). Patients with *CASR* mutations can display parathyroid hyperplasia or adenoma (3).

In 2014, the Fourth international workshop proposed indications for surgery in adults with asymptomatic PHPT more frequently than the last set of guidelines (8). This workshop published surgical management of asymptomatic primary hyperparathyroidism and stated that age below 50 years should be considered as one of indications for surgery (9). Children with sporadic PHPT, symptomatic MEN1 and HPT-JT,

should be treated surgically if there are no contraindications (9, 10). In other cases or before surgery, medical management is proposed, aiming at decreasing calcium levels, obtaining normal phosphate levels, repleting native vitamin D, and avoiding hypercalciuria that may further induce nephrolithiasis and/or nephrocalcinosis (8). In adults, the conventional management uses bisphosphonates, with a preference to alendronate, to improve Bone Mass Density (BMD), since it does not alter serum calcium and PTH concentration; and in an acute setting in case of symptomatic severe hypercalcemia, calcimimetics are combine (8). Restriction of calcium intake below established guidelines for all individuals is not recommended to avoid bone defects and impaired peak bone mass (11).

The calcimimetic cinacalcet inhibits PTH secretion through the sensitization of the parathyroid CaSR by enhancing signal transduction (12). In adults, the recent international guidelines recommend to use calcimimetics in PHPT patients unable to undergo surgical parathyroidectomy (8). A recent meta-analysis of 28 studies, including four randomized clinical trials (RCTs), has reported that serum levels of calcium and PTH levels were significantly reduced, and phosphate levels significantly increased with cinacalcet use in PHPT (13).

Cinacalcet was recently licensed in Europe in dialysis children above 3 years with secondary hyperparathyroidism due to end-stage kidney disease; European guidelines have delineated its use in this peculiar setting (14). Alternatively, the use of cinacalcet, as an off-label therapy in PHPT, has been reported only in few pediatric case reports and is considered to be a challenge in daily practice, because of the putative risk of hypocalcemia, increased QT interval and drug interactions (15, 16).

Thus, we aimed to retrospectively report the French experience of cinacalcet in pediatric PHPT. We hypothesized that cinacalcet improves calcium levels with a good safety profile.

Patients and methods

Patients

This is a retrospective multicenter series of 18 pediatric patients (below the age of 18 years), followed in seven tertiary centers of rare diseases of calcium and phosphate metabolism (Toulouse $N = 6$; Montpellier $N = 4$; Lyon $N = 2$; Bicêtre Paris Saclay $N = 2$; Rouen $N = 2$; Paris-Robert Debré $N = 1$; Limoges $N = 1$), in France, for PHPT. We included all children who started cinacalcet between November 2013 and January 2020, as an off-label therapy, with at least 1 year of follow-up for 17 patients.

Biochemicals and assessment of growth parameters

As part of the routine follow-up of these patients with PHPT, total calcium, phosphate, magnesium, alkaline phosphatase (ALP), PTH, creatinine, 25-OH vitamin D (25-OHD) and urinary calcium were regularly assessed. Calcium (Ca), phosphate (Ph), creatinine, and 25-OHD levels were locally measured by standard methods. Variables with age-dependent reference values (plasma phosphate concentration, ALP, urinary calcium/creatinine ratio) are expressed as z score, calculated as follows: $z \text{ score} = (\text{measured value} - \text{mean normal value})/\text{SD}$. Reference values for across corresponding age groups and/or gender were used from prior literature (17, 18) whereby a square root transformation and corresponding reference values for ALP (19). Estimated Glomerular Filtration Rate (eGFR) was estimated using the 2009 Schwartz formula (20). Laboratory parameters were collected at six different time points: initiation of cinacalcet therapy (baseline), and after 1, 3, 6, and 12 months; data at last follow-up were also recorded. In the youngest children in whom 24-h urinary calcium assessment was not possible, spot mictions were obtained to measure urinary calcium/creatinine ratio.

Because the local assays used for routine assessment of PTH were different, as illustrated in Table 1, we decided to present PTH data as xx-time the upper limit for normal (xx-ULN).

Main outcome parameters [height, weight and Body Mass Index (BMI)] were referenced to sex and age according to new French growth curves (www.afpa.org) and are reported as standard deviation scores (SDS).

Ethics

The study was approved by the local ethical committee (Comité d'Ethique des Hospices Civils de Lyon, session 18/02/2019, approval 19-26), and declared to the Information Technology and Liberty Commission (CNIL n°19-168). As per

French law, parents and patients do not need to give a written consent for retrospective studies.

Statistical analysis

Non-parametric Kruskal-Wallis tests followed by Dunn's tests compared to baseline were used. In all cases, p -values below 0.05 were considered statistically significant using GraphPad Prism software 8.0 (GraphPad, La Jolla, CA, USA). Results are presented as median (inter-quartile range, i.e., 25th–75th percentile) for biochemical routine parameters.

Results

Cinacalcet introduction

Relevant demographic, clinical and biochemical features of the 18 patients (8 girls) at the time of cinacalcet initiation are presented in Table 2. At a median age of 10.8 (2.0–14.4) years, cinacalcet was introduced at an estimated glomerular filtration rate (eGFR) of 120 (111–130) mL/min/1.73 m², plasma calcium of 3.04 (2.96–3.14) mmol/L, plasma phosphate of 1.1 (1.0–1.3) mmol/L, age-standardized (z score) phosphate of -3.0 (-3.5 ; -1.9), total ALP of 212 (164–245) UI/L age-standardized (z score) ALP of -2.4 (-3.7 ; -1.4), 25-OHD of 37 (20–46) ng/L, and PTH of 75 (59–123) ng/L corresponding to 1.2 (1.0–2.3)-time the ULN. The starting daily dose of cinacalcet was 0.7 (0.6–1.0) mg/kg, with a maximum dose of 1.0 (0.9–1.4) mg/kg per day (median in CaSR group: 1.1 (0.9–1.3) mg/kg/day; other etiologies: 1.7 (1.3–1.9) mg/kg/day). Six patients (32%) had received bisphosphonates (Pamidronate disodium) and no parathyroidectomy was performed before cinacalcet introduction. Seventeen patients had at least a 1-year follow-up. The median time of follow-up was 2.2 (1.3–4.3) years. No patients have received phosphate supplementation and active vitamin D analogs during the follow-up. Main outcome parameters (height, weight and BMI) remained stable during the follow-up (Table 3). Nephrocalcinosis was improved for one patient and another one displayed nephrolithiasis secondary to hypercalciuria (this patient had nephrolithiasis history before the Cinacalcet introduction).

Evolution of PTH and calcium levels in response to cinacalcet

The evolution of the main biochemical parameters at different time points under cinacalcet therapy is reported in Table 3. We observed a nearly 50% significant decrease in PTH levels between cinacalcet initiation [75 (59–123) ng/L] and the last follow-up [37 (34–54) ng/L]. The ULN PTH levels

TABLE 1 Assays used for routine assessment of plasma PTH levels in different contributing centers.

Commercial kit	Center	Assay	Normal range (ng/L)
Roche on a Cobas analyzer	Lyon, Montpellier, Toulouse	Electro-CLIA	15–65
Liaison 1-84 PTH Assay, DiaSorin	Limoges	CLIA	6.5–36.8
Liaison XL Assay, DiaSorin	Rouen	CLIA	5–40
Access Intact, Beckman Coulter	Paris-Robert Debré	CLIA	10–50
Centaur, Siemens, Deerfield	Paris-Bicêtre	CLIA	17–84

CLIA, chemi-luminescence-immuno assay.

significantly decreased between cinacalcet initiation and after 12 months ($p = 0.02$), and at the last follow-up ($p = 0.01$), as shown in [Figure 1A](#). We observed a significant decrease in calcium levels at 1, 6, 12 months and at the last follow-up as compared to baseline, with a similar pattern in the CaSR sub-group ([Figure 1B](#)). At the time of cinacalcet initiation, all patients were hypercalcemic ≥ 2.80 mmol/L; calcium levels remained above 2.8 mmol/L in 6 patients after initiation of cinacalcet therapy.

Evolution of eGFR and urinary calcium in response to cinacalcet

Renal function assessed by eGFR remained stable during follow-up ([Figure 1C](#)). Results were similar in patients with CASR mutations as compared to patients with other etiologies ([Figure 1C](#)). Urinary absolute calcium excretion and urinary calcium/creatinine ratio remained stable, whatever the underlying cause; as expected patients with CASR mutations displayed lower urinary calcium levels ([Figure 2](#)). Interestingly, one may discuss a trend toward decreased absolute urinary calcium levels in patients without CASR mutations at the end of the follow-up ([Figure 2A](#)); this could be relevant in practice since median urinary calcium at baseline in this sub-group is far higher (i.e., 6.5 mmol/L) than the crystallization threshold (i.e., 3.8 mmol/L).

Evolution of phosphate, ALP, and 25-OHD in response to cinacalcet

As shown in [Figure 3A](#), there has been no significant change of phosphate SDS levels before and after cinacalcet therapy. As illustrated in [Figure 3B](#), age-standardized (z score) ALP remained stable. Of note, following European recommendations guidelines (8), 12 children received native vitamin D supplementation to obtain normal 25-OHD levels between 20 and 40 ng/ and 25-OHD levels remained stable during the observational period ([Figure 3C](#)).

Side effects

No severe side effects were reported in 18 patients, and notably no iatrogenic hypocalcemia and no increased QT interval. Renal ultrasounds (when available, $N = 6$ at baseline; $N = 5$ at 1 year and $N = 6$ at the last follow-up) were performed during the follow-up. We observed a disappearance in the last ultrasounds for one patient who presented nephrocalcinosis at baseline. Cinacalcet was withdrawn in three patients: in one patient because of complete calcium normalization after 1 year of follow-up, in one patient because of secondary surgery of an adenoma, and for the last patient because of bad therapeutic compliance.

Discussion

This retrospective multicenter observational study evaluated the off-label use of cinacalcet to treat pediatric PHPT. The main results are the following: 1/ cinacalcet allows better control of calcium and PTH levels, without any substantial changes in urinary calcium excretion, and 2/ cinacalcet is safe and effective without notable side effects.

In pediatrics, data are scarce, and to our knowledge, only 50 pediatric patients (including the 18 patients presented here) out of a total of almost 1,200 pediatric patients reported in the literature with PHPT, have been treated with cinacalcet, as summarized in [Table 4](#) (15, 16, 21–25, 27–30, 32, 33, 35–37, 40, 43–45). The present study almost doubles the number of reported pediatric patients having received cinacalcet as an off-label drug for PHPT.

A recent meta-analysis has evaluated 28 studies including 722 adults receiving cinacalcet for PHPT (because of contraindication to surgical procedure or parathyroidectomy failure) (13). It reported a normalization of calcium levels in 90% of cases; PTH levels were significantly reduced but only 10% of cases normalized PTH levels (13). The effect of cinacalcet was greater when baseline calcium values were above 3 mmol/L. In our study, we observed a significant decrease of calcium levels after 1 month of therapy that was sustained until the last follow-up; no hypocalcemia was reported. All patients displayed calcium levels above 2.80 mmol/L at the time of cinacalcet

TABLE 2 Patient characteristics at Cinacalcet initiation and during follow-up.

No.	Etiology	Age at Cina intro (years)	Sex	SDS height	SDS weight	BMI (kg/m ²)	PTH (ng/L)	xx- ULN PTH	Ca (mmol/L)	SDS Ph	Biphosphonates (Pamidronate disodium), before Cinacalcet, dose (mg/kg); number of doses	Cinacalcet initiation (mg/kg/day)	Cinacalcet max dose (mg/kg/day)	Cinacalcet dose at last follow-up (μg/kg/day)
1	NSHPT Ho CASR mutation c.413C>T; p.Thr138Met	0.1	F	0	0	14.7	124	1.9	2.98	-3.9	No	1.0	1.0	0.6
2	NSHPT Ho CASR mutation c.1972C>T; p.Arg648Ter	0.1	F	-2	-0.5	14.3	459	7.1	3.04	-4.1	0.5; 2	0.1	0.7	0.6
3	NSHPT Ho CASR mutation c.1745G>A; p. Cys582Tyr	0.2	M	1	-0.5	13.9	34	0.9	3.16	-4.3	NA; 2	0.4	0.9	0.1
4	FHH He CASR mutation c.2425T>C; p.Phe809Leu	1.1	M	1	-1	14.8	53	0.8	3.12	-3.1	No	1.6	3.2	1.8
5	FHH He CASR mutation c.1972C>T; p.Arg648Ter	1.4	F	0	0.5	17.1	14	0.2	2.88	-1.6	1; 5	0.4	1.4	0.2
6	FHH He CASR mutation c.2425T>C; p.Phe809Leu	3.6	M	0.5	1	16.4	80	1.2	3.07	-3.6	NA; 1	0.9	1.4	1.3
7	FHH He CASR mutation c.2425T>C; p.Phe809Leu	6.6	F	1	2	22.1	59	0.9	2.96	-3.2	No	0.7	1.3	1.3
8	FHH He CASR mutation c.2425T>C; p.Phe809Leu	7.3	M	-1.5	-0.5	15.5	67	1	2.86	-2.9	No	1.2	1.5	1.5
9	FHH He CASR mutation c.1745G>A; p. Cys582Tyr	10.1	F	1	2	24.2	85	1.3	3.10	-1.2	No	1.1	1.1	1.1

(Continued)

TABLE 2 Continued

No.	Etiology	Age at Cina intro (years)	Sex	SDS height	SDS weight	BMI (kg/m ²)	PTH (ng/L)	xx- ULN PTH	Ca (mmol/L)	SDS Ph	Biphosphonates (Pamidronate disodium), before Cinacalcet, dose (mg/kg); number of doses	Cinacalcet initiation (mg/kg/day)	Cinacalcet max dose (mg/kg/day)	Cinacalcet dose at last follow-up (μg/kg/day)
10	FHH He CASR mutation c.2425T>C; p.Phe809Leu	11.5	F	1	1	21.8	60	0.9	3.23	-1.5	No	0.6	0.6	NA
11	FHH He CASR mutation c.413C>T; p.Thr138Met	12.2	M	0	-1	16.9	72	1.1	2.80	-1.8	No	0.9	0.9	NA
12	FHH Ho CASR mutation c.482A>G; p.Tyr161Cys	12.6	M	-0.5	0	19.1	59	1.2	2.99	-2.5	No	0.7	1.0	0.9
13	FHH Ho CASR mutation c.482A>G; p.Tyr161Cys	14.7	F	-0.5	0	20.5	243	4.9	3.40	-3.6	No	0.6	1.2	1.2
14	Hereditary hyperparathyroidism-jaw tumor syndrome CDC33 mutation	14.3	F	-1	-0.5	20.0	171	2.6	2.86	-2.8	No	0.6	1.9	1.8
15	MEN type 1 MEN1 He mutation	14.4	M	-1.5	-2	16.9	77	1.2	2.87	-1.5	No	0.8	1.3	1.2
16	Unknown	15.3	M	NA	-2	15.3	199	2.4	3.33	-3.3	NA; 2	1.1	1.9	2.1
17	Unknown	15.5	M	0.5	-1	23.0	38	1	3.04	-3.0	No	1.7	1.7	NA
18	Unknown	17.5	M	1	0.5	24.9	120	3	2.96	-2.0	No	0.7	0.7	NA

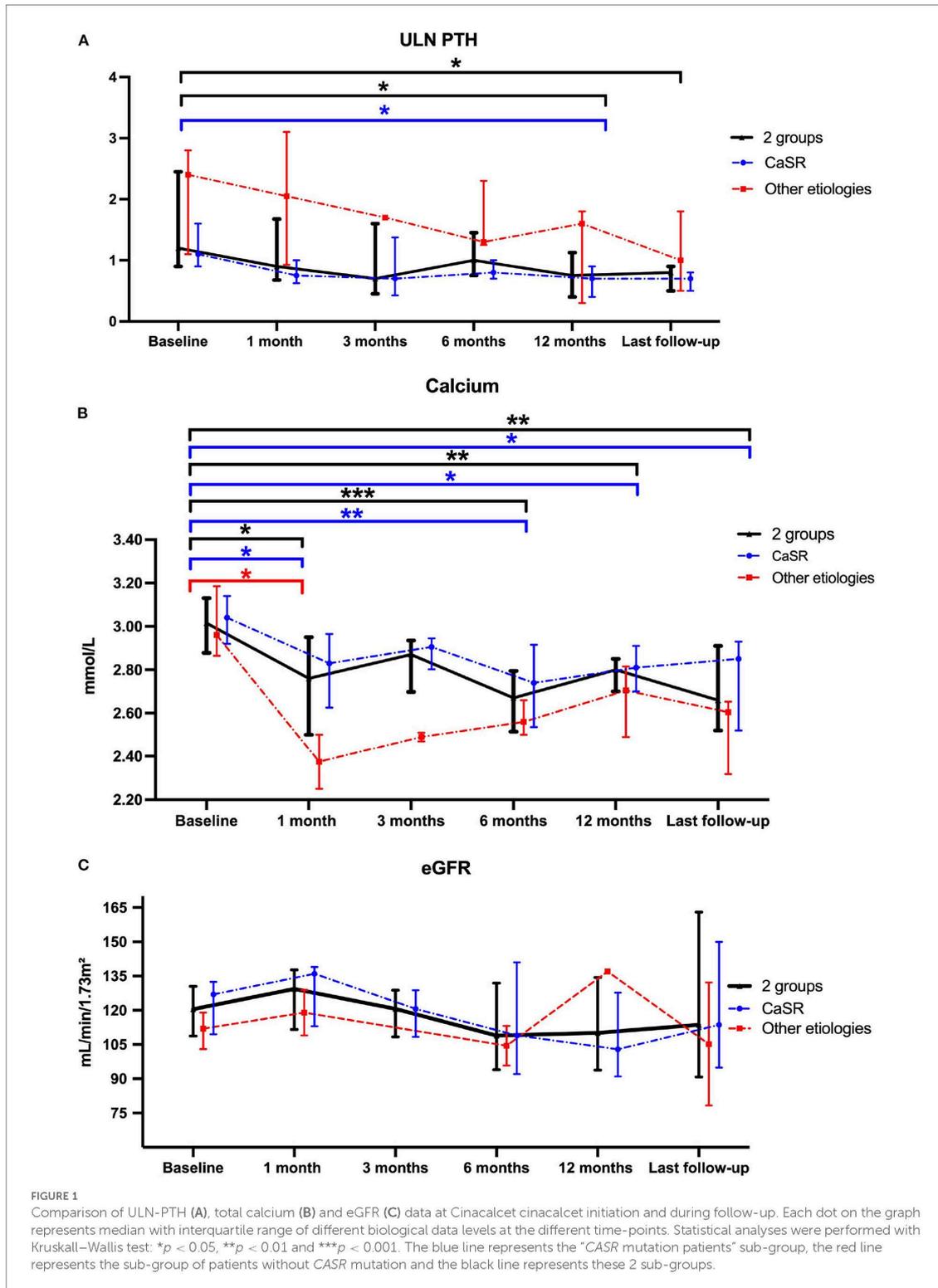
NA, number; NSHPT, Neonatal Severe Hyperparathyroidism; FHH, Familial Hypocalcemia hypercalcemia; He, heterozygous; CASR, Calcium sensing receptor; MEN, Multiple Endocrine Neoplasia; Cina, Cinacalcet; Ca, Calcium; SDS, Standard Deviation; NA, not available; Intro, Introduction; ULN, upper limit for normal; SDS Ph, phosphate levels were normalized and expressed as Z-score for age.

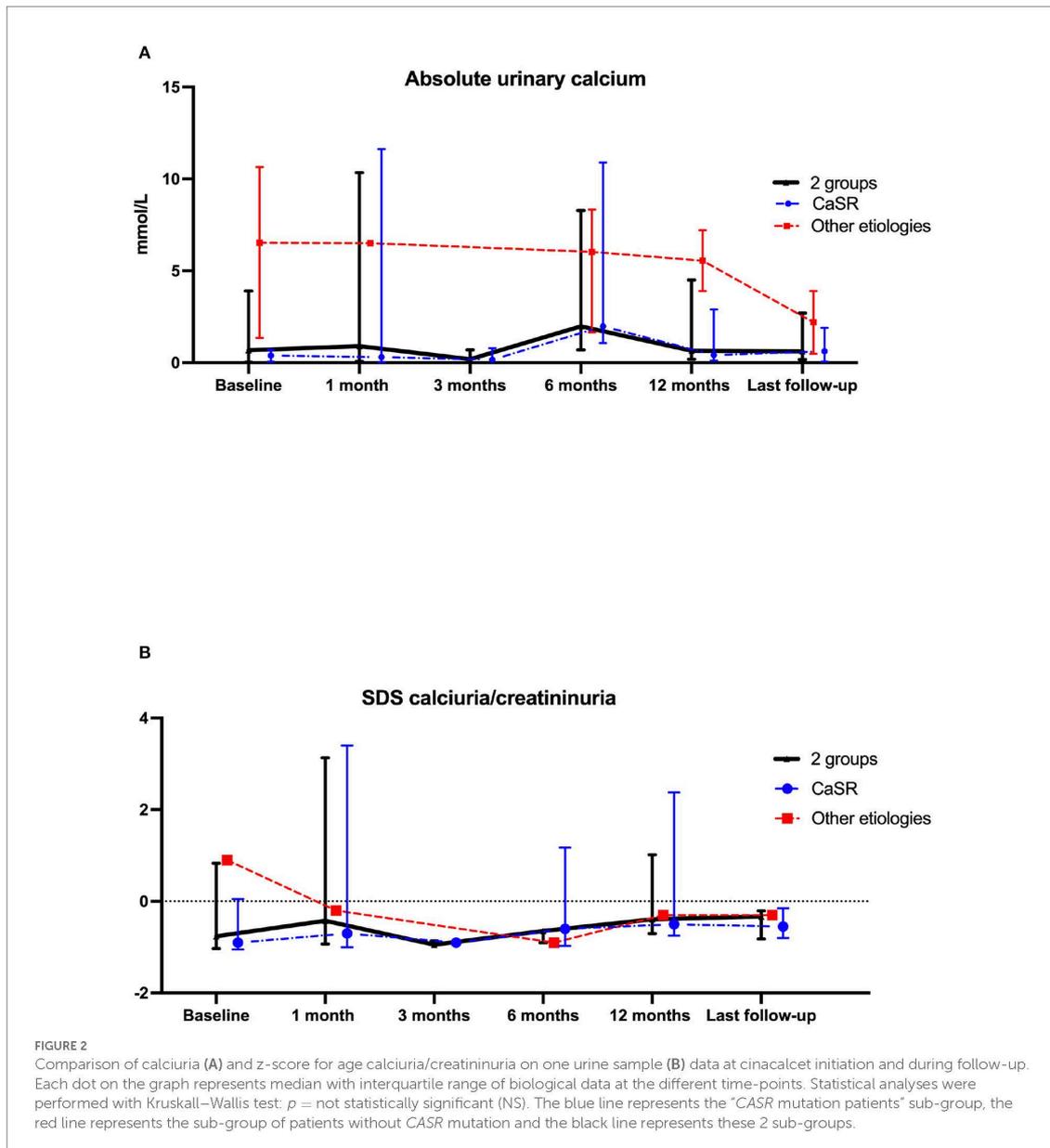
TABLE 3 Summary of the biochemical data available in patients at the different follow-up.

		Baseline		1 Month		3 Months		6 Months		12 Months		Last follow-up	
Dose		0.7		1.2		0.8		1.1		1.1		1.3	
mg/kg/j		(0.6–1.0)		(0.6–1.4)		(0.6–0.9)		(0.8–1.4)		(0.8–1.3)		(0.9–1.7)	
CaSR	Others	0.7	0.8	1.0	1.4	0.9	0.7	1.0	1.6	1.0	1.8	1.2	1.7
PTH		75		59		46		53		41*		37*	
g/L		(59–123)		(46–82)		(27–100)		(46–98)		(30–63)		(34–54)	
CaSR	Others	67	120	51	159	46	110	48	98	38	65	37	87
xx-ULN		1.2		0.9		0.7		1.0		0.8*		0.8*	
PTH		(1.0–2.3)		(0.7–1.2)		(0.5–1.5)		(0.8–1.4)		(0.5–1.1)		(0.5–0.8)	
CaSR	Others	1.1	1.9	0.8	3.0	0.7	1.7	0.8	1.5	0.7*	1.7	0.7	1.0
Calcium		3.04		2.80*		2.90		2.67***		2.80**		2.66**	
mmol/L		(2.96–3.14)		(2.60–2.93)		(2.79–2.93)		(2.55–2.80)		(2.70–2.84)		(2.55–2.90)	
CaSR	Others	3.04	2.96	2.83*	2.25*	2.91	2.49	2.76**	2.56	2.81*	2.71	2.85*	2.61
Ph		1.1		1.4		1.2		1.3		1.2		1.2	
mmol/L		(1.0–1.3)		(0.9–1.6)		(1.1–1.5)		(1.1–1.4)		(1.1–1.4)		(1.1–1.2)	
CaSR	Others	1.2	0.9	1.4	1.4	1.3	1.2	1.3	0.9	1.2	0.8	1.2	1.1
Ph		–3.0		–2.0		–2.1		–1.9		–2.3		–2.2	
Z-score		(–3.6 to –1.9)		(–3.4 to –1.4)		(–3.4 to –1.7)		(–2.6 to –1.6)		(–2.9 to –1.8)		(–2.6 to –1.4)	
CaSR	Others	–3.1	–2.9	–2.0	–1.8	–2.7	–1.5	–1.9	–2.8	–2.2	–3.1	–2.4	–1.7
eGFR		120		130		121		109		110		114	
mL/min/1.73 m ²		(111–130)		(114–136)		(115–125)		(96–123)		(100–130)		(95–121)	
CaSR	Others	127	112	136	119	120	NA	109	105	103	137	114	96
25-OHD		37		35		25		34		35		33	
ng/L		(20–46)		(29–38)		(21–36)		(24–45)		(24–45)		(21–41)	
CaSR	Others	42	18	35	NA	25	NA	38	26	35	29	33	30
ALP		212		253		304		185		254		170	
UI/L		(164–245)		(220–287)		(277–369)		(139–240)		(174–305)		(158–196)	
CaSR	Others	220	217	253	NA	304	NA	204	130	295	139	170	NA
ALP		–2.4		–2.8		–1.9		–2.6		–1.5		–3.0	
Z-score		(–3.7 to –1.4)		(–3.1 to –2.6)		(–2.6 to 0)		(–3.4 to –1.8)		(–2.7 to –0.9)		(–3.3 to –2.3)	
CaU		0.6		0.9		0.2		2.0		0.7		0.6	
mmol/L		(0.1–1.6)		(0.1–7.8)		(0.2–0.6)		(1.0–7.1)		(0.3–4.1)		(0.5–2.3)	
CaSR	Others	0.1	6.5	0.3	6.5	0.2	NA	1.1	6.0	0.4	5.6	0.6	2.2
Ca/CreatU		0.1		0.2		0.1		0.3		0.2		0.2	
mmol/mmol		(0.0–0.6)		(0.1–0.8)		(0.1–0.1)		(0.1–0.4)		(0.1–0.4)		(0.1–0.2)	
CaSR	Others	0.0	0.3	0.1	0.2	0.1	NA	0.2	0.4	0.2	0.2	0.2	0.2
Ca/CreatU		–0.8		–0.6		–0.9		–0.6		–0.4		–0.3	
Z-score		(–1.0 to 0.3)		(–0.9 to 2.9)		(–0.9 to –0.9)		(–0.9 to –0.6)		(–0.7 to 0.4)		(–0.8 to –0.3)	
CaSR	Others	–0.9	0.9	–0.6	–0.2	–0.9	NA	–0.6	–0.9	–0.5	–0.3	–0.6	–0.3
SDS		0		0		–0.5		–0.5		0		0	
height		(–0.5 to 1)		(–0.5 to 1)		(–1 to 0)		(–1 to 0.5)		(–1 to 0.5)		(–1 to 1)	
CaSR	Others	0	0.5	0	–0.5	0	–1	–0.5	–1	0	–1	0	–1
SDS		0		–0.5		0		0		–0.5		0	
weight		(–1 to 0.5)		(–1 to 1)		(–0.5 to 0)		(–0.5 to 0.5)		(–1 to 0)		(–0.5 to 1.5)	
CaSR	Others	0	–1	0.5	–1	0	–1	0	–0.5	0	–0.5	–0.5	0

CaSR, Calcium Sensing receptor patients; ULN, upper limit for normal; eGFR, Estimated Glomerular Filtration Rate; Ph Z-score, phosphate Z-score; 25-OHD, 25-OH vitamin D; CaU, urinary calcium; CreatU, creatininuria; ALP, total Alkaline Phosphatase; NA, not available; SDS, Standard Deviation. The results are presented as median (interquartile range) and median is presented for CaSR mutation patients and patients with other etiologies.

Gray cellules were different statistical data between baseline and different follow-ups after this initiation of Cinacalcet for biological results. Statistical analyses were performed with Kruskal–Wallis test: * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.





initiation; twelve patients normalized their calcium levels after cinacalcet therapy with doses that remained stable.

In the present study, different PTH assays were used in the different centers, with different normal ranges, explaining why we presented PTH data as ULN-PTH. The ULN-PTH significantly decreased after 12 months under cinacalcet treatment, and we observed a nearly 50% decrease of PTH levels between cinacalcet initiation and the last follow-up. The patients

nevertheless remained within the “normal” range during follow-up, even though the normal range in case of hypercalcemia remains quite challenging to define. The main objective for physicians was to obtain acceptable calcium levels allowing to obtain optimal growth with adequate nutritional calcium intake for age.

Hypercalcemia due to neonatal severe HPT (NSHPT) secondary to homozygous or heterozygous mutations of

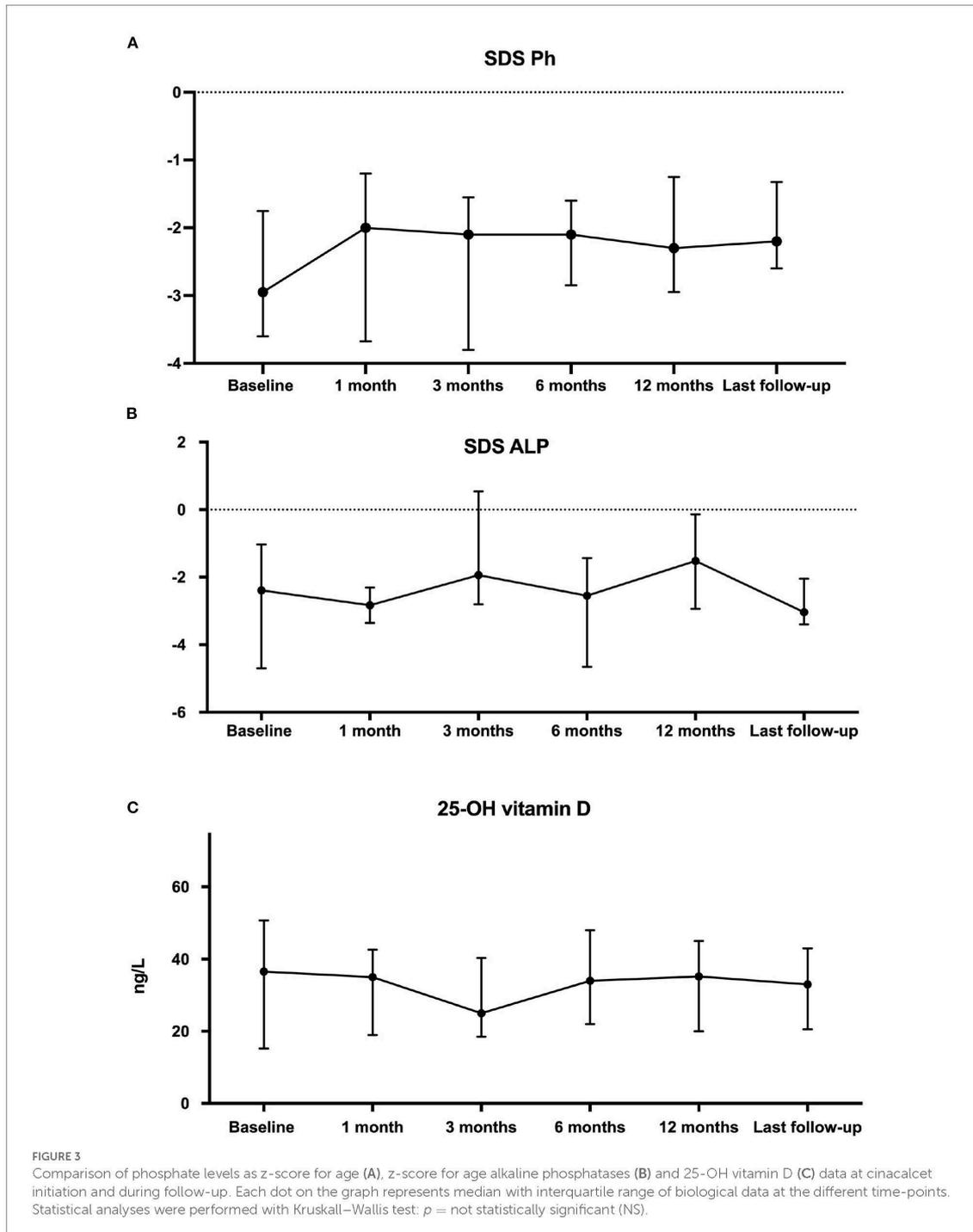


TABLE 4 summary of the pediatric studies reporting the use of cinacalcet in children.

References	No. cases	Etiology	CASR mutation protein	Age at inclusion	Follow-up	Cinacalcet dose	Main conclusions: calcium/phosphate homeostasis	Main conclusions: calciuria/nephrocalcinosis	Main conclusions: bone turnover	Side effects
Henrich et al. (21)	1	Not available	Not available	16 years	8 weeks	30 mg/day during 4 weeks; then 60 mg/day during 4 weeks	Normalization of calcium; PTH changes were assay dependent	Not evaluated	Not evaluated	No side effects except for mild headache
Alon (22)	1	He CASR mutation	c.659G>A p.Arg220Gln	6 years	12 months	30 mg/day during 2 weeks; then 60 mg/day	PTH and ionized Ca decreased; near-normal levels	Not evaluated	Not evaluated	No
Rehet et al. (23)	1	He CASR mutation	c.554G>A p.Arg185Gln	23 days	31 months	20 mg/m ² /day	PTH decreased	Urine calcium remained undetectable	ALP slightly above normal; at 17 months, osteopenia greatly decreased	No
Wilhelm-Bals et al. (24)	1	Ho CASR mutation	c.206G>A p.Arg69His	6 years	5 years	1.4 mg/kg/day increased to 3.5 mg/kg/day and 1.4 mg/kg/day on alternating days	Normalization of calcium, PTH and phosphate levels	Urine calcium levels decreased at the normal range	Osteocalcin and ALP: normal values	No
García Soblechero et al. (25)	1	Ho CASR mutation	c.1392_1404del13 p.Arg465Leufs*9	2 months	49 days	20 mg/m ² /day	Calcaemia was maintained at acceptable levels; no significant decrease in the PTH levels	Not evaluated	Not evaluated	No
Atay et al. (26)	1	Ho CASR mutation	c.222_226delGATAE1	days	14 months	30 mg/m ² /d up to 90 mg/m ² /d	Persistent hyperPTH	Not evaluated	Hungry bone	No
Gannon et al. (27)	1	He CASR mutation	c.554G>A p.Arg185Gln	2 days	18 months	6–202 mg/m ² /day	Reductions in serum concentrations of ionized calcium and intact PTH over the next 4 days	Fractional excretion of calcium remained low	Improvement in bone mineralization and no further fractures	No

(Continued)

TABLE 4 Continued

References	No. cases	Etiology	CASR mutation protein	Age at inclusion	Follow-up	Cinacalcet dose	Main conclusions: calcium/phosphate homeostasis	Main conclusions: calciuria/nephrocalcinosis	Main conclusions: bone turnover	Side effects
Tenhola et al. (28)	1	22q11.2 deletion syndrome and AP2S1 mutation	Not applicable	10 years	3 years	30 mg/day to 30 mg twice daily	Plasma calcium levels normalized, and PTH levels decreased slightly	Fractional excretion of calcium remained low	BMD remained stable during the follow-up	Yes: occasional numbness and tingling in his legs
Fisher et al. (29)	2	He CASR mutation	c.554G>A p.Arg185Gln	Case 1: 13 months Case 2: 4 months	Case 1: 32 months Case 2: Not available	Case 1: 3.7 mg/kg/day Case 2: 2 mg/kg/day	Cases 1 and 2: Normalization of calcium and PTH levels	Not evaluated	Not evaluated	Case 1: mild nausea and vomiting after one dose increase Case 2: mild nephrocalcinosis
Srivastava et al. (30)	1	Pseudo hypoPTH type 1b	Not applicable	4.8 years	32 months	0.8 mg/kg/day	Serum calcium, phosphorus, PTH and ALP concentrations improved	Kidney function and urine calcium level remained normal	Bone turnover markers and radiographic abnormalities improved under Cinacalcet	No
Savas-Erdeve et al. (31)	1	Ho CASR mutation	c.1630C>T p.Arg544*	12 days	18 months	10 mg to 25 mg/kg/m ²	Persistent hypercalcaemia and hyperPTH	Not evaluated	Not evaluated	No
Murphy et al. (32)	1	Ho CASR mutation	c.206G>A p.Arg69His	4 days	46 days	0.4 mg to 8.5 mg/kg/day	Persistent hypercalcaemia and hyperPTH	Not evaluated	Not evaluated	No
Alon et al. (33)	1	XLH Rickets	Not applicable	5 years	18 months	1 mg/kg/day	Normalization of calcium, PTH and phosphate levels	No nephrocalcinosis	BMD remained stable during the follow-up	No
Ahmad et al. (34)	1	Ho CASR mutation	C.1378-2A>G	60 days	16 months	4 mg/kg/day	Persistent hypercalcaemia and hyperPTH	Not evaluated	Not evaluated	No

(Continued)

TABLE 4 Continued

References	No. cases	Etiology	CASR mutation protein	Age at inclusion	Follow-up	Cinacalcet dose	Main conclusions: calcium/phosphate homeostasis	Main conclusions: calciuria/nephrocalcinosis	Main conclusions: bone turnover	Side effects
Mogas et al. (35)	2	He CASR mutation	Case 2: c.2446A>G+ p.Ile816Val	13 years	Case 1: 18 months Case 2: 12 months	Case 1: 1.1 mg/kg/day Case 2: 30 mg/day	Normalization of calcium and PTH levels	Urine calcium levels decreased at the normal range	Not evaluated	No
Sun et al. (16)	1	Ho CASR mutation	c.242T>A p.Ile811Lys	72 days	10 months	30–45 mg/m ² /day	Total calcium was maintained within the high-normal range and PTH was normalized	Not evaluated	ALP: normal values	No
Capozza et al. (36)	1	Ho CASR mutation	IVS5+1G>A c.1608+1G>A Splice site-skipped Exon 5	18 days	10 days	0.4 mg/kg/day	Normalization of calcium and PTH	Not evaluated	Not evaluated	No
Scheers et al. (37)	1	<i>SPINK1/AP2S1</i> mutation	Not applicable	13 years	6 years	40 mg/day	Normalization of calcium and PTH	Urine calcium levels remained stable at the normal range	Not evaluated	No
Hashim et al. (38)	1	Ho CASR mutation	c.679 C>T p.Arg227Ter	10 days	18 months	0.5 mg/kg/d up to 11 mg/kg/d	Persistent hypercalcemia and hyperPTH	Not evaluated	Not evaluated	No
Forman et al. (39)	1	He CASR mutation	c.554G>A p.Arg185Gln	3 days	14 months	0.4–5 mg/kg/day	Normalization of calcium, PTH, and phosphate	No nephrocalcinosis	Not evaluated	No
Sadacharan et al. (40)	4	Ho CASR mutation for three patients	c.1608+1G>A	Case 1: 80 days Case 2: 5 months Case 3: 48 days Case 4: 29 days	Case 1: 1 month Case 2: 5 years Case 3: 12 days Case 4: 4 days	Cases 1, 2, and 4: Not indicated Case 3: 30 mg/day	Cases 1, 2, and 3: Not detailed Case 4: drop in PTH	Not evaluated	Not evaluated	No

(Continued)

TABLE 4 Continued

Reference	No. cases	Etiology	CASR mutation protein	Age at inclusion	Follow-up	Cinacalcet dose	Main conclusions: calcium/phosphate homeostasis	Main conclusions: calciuria/nephrocalcinosis	Main conclusions: bone turnover	Side effects
Gulcan-Kersin et al. (41)	1	Ho CASR mutation	c.1836 G>A p.Gly613Glu	1 day	18 months	1.5 mg/kg/d up to 8 mg/kg/d	Normalization of calcium and PTH	Urine calcium levels remained stable at the normal range	Not evaluated	No
Abdullayev et al. (42)	1	Ho CASR mutation	c.679 C>T p.Arg227Ter	10 days	18 months	1.5 mg/kg/d up to 11 mg/kg/d	Persistent hyperPTH	Not evaluated	Not evaluated	No
Leunbach et al. (43)	1	He CASR mutation	c.1745G>A p.Cys582Tyr	29 days	8 months	0.5 mg/kg/day	Normalization of calcium	Not evaluated	Not evaluated	No
Tuli et al. (15)	1	Not available	Not applicable	14 years	3 months	1.3–8.5 mg/kg/day	Normalization of calcium and phosphate; persistent elevated PTH levels	Not evaluated	Not evaluated	No
Aubert-Mucca et al. (44)	1	He CASR mutation	c.554G>A p.Arg185Gln	22 days	11 months	0.5–3 mg/kg/day	Normalization of calcium and PTH	Nephrocalcinosis at 6 months	Normalization of bone abnormalities	No
Sunuwat et al. (45)	1	VDR Type II	Not applicable	2.5 years	12 months	0.25–0.5 mg/kg/day	Normalization of calcium, phosphate and PTH	Not evaluated	Resolution of wrist swelling	No

CaSR, Calcium Sensing receptor; He, Heterozygous; Ho, Homozygous; VDR, Vitamin-D Dependent Rickets.

CASR, has been treated since 2011 with cinacalcet, in association with biphosphonates, in order to avoid post-surgical hypoparathyroidism (2, 16, 23, 25, 36, 40). Clinical presentation or response to treatment, such as Cinacalcet, may differ depending to CASR mutation (homozygous or heterozygous) and interindividual differences (46, 47). Some mutations only partially affect the CaSR function secondary to an alteration of the configuration of the calcium binding domain or a partial loss of the transduction domain. As suggested by Gulcan-Kersin et al. a therapeutic test with Cinacalcet could be started before genetic result to treat NSHPT or severe acute hypercalcemia and suspended if we do not observe hypercalcemia correction (41).

Maximum doses of cinacalcet were similar between the group with "CASR mutation" and "other etiologies" (1.0 mg/kg/day). The European consensus in pediatric dialysis recommends ≤ 0.2 mg/kg per day for the starting dose and 2 mg/kg per day for the maximal dose of cinacalcet (14). However, this consensus was established to stay on the safe side because of the risk of hypocalcemia in dialysis; here, in patients with hypercalcemia due to PHPT and normal renal function at baseline, the risk of hypocalcemia is lower.

In a meta-analysis of studies in adults, 23% of 722 patients suffered from nausea or vomiting and 3% had hypocalcemia (most of them are mild or asymptomatic); the majority of these adverse reactions were not severe enough to stop the treatment (13). In pediatrics (Table 4), one paper described gastrointestinal symptoms (29). Other reported adverse effects included mild headache and occasional numbness and tingling in legs but were not observed in our cohort (21, 28). Despite these favorable outcomes, we believe that it is of utmost importance to provide a global information to children and their caregivers on the risk and symptoms of hypocalcemia, on the risk of drug interactions and further QTc interval prolongation and not only through hypocalcemia with cinacalcet treatment. Indeed, a special caution should be given in children to the association between cinacalcet and macrolides or ondansetron, that is contra-indicated (48).

In the kidney, CaSR is expressed in all nephron segments; it plays a crucial role in calcium excretion (49, 50). Activating CASR mutations lead to hypercalcemia that reduces calcium and sodium transport in the Henle loop (Na-K-2Cl and paracellular pathways) through claudins, in association with a decreased urinary concentrating ability (51, 52). Our results are similar to previous reports, demonstrating stable renal function and no direct effects of cinacalcet on urinary calcium (22–24, 27, 28, 30, 35, 37). Only one paper has reported the onset of nephrocalcinosis after 6 months of cinacalcet therapy in a patient with heterozygous CASR mutation (44). Multiple CASR mutations have been identified (53) which reduce receptor function or produce truncated inactive CaSR and heterogeneous response to cinacalcet have been observed and influence urinary calcium excretion (22–24, 27, 28, 30, 35, 37). *In vitro* studies showed that calcimimetics were able to restore sensitivity on intra- and extracellular CASR mutations and deactivations

mutations (54, 55). This mechanism in the kidney is still under investigation and requires further studies.

This study has some limitations, and notably the (relatively) small number of patients. However, we are in the field of orphan diseases in pediatrics, with an off-label use of novel therapies; here, we almost double the number of reported pediatric patients having received cinacalcet as an off-label drug for PHPT. Data on bone metabolism (such as CTX, bone density) and calcium intake are unfortunately not available after cinacalcet treatment in the present study; however in most studies, growth improved or remained stable at the end of the follow-up, similarly to what is observed here (16, 23, 24, 27).

In conclusion, our observational data suggest that cinacalcet could be a safe alternative therapy in pediatric PHPT to treat hypercalcemia without major side effects. Even though European guidelines already propose this management as a second-line off-label therapy in adults with PHPT, larger international studies may evaluate its effect with the main objective to treat hypercalcemia and obtain optimal growth with normal calcium intake and vitamin D supplementation. Its long-term consequences especially for the potential risk of hypercalciuria and nephrocalcinosis later in life of these children should be further assessed.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Comité d'Ethique des Hospices Civils de Lyon, session 18/02/2019, approval 19-26), and declared to the Information Technology and Liberty Commission (CNIL n°19-168). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

JBe and JBa: data collection and writing the manuscript. SF: data collection. J-PS, CA, MC, ALie, LM, and ALin: physicians of patients from different center and proofreading of the manuscript. ID: statistical analysis required for the review. All authors contributed to the article and approved the submitted version.

Conflict of interest

JBa is a clinical investigator for industry-sponsored clinical trials on the use of calcimimetics

(cinacalcet and etelcalcetide) in pediatric dialysis (Amgen). JBa has received research grants from Amgen (RENOCLASTE study: ID-RCB 2017-A03241-52).

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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V- DISCUSSION

La physiopathologie et le traitement du CKD-MBD restent complexes et impactent la morbi-mortalité du patient insuffisant rénal en pédiatrie.

Notre étude RENOCLASTE est la première à étudier la biologie ostéoclastique chez des témoins et en MRC pédiatrique, et à évaluer l'impact direct osseux des traitements les plus fréquemment utilisés pour l'hyperparathyroïdie du CKD-MBD. Nos observations confirment les résultats précédemment décrits par notre équipe [67] : la $1\alpha,25(\text{OH})_2\text{D}$ est un inhibiteur puissant de l'ostéoclastogénèse avec un effet dose-dépendant chez le témoin sain. Nous avons également mis en évidence une résistance à la $1\alpha,25(\text{OH})_2\text{D}$ associée à une diminution de l'expression du VDR lorsque le DFG diminue. Il est important de souligner que cette résistance à la $1\alpha,25(\text{OH})_2\text{D}$ a aussi été observée dans les ostéoblastes [157]. Ces résultats soulèvent des questions sur l'efficacité globale des analogues actifs de la $1\alpha,25(\text{OH})_2\text{D}$ dans la promotion de la santé osseuse en MRC pédiatrique et va dans le sens de la réduction du nombre de VDR, au cours de la progression de la MRC, déjà rapporté dans la littérature [48–51].

Après analyse chez les témoins sains et les patients pédiatriques en MRC, le KP2326 inhibe la différenciation ostéoclastique par l'activation de la voie ERK, mais de manière moindre que la $1\alpha,25(\text{OH})_2\text{D}$ sans différence significative dans l'expression du CaSR. On note cependant que l'effet de ce calcimimétique varie en fonction des différentes concentrations mais n'est pas modifié par le stade de la MRC. Plusieurs phénomènes peuvent expliquer cette modulation de l'action du CaSR. D'une part, les concentrations de $[\text{Ca}^{2+}]$ peuvent modifier la coopérativité du récepteur, comme décrit dans l'introduction, au sein du microenvironnement osseux en répondant à d'autres stimuli extracellulaires, tels que des cations inorganiques comme le strontium ou l'aluminium ; certaines polyamines ; des antibiotiques comme les aminoglycosides et également le magnésium [16]. Il est également intéressant de préciser, que de possibles variabilités interindividuelles et polymorphismes du CaSR existent, mais également que le statut en vitamine D et en calcium des témoins sont inconnus et peuvent influencer l'ostéoclastogénèse de nos patients étudiés.

Par analyse en western-blot sur les ostéoclastes d'un témoin sain, réalisé après induction au KP2326 à 10^{-6}M , nous avons confirmé l'activation de la voie ERK-pERK. Récemment, l'action inhibitrice directe du PGE2 sur la différenciation des précurseurs ostéoclastiques a pu être mise en évidence, sans effet sur les ostéoclastes matures [19]. On peut alors supposer que la diminution du nombre d'ostéoclastes

soit liée à l'augmentation de la concentration de PGE2 via la voie ERK-pERK par activation du CaSR par le KP2326. En effet la voie ERK/pERK active la phospholipase A2 stimulant la formation d'acide arachidonique et du PGE2 après activation du CaSR, comme illustré sur la **Figure 3**. Une deuxième hypothèse peut être également émise : la diminution du nombre d'ostéoclastes est liée à l'activation de l'apoptose, diminuant ainsi la durée de vie des ostéoclastes [7]. Cette hypothèse a été confirmée par Chiu et al. qui rapporte que le cinacalcet améliore les paramètres osseux en régulant le stress du réticulum endoplasmique des ostéoclastes, l'autophagie et l'apoptose [158].

Nous avons rapporté que le KP2326 inhibait la résorption osseuse chez les témoins sains contrairement à la 1 α ,25(OH) $_2$ D. Toutefois, nous n'avons pas pu effectuer cette analyse sur notre groupe pédiatrique atteint de MRC en raison de la quantité de sang requise pour l'expérience et de l'anémie résultant de la MRC chez nos patients. Les contraintes éthiques ont limité la possibilité de réaliser des biopsies osseuses ou d'obtenir des échantillons de tissu adipeux pour analyser le versant ostéoblastique après transdifférentiation.

En se basant sur la publication de Diaz-Tocados *et al.*, l'etelcalcétide exerce un effet anabolique direct sur l'os en favorisant la différenciation ostéoblastique dans un modèle animal de rat avec MRC d'une part, et la minéralisation dans les cellules souches mésenchymateuses humaines *in vitro* d'autre part. Les chercheurs ont constaté que l'administration de ce médicament augmentait le nombre d'ostéoblastes et la surface des ostéoïdes chez les rats urémiques soumis au « PTH clamp » pour maintenir une concentration constante de PTH [159]. Ces résultats sur les effets directs sur ostéoblastes et les ostéoclastes montrent un effet bénéfique des calcimimétiques sur l'os, favorisant la formation osseuse et pourrait présenter une implication clinique importante, en particulier chez les patients dialysés, susceptibles de développer une ostéodystrophie rénale.

Les guidelines KDIGO 2017, codifiant le diagnostic, l'évaluation, la prévention et le traitement du CKD-MBD ont mis à jour leur ligne directrice de 2009 pour pallier l'hyperphosphatémie et l'hypercalcémie mais également réduire le risque de calcifications vasculaires et la morbi-mortalité. Elles préconisent l'utilisation de 1 α ,25(OH) $_2$ D seule, de calcimimétique seul, ou d'un possible traitement combinatoire (1 α ,25(OH) $_2$ D et calcimimétique), en cas d'hyperparathyroïdie de la MRC, à évaluer au cas par cas, et sans grand rationnel clinico-expérimental pour justifier cette approche. A notre connaissance, l'effet combinatoire de la 1 α ,25(OH) $_2$ D du KP2326 sur l'ostéoclaste n'avait jamais été étudiée. En se basant sur nos observations, le KP2326 ne majore pas la forte inhibition de l'ostéoclastogénèse de la 1 α ,25(OH) $_2$ D à différentes concentrations chez des témoins sains. L'absence d'effet synergique de ces deux traitements peut être intéressante dans le cas de la MRC : en effet, plusieurs essais prospectifs

évaluant l'utilisation de la 1 α ,25(OH) $_2$ D chez l'IRC pédiatrique mettent en évidence des anomalies de la structure osseuse chez certains patients à type d'os adynamique, et ce malgré des concentrations de PTH améliorées [56, 160]. On peut alors supposer que l'absence d'effet synergique de ces deux molécules ne majore pas les anomalies du turnover osseux et permettrait d'améliorer la croissance de l'enfant. Des études complémentaires seraient nécessaires pour confirmer cette hypothèse mais il est important de rappeler les difficultés de la réalisation d'un essai clinique sur ce sujet en pédiatrie et plus particulièrement dans le contexte des maladies rares.

Nous nous sommes ensuite intéressés à l'utilisation hors AMM du cinacalcet en MRC pédiatrique ; nous avons réalisé deux études qui apportent de nouvelles données cliniques sur les bénéfices et les risques associés au traitement par cinacalcet dans la prise en charge du CKD-MBD.

La première étude, une analyse rétrospective multicentrique européenne, confirme l'effet du cinacalcet sur le sHPT avec un effet bénéfique sur la croissance chez les enfants dialysés âgés de moins de trois ans. Une proportion significative de patients (>50%) a réussi à atteindre des concentrations de PTH dans la fourchette cible après neuf mois de traitement, reflétant ainsi les résultats observés chez les patients pédiatriques dialysés plus âgés [84].

Certains effets indésirables ont été observés, en particulier l'hypocalcémie, justifiant une surveillance clinique et biologique rapprochée en particulier dans cette population où la croissance est accélérée. Les apports calciques apportés par l'alimentation, les médicaments et la dialyse doivent être considérés et calculés par le médecin prescripteur. Trois patients ont présenté une puberté précoce. Deux d'entre elles présentaient des mutations de *TCF2/HNF1 β* ; le test génétique n'a malheureusement pas été réalisé chez la troisième patiente, mais elle présentait également un CAKUT, souvent associée aux mutations *TCF2/HNF1 β* . Le diabète sucré peut être également retrouvé dans cette maladie génétique [161], mais à l'exception des malformations génitales qui ne sont pas rares [162], aucune anomalie de la puberté ou de dysgonadisme n'a été rapportée jusqu'à présent. Il est difficile de conclure un lien entre le cinacalcet et l'apparition d'une atteinte endocrinienne mais il est important de reconnaître et rechercher ces signes dans ce contexte, en particulier chez les enfants présentant des variants *TCF2/HNF1 β* .

La seconde étude s'est intéressée à l'effet du cinacalcet hors AMM en post-transplantation pédiatrique. Cette première étude multicentrique et rétrospective a rapporté une diminution statistiquement significative de la PTH de la calcémie. Le traitement du CKD-MBD en post-

transplantation est peu codifiée en pédiatrie mais ces résultats permettraient de prévenir certaines atteintes du greffon.

Ces deux études ont permis de rapporter l'efficacité du cinacalcet sur le sHPT. Pour prévenir le risque d'hypocalcémie, nous avons rappelé que le consensus européen recommande $\leq 0,2$ mg/kg de cinacalcet par jour comme dose initiale et seulement si la calcémie est supérieure à 2,40mmol/L et 2 mg/kg par jour pour la dose maximale chez l'enfants dialysés. Ces études présentent cependant des limites, en particulier, en lien avec l'analyse rétrospective, l'absence d'un groupe témoin et le nombre limité de patients inclus justifiant une interprétation prudente de ces résultats.

Après avoir évalué l'effet du cinacalcet en MRC pédiatrique, nous avons analysé son effet en HPP pédiatrique et avons rapporté l'expérience multicentrique française de son utilisation hors AMM dans ce contexte clinique.

Le cinacalcet a démontré son efficacité sur les concentrations de PTH et le traitement de l'hypercalcémie sans effets secondaires significatifs. Ces diminutions de la calcémie et de la PTH permettraient ainsi de prévenir certaines complications comme la néphrocalcinose, les calculs rénaux et la perte osseuse chez ces enfants en pleine croissance. Centino *et al.* se sont intéressés au rôle du phosphate extracellulaire dans la régulation de son propre excrétion rénale à travers la stimulation de la sécrétion de l'PTH. Ils se sont concentrés sur l'interaction phosphate/CaSR. Les résultats clés révèlent que les concentrations élevées de phosphate, observées en MRC, inhibent l'activité du CaSR par antagonisme non compétitif, entraînant une augmentation de la sécrétion de PTH. De plus, l'étude identifie des sites de liaison d'anions multivalents sur le domaine extracellulaire du CaSR, où le phosphate se lie et stabilise la forme inactive du récepteur, augmentant ainsi la sécrétion de PTH. La compréhension du rôle du phosphate et son interaction avec le CaSR, comme montre cette étude, pourrait ainsi potentiellement influencer les stratégies thérapeutiques impliquant l'utilisation de calcimimétiques [163].

Comme pour nos deux études cliniques précédentes, le recueil des données de façon rétrospective n'offre pas le même niveau de preuve qu'un essai contrôlé randomisé et ces résultats, bien que significatifs, doivent être interprétés avec prudence. Des études complémentaires sont nécessaires pour évaluer l'effet de ce traitement à long terme, et en particulier son effet sur l'hypercalciurie et le risque de néphrocalcinose pouvant altérer la fonction rénale ; mais également sur la santé osseuse de l'enfant. Bien que les directives européennes aient déjà approuvé son utilisation comme thérapie hors

AMM de deuxième ligne chez les adultes atteints de HPP, ces résultats confirment son utilisation comme une alternative thérapeutique dans ce contexte particulier.

VI- CONCLUSIONS ET PERSPECTIVES

La MRC pédiatrique nécessite une prise en charge précoce en raison des complications liées au trouble de la croissance et des conséquences cardiovasculaires (calcifications vasculaires) secondaires au CKD-MBD. Les calcifications vasculaires apparaissent précocement dans la MRC chez l'enfant, expliquant en partie que la mortalité d'un enfant dialysé soit 30 fois plus élevée que celle d'un enfant en bonne santé et que l'état vasculaire d'un patient dialysé âgé de 20 ans soit égale à celui d'un adulte de 80 ans sans maladie rénale [164]. En 2019, Freise *et al.* ont mis en évidence une artériopathie chez des enfants dialysés avec une teneur en calcium des artères 5 fois plus élevée que les patients contrôles [165].

Nos résultats ont permis de progresser sur l'impact direct des calcimimétiques et de la vitamine D sur les ostéoclastes chez les patients pédiatriques atteints de MRC à différents stades et confirmer son efficacité sur les concentrations de PTH et de calcémie en MRC mais aussi en HPP. En l'associant à des doses réduites d'analogues de vitamine-D, ces calcimimétiques peuvent influencer la minéralisation et le renouvellement osseux. Plus important encore, ils pourraient moduler l'axe os-vasseaux, et donc la progression des calcifications vasculaires dans la MRC pédiatrique.

Pour compléter ces recherches, je me suis intéressée au versant vasculaire du CKD-MBD. Au cours de ma mobilité au sein du laboratoire de néphrologie pédiatrique du Prof. Claus Peter Schmitt, à Heidelberg en Allemagne, j'ai réalisé des analyses protéomiques et transcriptomiques d'artérioles microdisséquées d'épiploon provenant d'enfants atteints de MRC, inclus dans *l'International Peritoneal Biobank*.

Après analyse histomorphométrique, nous avons mis en évidence une vasculopathie chez les enfants atteints de MRC caractérisée par un épaissement de l'intima et de la média, ainsi qu'une infiltration sub-endothéliale importante de cellules inflammatoires type macrophages. A partir d'une bibliothèque de gènes incluant des voies connues impliquées dans la physiopathologie des calcifications vasculaires, 21/442 voies étaient significativement régulées à l'échelle transcriptomique en rapport avec l'activation du complément, la signalisation de la voie Wnt, l'organisation de la matrice extracellulaire, le stress du réticulum endoplasmique, l'apoptose/autophagie et l'ossification. A l'échelle protéomique, après analyse par *Gene Set Enrichment Analysis* (GSEA), les principales voies régulées identifiées comprenaient l'activation du complément, l'organisation de la matrice extracellulaire, le métabolisme des acides gras, la liaison du calcium et l'apoptose. Nous avons confirmé ces résultats par immunohistochimie dans une cohorte indépendante de 30 patients atteints de CAKUT et 20 patients contrôles (cf **Figure 10**).

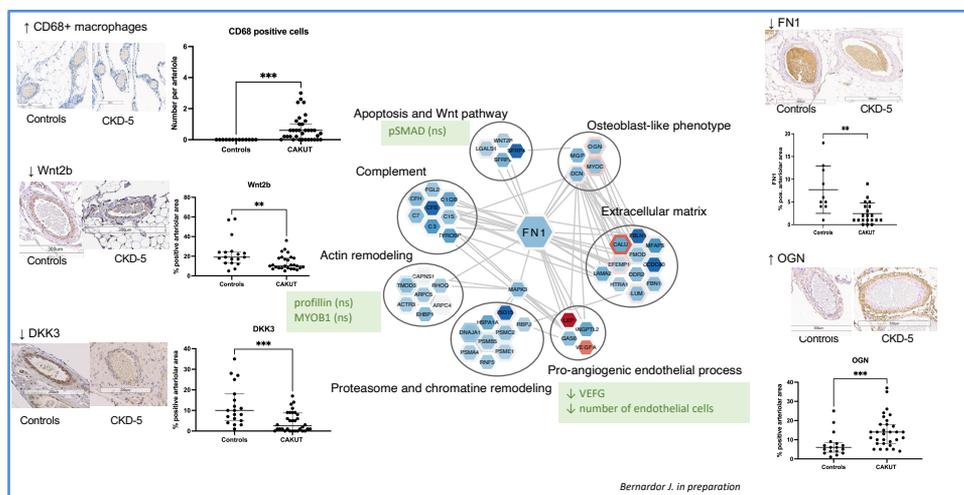


Figure 10 : Voies de régulation et gènes impliqués dans la physiopathologie de la calcification vasculaire chez l'enfant insuffisant rénale chronique

La coloration de Van Kossa et l'analyse par PETScan ^{18}FNa n'ont pas permis de mettre en évidence des microcalcifications chez nos patients pédiatriques et suggère que les mécanismes impliqués dans cette vasculopathie apparaissent à un stade précoce de la maladie.

Les vaisseaux sont constitués de différentes cellules et unités fonctionnelles comme l'endothélium, les cellules musculaires lisses vasculaires, les cellules immunitaires, les cellules de transition endothéliale-mésenchymateuse et les cellules de type ostéoblaste. L'analyse transcriptomique et protéomique ne permettent pas de connaître la proportion et la fonction des cellules présentes des vaisseaux. Pour pallier cette limite, nous réalisons actuellement une analyse en *Single nuclei RNA sequencing* (SnRNAseq) des vaisseaux en utilisant des artérioles microdisséquées provenant de 5 patients pédiatriques atteints de MRC et ceux de patients contrôles avec une fonction rénale normale en coopération avec le laboratoire LP2M de l'Université Côte d'Azur (Nice). Ces études seront poursuivies au LP2M. Il s'agit d'une alternative au séquençage de l'ARN d'une seule cellule et permet ainsi d'étudier des cellules uniques difficiles à isoler. Cette technique peut être appliquée à des tissus congelés facilement disponibles et permet l'exploration des altérations spécifiques à un type de cellule. Cette technique permettra de définir un « cellulome » des vaisseaux en MRC pédiatrique et d'identifier de nouveaux mécanismes moléculaires et cellulaires impliqués dans la vasculopathie de l'enfant avec MRC. Ces analyses permettront d'obtenir des résultats préliminaires pour de futures études *ex-vivo* et expérimentales, et identifier de futures voies potentielles thérapeutiques.

En utilisant un modèle d'organoïdes de vaisseaux obtenus à partir de lignées de cellules souches induites pluripotentes (iPSc) de donneurs sains [166], en cours d'implémentation au laboratoire, nous

envisageons d'évaluer l'effet de différents marqueurs identifiés grâce aux analyses par multiomics et le snRNA en présence de conditions pro-calcifiantes et de toxines urémiques mimant la MRC.

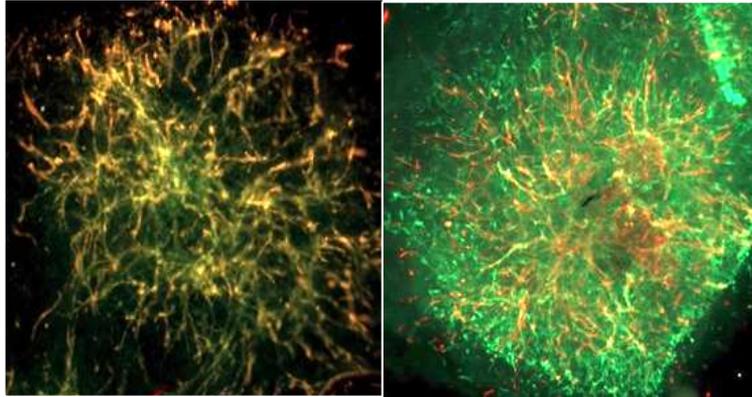


Figure 11 : Expression des marqueurs de vaisseaux dans un modèle organoïde
immunofluorescence : à gauche : CD31 (Rouge) et PDGFRb (vert) ; à droite CD31 (rouge)- aSMA
(vert)

Les résultats présentés et ces différentes analyses permettront une meilleure compréhension des mécanismes physiopathologiques de la CV et s'intégreront dans la prise en charge de l'axe os-vaisseaux du CKD-MBD pour réduire la morbidité à long terme tout en permettant une croissance optimale chez l'enfant atteint de MRC.

VII- ANNEXE : CURRICULUM VITAE

CURRICULUM VITAE Dr Julie BERNARDOR

Section CNU : 5401-Pédiatrie

Établissement : Faculté de médecine de Nice, Université Nice Côte d'Azur

Date de naissance : 14/10/1989 ; Lieu de naissance : Saint-Étienne, Nationalité : Française

Courriel : bernardor.j@chu-nice.fr, Téléphone : 04-92-03-63-65

Ordre : 06/14199, N°RPPS: 10101978707

Fonctions actuelles

- **Maitre de Conférences des Universités-Praticien Hospitalier (MCU-PH)**, Unité de néphrologie pédiatrique et hémodialyse pédiatrique, CHU de Nice depuis Septembre 2023
- Étudiante en thèse de sciences, 4^{ème} année, École Doctorale Interdisciplinaire Sciences-Santé (EDISS), Université Claude Bernard Lyon 1

Équipes de recherche :

- Depuis Septembre 2020 : **Doctorante**, : « Les calcimimétiques : des mécanismes d'action dans les cellules ostéoclastiques aux nouvelles indications thérapeutiques en pédiatrie », co-encadrements : Dr Irma Machuca-Gayet et Pr Justine Bacchetta, **INSERM 1033**, Lyon physiopathologie, diagnostic et traitements des maladies osseuses ; Team 3 : Olivier Peyruchaud, UFR de Médecine Lyon Est
- Novembre 2021-Août 2023 : **Guest research visitor : laboratoire de Néphrologie Pédiatrique**, Pr Claus Peter Schmitt, Université d'Heidelberg, Allemagne
- Depuis Novembre 2022 : **Doctorant visiteuse : Laboratoire de physioMédecine Moléculaire (LP2M)**, UMR 7370, team ostéoimmunologie, niches et inflammation, Claudine Blin, Nice

Diplômes Universitaires

- 2023 : Diplôme Inter-Universitaire de Maladies Auto-inflammatoires, Université Paris-Saclay
- 2023 : DU de Pédagogie et enseignement par la simulation en sciences de la santé, Université Nice Côte d'Azur
- 2020 : Diplôme Inter-Universitaire de Rhumatologie Pédiatrique
- 2019 : Diplôme d'Études Spécialisées de Pédiatrie, Université Nice Côte d'Azur
- 2019 : **Docteur en Médecine, spécialité Pédiatrie, Université Nice Côte d'Azur**
- 2018 : Master 2 Biologie Cellulaire Physiologie Pathologie, Spécialité : Biominéralisation morphogénèse et pathologies inflammatoires, Paris 13
- 2017 : Diplôme Inter-Universitaire de Néphrologie Pédiatrique
- 2016 : Diplôme Inter-Universitaire Dermatologie Pédiatrique

Activités de diagnostic et de soins

Unité de néphrologie pédiatrique et hémodialyse pédiatrique, Hôpital Archet 2, CHU de Nice ; Chef de service : Dr C. Faudeux

Activité de néphrologie pédiatrique, rhumatologie pédiatrique et médecine interne

- **Supervision de l'hémodialyse** : 2 patients, séance de 4 heures (2 séances/jour), 6 jours/7
- **Consultations** : néphrologie pédiatrique et pathologies phosphocalciques (2 demi-journées/semaine) ; rhumatologie pédiatrique (2 demi-journées par semaine)
- **Avis en néphrologie et rhumatologie pédiatrique** : >3avis/jour ; participation aux staffs hebdomadaires du Centre Pluridisciplinaire de Diagnostic Prénatal
- Participation à la rédaction des **protocoles** d'urgence et de parcours de soins

Activité d'Enseignement

- Score SIAPS (2023) : 252

- **2ème cycle**

- Cours type ARC aux étudiants de DFASM2, DFASM3 depuis 2020 (2heures/an)
- Depuis Novembre 2022 : Tutorat pour les stations ECOS pour les DFSAM1 et DFSAM2 en stage de pédiatrie (8 heures/mois)

- **3ème cycle**

- Depuis 2019: DES de pédiatrie : néphrologie pédiatrique et rhumatologie pédiatrique Faculté de médecine, Nice Côte d'Azur (8 heures/an)
- Autres
 - 2021: Enseignement au DIU de Rhumatologie pédiatrique (1heure)
 - Enseignement au DIU de Néphrologie pédiatrique (2 heures/an)
 - Depuis 2021: Enseignement de Néphrologie pédiatrique, École de puériculture depuis 2021 (3 heures/an)

- **Articles didactiques** : 7

- **Encadrement**

- Directrice de thèse de médecine (x3) ; directrice de mémoire de DIU de néphrologie pédiatrique (x1) et DIU de pathologies infectieuses pédiatriques (x1)
- Depuis 2020 : Rédactrice de **dossiers progressifs SIDES** et **Tests de Concordance de Script** (TCS)
- **Certifications ECOS** : parcours tuteur ; patients standardisés et évaluateur
- Membre du **groupe de travail station ECOS**, antenne pédiatrique, du CNU de pédiatrie ; responsable du groupe calibrage station ECOS, faculté de médecine, Université Nice Côte d'Azur.
- 2022- : Rédaction de stations d'ECOS, surveillance des ECOS et responsable de circuits

Activités de recherche

- **Score SIGAPS personnel total: 255; Indice H : 5** (Web of science)

- 17 publications référencées PubMed

Période : 2018 - 2023											
Année	Total	A+	A	B	C	D	E	NC	Score	Score avec Inv.	Score Frac.
2018	1	0	0	0	0	0	1	0	8	8	0.40
2019	2	0	0	2	0	0	0	0	48	48	2.53
2020	2	0	0	2	0	0	0	0	48	48	2.26
2021	6	1	0	0	3	2	0	0	54	68	3.21
2022	4	0	0	0	3	1	0	0	51	51	2.90
2023	2	0	0	0	2	0	0	0	32	32	1.87
Total	17	1	0	4	8	3	1	0	241	255	13.17

Période : 2018 - 2023								
Position	Total	A+	A	B	C	D	E	NC
1	10	0	0	4	5	0	1	0
2	2	0	0	0	0	2	0	0
Inv	1	1	0	0	0	0	0	0
k	2	0	0	0	1	1	0	0
DA	2	0	0	0	2	0	0	0
Total	17	1	0	4	8	3	1	0

k = Toute position intermédiaire entre 3 et ADA

ADA = Avant Dernier Auteur

DA = Dernier Auteur

Inv = Investigateur

Communications :

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- Battista J., De Luca D., Eleni Dit Trolli S., Allard L., Ranchin B., Bouhamri N., Enoch C., Faudeux C., Bacchetta J., **Bernardor J.**, Épuration extra-rénale continue par le CARPEDIEM[®] chez le nouveau-né et le nourrisson : une étude française multicentrique , Congrès annuel de la Société de Néphrologie Pédiatrique, Annecy, 3 Juin 2022
- **Bernardor J.**, Bartosova M., Zhang C., Marinovic I., Schaefer B., Herzog R., Melk A., Oh J., Klaus G., Arbeiter K., Cerkauskiene R., Drozd D., Vondrak K., Farlay D, Mehrabi A., Kratochwill K., Schmitt C.P, Étude du profil transcriptomique et protéomique d'artéioles d'insuffisants rénaux pédiatriques: quels mécanismes sont impliqués dans le processus procalcifiants ?, Nephropedies Lyon, 10 Mai 2022
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- **Bernardor J.**, Rédaction et présentation du Pas en pas en pédiatrie, Hyperkaliémie de l'enfant, Congrès de la Société Française de Pédiatrie, congrès virtuel, 4 Novembre 2020
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- **Bernardor J.**, Machuca-Gayet I., Peyruchaud O., Flammier S., Bacchetta J. Vitamine D et Etelcalcetide: leurs effets sur l'ostéoclastogénèse d'insuffisants rénaux, Congrès de la Société Française de Pédiatrie, Paris, 20 Juin 2019
- **Bernardor J.**, Gourdan P., Faudeux C., Bérard E. "Hypertension artérielle : une histoire de famille", à propos de cas cliniques d'un syndrome de Liddle, DES Day, Marseille, 7 Juin 2019
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- **Bernardor J.**, Herrissé AL, Isoardo M., Chami L., Haas H., A propos d'un cas d'atteinte systémique hépatosplénique de la maladie des griffes du chat, DES Day, Marseille, 9 Avril 2016
- **Bernardor J.**, Bailly C., Blanc S., Lecompte JF., Chami L., "une bronchiolite trainante", cas clinique et revue de la littérature du kyste bronchogénique, 12^{ème} journée du Groupe Inter-régional de Pneumopédiatrie, Nice, 4 Mars 2016

- **Bernardor J.**, Morand A., Minodier P., "une histoire d'hommes", cas clinique d'infection à *Mycoplasma hominis*. Journée des pédiatres infectiologues du Sud de la France, Montpellier, 12 Juin 2015

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- **Bernardor J.**, Flammier S., Bertholet-Thomas A., Tanné C., Bacchetta J. Injection bi-quotidienne de tériparatide sous-cutanée chez les enfants atteints d'hypoparathyroïdie: expérience lyonnaise, Congrès de la Société de Néphrologie Pédiatrique, Bruxelles, 29 Novembre 2019
- Faudeux C., **Bernardor J.**, Lemeignen M., Berard E. Un diagnostic différentiel du SHU à ne pas négliger : L'Hémoglobininurie Paroxystique Nocturne, Congrès de la Société Française de Pédiatrie, Marseille, 17-19 Mai 2017
- **Bernardor J.**, Dubois M-A., Haas H., Pitelet G., Popoff M-R., Mazuet C. Hypotonie, ptosis bilatéral et constipation : quel est votre diagnostic ? Congrès de la Société Française de Pédiatrie, Marseille, 17-19 Mai 2017
- Faudeux C., **Bernardor J.**, Marty P., Berard E. Une aire nouvelle de contamination par les schistosomes, Congrès de la Société Française de Pédiatrie, Marseille, 17-19 Mai 2017
- **Bernardor J.**, Faudeux C., Chausseot A., Berard E. Syndrome néphrotique congénital et atteinte mitochondriale : à propos d'un cas, Congrès de la Société Française de Pédiatrie, Marseille, 17-19 Mai 2017
- Faudeux C., Marchal S., Giovannini-Chami L., **Bernardor J.**, Triolo V., Hoflack M., Bérard E, Atteinte polyviscérale auto-immune compliquée de tubulopathie et déficit en STAT3, Séminaire Pierre Royer, Institut Imagine, Paris, 21 Mars 2017

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- **Bernardor J.**, Flammier S., Salles J-P, Castanet M., Martinerie L., Lienhardt A., Amouroux C., Linglart A., Bacchetta J. Off-label use of cinacalcet in pediatric primary hyperparathyroidism: a French multi-centre experience, 59th Annual Meeting of European Society for Paediatric Endocrinology, Online, 22-26 Septembre 2021
- **Bernardor J.**, Flammier S., Salles J-P, Castanet M., Martinerie L., Lienhardt A., Amouroux C., Linglart A., Bacchetta J. Off-label use of cinacalcet in pediatric primary hyperparathyroidism: a French

multi-centre experience, 53rd annual scientific meeting of the European Society for Paediatric Nephrology, Amsterdam, 16-19 Septembre 2021

- **Bernardor J.**, Regiroli G., Faudeux C., François-Garret B., Damez-Fontaine S., Eleni Dit Trolli S., De Luca D., CARPEDIEM continuous renal replacement therapy for very low birth weight neonates: first experience in Europe, 53rd annual scientific meeting of the European Society for Paediatric Nephrology, Amsterdam, 16-19 Septembre 2021
- **Bernardor J.**, Flammier S., Ranchin B., Gaillard S., Peyruchaud O., Machuca-Gayet I., Bacchetta J. Inhibition of osteoclast differentiation by 1.25-D and the calcimimetic KP2326 reveals 1.25-D resistance in advanced CKD, 57th ERA-EDTA congress fully virtual, Milan, 6-9 Juin 2020
- **Bernardor J.**, Flammier S., Ranchin B., Gaillard S., Peyruchaud O., Machuca-Gayet I., Bacchetta J. The calcimimetic KP2326 inhibits osteoclastic differentiation and bone resorption in healthy controls and in patients with chronic kidney disease, 18th Congress of the International Pediatric Nephrology Association, Venise, 17-21 Octobre 2019
- **Bernardor J.**, Leclerc AL., Schmitt C., Tönshoff B., Bacchetta J., Cinacalcet use after pediatric renal transplantation, data from the CERTAIN registry, CERTAIN meeting, 18th Congress of the International Pediatric Nephrology Association, Venise, 17-21 Octobre 2019
- **Bernardor J.**, Flammier S., Ranchin B., Gaillard S., Peyruchaud O., Machuca-Gayet I., Bacchetta J. The calcimimetic KP2326 inhibits osteoclastic differentiation and bone resorption in healthy controls and in patients with chronic kidney disease, Nephropedies Lyon, 16 Avril 2019
- **Bernardor J.**, Leclerc AL., Schmitt C., Tönshoff B., Bacchetta J., Cinacalcet use after pediatric renal transplantation, data from the CERTAIN registry, 40th Symposium Interdisciplinary Study Group Renal Transplantation in Pediaetrics, Heidelberg, Allemagne, 8 Décembre 2018

Communications affichées dans des congrès internationaux

- Bartosova M., **Bernardor J.**, Zhang C., Herzog R., Marinovic I., Melk A., Peters V., Kratochwill K., Schmitt CP., Molecular pathways underlying vascular disease in children with end stage kidney disease (ESKD), Kidney Week, American Society of Nephrology, Orlando, Floride, 3-6 Novembre 2022
- **Bernardor J.**, Regiroli G., Faudeux C., François-Garret B., Damez-Fontaine S., Eleni Dit Trolli S., De Luca D., CARPEDIEM continuous renal replacement therapy for very low birth weight neonates: first experience in Europe, European Society of Paediatric Neonatal Intensive Care Congress 2021, virtual congress, 15-18 Juin 2021
- **Bernardor J.**, Leclerc AL., Schmitt C., Tönshoff B., Bacchetta J., Cinacalcet use after pediatric renal transplantation, data from the CERTAIN registry, abstract soumis, 18th Congress of the International Pediatric Nephrology Association, Venise, 17-21 Octobre 2019

Communications orales sur invitation

- **Bernardor J.**, Table ronde : Suivi libéral de l'enfant vulnérable: prématuré et RCIU; focus sur le suivi néphrologique, congrès national de l'Association Française de Pédiatrie Ambulatoire, Hyères, 25 Novembre 2023
- **Bernardor J.** Cinacalcet in real-life clinical practice, 55th annual meeting of the European Society for Paediatric Nephrology, Pre-Congress, CME-1, Vilnius, 28 Septembre 2023
- **Bernardor J.** Prevention of vascular calcifications in children with chronic kidney disease, Controversies session, 55th annual meeting of the European Society for Paediatric Nephrology, Vilnius, 30 Septembre 2023
- **Bernardor J.** Vitamine D et calcium chez l'enfant et l'adolescent, les nouvelles recommandations 2022, Congrès Preuves et pratiques, Antibes, 18 Mars 2023
- **Bernardor J.** Vitamine D et calcium en pédiatrie générale: les nouvelles recommandations 2022, Formation Médicale Continue de l'association des pédiatres de Nice Côte d'Azur, Nice, 13 Décembre 2022

- **Bernardor J.**, Eleni Dit Trolli S., Session Néphrologie: indications et méthodes d'épuration extra-rénale chez le nouveau-né et nourrisson de 5 kilogrammes, 10^{ème} journée Réa néonatale et pédiatrique InterCHU Grand Sud, Toulouse, 18 Novembre 2022
- **Bernardor J.** Pediatric urolithiasis: What may be the underlying diseases? What is expected from imaging? What medical treatment? 42nd post-graduate course, European Society of Pediatric Radiology Congress, Marseille, 7 Juin 2022

Implication dans PHRC/STIC/contrats recherche/essais cliniques/gestion cohorte

- Co-investigatrice principale, CHU de Nice, de l'étude PIN'SNP, Cohorte prospective nationale de patients suivis pour un syndrome néphrotique idiopathique ayant débuté à l'âge pédiatrique
- Investigatrice principale de l'étude NEPHRO-DREPA : Dépistage des complications rénales chez l'enfant drépanocytaire majeur, AOI CHU de Nice 2021, 8k€

Investigatrice principale : Reins hyperéchogènes sans kystes diagnostiqués en période anténatale : quelle place pour des anomalies de *SLC34A1* ? Appel à projets OSCAR 2021, 20 k€1. Bacchetta J, Bardet C, Prié D (2019) Physiology of FGF23 and overview of genetic diseases associated with renal phosphate wasting. *Metabolism*. <https://doi.org/10.1016/j.metabol.2019.01.006>

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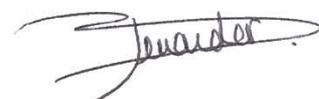
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