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## **Mineralocorticoid receptor antagonists and kidney diseases: pathophysiological basis**

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## **Abstract**

Chronic kidney disease (CKD) represent a global health concern, and its prevalence is increasing. The ultimate therapeutic option for CKD is kidney transplantation. However, the use of drugs that target specific pathways to delay or halt CKD progression such as ACEis, ARBs and SGLT-2 inhibitors is limited in the clinical practice. Mineralocorticoid receptor (MR) activation in non-classical tissues, such as the endothelium, smooth muscle cells, inflammatory cells, podocytes, and fibroblasts may have deleterious effects on kidney structure and function. Several pre-clinical studies have shown that mineralocorticoid receptor antagonists (MRAs) ameliorate or cure kidney injury and dysfunction in different models of kidney disease. In this review, we will present the pre-clinical evidence showing a benefit of MRAs in acute kidney injury (AKI), the AKI to CKD transition, hypertensive and diabetic nephropathy, glomerulonephritis, and kidney toxicity induced by calcineurin inhibitors. We also discuss the molecular mechanisms responsible for renoprotection related to MRAs that lead to reduced oxidative stress, inflammation, fibrosis, and hemodynamic alterations. The available clinical data support a benefit of MRA in reducing proteinuria in diabetic kidney disease and improving cardiovascular outcomes in CKD patients. Moreover, a benefit of MRAs in kidney transplantation has also been observed. The past and present clinical trials describing the effect of MRAs against kidney injury are presented and the risk of hyperkalemia and options, such as potassium binding agents or non-steroidal MRAs, are also addressed. Altogether, the available pre-clinical and clinical data support a benefit of MRA in CKD which has to be further explored in future clinical trials.

## **Introduction**

The classic effect of aldosterone is exerted in the epithelium of the aldosterone-sensitive distal nephron (ASDN), where it binds to the mineralocorticoid receptor (MR) and controls sodium reabsorption and potassium secretion. In these cells, specificity is conferred by the expression of 11 $\beta$ -hydroxysteroid dehydrogenase type 2 (11 $\beta$ -HSD2).<sup>1</sup> MR expression has been detected in non-classical tissues, where its activation may be pathophysiological. MR activation in non-classical tissues is not always due to the action of aldosterone, as 11 $\beta$ -HSD2 is not always expressed.<sup>1</sup> Table 1 summarizes the cell types in the kidney in which MR and/or 11 $\beta$ -HSD2 are expressed and whether MR-activation effects are observed. MR expression may be upregulated in some pathological conditions, such as diabetes<sup>2</sup>, heavy proteinuria<sup>3</sup>, vascular ageing<sup>4</sup> and hypertension<sup>5</sup>, thus amplifying MR signaling.

Steroidal mineralocorticoid receptor antagonists (MRAs) such as spironolactone or eplerenone have been used for decades as diuretics drugs for the management of hypertension, primary aldosteronism and heart failure. Canrenone is the active metabolite of spironolactone and is available in some countries. An injectable preparation of potassium canrenoate is also available in some countries. More recently, novel non-steroidal MRAs with improved safety profile have been developed including, BR-4628 (Bayer), finerenone (Bayer), PF-03882845 (Pfizer), SM-368229 (Dainippon Sumitomo), AZD9977 (AstraZeneca) among others. None of them is currently marketed.<sup>1,6,7</sup>

### **Pre-clinical data that support a benefit of MR antagonists (MRAs) in kidney diseases**

*Pre-clinical data: Benefit of MRAs on acute kidney injury (AKI) induced by ischemia/reperfusion (IR)*

Several clinical conditions can lead to a reduction in blood supply to the kidney, resulting in AKI. Indeed, a major cause of AKI is renal IR. In the clinical setting, there is no pharmacological approach to treat this condition. In 2007, *Mejía-Vilet et al.* showed that prophylactic MR antagonism with spironolactone efficiently prevents acute kidney dysfunction and tubular injury induced by bilateral renal IR injury in the rat.<sup>8</sup> The sustained reduction in renal blood flow observed in IR-untreated rats was prevented in the spironolactone treated groups and was accompanied by a reduction in oxidative stress and increased activation of endothelial nitric oxide synthase (eNOS).<sup>8</sup> Adrenal gland removal had similar protective effects against ischemic injury.<sup>9</sup> In common clinical situations, ischemic AKI cannot be anticipated. Thus, it was further shown that spironolactone administration at the time of ischemia and even after three hours of reperfusion is fully protective, whereas the protection was only partial when spironolactone was administered six hours after ischemia induction.<sup>10</sup> The non-steroidal MR antagonists (MRAs) BR-4628 and finerenone have also shown beneficial effects in rat and mouse models of ischemic AKI.<sup>11,12</sup> It was demonstrated that the benefit of MR antagonism in kidney IR is dependent on endothelin-B receptor signaling and that a sulfenic acid modification that functionally inactivates this receptor is responsible for reduced eNOS activation and therefore sustained vasoconstriction after IR.<sup>11,12</sup> Cell-specific MR knockout mice in which MR expression in endothelial and smooth muscle cells (SMC) was targeted were developed to delineate the role of vascular MRs in renal IR injury. MR deficiency in endothelial cells had no effect on the severity of AKI. However, SMC-specific MR gene deletion prevented renal dysfunction and structural injury induced by IR.<sup>13</sup> This protective effect was related to reduced Rac1-induced oxidative stress in SMCs, thus preventing the sulfenic acid modification of the endothelin-B (ETB) receptor, leading to inefficient ETB receptor signaling and defective

eNOS activation.<sup>13</sup> Therefore, the SMC MR signaling pathway could potentially be specifically targeted to prevent the acute consequences of an ischemic kidney episode.

The benefit of MRAs in ischemic AKI was translated into the White Large Pig pre-clinical model in which MR antagonism with Soludactone® (potassium Canrenoate, a soluble MRA used in the clinic, mainly in intensive care units) prevented the effects of AKI, as shown by reduced urinary NGAL, Kim-1, and oxidative stress levels, as well as the remarkable preservation of kidney function and structure.<sup>13</sup>

The benefit of MRAs in ischemic diseases is not limited to the kidney, as the protective effects of MRAs have also been described in intestinal, cardiac, and brain ischemic injury.<sup>1,14,15,16</sup>

#### ***Pre-clinical data: MR antagonism prevents the AKI to CKD transition***

Clinical and experimental studies have demonstrated that AKI is independently associated with an increased risk of CKD. Comprehensive research has targeted the mechanisms of the AKI to CKD transition, but no therapeutic options are yet available in the clinical setting.<sup>17</sup> In 2013, *Barrera-Chimal et al.* showed, that a single episode of ischemic AKI can induce CKD in rats subjected to bilateral kidney IR and followed up to nine months, characterized by proteinuria, kidney dysfunction, and severe structural injury. Prophylactic spironolactone administration prevented all these alterations.<sup>18</sup> The protective effect of spironolactone was also observed when administered in one dose up to 1.5 hours after ischemia induction or when the administration was started 24 hours after the AKI episode.<sup>18,19</sup> MR antagonism was also able to prevent CKD induced by a short ischemic period (20 min) even when administered three hours after IR.<sup>20</sup> The benefit of the non-steroidal MRA finerenone in this setting was also demonstrated in both rat and mouse

models of the AKI to CKD transition.<sup>12,19</sup> Inflammatory cells play a crucial role for efficient repair after an ischemic episode. We therefore explored the impact of MRAs and targeted deletion of MRs in myeloid cells in inflammation and the development of CKD after AKI. Mice treated with finerenone or those with myeloid-specific MR deletion were protected against the AKI to CKD transition. This was due to a reduction of pro-inflammatory macrophage infiltration and the promotion of macrophage polarization to an M2-wound-healing phenotype in the acute phase after the ischemic injury. MR inhibition promoted M2 macrophage polarization by increasing macrophage interleukin-4 receptor expression and activation.<sup>19</sup> Importantly, for translation to the clinic, Soludactone® administration in the White Large Pig model, from two days before and up to two days after the ischemic insult, was also able to prevent AKI-induced CKD progression, with reduced kidney fibrosis and plasma creatinine levels 90 days after the AKI event. This effect was associated with decreased parenchyma inflammation at day 14.<sup>19</sup> Altogether, the pre-clinical evidence proposed that the pro-inflammatory signal transduction mediated by MR is a plausible target to prevent the chronic alterations induced by ischemic AKI.

The benefit of MRAs in wound healing, favoring M2 repair macrophages over inflammatory M1 macrophages extends beyond the kidney, as reported for stroke<sup>21,22</sup> aldosterone-salt induced hypertension<sup>23,24</sup>, cardiac remodeling<sup>25-27</sup>, and skin injury (*Jaisser F, personal data*).

#### ***Pre-clinical data: MR antagonism in kidney fibrosis and CKD progression***

Kidney fibrosis is the common end-point of CKD from various etiologies. Several experimental models have been used to mimic the human disease, leading to kidney fibrosis, in which the role of aldosterone and MR activation has been evaluated. A pioneer

study by *Greene et al.* reported that ACE and AT1 receptor inhibition ameliorated the proteinuria and glomerulosclerosis in the subtotal nephrectomy model.<sup>28</sup> The addition of aldosterone to rats with subtotal nephrectomy and ACE/AT1 antagonist treatment reversed their protective effects. Moreover, spironolactone treatment of subtotal nephrectomized rats transiently reduced proteinuria, lowered blood pressure, and reduced cardiac hypertrophy.<sup>28</sup> MR antagonism not only prevents glomerulosclerosis in this remnant kidney model but also induces its regression.<sup>29</sup> Recently, it was shown that the non-steroidal aldosterone antagonist Ly also protects against albuminuria and kidney hypertrophy in the remnant kidney model, with better efficacy than eplerenone. However, a beneficial effect on collagen deposition and macrophage infiltration was observed only when it was given together with losartan, possibly through a better antihypertensive effect.<sup>30</sup>

Since a common feature in the aforementioned studies is the positive effect of MRAs on proteinuria, it was suspected that MR inhibition was acting through an effect on podocyte health, therefore maintaining the glomerular filtration barrier. In the Dahl salt hypertensive rat model, eplerenone had a modest effect on reducing blood pressure, but it reduced podocyte damage and effacement and delayed the progression of proteinuria and glomerulosclerosis.<sup>31</sup> Similarly, the non-steroidal MRA CS-3150 prevented glomerulosclerosis, tubular injury, and fibrosis.<sup>32</sup> MR activation in podocytes may occur by aldosterone-independent mechanisms. In 2008, *Shibata et al.* showed that a constitutively active form of Rac1 induced MR nuclear translocation and activation in podocytes.<sup>33</sup> Moreover, mice with globally increased Rac1 activity presented severe albuminuria and podocyte injury, which were prevented by the MRA eplerenone.<sup>33</sup>

MR antagonism as a strategy to treat or delay glomerulonephritis progression has been evaluated. Spironolactone reduced proteinuria in a model of proliferative



glomerulonephritis induced by anti Thy1.1 antibody administration and this effect was potentiated to prevent fibrosis in combination with cilazapril.<sup>34</sup> In the same model, spironolactone treatment reduced TGF-beta staining and the injury score in the artery and glomerulus, and most importantly it also reduced proliferation.<sup>35</sup> More recently, it was questioned whether aldosterone blockade not only prevents glomerulonephritis due to the effect on proliferation but also because of an anti-inflammatory effect. Indeed, eplerenone administration in the anti-Thy1.1 model inhibited glomerular macrophage infiltration, MCP-1 expression, and mesangial phenotypic activation.<sup>36</sup>

The administration of anti-GBM antibodies is another strategy used to mimic glomerulonephritis. In this animal model, eplerenone administration reduced albuminuria, glomerular sclerosis, and glomerular crescents, accompanied by reduced infiltration of CD4<sup>+</sup> and CD8<sup>+</sup> T cells and macrophages.<sup>37,38</sup> *Huang et al.* assessed the severity of glomerulonephritis in mice receiving anti-GBM antibodies and specific deletion of the MR, either in podocytes or myeloid cells, to evaluate whether the MRs expressed in podocytes or inflammatory cells were responsible for the beneficial effect. Myeloid-specific MR deficiency or eplerenone treatment had no effect on the severity of proteinuria. However, a reduction in cystatin C levels and crescent numbers and reduced infiltration of macrophages and neutrophils to the kidney was observed. A reduction in pro-inflammatory cytokine levels, tubular damage, and renal fibrosis was also documented. Podocyte-specific MR deletion had no effect in ameliorating anti-GBM glomerulonephritis.<sup>38</sup> Therefore, MRs expressed in inflammatory cells play a key role in the progression of glomerulonephritis. The novel non-steroidal MRA BR-4628 also efficiently improved renal function, prevented glomerular tuft damage, and reduced glomerular crescents by 50%. The inflammatory component was also affected in the kidney, with lower levels of infiltrated macrophages

and T cells, associated with reduced pro-inflammatory cytokine expression. Therefore, these authors found that BR-4628 provided similar protection from glomerulonephritis as eplerenone without altering tubular salt handling.<sup>39</sup>

Hypertension is associated with kidney injury. The benefit of MR antagonism against hypertension-induced kidney injury has been tested in several rodent models and has proven to be independent from blood pressure control. In the stroke-prone spontaneously hypertensive rat, which develops nephrosclerotic lesions and proteinuria, spironolactone administration effectively prevented kidney damage, even if the increase in blood pressure was not affected.<sup>40</sup> A similar effect on proteinuria and glomerular sclerosis was described in the radiation injury hypertensive model, without an effect on blood pressure.<sup>41</sup> In spontaneously hypertensive rats, chronic NOS inhibition with L-NAME exacerbates nephrosclerotic lesions. Eplerenone administration ameliorated proteinuria and glomerular, arteriolar, and tubulointerstitial lesions, despite having no effect on systemic or renal hemodynamics.<sup>42</sup> In the same model of NOS inhibition, it was further observed that inflammatory cell infiltration to the kidney was inhibited by spironolactone.<sup>43</sup> In the hypertensive kidney injury induced by aldosterone-salt plus unilateral nephrectomy model, eplerenone attenuated hypertension and efficiently reduced albuminuria, kidney fibrosis, glomerular injury, and pro-inflammatory gene expression in Sprague-Dawley rats.<sup>44</sup> The novel non-steroidal MRAs SM-368229, CS-3150, and AZD9977 have also shown favorable effects on renal pathology in this context.<sup>45,46,47</sup> In a similar model that uses DOCA administration instead of aldosterone, specific MR deletion in the endothelium ameliorated cardiac remodeling, but not renal injury, suggesting that the MR in the endothelium does not play a crucial role in this type of kidney injury.<sup>48</sup>

A classic model of kidney fibrosis is unilateral ureteral obstruction (UUO). *Trachtman et al.* showed that spironolactone was able to reduce kidney fibrosis in C57BL/6 mice with two weeks of UUO.<sup>49</sup> In rats with UUO, eplerenone improved renal fibrosis and inflammation, reduced monocyte and macrophage infiltration and interstitial cell proliferation, and reduced oxidative stress.<sup>50</sup> The positive effects of eplerenone are not restricted to the obstructed kidney, as it also decreased macrophage infiltration, collagen deposition, and PCNA expression in the contralateral kidney.<sup>51</sup>

In summary, MRAs have beneficial effects in hypertensive, glomerular, and fibrotic kidney disease. The underlying mechanisms are detailed below.

#### ***Pre-clinical data: Benefit of MR blockade in diabetic nephropathy***

Kidney disease is a frequent complication in diabetic patients. It is estimated that 40% of end-stage renal disease cases are attributable to complications of diabetic kidney disease.<sup>52</sup> The most common model for type 1 diabetes in rodents is pancreatic beta-cell depletion by streptozotocin (STZ) administration. In 2004, *Fujisawa et al.* showed that spironolactone administration for three weeks reduced renal collagen deposition in the glomerular, tubulointerstitial, and perivascular areas in STZ-induced diabetic rats.<sup>53</sup> The protective effect of MRAs in diabetic nephropathy was corroborated in further studies.<sup>54,55,2,56</sup> Eplerenone also reduced glomerular volume and glomerular fibrosis in STZ-treated hypertensive rats.<sup>57</sup> The protective effect may be mediated by the restoration of G6PD activity and oxidative stress reduction by spironolactone.<sup>58</sup>

Type 2 diabetes is often studied in rodent models with genetic alterations that lead to metabolic disorders. Eplerenone reduced albuminuria, glomerular hypertrophy, and mesangial expansion in the db/db diabetic mouse model.<sup>54</sup> Similarly, The novel MRA

AZD9977 reduced albuminuria and kidney histology changes in db/db mice.<sup>46</sup> The addition of eplerenone to an ACE inhibitor in db/db mice had additional beneficial effects, improving albuminuria, glomerulosclerosis, podocyte injury, and renal fibrosis.<sup>59</sup>

In the rat, a commonly used model for diabetic kidney disease is the Otuska Long-Evans Tokushima Fatty rat (OLEFT). In this model, spironolactone decreased albuminuria and ameliorated glomerulosclerosis, macrophage infiltration, and MCP-1 upregulation.<sup>60</sup> Moreover, *Han et al.* showed that the antifibrotic effect of spironolactone in this model may be mediated by CTGF through a TGF-beta1-independent pathway.<sup>60</sup> A synergistic effect to prevent podocyte injury has been observed with a combination of enalapril and eplerenone<sup>61</sup> or with telmisartan.<sup>62</sup> In the Zucker-obese rat, finerenone reduced proteinuria and renal NGAL expression.<sup>63</sup> In a severe model that combines obesity, hypertension, and high salt (SHR/cps rat), massive proteinuria, severe renal lesions, and podocyte injury are observed; eplerenone dramatically ameliorated proteinuria and renal injury in these animals.<sup>64</sup> Mice with obesity induced by a high fat diet develop kidney injury, characterized by glomerular hypercellularity, mesangium expansion, albuminuria, and increased rho kinase activity; all of these alterations were attenuated by eplerenone.<sup>65</sup> In cultured mesangial cells, high glucose increases Rac1 and MR transcriptional activity.<sup>66</sup> In accordance with these observations, the inhibition of Rac1 reduced MR activity in the kidney and mitigated renal pathology in a mouse model of obesity-related type 2 diabetes (KKA(y) mouse), even if plasma aldosterone levels were unchanged.<sup>66</sup>

### ***Pre-clinical data: Calcineurin inhibitor toxicity and kidney transplantation***

Calcineurin inhibitors (CNI) are widely used in kidney-transplant patients as immunosuppressive drugs. Since the introduction of CNI in the clinic, the lifespan of the

grafts has been significantly extended. The use of CNI is often limited due to its acute or chronic nephrotoxic effects. Acute CNI nephrotoxicity is characterized by potent, but reversible, vasoconstriction of the afferent arteriole that leads to a reduction in renal blood flow and the GFR.<sup>67</sup> In 1987, it was shown that spironolactone treatment of rats with acute cyclosporine A (CsA) nephrotoxicity was able to preserve kidney function.<sup>68</sup> Later, *Perez-Rojas et al.* showed that spironolactone not only prevents the decrease in GFR but also prevents the fall in renal blood flow.<sup>69</sup> Similar effects were observed with eplerenone.<sup>70</sup> More recently, it was shown that the MRs in SMCs play a key role in the vasoconstriction induced by CsA. MR deletion in SMCs prevented the acute kidney dysfunction and injury induced by CsA and blunted the CsA-induced phosphorylation of contractile proteins, thus normalizing renal vascular resistance.<sup>71</sup>

During chronic CNI nephrotoxicity, tubulointerstitial fibrosis, arteriolopathy, proteinuria, and a progressive decrease in GFR occur and contribute to graft loss. The chronic reduction in renal blood flow and GFR induced by CNI are prevented by spironolactone.<sup>69</sup> This effect is due to modulation of the expression of prorenin, the endothelin-A receptor, and the endothelin-B receptor by the MRA, favoring vasodilation.<sup>69</sup> *Feria et al.* showed that CNI-induced tubulointerstitial fibrosis is prevented by spironolactone.<sup>72</sup> *Nielsen et al.* showed an increased volume fraction of the interstitial space in CsA-treated rats which was prevented by MR antagonism.<sup>73</sup> Moreover, *Pérez-Rojas et al.* showed that spironolactone can slow the progression of kidney injury in established CNI-induced kidney injury.<sup>74</sup> The up-regulation of TGF-beta, collagen I, and fibronectin mRNA levels induced by CsA was prevented by spironolactone.<sup>74</sup> MR antagonism also protected against CNI-induced arteriolopathy.<sup>72,73</sup>

Concerning kidney transplantation, spironolactone administration from two days before up to 12 weeks post-transplantation significantly ameliorated vasculopathy and glomerular macrophage influx and tended to reduce proteinuria and glomerulosclerosis in a model of chronic allograft dysfunction in the Dark Agouti to Wistar Furth rat.<sup>75</sup> However, interstitial fibrosis, creatinine clearance, and blood pressure were not affected. Other authors failed to detect any protection against glomerulosclerosis or interstitial fibrosis with MR antagonism or aldosterone synthase inhibition in a rat model of allograft injury in male Fisher-344 rats to male Lewis rats.<sup>76</sup>

***Pre-clinical data: Benefit of MRAs on the comorbidities of CKD***

The positive effect of MR inhibition may not be restricted to kidney protection, since spironolactone also prevented increases in cardiomyocyte size in both ventricles, despite no benefit on kidney function or blood pressure control in a subtotal nephrectomy CKD model in the rat.<sup>77</sup> Similarly, we reported that finerenone prevents cardiac diastolic dysfunction in mice with CKD despite maintained renal dysfunction.<sup>78</sup> In the Zucker rat, a model of metabolic syndrome cardiorenal injury, finerenone reduced cardiac hypertrophy, fibrosis, and dysfunction.<sup>63</sup> Cardiac diastolic dysfunction and vascular injury associated with diet-induced obesity are also prevented by MR inhibition.<sup>79,79,80</sup> MR antagonism prevented diabetic neuropathy, as shown by improved motor-nerve conduction velocity and mean myelin area after eight weeks of eplerenone treatment in STZ-induced diabetic rats.<sup>81</sup>

**Major pathophysiological mechanisms involved in the impact of MRs on kidney diseases:**

***Pre-clinical data: MRs and oxidative stress***

Multiple studies have shown the impact of aldosterone/MR-induced oxidative stress on detrimental consequences in the kidney. DOCA-salt administration for six weeks causes oxidative DNA damage and increases the mutagenic DNA modification 8-oxodG in the kidney.<sup>82</sup> Aldosterone infusion in the rat produces an MR-dependent increase in NADPH oxidase activity and ROS generation and increased expression of p67phox, p22phox, Nox4, Gp91phox, p47phox, and Rac1 in the kidney.<sup>83 84</sup> Mouse peritoneal macrophages and aortic segments from aldosterone-treated mice exhibit increased superoxide anion formation and increased translocation of the NADPH oxidase subunit p47phox to the macrophage plasma membrane.<sup>85</sup> The incubation of human mononuclear leukocytes with 10 nM aldosterone increases p22phox expression, an effect blocked by the MRA canrenone.<sup>86</sup> MR activation in SMCs and endothelial cells is associated with ROS production by increasing the expression and activity of NADPH oxidases.<sup>87,88,89,90</sup> The oxidative stress induced in SMCs in turn promotes cell senescence.<sup>91</sup> MRs in SMCs contribute to angiotensin II-induced vascular oxidative stress.<sup>92</sup> In endothelial cells, aldosterone decreases the expression of G6PD, which is a critical regulator of the redox state, shifting the balance towards increased oxidative stress.<sup>93</sup> In pulmonary artery endothelial cells, aldosterone increases ROS, which in turn modifies the cysteinyl thiols in the eNOS-activating region of the ET(B) receptor, thus decreasing endothelin-1 stimulated eNOS activity. These effects have physiological consequences on the local hemodynamics in pulmonary arterial hypertension<sup>94</sup> and in kidney ischemia/reperfusion injury.<sup>12,11</sup>

In rat mesangial cells, aldosterone directly stimulates superoxide anion generation, which is accompanied by an increase in NADPH oxidase activity and translocation of p47phox and p67phox to the cell membrane.<sup>95</sup> In addition, aldosterone induces mesangial-

cell apoptosis and the administration of an antioxidant or MRA attenuates the proapoptotic effects of aldosterone.<sup>96</sup>

In the White Large pig, MR antagonism reduced the increase in MDA levels induced by ischemic injury.<sup>13</sup> Patients with primary aldosteronism display higher NADPH oxidase Nox2 plasma and urinary isoprostane levels than essential hypertensive patients.<sup>97</sup> Moreover, reduced oxidative stress is observed in diabetic or kidney transplant patients treated with an MR antagonist.<sup>98,99</sup>

#### ***Pre-clinical data: MRs and inflammation***

A role for MR signaling in inflammation has been suggested since early studies showing that the treatment of rats with aldosterone/salt causes perivascular leukocyte infiltration and increased expression of osteopontin, MCP-1, IL-6, and IL-1 $\beta$  in the kidney, whereas MR antagonism prevents this pro-inflammatory state.<sup>44</sup> The endothelial MR participates in the inflammatory process by increasing adhesion molecule-1 expression.<sup>100</sup> There is a direct effect of MRAs on inflammatory cells in human PBMCs stimulated with LPS, as spironolactone inhibits the transcription of TNF-alpha, IL-6, and IFN-gamma.<sup>100</sup> Similarly, the pro-inflammatory effect of angiotensin II on human PBMCs is prevented by MRAs.<sup>101</sup>

Mice lacking the MR in myeloid cells displayed unaffected macrophage infiltration in the heart following DOCA treatment, showing that myeloid MRs are not involved in the migration capacity of the macrophages but probably in differentiation.<sup>23</sup> In accordance with this hypothesis, pharmacological or genetic MR inhibition in cultured macrophages reduced the levels of pro-inflammatory M1-markers and increased those of anti-inflammatory M2-markers.<sup>102</sup> In peritoneal macrophages, MR deficiency synergizes with IL-4 or an agonist of



PPARgamma to enhance alternative M2 activation.<sup>25</sup> *In vivo*, MR deficiency in macrophages mimics the effects of MRAs and protects against cardiac hypertrophy, fibrosis, and vascular damage caused by L-NAME/Ang II.<sup>25</sup> In cerebral ischemia, myeloid MR deficiency reduces the infarct volume and macrophage infiltration in the ischemic core, consistent with reduced M1 and increased M2 marker levels.<sup>21</sup> A recent report by our group showed that MR inhibition acts by influencing IL-4 receptor signaling, promoting macrophage differentiation to an M2 phenotype. These effects were protective against the AKI to CKD transition.<sup>19</sup> In macrophages, MR gene ablation prevents the activation of cJun and cFos in response to pro-inflammatory stimuli<sup>24,103</sup>. Aldosterone increases NGAL expression in macrophages, dendritic cells, and PBMCs. Mice depleted for NGAL in their immune cells are protected against aldosterone-salt-induced cardiac remodeling and inflammation, suggesting that NGAL mediates the pro-inflammatory effects of the MR.<sup>104</sup>

T-cell MR knockout mice showed lower cardiac hypertrophy, fibrosis, and dysfunction than littermate control mice after abdominal aortic constriction, suggesting that MRs in T cells may also play a pro-inflammatory role. *In vitro* studies showed that MR inhibition repressed T-cell activation, whereas MR overexpression had the opposite effect.<sup>105</sup> MR inhibition in T cells had a similar protective effect against hypertension in the AngII-salt model.<sup>105</sup> Furthermore, MR antagonism with spironolactone in a hypertension rat model reduced heart and kidney damage due to a blockade of Th17 polarization and the induction of regulatory T cells.<sup>106</sup> In dendritic cells, MR stimulation with aldosterone induces the secretion of IL-6 and TGF- $\beta$ , two proinflammatory cytokines that can polarize the adaptive immune response towards a Th17 phenotype.<sup>107</sup>

***Pre-clinical data: MR and renal hemodynamic alterations***

The MR contributes to the regulation of vascular tone.<sup>8,10,69</sup> We recently explored the role of SMC-MRs in kidney injury. We showed that SMC-MR-KO mice are protected against ischemia-induced renal injury through a mechanism involving oxidative stress and Rac1 activation<sup>13</sup>. In addition, MR deficiency in SMCs was also protective in an acute model of CsA nephrotoxicity.<sup>71</sup> This protection was attributed to blunted vascular L-type calcium channel activity in SMC-MR-KO mice, resulting in decreased renal artery vasoconstriction and an overall improvement in renal hemodynamics.<sup>106</sup>

#### ***Pre-clinical data: MR and fibrosis***

In uni-nephrectomized rats, aldosterone administration is associated with an increase in TGF- $\beta$ , collagen, and connective tissue growth factor expression that is accompanied by medullary and cortical fibrosis.<sup>108,109</sup> Aldosterone influences the production of plasminogen activator inhibitor-1 and this effect is associated with glomerulosclerosis.<sup>41</sup> Aldosterone has been reported to induce collagen synthesis in cultured fibroblasts<sup>110,111</sup> and glomerular mesangial cells.<sup>112</sup> In cultured fibroblast expressing MRs, aldosterone addition increases ERK1/2 phosphorylation and the mRNA levels of collagens I, III, and IV.<sup>113</sup> Moreover, aldosterone stimulates fibronectin synthesis through MR-dependent activation of the JNK kinase and subsequent phosphorylation of the AP1 transcription factor c-Jun.<sup>114</sup> Aldosterone induces OPN expression in kidney fibroblasts<sup>115</sup>; accordingly, OPN plasma levels are higher in patients with primary aldosteronism.<sup>116</sup> In addition, MR activation in renal fibroblasts results in rapid activation of growth-factor receptors and the induction of PI3K/MAPK signaling, which stimulates proliferation.<sup>117</sup> This evidence suggests that increased MR activation may promote kidney fibrosis by inducing fibroblast proliferation and the production of pro-fibrotic

molecules. MR activation in SMCs increases the expression of mediators of extracellular matrix deposition, such as integrin alpha 5, galectin-3, and placental growth factor.<sup>118-120</sup>

Mice deficient for NGAL are protected from aldosterone-induced increases in procollagen I expression and collagen fiber deposition in the coronary vessels and aorta<sup>121</sup> and kidney (*Jaisser F, unpublished data*). NGAL addition to human fibroblasts increases the production of galectin-3 and collagen I<sup>121</sup> and is required for the profibrotic effects of aldosterone.<sup>122</sup> Galectin-3 also mediates the pro-fibrotic effects of aldosterone through the MR, as galectin-3 KO mice are protected against aldosterone-induced kidney fibrosis.<sup>123</sup> Excessive SGK1 expression is observed in several kidney fibrotic diseases. MR-induced SGK-1 expression inhibits the degradation of Smad2/3, mediators of TGF-beta signaling, thus contributing to the development of fibrosis.<sup>124</sup> MRs may also act through the EGF receptor, directly contributing to fibrosis.<sup>125</sup>

Figure 1 summarizes the mode of action of MR antagonists and its effects on different cell types, as well as the clinical settings where MR antagonism may have a beneficial effect.

### **Clinical data supporting a benefit of MR antagonists in kidney diseases: past and current clinical trials**

#### ***Clinical data: Beneficial impact of MRAs in limiting the progression of CKD***

Over the past two decades, several RCTs have reported a substantial antiproteinuric effect and a possible major renoprotective effect of MRAs in CKD. Two meta-analyses including studies published from 2002 to 2011<sup>126</sup> and 2005 to 2014<sup>127</sup> showed that MRAs effectively reduce proteinuria when used in combination with angiotensin converting enzyme inhibitors (ACEis) and angiotensin receptor blockers (ARBs). As mentioned above,

the beneficial impact of MRAs may be of particular interest in the setting of diabetic nephropathy (DN). A meta-analysis analyzing the impact of spironolactone addition to ACEi or ARB in patients with DN confirmed a significant reduction of 24-hour urinary albumin/protein excretion in eight studies and a significantly higher reduction of the urinary albumin/creatinine ratio in three.<sup>128</sup> Of note, a significant reduction in blood pressure was also reported so one cannot exclude a reduction of proteinuria partly mediated by the blood pressure reduction. The ARTS-DN Study demonstrated a dose-dependent reduction in proteinuria in patients with DN receiving ACEis or ARBs in combination with finerenone.<sup>129</sup> In this trial, hypertension with mean sitting SBP  $\geq 160$  mmHg or mean sitting DBP  $\geq 100$  mmHg at the screening visit was considered as an exclusion criteria. At baseline, mean BP was  $< 140/80$  mmHg in the different arms of treatment. Moreover, the placebo least squares corrected mean differences in SBP from baseline to day 90 in the finerenone groups were slight. Finally, the post hoc analysis showed that no meaningful correlation was observed across all treatment groups between the proteinuria change and the change in SBP from baseline to day 90.

An added antiproteinuric effect of MRAs was also reported in cases of non-diabetic nephropathy, such as idiopathic chronic glomerulonephritis.<sup>130,131</sup>

Of note, the available studies were not designed to demonstrate a beneficial impact of MRAs on long-term renal outcomes, in particular time to renal death. Most of the studies evaluated the reduction of proteinuria with MRAs. The association between the reduction of proteinuria and the decrease of CKD progression is reported.<sup>132 133</sup> In 2009 the FDA and the National Kidney Foundation<sup>134</sup>, and again in 2012 the FDA and the American Society of Nephrology's glomerular disease advisory group, considered that for now, proteinuria could not be considered as an established surrogate endpoint and that further studies were

needed<sup>133</sup>. To date, proteinuria is included in the surrogate endpoints that were the basis of drug approval or licensure by the FDA only in the settings of the following renal pathologies: primary glomerular disease associated with nephrotic syndrome and primary immunoglobulin A nephropathy<sup>135</sup>.

The necessity to develop studies with surrogate outcomes is recognized in order to improve the prognosis of CKD patients.<sup>136</sup> In his editorial<sup>136</sup>, Roy-Chaudhury stressed again that nephrology is one of the medical specialties with the lowest number of RCT.<sup>137</sup> Moreover, he emphasized the fact that surrogate markers should be developed and validated in order to reduce the follow-up period of RCT. Outcomes such as renal death or doubling of creatinine preclude investigators from conducting RCT evaluating new therapeutic strategies. In trials evaluating interventions to slow CKD progression, the 30 to 40% reduction of estimated glomerular filtration rate (eGFR) probably is reliable surrogate marker.<sup>138</sup>

From a safety point of view, available data indicate a good short-term safety of MRAs in the setting of CKD. Indeed, the short-term impact of MRAs on the eGFR was considered to be either neutral in the meta-analysis of Boligiano et al. (n = 528 patients, mean difference of -2.55 mL/min/1.73 m<sup>2</sup>, 95% CI [-5.67 to 0.51]<sup>126</sup> or slightly deleterious in the meta-analysis of Currie et al. (n = 1,646 patients, mean difference of -3.2 mL/min/1.73 m<sup>2</sup> 95% CI [-5.4, to -1.0]).<sup>127</sup>

From an efficacy point of view, no data are currently available to evaluate the impact of MRAs on long-term renal protection. A RCT aiming to enroll a total of 4,800 patients with DN to assess the effect of finerenone on the progression of CKD is ongoing (FIDELIO NCT02540993). The primary outcome is time to the first occurrence of the composite

endpoint of the onset of kidney failure, a sustained decrease of eGFR  $\geq 40\%$  from baseline over at least four weeks, and renal death. A follow-up of up to 48 months is planned.

***Clinical data: Improvement of CV outcomes by MRAs in CKD/ESRD patients***

\* Potential beneficial impact of MRAs in heart failure among CKD patients

The life-saving effect of MRA in heart failure with altered left ventricular function and post-myocardial infarction is well established<sup>139</sup> and the prescription of MRAs is included in the international guidelines of treatment of heart failure<sup>140</sup>. MRAs possibly have also a beneficial effect in heart failure with preserved left ventricular function (TOPCAT<sup>141</sup>). Only few studies have assessed the beneficial impact of MRAs on CV outcomes in CKD or dialysis patients.

In a post-hoc analysis of the EMPHASIS-HF study<sup>142</sup>, Eschalier et al. reported that the HR for the primary outcome (hospitalization for HF or cardiovascular mortality) in the eplerenone group as compared with the placebo group was 0.69, 95 % CI [0.56-0.86]; in patients without CKD (defined by eGFR  $<60$  ml/min/1.73m<sup>2</sup>), and 0.62, 95% CI [0.49-0.79] in patients with CKD. The interaction test was not significant, so MRAs probably are as effective in CKD as in non-CKD patients for the treatment of heart failure. Of note, the EMPHASIS-HF trial enrolled patients at least 55 years old with heart failure and reduced ejection fraction (HF-REF), with an estimated glomerular filtration rate (eGFR)  $>30$  ml/min/1.73 m<sup>2</sup> and serum potassium  $<5.0$  mmol/l. In patients with CKD, the incidence of potassium  $>5.5$  mmol/l in patients receiving eplerenone increased in comparison to placebo (16.6% versus 9.3%,  $p=0.002$ ). There was no increase in the proportion of patients with a serum potassium  $>6.0$  mmol/l (1.9% vs 3.3%,  $p=0.29$  for the comparison between the eplerenone and the placebo groups). When comparing the patients treated with eplerenone

with or without CKD, there was a decrease in incident potassium >6.0 mmol/l, (1.9% vs 2.74%, p=0.01), but an increase in hyperkalemia leading to treatment discontinuation, (1.15% vs 1.08%, p=0.01, in eplerenone patients with CKD compared with patients without CKD).

A large recent Swedish registry trial reported that among patients with myocardial infarction and heart failure<sup>143</sup> (Swedish myocardial infarction registry, SWEDEHEART, 2005-2014), MRAs were under prescribed at discharge (only 9.9%), in particular among patients with eGFR<60mL/min (8.6%, vs 11.4% among patients with eGFR>60mL/min). The rate of prescription according to CKD stages is not available. Of note, the use of MRA was associated with better long-term survival (all-cause mortality up to 3 years) in patients with left ventricular ejection fraction (LVEF) <40% but not in those with LVEF ≥50%. Moreover, the mortality risk was similar in MRA-treated patients with (HR 0.92, 95% CI [0.85–0.99]) or without (HR 0.96, 95% CI [0.88–1.05]) CKD.

The safety of finerenone was evaluated in the setting of heart failure among patients with mild CKD (mean baseline eGFR  $69.1 \pm 8.43$  ml/min/1.73m<sup>2</sup>) and moderate CKD (mean baseline eGFR  $47.0 \pm 10.0$  ml/min/1.73m<sup>2</sup>) in the Miner Alcocorticoid-Receptor Antagonist Tolerability Study (ARTS).<sup>144</sup> Finerenone decreased the levels of B-type natriuretic peptide (BNP), amino-terminal proBNP, and albuminuria to the same extent as spironolactone. Finerenone was associated with significantly smaller increases in potassium levels than spironolactone (from 0.04 to 0.30 mmol/L, depending on the dose of finerenone, and 0.45 mmol/L for spironolactone, respectively, p < 0.0001 to 0.0107) and a lower incidence of hyperkalemia (5.3 and 12.7%, respectively, p = 0.048).

*\* Cardiac and vascular CKD-related abnormalities*

Scarce data are available concerning the potential beneficial impact of MRAs on CV outcomes among patients with CKD, either in dialysis or not.

In patients with stage 2-3 CKD, the administration of spironolactone for 40 weeks was associated with a significant improvement of left ventricular hypertrophy and arterial stiffness<sup>145</sup>, as well as markers of regional LV systolic and diastolic function, relative to placebo.<sup>146</sup> In patients with stage 3-4 CKD (mean eGFR 36 +/- 11 mL/min/1.73m<sup>2</sup>), the addition of eplerenone to standard medication did not improve pulse wave velocity after 24 weeks, but significantly attenuated pulse wave reflections (as measured by the Augmentation Index).<sup>147</sup> The increased pulse wave velocity observed in CKD is mainly related to higher arterial stiffness of larger conduit vessels. If structural changes of the arterial wall are partly mediated by the MR<sup>148</sup>, but the benefit from MRAs on pulse wave velocity probably need longer interventions to be detected. In contrast, improvement of the Augmentation Index probably reflects the rapid functional effect of eplerenone on the peripheral resistance of the small resistance vessels. Whether long-term use of MRAs will modify vessel structure is still to be determined in clinical trials.

In a limited number of patients with reduced ejection fraction, the administration of spironolactone during 6 months significantly improved the ejection fraction and the left ventricular mass in hemodialysis patients in comparison with placebo<sup>149</sup>, and improved the ejection fraction in patients on continuous ambulatory peritoneal dialysis<sup>150</sup>. Of course, the limited number of patients precludes drawing definitive conclusions and larger studies are needed to evaluate the effects of MRAs among patients with chronic heart failure in dialysis. Of note, from a clinical and methodological point of view, chronic heart failure may be difficult to define in dialysis because the ejection fraction is closely dependent on the dry weight<sup>151</sup>.



In hemodialysis, the recently published MIREnDa RCT (NCT01691053) did not reported a significant change in left ventricular mass index measured by cardiac magnetic resonance imaging with spironolactone administrated for 9 months (in press 10.1016/j.kint.2018.11.025). Moreover, the 6 months administration of spironolactone did not significantly change the diastolic function of patients in hemodialysis in the recent SPin-D RCT<sup>152</sup>.

Large RCT are needed to investigate the effect of MRAs on hard CV outcomes. The ongoing RCT ALCHEMIST (NCT01848639) will provide very informative data.

*\* Cardiovascular morbi-mortality among CKD/ESRD patients*

Regarding CV morbi-mortality, a meta-analysis of nine trials (829 patients) published between 2005 and 2015 on patients undergoing dialysis demonstrated a decreased risk of CV mortality (RR 0.34 (95% CI, 0.15-0.75)) for MRA-treated patients relative to controls.<sup>153</sup> Nevertheless, the trials included a limited number of patients and the conclusions are still uncertain. A large multicenter RCT is currently being conducted among patients on hemodialysis (ALCHEMIST: NCT01848639) to assess the efficacy of spironolactone in reducing CV-associated morbidity and mortality. In CKD, a RCT aiming to enroll a total of 6,400 patients with DN is ongoing to assess the effect of finerenone on the occurrence of CV outcomes (FIGARO NCT02545049). The primary outcome is time to the first occurrence of the composite endpoint of CV death and non-fatal CV events (myocardial infarction, stroke, or hospitalization for heart failure). The ongoing RCT BARACK D (Benefits of Aldosterone Receptor Antagonism in Chronic Kidney Disease) is an ongoing 36-month study of 2,616 patients with stage 3b CKD to compare the efficacy of the addition of spironolactone 25 mg once daily to routine care on mortality and CV

outcomes *versus* routine care alone.<sup>154</sup> CKD progression will be evaluated as a secondary outcome. Of note, another RCT (STOP-CKD<sup>155</sup>) aimed to evaluate the change in arterial stiffness (pulse wave velocity) as a surrogate marker of CV risk among stage 3 CKD patients with spironolactone in the setting of primary care, but ended prematurely due to limited recruitment.<sup>156</sup> The ongoing clinical trials evaluating cardiovascular outcomes with MRAs among CKD patients are summarized in Table 2.

***Clinical data: Focus on kidney transplantation (KT): evidence for IRI prevention and prevention of chronic graft dysfunction by MRAs***

***\* Prevention of IRI***

A pilot clinical trial on living-donor KT demonstrated a beneficial effect of spironolactone on renal oxidative stress when initiated in recipients one day prior to KT and administered for three days following KT.<sup>98</sup> Given that graft function was good in both arms (as expected in the setting of living donor donation), the investigators did not observe significantly better short-term renal function in the MRA group relative to that of the placebo group. Nevertheless, urinary levels of hydrogen peroxide (a biomarker of oxidative stress) were lower in the MRA group.

***\*Limiting IRI-induced chronic kidney dysfunction after KT***

We are currently conducting a multicenter RCT to assess the impact of short-term administration of eplerenone (immediately before and four days after KT) on three-month renal graft function in patients receiving a graft from an expanded-criteria donor (EPURE: NCT02490904)<sup>157</sup>. We plan to randomize 132 patients. The graft GFR is assessed at three months by measuring iohexol clearance. Secondary outcomes include the occurrence of

delayed graft function and immediate or slow graft recovery, as well as the occurrence of hyperkalemia.

***\*Limiting cyclosporine-induced nephropathy***

A recent RCT with eplerenone reported promising results in limiting the progression of biopsy-proven chronic graft nephropathy in children.<sup>158</sup> A RCT (SPIREN trial NCT01602861) is currently being conducted to evaluate the impact of the administration of spironolactone for three years among 170 prevalent kidney transplant patients. The primary endpoint is GFR measured by chrome-EDTA clearance. Secondary outcomes are proteinuria, the amount of interstitial fibrosis in graft biopsies, and cardiovascular events.<sup>159</sup>

***Clinical data: MRAs for IRI prevention outside the scope of kidney transplantation***

***\*Cardiac surgery***

In the setting of cardiac surgery, the administration of 100 mg spironolactone the day before the intervention and 25 mg/d for three days after failed to prevent post-operative AKI.<sup>160</sup> Indeed a trend towards risk mediated by the use of spironolactone was observed.<sup>160</sup> However, the association of the MRA with outcome in this trial may have been underestimated because of the higher proportion of diabetic patients in the MRA arm (31 vs 18%,  $p = 0.02$ ), AKI was mostly driven by AKI stage 1 and the planned sample size was not achieved, potentially underpowering the study. In a RCT aimed to test if RAAS inhibition reduced atrial fibrillation prevalence after cardiac surgery, spironolactone was protective against AKI while failing to decrease atrial fibrillation<sup>161</sup>.

***\* Critically ill patients***

The ongoing trial “Usefulness of Spironolactone for the Prevention of Acute Kidney Injury in Critically Ill Patients with Invasive Mechanical Ventilation (NCT03206658)” was designed to evaluate the effect of spironolactone administration on the incidence and severity of AKI in critically ill patients with invasive mechanical ventilation in the critical care unit. Patients receive the medication during the first five days of stay in the critical care unit. The primary outcome is the level of urinary biomarkers of AKI (concentrations of NGAL, KIM-1, oxidative stress, and Hsp72 in urine). The “Spironolactone Administration to Prevent Ischemic Kidney Injury in Critically Ill Cancer Patients (SPIROCAN) (NCT02531412)” study aims to evaluate the impact of short-term spironolactone administration on the prevention of AKI among patients admitted to the intensive care unit in the immediate postoperative period (first 24 hours) following major surgery.

***\* Coronary angiography/contrast-induced nephropathy***

In the RCT “Effect of Spironolactone on Acute Kidney Injury in Patients Undergoing Coronary Angiography (NCT03329443),<sup>162</sup> patients will receive either spironolactone 200 mg single dose or placebo in addition to their usual premedication. The primary end point will be CIN, defined as a more than 25% or 0.3mg/dl elevation in the level of serum creatinine from baseline during the first two to three days after the procedure.

***Clinical data: Benefit/risk over other therapies***

***MRAs as an adjunct therapy***

From a pathophysiological point of view, the phenomenon called “aldosterone breakthrough” favors the combinations MRA+ACEi or MRA+ARB. Indeed, RAS blockers initially decrease the plasma level of aldosterone, but this phenomenon is not sustained

after long-term administration in 10 to 53% of patients, depending on the definition.<sup>163</sup> Aldosterone levels may later reach or sometimes exceed pretreatment values. “Aldosterone breakthrough” limits the clinical efficacy of ACEis and ARBs, as it is associated with a poorer antiproteinuric response in patients with diabetic nephropathy.<sup>164</sup> It was reported that aldosterone breakthrough is associated with an enhanced decline in GFR in patients with type 1 diabetes and diabetic nephropathy on long-term ARB treatment.<sup>165</sup> This may be related to the direct deleterious effect of aldosterone. Nevertheless, in a *post-hoc* analysis of the AMADEO study,<sup>166</sup> conducted among type 2 diabetic patients, the authors did not observe an association between aldosterone breakthrough at six months and the decline of the GFR between six months and one year.<sup>167</sup> In this study, aldosterone breakthrough was defined as an increase of serum aldosterone levels > 10% over baseline values at six and 12 months of follow-up. The incidence of aldosterone breakthrough was 28% at one year. Aldosterone breakthrough at six months was not sustained at one year for 69% of cases.

In CKD, all available studies evaluated the effect of adding an MRA to an ACEi and/or ARB, RAS blockade being the gold-standard treatment of hypertension or proteinuria in these patients. There have been no placebo-controlled studies on MRAs without RAS blockers for the prevention of CKD progression. Moreover, there is no head-to-head comparison of the efficacy of ACEis or ARBs with MRAs in CKD.

A RCT compared the combination of spironolactone/ARB *vs* ACEi/ARB in patients with DN.<sup>168</sup> ACEi was discontinued in half of the patients with dual blockade (ACEi/ARB) of RAS and spironolactone was started after two weeks of washout. Patients receiving spironolactone and ARB showed a greater reduction of proteinuria after 18 months than those receiving ARB and ACEi. The eGFR declined significantly in both groups during follow-up and the decline rate did not differ significantly between the two.

Clinical data thus support the interest of MRAs as an add-on therapy among patients with CKD to reduce proteinuria and optimize blood pressure control.

***Clinical data: Risk of hyperkalemia with MRAs in CKD***

Despite international guidelines, MRAs are still under-prescribed in the field of heart failure<sup>140,169</sup> and resistant hypertension.<sup>170</sup> Physicians are still reluctant to prescribe MRAs because of the risk of hyperkalemia and worsening renal function.<sup>169</sup>

*\* Risk of hyperkalemia in CKD patients*

In the meta-analysis of Currie et al. (16 trials; n=1356) evaluating the impact of the addition of MRA to ACEi and/ARB among CKD patients, the mean increase of potassium from baseline to end of trial only was 0.19 mmol/L, 95 % CI [0.12–0.27].<sup>127</sup> Of note, the authors reported no effect of the baseline level of creatinine on the increase of potassium or the risk of hyperkalemia. Nevertheless, in these studies, the relative risk of hyperkalemia (whom threshold is varying according to the studies) was 3.02, 95 % CI [1.75-5.18], and the relative risk of being withdrawn from active treatment due to MRA induced hyperkalemia was 3.21, 95 % CI [1.19, 8.71].

In the meta-analysis from Bolignano et al., similar results were found.<sup>126</sup> Spironolactone in combination with ACEi and/or ARB increased the risk of hyperkalemia in CKD patients (RR=2.00, 95% CI [1.25-3.20]) compared to ACEi and/or ARB (11 trials, n=632). The risk of hyperkalaemia also increased when spironolactone was used in addition to either an ACEi or ARB compared to ACEi or ARB alone (9 trials, n=483): RR 1.80, 95% CI [1.09 to 2.96]. Spironolactone combined with ACEi and/or ARB increased serum potassium (mean difference 0.26 mmol/L, 95% CI [0.13-0.39]).

The results of the meta-analyses should be interpreted with caution, given the heterogeneity of the included studies concerning: 1) the threshold of baseline serum potassium levels as an exclusion criterion, 2) the definition of hyperkalemia during follow-up, 3) the predefined procedures in case of hyperkalemia, including MRA dose reduction or withdrawal from active treatment. Importantly, patients with a theoretical indication of a MRA (heart failure, CKD) generally have an increased risk of hyperkalemia independent of the prescription of the MRA and some observed hyperkalemia events may not be related to the treatment.<sup>171</sup> It is therefore necessary to interpret the frequency of hyperkalemia in RCTs relative to the placebo group.<sup>172</sup> Nevertheless, dedicated studies are needed to precisely evaluate the long-term risk/benefit balance of MRAs in the setting of CKD.

*\* Risk of hyperkalemia in patients on dialysis*

In the meta-analysis of nine trials (829 patients) published between 2005 and 2015<sup>153</sup>, the RR for hyperkalemia among patients in dialysis for MRA treatment was 3.05 (95% CI, 1.21-7.70). However, the broad range of spironolactone doses used in the included studies (from 25 mg every other day to 50 mg twice a day) and the varying threshold of baseline serum potassium levels as exclusion criteria make it difficult to draw definitive conclusions concerning the safety of MRA for patients on dialysis. The ongoing ALCHEMIST RCT (NCT01848639) will be highly informative concerning the safety profile of long-term MRA administration among patients on hemodialysis. It is planned that the patients will receive up to 25 mg/day spironolactone or placebo for two years. The primary outcome is the time to onset of the first incident, including non-fatal myocardial infarction, acute coronary syndrome, hospitalization for heart failure, nonfatal stroke, or

CV death. The investigators plan to randomize 825 participants in this large multicenter RCT.

*\* Risk of hyperkalemia among kidney transplant recipients*

*Gonzales Monte et al.* reported a good safety profile for the addition of spironolactone 25mg/day to ACEis and ARBs among 11 kidney transplant recipients with a baseline eGFR of  $52 \pm 12.7$  mL/min.<sup>173</sup> After six months under MRA therapy, they reported a slight increase in serum potassium levels ( $4.6 \pm 0.4$  vs  $5 \pm 0.62$  mmol/L), with no case of severe hyperkalemia. There was no MRA discontinuation during the study. *Bertocchio et al.* reported a good safety profile for the administration of eplerenone 25mg/day for two months among 31 kidney transplant recipients with moderate graft impairment (eGFR 30 to 50 mL/min).<sup>174</sup> Mild hyperkalemia ( $> 5$  mmol/L) was reported in less than 25% of patients and only one patient experienced moderate hyperkalemia, defined as serum potassium  $> 5.5$  mmol/L. There was no severe hyperkalemia ( $> 6$  mmol/L) during follow-up. The ongoing SPIREN RCT will be highly informative concerning the safety profile of long-term MRA administration among kidney transplant recipients, with the patients planned to receive spironolactone 25-50 mg/day or placebo for three years.<sup>159</sup>

It is also important to analyze the risk of hyperkalemia from observational retrospective data, which may reflect “real life” more accurately than RCTs. An observational study included all citizens of Stockholm initiating MRA therapy from 2007 to 2010 ( $n = 13,726$ ), with the identification of hyperkalemia events ( $> 5$  mmol/L) from all potassium measurements in healthcare.<sup>175</sup> Overall, 18.5% of patients experienced at least one episode of hyperkalemia within a year, most within the first three months of therapy. CKD, as well as other comorbidities, was associated with an increased risk of



hyperkalemia. After an hyperkalemia event, 47% discontinued MRA and 10% reduced the prescribed dose. CKD patients had the highest risk of MRA discontinuation.<sup>175</sup> Of note, there was no reintroduction of MRA during the subsequent year for 76% of the patients who discontinued MRA .<sup>175</sup>

*\* Prevention of hyperkalemia*

Close monitoring of serum potassium levels is required for patients under MRA treatment. A large retrospective cohort study conducted among Medicare patients with heart failure reported that the rate of appropriate monitoring of serum potassium levels was very low.<sup>176</sup> Appropriate monitoring was conducted more frequently among CKD patients but was still low.<sup>176</sup> Such non-appropriate serum potassium monitoring certainly contributes to adverse events because clinicians are unable to adjust their prescription according to laboratory findings. Prevention of hyperkalemia among patients under MRA treatment should include dietary counseling, the avoidance of co-administration of other medications that could potentially induce hyperkalemia, and the correction of metabolic acidosis.<sup>177</sup> Treatment of hyperkalemia may include potassium-binding resins<sup>177</sup>, including the new binders patiromer<sup>178-180</sup>, or sodium zirconium cyclosilicate (ZS-9)<sup>180-182</sup> that are now both approved in the EU and the FDA. Of note, the development of the new potassium binders offers now opportunities to conduct trials among patients with advanced CKD to test the efficacy of MRAs either on CKD progression or on CV morbi-mortality.<sup>183 178</sup> Finally, the reduction of the MRA dose may be necessary.<sup>177</sup> Nevertheless, dose reduction obviously exposes patients to reduced efficacy of the MRAs.

*\* Risk of hyperkalemia depending on the type of MRA*

The risk of hyperkalemia may decrease with the use of finerenone. This non-steroidal MRA has higher selectivity than spironolactone (first generation steroidal MRA) and stronger affinity for the MR than eplerenone (second generation steroidal MRA).<sup>184,185</sup> Potential higher selectivity for non-distal tubule cell targets may lead to lower potassium reabsorption in the distal nephron and reduced risk of hyperkalemia, with higher efficacy in other cell types. The higher potency *versus* that of spironolactone and eplerenone may also allow dose reduction. The therapeutic index may therefore be better with finerenone than spironolactone or eplerenone.<sup>186,187</sup>

### **Conclusion**

Pre-clinical evidence shows the potential of MR antagonism to treat or delay kidney disease due to various etiologies, including ischemic kidney disease, diabetic and hypertensive nephropathy, glomerulonephritis, and calcineurin inhibitor toxicity in the context of kidney transplant. Promising clinical evidence shows a benefit of MRAs in proteinuria reduction in CKD. Further and larger studies to investigate the effectiveness of steroidal and novel non-steroidal MR antagonists to maintain kidney health in several clinical settings are urgently needed.

## **Disclosure**

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JBC, SG, and FJ wrote the review.

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## Tables

	Distal nephron	Endothelial cells	Smooth muscle cells	Podocytes	Mesangial cells	Fibroblasts	Macrophages	T cells
MR protein expression	Yes <sup>1</sup>	Yes <sup>1,100</sup>	Yes <sup>1</sup>	Yes <sup>1,38</sup>	Yes, primary culture <sup>188</sup>	Yes, cell line, NRK-49F, rat <sup>113</sup>	Yes bone marrow derived <sup>23,25</sup>	Yes Isolated T cells <sup>105,189</sup>
Presence of 11 $\beta$ -hydroxysteroid dehydrogenase type 2	Yes <sup>190</sup>	Yes <sup>100,191</sup>	Controversial <sup>192</sup>	No	Yes (In vitro) <sup>193</sup>	No	No <sup>194,195</sup>	ND
Aldosterone effects in vitro	Yes <sup>1</sup>	Yes <sup>93,94,87</sup>	Yes <sup>90</sup>	Yes <sup>83</sup>	Yes <sup>95,96</sup> 112	Yes <sup>110,111</sup> 115	Yes <sup>86</sup> 104	ND
KO mice available	Yes <sup>196</sup>	Yes <sup>196</sup>	Yes <sup>196</sup>	Yes <sup>38</sup>	No	No	Yes (Myeloid) <sup>196</sup>	Yes <sup>105</sup>

**Table 1.** Summary of cell types expressing the MR, 11 $\beta$ -hydroxysteroid dehydrogenase type 2, presenting aldosterone effects and for which the KO mice is available.

Trial	Drug name and dosage	Inclusion criteria	Exclusion criteria	Patient numbers	Endpoints	Fol
BARACK D ISRCTN44522369	Spironolactone 25 mg per day	<ul style="list-style-type: none"> <li>- Aged 18 years or above</li> <li>- CKD stage 3b (eGFR 30-44 mL/min/1.73 m<sup>2</sup>)</li> </ul>	<ul style="list-style-type: none"> <li>- Type 1 diabetes mellitus</li> <li>- Clinical diagnosis of chronic heart failure or known LV systolic dysfunction with ejection fraction <math>\leq</math> 40%.</li> <li>- Recent myocardial infarction (within 6 months).</li> <li>- Documented previous hyperkalaemia not thought to be spurious, or intolerance of spironolactone.</li> <li>- Serum potassium at baseline over 5 mmol/L.</li> <li>- Documented symptomatic hypotension or baseline systolic BP under 100 mmHg.</li> <li>- Recent acute kidney injury or admission for renal failure.</li> <li>- Albumin/creatinine ratio (ACR) &gt;70 mg/mmol.</li> </ul>	2616 (1,308 participants per arm)	<p><b>PRIMARY:</b> Time from randomization until the first occurring death, first onset, or hospitalisation for heart disease (coronary heart disease, arrhythmia, new onset/first recorded atrial fibrillation, sudden death, failed sudden death), stroke, or heart failure</p> <p><b>SECONDARY:</b></p> <ul style="list-style-type: none"> <li>- Change in BP annually and at final visit</li> <li>- Rates of hypotension</li> <li>- Changes in B-type natriuretic peptide (BNP)</li> <li>- Change in urine ACR</li> <li>- Changes in eGFR</li> <li>- Change in health-related quality of life</li> <li>- Incremental cost effectiveness analysis</li> <li>- Transient ischaemic attack</li> <li>- Rates of AEs</li> <li>- Rates of hyperkalaemia</li> </ul>	36 months
ALCHEMIST NCT01848639	Spironolactone titrated over one month to a maximum single dose of 25 mg/d	<ul style="list-style-type: none"> <li>- Aged 18 years or above</li> <li>- On HD for at least 45 days for ESRD regardless of the aetiology including diabetes</li> <li>- Presenting at least one of the follow comorbidities, CV abnormalities or CV risk factors: <ul style="list-style-type: none"> <li>◆ Left ventricular hypertrophy</li> <li>◆ OR left ventricular ejection fraction &lt;40%</li> <li>◆ OR Left bundle branch block</li> <li>◆ Diabetes;</li> <li>◆ OR history of CV disease: coronary artery disease, symptomatic lower limb peripheral arterial disease, carotid or renal artery stenosis &gt; 50%, stroke, hospitalization for heart failure, permanent atrial fibrillation (AF), oral anticoagulant treatment for AF, valvular heart prosthesis,</li> <li>◆ OR CRP &gt; 5 mg/l for 3 months without infectious or neoplastic disease</li> </ul> </li> </ul>		825 participants	<p><b>PRIMARY:</b> The time to onset of the first incident: non-fatal MI or acute coronary syndrome or hospitalization for heart failure or nonfatal stroke or cardiovascular (CV) death</p> <p><b>SECONDARY:</b></p> <ul style="list-style-type: none"> <li>- The cumulate rate of non-fatal MI or acute coronary syndrome, hospitalization for heart failure, nonfatal stroke or CV death</li> <li>- The time to onset of death from i) any cause and ii) from a CV event and iii) from a non-CV cause</li> <li>- The time of survival without a major CV event (non-fatal MI, acute coronary syndrome, hospitalization for heart failure, non-fatal stroke, cardiac arrest resuscitation)</li> <li>- Incidence of procedures related to stenosis or vascular access thrombosis for hemodialysis (HD)</li> <li>- Incidence of coronary or peripheral revascularizations (including lower limb amputations)</li> <li>- BP and its inter visit variability</li> <li>- The occurrence of AF</li> <li>- Incidence of hyperkalemia &gt; 6 mmol/l</li> <li>- Estimation of the effect of treatment on quality of life.</li> </ul>	24 months

<p>FIGARO NCT02545049</p>	<p>Finerenone 10 mg or 20 mg once daily</p>	<ul style="list-style-type: none"> <li>- Aged 18 years or above</li> <li>- Type 2 Diabetes Mellitus</li> <li>- Diagnosis of Diabetic Kidney Disease with persistent high or very high albuminuria</li> <li>- Pretreated with either ACEi or ARB at maximal tolerated labelled dose</li> <li>- Serum potassium <math>\leq</math>4.8 mmol/L</li> </ul>	<ul style="list-style-type: none"> <li>- Hyperkalaemia <math>&gt;</math> 5.5mmol/l during the two weeks prior to enrolment</li> <li>- History of unscheduled haemodialysis for hyperkalaemia during the last six months</li> <li>- Hospitalization for hyperkalaemia during the last six months</li> <li>- Patients with imperative indication of a combination of ACEI and ARB or renin inhibitor (each being authorized separately)</li> </ul>	<p>6400 participants</p>	<p>PRIMARY: Time to the first occurrence of the composite endpoint of CV death and non-fatal CV events (myocardial infarction, stroke, or hospitalization for heart failure)</p> <p>SECONDARY:</p> <ul style="list-style-type: none"> <li>- Time to first occurrence of the following composite endpoints: onset of kidney failure, a sustained decrease in estimated glomerular filtration rate (eGFR) of <math>\geq</math>40% from baseline over at least 4 weeks and renal death</li> <li>- Time to all-cause mortality</li> <li>- Time to all-cause hospitalization</li> <li>- Change in urinary albumin-to-creatinine ratio (UCAR) from baseline to month 4</li> <li>- Time to first occurrence of the following composite endpoint: onset of kidney failure, a sustained decrease in estimated glomerular filtration rate (eGFR) of <math>\geq</math> 57% from baseline over at least 4 weeks or renal death.</li> </ul>	<p>53 months</p>
<p>FIDELIO NCT02540993</p>	<p>Finerenone 10 mg or 20 mg once daily.</p>	<ul style="list-style-type: none"> <li>- Aged 18 years or above</li> <li>- Type 2 diabetes mellitus</li> <li>- Diagnosis of diabetic kidney disease (DKD) with persistent high or very-high albuminuria</li> <li>- Pretreated with either ACEi or ARB at maximal tolerated labelled dose without adjustments</li> <li>- Serum potassium <math>\leq</math>4.8 mmol/L.</li> </ul>	<ul style="list-style-type: none"> <li>- Uncontrolled arterial hypertension</li> <li>- Clinical diagnosis of chronic heart failure with reduced ejection fraction (HFrEF) and persistent symptoms New York Heart Association (NYHA class II - IV) at run in visit (class 1A recommendation for mineralocorticoid receptor antagonist (MRAs)</li> <li>- Dialysis for acute renal failure within 12 weeks of run in visit</li> <li>- Renal allograft in place or scheduled kidney transplant within next 12 months</li> <li>- Glycated haemoglobin HbA1c <math>&gt;</math> 12%.</li> </ul>	<p>5734 participants</p>	<p>PRIMARY: Time to the first occurrence of the composite endpoint of onset of kidney failure, a sustained decrease of eGFR <math>\geq</math> 40% from baseline over at least 4 weeks and renal death.</p> <p>SECONDARY:</p> <ul style="list-style-type: none"> <li>- Time to first occurrence of the composite endpoint: CV death or non-fatal CV events (myocardial infarction, stroke, hospitalization for heart failure)</li> <li>- Time to all-cause mortality</li> <li>- Time to all-cause hospitalizations</li> <li>- Time to first occurrence of the following composite endpoint: onset of kidney failure, a sustained decrease in eGFR of <math>\geq</math> 57% from baseline over at least 4 weeks or renal death.</li> <li>- Change in urinary albumin-to-creatinine ratio (UACR) from baseline to month 4</li> </ul>	<p>48 months</p>

**Table 2.** Ongoing clinical trials evaluating cardiovascular outcomes with MRAs among CKD patients.