CASE HISTORY

Aliskiren: the first renin inhibitor for clinical treatment

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Abstract | The first evidence of the existence of renin was presented over 100 years ago. However, the importance of renin and the renin–angiotensin system in the pathogenesis of cardiovascular disease was only fully realized in the 1970s. It was another 20 years before the first inhibitors of renin were available for clinical research. Here, we describe the discovery and development of aliskiren, an orally active renin inhibitor, which became the first drug in its class to receive regulatory approval. In 2007, it was approved for the treatment of hypertension by the US Food and Drug Administration and the European Medicines Agency.

Hypertrophy
An increase in ventricular mass, which is typically characterized by an increased cardiac myocyte size in conjunction with cardiac fibrosis.

Fibrosis
The formation of excess connective tissue.

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Aliskiren almost never was. Following the merger of Ciba–Geigy with Sandoz to form Novartis in 1996, and the launch of the antihypertensive drug valsartan (Diovan; Novartis) in 1997, the fate of the investigational renin inhibitor aliskiren seemed to be a forgotten back shelf of a research laboratory. However, a small group of former Ciba–Geigy employees convinced the new management of Novartis to out-license aliskiren to Speedel, a start-up biopharmaceutical company established in 1999, to keep aliskiren in clinical development (FIG. 1).

The discovery of renin[1] by Tigerstedt and Bergman had itself remained unnoticed for more than 40 years, partly because the authors never published any further work in this area[2]. It was not until Goldblatt published his ground-breaking work in renal ischaemia in 1934 that the idea of an active substance secreted by the kidney came into focus again[3]. Milestones in the understanding of the kidney and the renin–angiotensin system (RAS) were sporadic until the 1950s when the molecular structure of angiotensin II was elucidated[4,5] (TIMELINE). In the past 30 years, during which drugs to block the RAS were successfully developed[6–8], the importance of the RAS in the control of fluid balance, hypertension and cardiovascular disease has become clear.

The most logical drug target in the RAS has long been considered to be renin — which is at the top of the enzymatic cascade (FIG. 2) — but renin has also proved to be a highly challenging enzyme to target. This article describes how these challenges were addressed in the discovery and development of aliskiren (Tekturna (USA)/Rasilez (EU)), which was approved for the treatment of hypertension in 2007.

The renin–angiotensin system in disease

The RAS cascade starts with angiotensinogen, a protein that is secreted by the liver (FIG. 2). From this substrate, the decapeptide angiotensin I is then cleaved by the enzyme renin, which is produced in the kidney (the activity of renin in the plasma is referred to as plasma renin activity (PRA)). Angiotensin I is then further transformed by the angiotensin-converting enzyme (ACE) to produce the octapeptide angiotensin II, which binds to angiotensin II subtype 1 receptors (AT_1 receptors). This leads to a narrowing of blood vessels and an increase in blood pressure (BP), changes in renal glomerular and tubular function, inflammation and fibrosis in the kidney, as well as hypertrophy, fibrosis and vasoconstriction in the heart.

Clinical intervention in the RAS was first achieved with the introduction of ACE inhibitors[6,9], developed in the 1970s and 1980s. These inhibit the conversion of angiotensin I into angiotensin II (FIG. 2). Then, in the 1990s, the angiotensin II receptor blockers (ARBs), which are specific for the AT_1 receptor, were introduced[10,11]. Both classes of drugs are now widely used in the treatment of hypertension, and are also used for treating heart failure and diabetic nephropathy.

Blockade of the RAS leads to a feedback increase in renin secretion and synthesis[12] (FIG. 2). In the case of the ACE inhibitors, large increases in PRA and angiotensin I can be observed[12,13]. Enzymes such as cathepsin G and elastase are thought to directly convert angiotensinogen to angiotensin II, and chymases and cathepsin G are thought to provide an alternative pathway for the conversion of angiotensin I to angiotensin II[14–16]. It has been suggested that owing to the existence of collateral or 'escape' pathways that are not mediated by ACE, angiotensin II can be

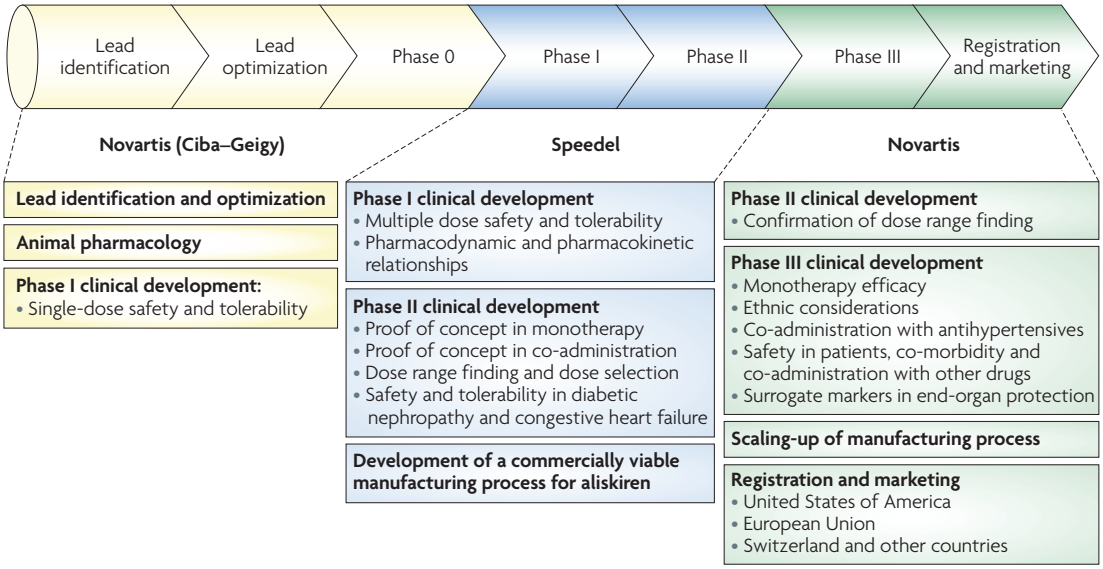


Figure 1 | **Aliskiren: from bench to marketplace.** The discovery and optimization of aliskiren using X-ray crystallography techniques, biochemical and animal pharmacological characterization was performed at Ciba–Geigy[32,33]. A single-dose safety and tolerability study was performed in healthy subjects that showed the potential of the compound. However, the synthesis of aliskiren remained a central problem — it was simply too expensive for the marketplace. Novartis out-licensed the compound to Speedel in 1999 for Phase I and Phase II development and to invent a new synthesis process. Speedel successfully overcame this major technical hurdle, which was critical for advancing the development of aliskiren. During the period 1999–2002, Speedel also established the clinical efficacy of aliskiren in over 500 subjects with mild-to-moderate hypertension in 18 Phase I and II trials and selected the doses to be used later in the Phase III studies. Novartis licensed back the compound in June 2002. The subsequent clinical programme carried out by Novartis, which included over 8,000 patients, provided the evidence for the safety and efficacy of aliskiren in treating hypertension, leading to its regulatory approval in the United States and Europe in 2007.

produced and the effect of ACE inhibitors on BP decreases over time[17,18]. In addition, through inhibition of other pathways, administration of ACE inhibitors can cause dry cough or angioedema, which can lead to a life-threatening constriction of the airways[19,20]. With the ARBs, increases in PRA and in angiotensin II are observed[21,22]. Diuretics, another effective class of antihypertensive agents, also enhance PRA[23]. Increases in PRA have been associated with four- to six-times higher mortality rates due to heart attacks and accelerated renal failure[24,25].

Renin and prorenin

Renin is an aspartic protease that consists of two homologous lobes, with a cleft in between that contains the two active-site catalytic aspartic residues[26]. Unlike other aspartic proteases, such as pepsin or cathepsin D, renin is highly specific and only cleaves angiotensinogen. Renin is often described as 'active' renin to distinguish it from its inactive precursor, prorenin[27]. Prorenin can be activated at a low temperature and pH, as well as being activated by binding to the prorenin/renin receptor[28,29]. The role of the receptor is not yet clear, although it has been suggested that the receptor helps to localize the action of renin and prorenin to the cell surface[29]. In human cultured mesangial cells, binding of renin to the receptor increases angiotensin I and induces intracellular signalling[28]. Peptidic antagonists of the receptor 'handle region' or decoy peptides prevent receptor-mediated prorenin activation[30], but in human renin animal models, the decoy peptides had no effect[31].

Discovery of aliskiren

The chemical development of renin inhibitors, leading to the discovery of aliskiren[32,33], can be divided into three generations of compounds (FIG. 3a): first, peptide analogues of angiotensinogen to block the enzymatic action of renin[34]; second, peptidomimetic compounds that were dipeptide transition-state analogue inhibitors of the active site[35–38]; and third, non-peptide-like compounds, of which aliskiren is the most successful example[39,40]. It was not until the early 1980s, when medicinal chemistry produced compounds that were more drug-like rather than substrate-like, that hopes of a breakthrough appeared. First-generation renin inhibitors were not very potent and were metabolically unstable. The second-generation compounds were potent (with activity in the nanomolar range) and, when administered by a parenteral route, lowered BP both in animals and humans[35–38]. These studies gave an insight into the endocrine and haemodynamic effects of renin inhibitors in animal models. Further development of the second-generation molecules led to longer durations of action and oral activity, although very high doses were needed in humans. Structural changes in the molecules to improve metabolic stability were successful, but oral bioavailability and the associated lowering of BP remained low[38,41]. Their clinical use was limited not only by their lack of oral activity but also by their short duration of action[41].

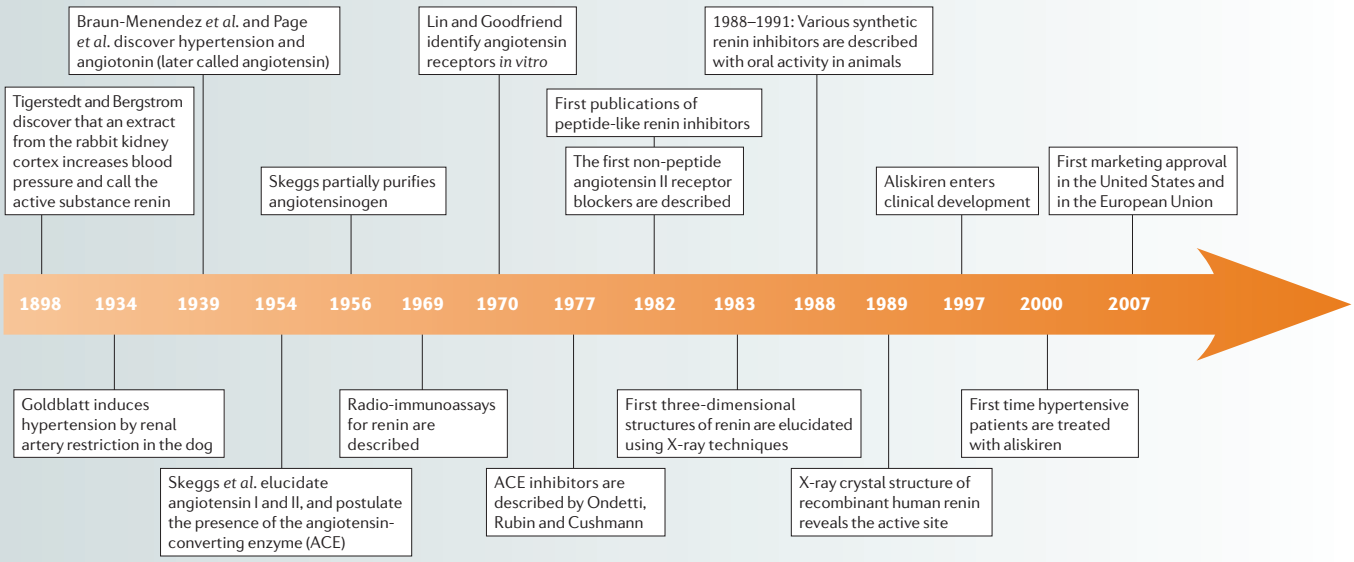
The third generation of compounds benefited from advances in crystallography and a structure-based

Angioedema
A rapid swelling of the skin, mucosa or submucosal tissues.

Diuretics
Drugs that increase the net renal excretion of solute and water.

Peptidomimetic
Small-molecule chemicals that mimic the effects of short peptides.

Timeline | **Milestones on the pathway to aliskiren**



approach to drug design, and led to the discovery of aliskiren. As shown schematically in FIG. 3b, aliskiren interacts with several binding pockets in distinct regions around the active site of renin. In particular, aliskiren was found to bind to the previously unrecognized sub-pocket $S3^{sp}$ of renin that extends from the S3-binding site. Binding to this sub-pocket is essential for strong renin inhibition by aliskiren[39,40]. The *in vitro* specificity of aliskiren for human renin over other aspartic proteinases and renin from other species is shown in FIG. 3c.

Renin inhibitors have traditionally had prohibitively high manufacturing costs at commercial volumes. Indeed, because aliskiren has four chiral centres, the initial synthesis of the compound was both difficult and expensive. This issue, which was a key factor hampering the further development of aliskiren (FIG. 1), was resolved through the development of a new and innovative synthetic route based on the synthon approach for commercial production (FIG. 4). The process developed and patented by Speedel is now used by Novartis on a multiton scale and represents a landmark in the history of renin inhibitors.

Animal pharmacology

Owing to species specificity, the standard rodent models (spontaneously hypertensive rats; SHRs) have not been helpful in establishing dose–response relationships for human renin inhibitors. Indeed, many of the early animal studies with aliskiren used primates, such as marmosets[42–45], which are much closer to man in their IC_{50} of aliskiren. Recently, double transgenic rat models (dTGRs)[46], which incorporate the human genes for angiotensinogen and renin, have been very useful in screening newer generations of renin inhibitors. Not only do these animals exhibit hypertension, but they also develop severe kidney and heart dysfunction when not treated with drugs that affect the RAS, and so they can be used to study end-organ protection[46,47].

Aliskiren was first studied in sodium-depleted marmosets and in SHRs[44]. The effects of aliskiren on the RAS could be measured at several points along the cascade. Oral administration of a renin inhibitor causes the PRA to decrease, which is reflected by a decrease in angiotensin I and angiotensin II levels in the plasma. At the same time, the total amount of renin increases in the plasma[40].

In the sodium-depleted marmoset, oral administration of aliskiren led to a sustained decrease in BP, an increase in plasma renin concentration and a decrease in renin activity[40,44]. The decrease in BP was dose-dependent over a range of 1–30 mg per kg following oral administration[44]. A single oral dose of 3 mg per kg of aliskiren was more powerful than equivalent doses of the renin inhibitors remikiren or zankiren, and 10 mg per kg doses of aliskiren were more potent than the ACE inhibitor benazepril or the ARB valsartan in lowering BP[44].

Aliskiren was also shown to effectively lower BP in SHRs, although the doses needed were up to 10-times higher than those used in marmosets[44], which reflects the differences in the *in vitro* inhibitory activity of human renin inhibitors between species (FIG. 3c). Aliskiren lowered BP in a dose-dependent, long-acting and persistent manner during 14 days of once-a-day dosing, and combination of suboptimal doses of aliskiren with valsartan or benazepril potentiated the antihypertensive effects of both agents[44].

Recent studies using dTGRs with human genes for angiotensinogen and renin have corroborated early work in the marmoset and SHRs. Subcutaneous administration of aliskiren for 14 days using an osmotic minipump decreased BP in a dose-dependent manner as well as proteinuria (a marker for kidney damage) and cardiac hypertrophy[47]. The decrease in macrophage infiltration in the heart and kidneys of the aliskiren-treated dTGRs is suggestive of a decrease in angiotensin II-mediated inflammation.

Chiral centre
Any atom in a molecule bearing at least four different substituents.

Synthon
A synthon is defined as a structural unit within a molecule that is related to a possible synthetic operation.

Proteinuria
The presence of excess serum proteins in the urine.

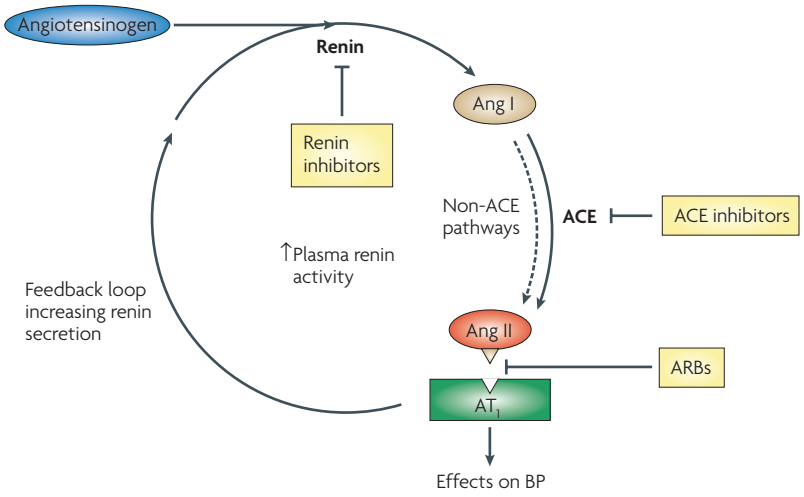


Figure 2 | **Schematic of the renin–angiotensin system (RAS).** Cleavage of angiotensinogen by the protease renin produces the decapeptide angiotensin I (Ang I). Ang I is then further transformed by the angiotensin-converting enzyme (ACE), producing the octapeptide angiotensin II (Ang II). Ang I can also be converted by other non-ACE pathways. Ang II is the end product of the cascade and binds to Ang II subtype 1 receptors (AT_1), thereby narrowing blood vessels and increasing blood pressure (BP). Direct perturbation of the RAS by ACE inhibitors, angiotensin receptor blockers (ARBs) and renin inhibitors, or indirect changes due to a fall in BP brought about by calcium-channel blockers or diuretics such as hydrochlorothiazide (not shown), lead to feedback stimulation of renin secretion and an increase in plasma renin activity.

Clinical pharmacology

The first clinical trial of aliskiren to study the effects of single and multiple doses in healthy volunteers under controlled conditions was performed in 2000 (REF. 12). In this study, 18 healthy male subjects received 40 and 80 mg or 160 and 640 mg of aliskiren (fumarate salt) once-daily for 8 days while on a constant salt intake of 100 mmol per day of sodium (a diet that is known to slightly stimulate the RAS). The ACE inhibitor enalapril (20 mg once-daily) was used as a positive control.

A dose-dependent decrease in PRA, angiotensin I and II levels was shown across all doses of aliskiren[12]. As expected, there was an increase in total amount of plasma renin after aliskiren administration. Following enalapril administration, there was a decrease in angiotensin II, an increase in angiotensin I and a marked increase in both total amount of plasma renin and in PRA. PRA inhibition by aliskiren was maintained over the 8-day dosing period. In the same time period, PRA increased threefold during treatment with enalapril. There was a decrease in urinary aldosterone excretion at doses of 80 mg (or above) of aliskiren and 20 mg of enalapril. As expected in a normotensive population, there were no changes in BP and heart rate; the drug was well tolerated by all subjects at all doses.

Plasma drug concentrations of aliskiren increased in a dose-dependent manner, with maximal plasma concentrations reached between 3 and 6 hours after administration[12]. The increase in aliskiren plasma levels following oral administration correlated well with the increase in total plasma renin and the decrease in PRA and angiotensin II (FIG. 5).

Aldosterone
A steroid hormone that acts to reabsorb sodium and increases blood pressure.

A subsequent study confirmed these results at doses of 150 and 300 mg of aliskiren[48]. In addition, the synergistic effect of aliskiren with valsartan, as measured by the reactive rise in total plasma renin, was observed. However, these effects were measured at suboptimal doses of both valsartan (80 mg) and aliskiren (150 mg)[48].

Clinical research

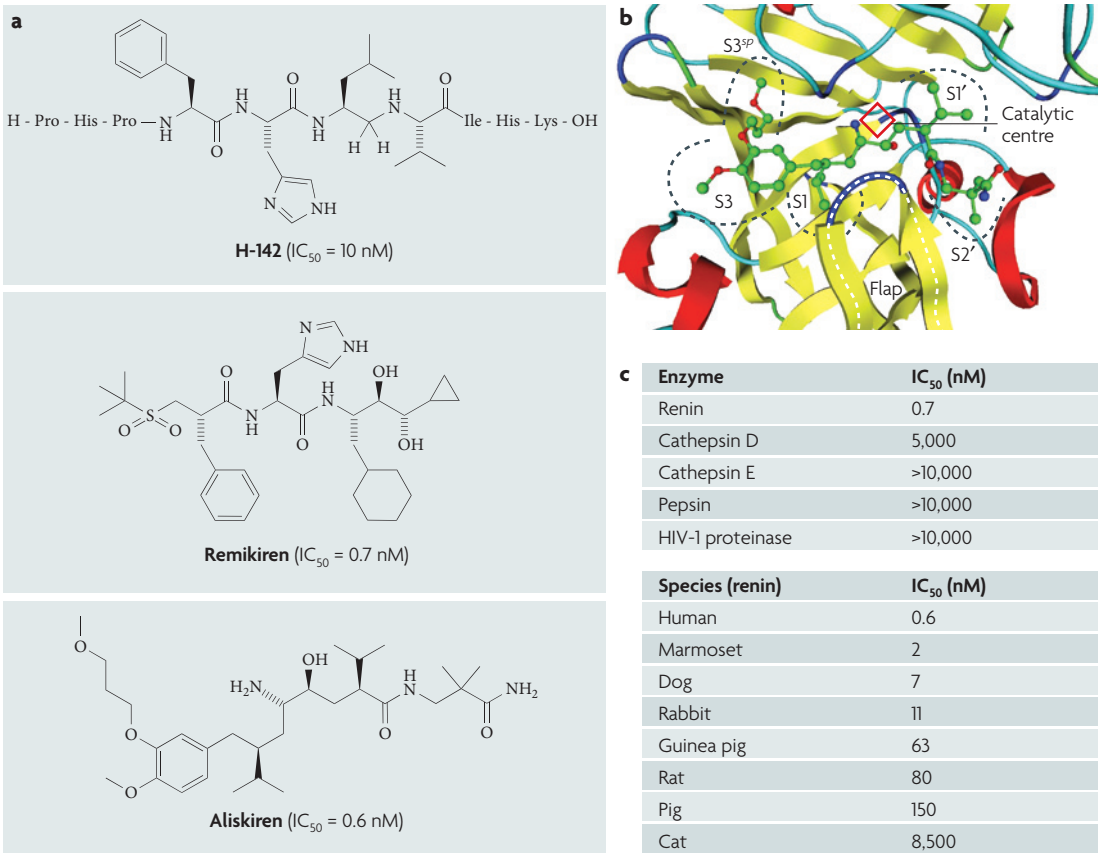
The first Phase I/II clinical trials of aliskiren in patients were performed by Speedel between 2000–2002. Since then, over 8,000 patients have been studied in a number of large, controlled, Phase II and Phase III clinical trials by Novartis. These studies have addressed:

- The dose–response relationship of aliskiren in decreasing BP.
- Its efficacy as monotherapy (Supplementary information S1 (table)).
- Its efficacy in combination with other antihypertensives.
- The safety and efficacy of aliskiren in various patient populations (the elderly, different racial groups, diabetic patients, obese patients and patients with reduced renal and hepatic function).
- The pharmacokinetics of aliskiren and the potential for drug–drug interactions.
- Its effect on surrogate markers of end-organ disease, such as diabetic kidney disease and congestive heart failure.

Dose–response and monotherapy. The first proof of concept was obtained in patients with mild-to-moderate hypertension treated with 75 and 150 mg of aliskiren each (forced titration) for 14 days[49]. Ambulatory systolic and diastolic BP (aSBP and aDBP) were measured over 24 hours and the daytime and night-time averages were calculated. This was a milestone in the development of renin inhibitors, showing a clinically significant decrease in BP at reasonable oral doses. Aliskiren was well tolerated in this trial and there were no important adverse effects.

Following this success, a second study was initiated over a range of doses that had been shown to be effective in inhibiting PRA in healthy subjects[50].The aim of this randomized, double-blind, active comparator trial was to assess the BP-lowering efficacy and safety of aliskiren. The ARB losartan was used as a positive control.

Two hundred and twenty-six patients, 21–70 years of age, with mild-to-moderate hypertension, were randomly assigned to receive 37.5 mg, 75 mg, 150 mg or 300 mg aliskiren, or 100 mg losartan once a day for 4 weeks[50]. The 100 mg dose of losartan was chosen to ensure that it had a maximal effect on BP in comparison with aliskiren. The daytime aSBP was the primary efficacy parameter. In addition, daytime aDBP, night-time aSBP and aDBP, as well as sitting systolic and diastolic BP (sSBP and sDBP), were analysed. aSBP measurements were used because of the overwhelming evidence of the importance of systolic pressure as a prognostic indicator, the enhanced reproducibility of ambulatory BP parameters over clinic BP measurements and because it provides important additional information about the extent and duration of the BP-lowering effects in real-life conditions.



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Enzyme	IC_{50} (nM)
Renin	0.7
Cathepsin D	5,000
Cathepsin E	>10,000
Pepsin	>10,000
HIV-1 proteinase	>10,000

Species (renin)	IC_{50} (nM)
Human	0.6
Marmoset	2
Dog	7
Rabbit	11
Guinea pig	63
Rat	80
Pig	150
Cat	8,500

Figure 3 | **Renin inhibitors. a** | Chemical structures of three generations of renin inhibitors: the peptide analogue H-142, the peptidomimetic remikiren and the non-peptidic aliskiren. **b** | Graphic depiction of the binding of aliskiren to the active site of renin. S1, S1′, S2′, S3 and $S3^{sp}$ all represent binding pockets in the active site of renin. In this model of renin, the flap is in the closed position. **c** | Tables showing the potency, enzyme specificity and species specificity of aliskiren[40].

Dose-dependent reductions in daytime aSBP were observed with 37.5, 75, 150 and 300 mg aliskiren, and the best 24-hour effect of aliskiren on ambulatory BP was shown for the 300 mg dose[50]. The clinic sitting BP decreased with increasing doses of aliskiren (FIG. 6a) and trough plasma levels of aliskiren also increased with dose. Inversely, PRA decreased with increasing doses of aliskiren from –55% to –83% of baseline activity. As expected for ARBs, treatment with losartan increased PRA by 110%.

A second dose-finding study extended the dose range up to 600 mg of aliskiren; 652 mild-to-moderate hypertensive patients were treated for 8 weeks with once-daily doses of 150, 300 or 600 mg of aliskiren, 150 mg of the ARB irbesartan or placebo[51]. Once-daily treatment with all three doses of aliskiren significantly reduced sDBP compared with placebo. The two upper doses, 300 mg and 600 mg, were significantly better than 150 mg of irbesartan (FIG. 6b). Maximal decreases in BP, measured at 2-week intervals throughout, were reached by the second week of treatment with aliskiren and irbesartan. Peak-to-trough ratios, used as an index of duration of action, ranged from 0.62 to 0.92 for aliskiren and 0.63 for irbesartan, indicating the long duration of action of aliskiren. Across all observations, the effects of aliskiren at 300 and 600 mg were similar. Aliskiren treatment was well tolerated across all doses, and the incidence of adverse events and the number of discontinuations in the aliskiren treatment groups were similar to those of irbesartan and placebo. There was a higher incidence of diarrhoea at the 600 mg dose of aliskiren compared with placebo (6.9% versus 1.5%).

Combination with other antihypertensives. The treatment of hypertensive patients currently involves the use of several therapeutic approaches[52–54]. Despite current treatments, BP is not adequately controlled in the majority of patients that are diagnosed and treated for hypertension, and so it is expected that patients need to be treated with three to five medications simultaneously. Phase II and III studies of aliskiren were performed to test not only the additive effects in decreasing BP by adding aliskiren to established therapies, such as ACE inhibitors (ramipril), ARBs (irbesartan and valsartan), calcium-channel blockers (amlodipine) and diuretics (hydrochlorothiazide; HCTZ), but also to provide assurance that these combinations would be safe.

Proof-of-concept combination studies, in which open-label aliskiren was combined with HCTZ, ramipril or irbesartan in small cohorts of patients, were designed to test whether aliskiren could prevent the reactive rise in PRA that is caused by these drugs and, at the same

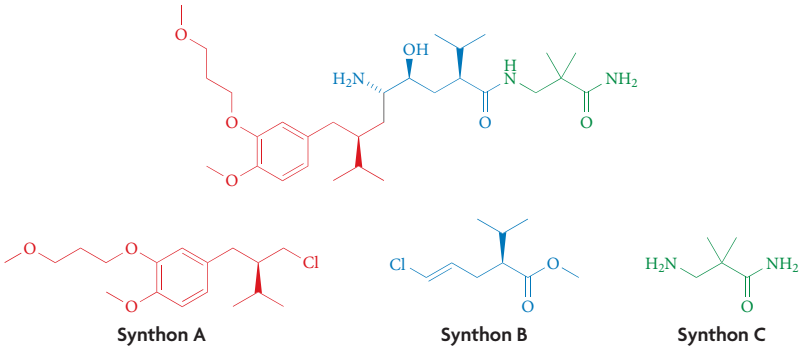

Figure 4 | **The synthon approach to produce aliskiren at an acceptable cost-of-goods.** Retrosynthetic analysis resulted in a novel convergent synthesis concept for aliskiren based on the synthon approach. The key elements of this approach are the three different building blocks — synthons A, B and C — which can be synthesized independently. Reactions involved include a rhodium-catalysed enantioselective hydrogenation (synthon A), a pig liver esterase-catalysed enzymatic resolution of a racemic ester (synthon B) and a one-pot nitrile reduction/ester aminolysis (synthon C) as the key steps. Further steps in the final linear and straightforward synthetic sequence for aliskiren involve three parts: first, a nickel-catalysed cross-coupling reaction of synthon A with synthon B. Second, build-up of the remaining two chiral centres with the help of the two already existing chiral centres (synthon A and B) by a highly diastereoselective halolactonisation. And third, integration of synthon C by lactone aminolysis. The synthon approach is convergent, flexible, more elegant (from a synthetic point of view) and most importantly, in the case of aliskiren, it turned out to be very efficient, resolving the long-standing problem of over-expensive manufacturing costs. Each chemical step and each of the different chemical reactions underwent feasibility studies, optimization steps and stepwise scale-up experiments from milligram to multigram to kilogram scale[95–100].

time, further enhance their effects on BP lowering[55]. The studies were performed with doses of 75 and 150 mg aliskiren, and ambulatory BP measurement was used to study the extent and duration of the BP-lowering effect.

The usefulness of combining aliskiren with ACE inhibitors, ARBs and diuretics was confirmed in these studies[55]. The addition of 25 mg of HCTZ to 150 mg of aliskiren led to further significant reductions in daytime and night-time ambulatory BP. Aliskiren sustained BP lowering alone and in combination over the 24-hour period studied. PRA was inhibited by aliskiren alone and in the presence of the diuretic. The addition of aliskiren to ramipril resulted in significant reductions of both daytime and night-time aSBP and aDBP, again reflecting the long half-life of aliskiren (FIG. 7a). An adequate control of night-time BP by aliskiren, even at the lower 75 mg dose, was also shown. Similar results were reported for the combination of aliskiren with irbesartan. PRA was increased by 90% and 175% by ramipril and irbesartan, respectively. The addition of aliskiren resulted in PRA levels equal to or lower than baseline levels. The authors concluded that the reduction in BP tended to correlate with the extent of PRA inhibition upon commencement of aliskiren treatment and this effect was dose dependent[55]. Overall, no significant changes in laboratory parameters were noted. Individual serum potassium levels did not exceed 5.3 and 5.4 mmol per L with co-administration of ramipril or irbesartan, respectively. Adverse events were distributed evenly across treatment groups and all treatments were well tolerated.

Retrosynthetic analysis
A synthesis that is planned in reverse, beginning with the final product.

A Phase III trial using ramipril confirmed these results and added some important observations on efficacy and adverse events in diabetic patients with mild-to-moderate hypertension[56]. The study included 837 patients treated either with 150 mg of aliskiren, 5 mg ramipril or 150 mg aliskiren and 5 mg ramipril. After 4 weeks, dosages were force-titrated to 300 mg aliskiren, 10 mg ramipril or 300 aliskiren and 10 mg ramipril for an additional 4 weeks. After 8 weeks of treatment, the reduction in clinic mean sSBP was significantly greater with aliskiren monotherapy compared with ramipril alone ($p < 0.05$). Significantly more patients responded to aliskiren or the combination of aliskiren and ramipril than to ramipril alone for the reduction of clinic sSBP and sDBP. Ambulatory BP measurements in a subset of 173 patients showed that the addition of aliskiren significantly improved BP control over 24 hours.

Of particular interest was the incidence of ACE inhibitor-induced cough in all treatment groups. It was expected that, owing to the specificity of aliskiren for human renin, the incidence of cough would be lower with aliskiren monotherapy than with ramipril, which was confirmed. However, with combination therapy, the incidence of cough was also lower than with ramipril alone, suggesting an attenuation of ACE inhibitor-induced cough[56].

A large Phase III trial tested all combinations of 6.25, 12.5 or 25 mg of HCTZ with 75, 150 and 300 mg of aliskiren (with the exception of 300 mg of aliskiren with 6.25 mg of HCTZ) in 2,776 patients with mild-to-moderate hypertension[23]. All groups with active treatment (receiving monotherapy or combination therapy) were compared with placebo, and the primary efficacy parameter was clinic mean sDBP. Both aliskiren and HCTZ contributed to lowering BP, and all combinations were better than placebo. The greatest reductions in BP were observed with the combination of 300 mg aliskiren and 25 mg HCTZ. In addition, the reactive rise in PRA induced by HCTZ was neutralized by aliskiren. All active treatments were well tolerated and the incidence of adverse events was not related to dose.

A landmark study of aliskiren in combination with valsartan used high doses of each compound (most prior trials had used submaximal doses of aliskiren and the combined drug). In this double-blind Phase III study, 1,797 patients with hypertension (mean sDBP 95–109 mm Hg and 8-hour daytime aDBP ≥90 mm Hg) were randomly assigned to receive once-daily 150 mg doses of aliskiren ($n = 437$), 160 mg valsartan ($n = 455$), a combination of 150 mg aliskiren and 160 mg valsartan ($n = 446$) or placebo ($n = 459$) for 4 weeks[21]. This was followed by forced titration to double the dose to the maximum recommended dose for another 4 weeks. The primary end point was change in mean sDBP. The combination of 300 mg aliskiren and 320 mg valsartan lowered mean sDBP from baseline by –12.2 mm Hg; significantly more than either monotherapy (300 mg aliskiren, –9.0 mm Hg; 320 mg valsartan, –9.7 mm Hg) or placebo (4.1 mm Hg) after 8 weeks of treatment (FIG. 7b). The incidence of adverse events and laboratory abnormalities were similar in all treatment groups.

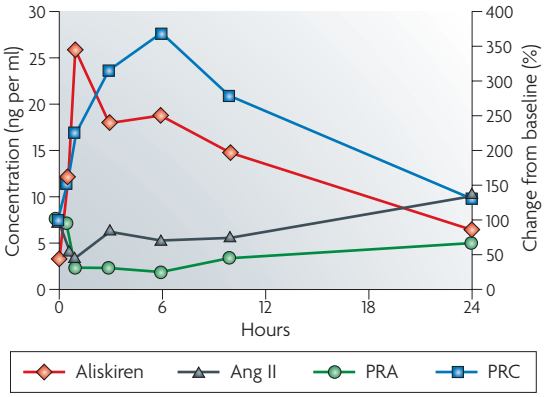

Figure 5 | **Clinical pharmacology of aliskiren.** Relationship between aliskiren plasma concentrations and plasma renin concentration (PRC), plasma renin activity (PRA) and angiotensin II plasma concentrations (Ang II). Healthy subjects were given 160 mg of aliskiren once-daily for 8 days. Ingestion of aliskiren at time 0 h leads to a reactive rise in PRC. However, owing to the potent inhibition of renin by aliskiren, the PRA and Ang II levels in plasma decrease. Samples were taken over 24 hours on day 8 ($n = 9$). Modified, with permission, from REF. 12 © (2002) American Heart Association.

In addition, aliskiren in combination with valsartan inhibited PRA by 48% despite the high circulating levels of renin.

These studies not only provided the first results on high-dose combinations of aliskiren with ARBs, but also provided a clear rationale for combining aliskiren with other drugs for more effective control of BP. In a 6-week randomized, parallel group study, patients with mild-to-moderate hypertension treated with 5 mg of amlodipine for 4 weeks who still had 90 mm Hg or higher diastolic BP were randomized to either continue on 5 mg amlodipine, to receive double the dose of amlodipine (10 mg) or receive 150 mg of aliskiren together with 5 mg of amlodipine[57]. Aliskiren significantly increased the BP-lowering effect of 5 mg amlodipine, and the combination was numerically, but not significantly, better than 10 mg amlodipine. Not surprisingly, the combination of 5 mg amlodipine with 150 mg aliskiren was associated with lower rates of oedema than seen with 10 mg amlodipine.

Efficacy and safety in special populations. Of particular importance for any new therapeutic approach is performance in special populations of patients. These special populations can include those distinguished by ethnicity, gender, age and comorbidities, such as diabetes, renal and hepatic disease.

Obesity is a common comorbidity in hypertensive patients. It has been estimated that nearly 75% of obese patients are hypertensive but that less than 20% of those are well controlled[58]. In a large multi-centred trial, 489 patients who were non-responders to a 4-week treatment with HCTZ were randomly assigned to double-blind, once-daily aliskiren (150 mg), irbesartan (150 mg), amlodipine (5 mg) or placebo in addition to continuing HCTZ (25 mg) once daily[59]. After 4 weeks, the doses of aliskiren, irbesartan and amlodipine were doubled, and treatment continued for a further 8 weeks. Significantly more patients were found to respond to the co-administration of aliskiren and HCTZ than to HCTZ alone. The response to aliskiren in combination with HCTZ was similar to the combinations with irbesartan or amlodipine. All combinations were considered to be safe and well tolerated, although there was a higher incidence of oedema in the amlodipine-treated patients. The authors concluded that aliskiren was an effective and well-tolerated treatment for obese patients that do not respond to HCTZ.

Diabetes is also a common comorbidity in hypertensive patients. Aliskiren monotherapy (300 mg) or aliskiren plus ramipril (300 mg and 10 mg, respectively) has been shown to be more effective than ramipril alone in controlling BP in hypertensive diabetic patients, particularly during the early morning BP surge 21 to 24 hours after the last dose[56].

Hypertension affects almost 50% of the Japanese population[60,61], and in patients over 40 years old, less than half reach BP treatment targets[62]. Aliskiren was shown to be as effective in the Japanese population as in the Caucasian population at doses of 75, 150 and 300 mg once-daily in 455 patients with mild-to-moderate hypertension[63]. The incidence of adverse events in the aliskiren-treated groups was similar to that of placebo. The pharmacokinetic profile of aliskiren in Japanese subjects has also been reported to be similar to that of Caucasians when single oral doses of 300 mg were compared[64].

Differences in BP control with gender or age have not been found in aliskiren-treated patients. It has, however, been reported that plasma levels of aliskiren could be up to 30% higher in elderly patients following a single oral dose of 300 mg of aliskiren[65]. Pooled data from seven clinical trials did not show a difference in adverse event profiles for elderly patients compared with younger patients[66].

Pharmacokinetics of aliskiren. The pharmacokinetic profile of aliskiren has been examined in healthy subjects and in patients with hepatic and renal disease following single oral and intravenous doses[67–69]. Aliskiren is absorbed rapidly following oral doses, with maximal plasma concentrations reached between 1 and 3 hours after dosing[67]. The absolute bioavailability is low, with less than 3% of the dose being absorbed, but it is predictable and constant. Aliskiren displays linear pharmacokinetics across a dose range of 75 to 600 mg. Following the administration of [^{14}C]-aliskiren, 90% of the absorbed dose was eliminated by the faecal route and less than 0.6% was recovered in the urine, and the estimated elimination half-life was 44 hours. Very little metabolism of aliskiren occurs; the parent compound represents more than 80% of the radioactivity in plasma[67].

Following a single oral 300 mg dose of aliskiren, the elimination pharmacokinetics were similar in patients with hepatic disease compared with matched healthy subjects, as measured by the area under the measured concentration curve (AUC)[69]. The half-life tended to be slightly longer in hepatic patients (70.1 hours) versus healthy subjects (50.1 hours).

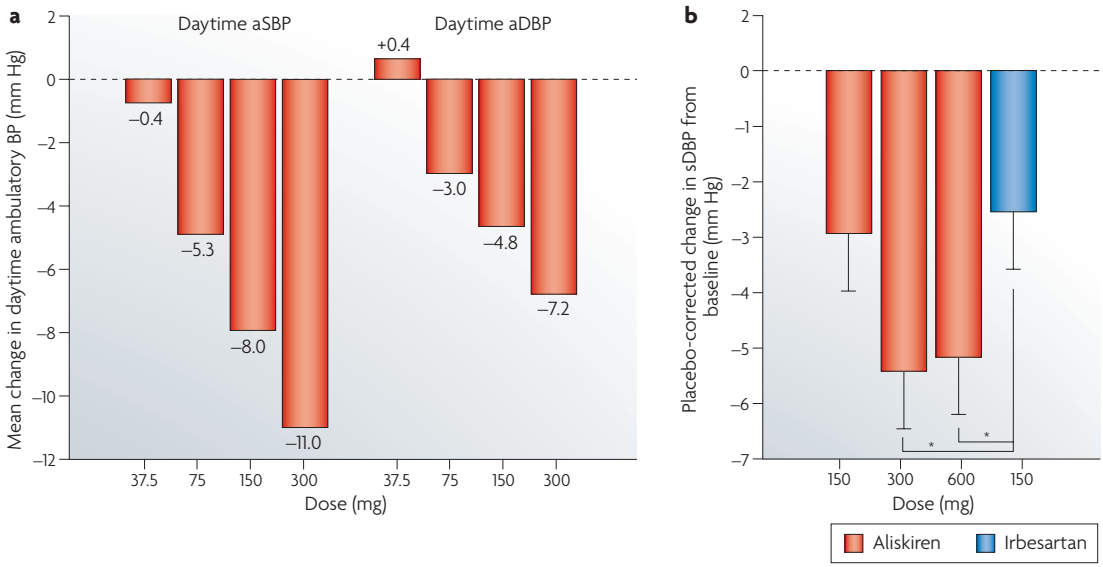

Figure 6 | **Aliskiren as a monotherapy. a** | Mean change in daytime ambulatory systolic and diastolic blood pressure (BP) (aSBP and aDBP, respectively) after 4 weeks of treatment with 37.5, 75, 150 and 300 mg of aliskiren. **b** | Mean (and standard error) of placebo-corrected changes in sitting diastolic blood pressure (sSBP) from baseline. *$p < 0.05$ compared with irbesartan. Panel **a** was adapted from REF. 50. Panel **b** was adapted from REF. 51.

The steady-state pharmacokinetics of aliskiren have also been studied in patients with various degrees of renal insufficiency[68]. Renal impairment was associated with a modest increase in exposure (a 65% increase in AUC at steady-state). However, the increase in exposure did not correlate with the severity of renal disease. The authors concluded that the effects observed might be due to differences in non-renal drug distribution.

Aliskiren exhibited similar pharmacokinetics in patients with type 2 diabetes and in healthy volunteers[70]. Exposure to aliskiren was slightly higher in patients with type 2 diabetes compared with healthy volunteers, but there were no statistically significant differences for any pharmacokinetic parameters.

Overall, the results of these trials indicate that there is no need for dose adjustments of aliskiren in patients with hepatic and renal disease, or for elderly patients or patients with diabetes.

Safety and tolerability. Safety considerations in the development of aliskiren can be divided into two categories: those due to structure-related toxicity and those that are pharmacologically predictable. Preclinical safety pharmacology studies with aliskiren revealed no significant effects on the central nervous system, cardiovascular, respiratory or renal systems in animal models[71]. Local irritation at the site of administration was a key feature of the toxicity studies and was thought to be related to the increased incidence of inflammatory and proliferative changes in the gastrointestinal tract during the carcinogenicity studies, which use doses that are well in excess of the maximum tolerated dose in humans.

For the European Medicines Agency (EMEA) submission[71], clinical safety assessment was based on observations obtained in a total of 11,566 treated patients. These included two dose-selection studies, nine efficacy and safety studies (four placebo-controlled and five active-controlled) and four long-term studies, including an open label 12-month study with a placebo-controlled withdrawal period and a 4-month extension and two double-blind, active-controlled 6-month studies. Additional safety data were obtained from pilot efficacy studies, clinical pharmacology and biopharmaceutical studies, as well as ongoing studies from 13 clinical trials and six clinical pharmacology studies.

The overall number of adverse events was lower in patients treated with aliskiren than in patients treated with placebo (37.7% versus 40.2%). The most common adverse events in patients treated with aliskiren compared with those treated with placebo include diarrhoea, cough, peripheral oedema, fatigue, rash and influenza. Among these adverse events, diarrhoea was the most common and was two-times more frequent in patients treated with aliskiren than patients treated with placebo (2.4% versus 1.2%). Cough, the second most common adverse event, was substantially less frequent in patients treated with aliskiren compared with patients treated with ACE inhibitors (1.0% versus 3.8%), and peripheral oedema was substantially less frequent in patients treated with aliskiren compared with those treated with amlodipine (0.9% versus 7.3%).

As far as laboratory-test data are concerned, the effects of an 8-week treatment with aliskiren were of minor statistical or medical significance. Transient elevations in serum potassium, above 5.5 mmol per L, have been observed with aliskiren. Mild increases in potassium are expected with agents that block the RAS (including ACE inhibitors and ARBs) by multiple mechanisms, including sudden changes in both glomerular filtration rate and aldosterone secretion[72,73].

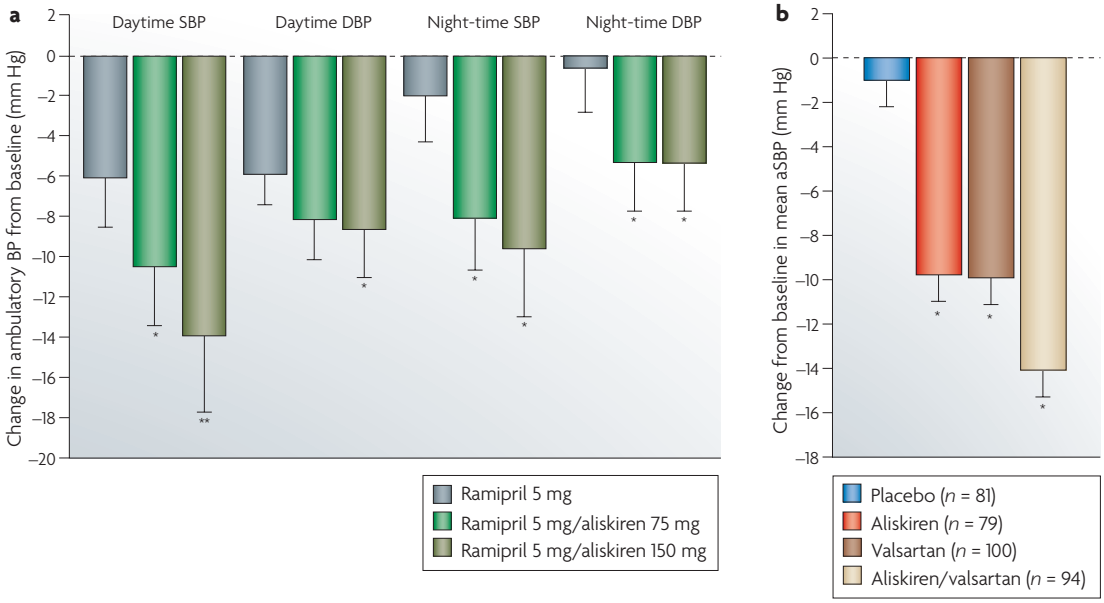

Figure 7 | **Combination of aliskiren with other antihypertensives. a** | Effects of ramipril alone and in combination with aliskiren on daytime and night-time ambulatory systolic and diastolic blood pressure (SBP and DBP). *$p < 0.05$ **$p < 0.001$. **b** | Change from baseline in mean 24-hour ambulatory systolic blood pressure (aSBP) after 8 weeks of treatment alone and in combination with valsartan. Panel **a** was adapted from REF. 55. Panel **b** was adapted from REF. 21.

It has been speculated that increases in renin could result in hypertensive episodes or limit the efficacy of aliskiren[74]. However, long-term studies have shown that the BP-lowering effects are constant over a 12-month period[75]. In addition, the initial increase in plasma renin concentration due to RAS inhibition was also steady over the same period of treatment[76]. Indeed, upon cessation of treatment with aliskiren, the BP-lowering effect persists for at least 2 weeks and there is no rebound effect due to increased plasma renin concentrations[77].

Drug–drug interactions. A number of pharmacokinetic studies have been performed to assess the potential of aliskiren to interact with other drugs. As aliskiren does not interact with the cytochrome P450 system[71,78], it is unlikely that aliskiren would have significant interactions with agents that are metabolized by this system.

Aliskiren has no known clinically relevant interactions with commonly used medicinal products for the treatment of hypertension or diabetes[71]. Co-administration with furosemide led to a 30% decrease in furosemide AUC and a 50% decrease in C_{max} in healthy subjects following single doses[79]; however, in clinical trials there was no adjustment of furosemide dose when the two drugs were administered together. Co-administration of aliskiren with either valsartan, metformin, amlodipine or cimetidine resulted in a 20–30% change in C_{max} or AUC of aliskiren[80]. When administered with atorvastatin, steady-state aliskiren AUC and C_{max} increased by 50%. Co-administration of aliskiren had no significant effect on atorvastatin, valsartan, metformin or amlodipine pharmacokinetics[80]. The EMEA has concluded that no dose adjustments for aliskiren or these co-administered medicinal products are necessary[71].

Cytochrome P450 system
A family of haem proteins responsible for oxidative drug metabolism.

End-organ protection. Although the rate of hypertension-related morbidity has slowed in the past decade, the incidence of chronic heart failure and end-stage renal disease (ESRD) is now increasing[52]. The RAS is strongly implicated in the development of hypertension-related end-organ damage. Despite the success of drugs such as the ACE inhibitors and ARBs in controlling hypertension, compensatory increases in plasma renin levels can lead to adjustments in angiotensin I and II production and might limit the effects of these drugs. The possibility of more complete control of the RAS by renin inhibitors has been suggested for many decades. The blockade of the RAS by ACE inhibitors or ARBs is often incomplete, partly owing to low doses or the short duration of drug action. Although some improvements in clinical outcome have been reported with dual blockade, there is still room for further improvement[81,82].

Experiments in dTGRs, which develop kidney and heart damage due to an overactivated and non-responsive human renin system, have demonstrated that treatment with ACE inhibitors or ARBs can provide considerable organ protection[83]. This protective effect is independent of BP changes[84]. Treatment of these animals with aliskiren (3 and 0.3 mg per kg) lowered BP to a similar extent as valsartan (10 and 1 mg per kg). However, whereas 100% of animals in the placebo-treated group died by the ninth week of the experiment and 26% survived in the valsartan-treated group, 100% of the rats survived in the aliskiren-treated group[47]. Another experiment showed that aliskiren ameliorates complement activation of C3c and C5b–9, with improvements in target-organ damage[85].

In healthy human subjects, dose-dependent increases in renal plasma flow following single-dose treatment with 75, 150 and 300 mg of aliskiren have been

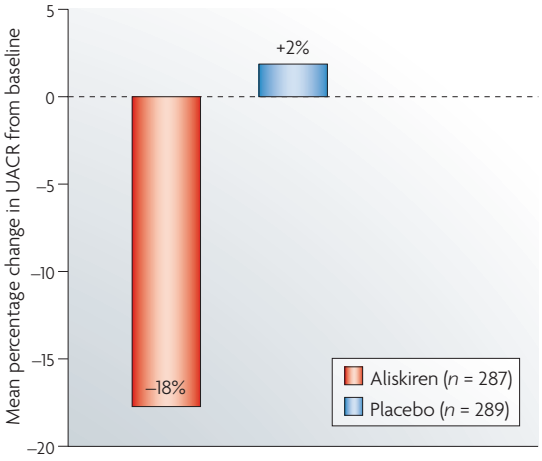

Figure 8 | **Aliskiren and end-organ protection.** Percentage change in urinary albumin creatinine ratio (UACR) in diabetic patients treated with aliskiren or placebo on top of optimized blood-pressure control. Adapted from REF. 91.

reported[86]. The authors concluded that aliskiren induced a renal vasodilatation that was substantially larger than what has been previously seen with other agents, such as ACE inhibitors or ARBs. There is considerable potential for renin inhibitors to improve organ damage beyond BP control, and owing to the inhibition of the RAS at the top of the cascade, this potential for improvement might even be greater than that of the ACE inhibitors and ARBs. Clinical trials with aliskiren have been initiated to study primary and secondary prevention in more than 20,000 subjects with both cardiovascular and renal risk[87]. Within this programme, several clinical trials are being carried out to explore the effects of aliskiren on surrogate markers and outcomes involving patients with left-ventricular hypertrophy, diabetes and various cardiovascular and renal disorders.

So far, results from trials known as ALOFT (aliskiren observation of heart failure treatment) and AVOID (aliskiren in the evaluation of proteinuria in diabetes) are available. ALOFT was designed to assess the safety of adding 150 mg aliskiren to standard therapy in hypertensive patients with stable heart failure[88–90]. Efficacy evaluations were also included, such as changes in B-type natriuretic peptide (BNP) and N-terminal (NT)-pro BNP levels, in PRA and in echocardiographic measurements. A total of 302 hypertensive patients with a mean age 68 years who had had stable New York Heart Association (NYHA) heart failure class II–IV for 1 month or longer were recruited. Patients were randomized to 12 weeks of treatment with 150 mg aliskiren or placebo, in addition to the stable doses of ACE inhibitor, ARB, aldosterone antagonist or β-blocker that they were already taking. The mean plasma BNP level in these patients was above 100 pg per ml (28.9 pmol per L) at baseline. Compared with placebo, aliskiren reduced plasma NT-pro BNP by 25% ($p = 0.0106$); plasma BNP by 25% ($p = 0.016$) and urinary aldosterone by 21% ($p = 0.015$). There was also a favourable change in a Doppler-echocardiographic measurement of left-ventricular filling pressure. Aliskiren was well tolerated and there was no significant excess of hypotension or renal dysfunction. These improvements in key indicators of heart-failure severity, namely BNP, NT-pro BNP and urinary aldosterone, warrant further investigation in larger clinical trials.

The AVOID trial assessed whether dual renin system intervention with aliskiren added to current optimal treatment with losartan could provide additional renal protection compared with the addition of placebo in hypertensive patients with type 2 diabetes and macro-albuminuria[91]. Male and female patients aged 18–85 years with hypertension (mean sSBP >140 mm Hg and/or mean sDBP > 90 mm Hg), proteinuria (urinary albumin creatinine ratio (UACR) ≥ 200 mg per g to ≤ 3,500 mg per g) and type 2 diabetes mellitus were included in the trial. All patients were started on 100 mg of losartan for 12 to 14 weeks, in addition to optimal hypertension therapy, to reach a target BP of 130/80 mm Hg. Following the run-in period, eligible patients with BPs below 150/95 mm Hg and UACRs ≥ 100 mg per g were randomized to double-blind, once-daily doses of 150 mg aliskiren or placebo in addition to losartan and optimal anti-hypertensive therapy. After 12 weeks, patients receiving aliskiren were force titrated to 300 mg aliskiren once-daily and treatment continued for all patients for a further 12 weeks. The primary efficacy criterion was the change in UACR with the secondary criterion of BP control. Six hundred patients were evaluated at the end of the treatment period. Aliskiren provided an 18% reduction in UACR compared with placebo at week 24 ($p = 0.0009$; FIG. 8). By week 12, 150 mg aliskiren had reduced UACR by 11% ($p < 0.02$) compared with placebo. BP control was similar between the two treatment groups and remained controlled throughout the study. Treatment with aliskiren was well tolerated; the incidence of adverse events and serious adverse events was similar between the aliskiren and placebo groups — the most commonly reported adverse events were headache, nasopharyngitis and dizziness. The effect of aliskiren on decreasing albuminuria was observed within 3 days and reached a maximal effect within 28 days of treatment with doses of 300 mg once-daily[92].

These two trials are notable because aliskiren treatment was initiated on top of standardized BP control. For example, in the RENAAL (reduction of endpoints in NIDDM with the angiotensin II antagonist losartan) and IDNT (irbesartan in diabetic nephropathy) trials, treatment with losartan or irbesartan decreased proteinuria by 35% and 33%, respectively[93,94]. However, in both of these studies, drug treatment was compared with placebo, whereas in AVOID and ALOFT, treatment was on top of optimal BP control.

Next-generation renin inhibitors

Two companies have publicly announced that they have renin inhibitors in clinical research: Actelion (partnered with Merck) and Speedel. Speedel has presented data

BNP
Brain natiuretic protein is secreted by the heart in response to stretching of the heart muscle cells. It is used as a biochemical measure of heart failure.

Macro-albuminuria
The urinary excretion of >300 mg per day of serum albumin.

Urinary albumin creatinine ratio
A biochemical measure of chronic kidney disease.

on three compounds from two series of compounds, SPP600 and SPP1100. Two compounds are in Phase I, SPP676 and SPP1148, with one compound in Phase II, SPP635. SPP635 has been shown to lower BP in mild-to-moderate hypertensive patients, but the dosage has not been disclosed. The compound is now being studied in diabetic patients with albuminuria. At the end of 2007, Actelion/Merck announced that a lead compound was entering Phase II trials.

Conclusions

Aliskiren, the first renin inhibitor available for clinical use, has been shown in experimental and clinical studies to be effective in lowering BP and to hold considerable potential for organ protection beyond BP reduction. These important cardiovascular effects occur without any limiting adverse events, and the long half-life of the compound justifies once-daily administration.

At first inspection, the absolute BP changes with aliskiren monotherapy do not appear to be different to those with the ACE inhibitors or ARBs. However, combining aliskiren with various other antihypertensive drugs does result in a greater therapeutic efficacy, without any adverse drug interactions being observed. Blockade of the RAS obtained with ACE inhibitors or ARBs can be further enhanced by adding aliskiren to these agents. This combination improves BP control, and surrogate end points indicate that it might provide greater organ protection.

The role of prorenin and renin receptors in hypertension and organ damage[29] remains speculative, and we anticipate that it will be one of the most active areas in RAS research in years to come. Overall, renin inhibition opens up new therapeutic potential for more complete blockade of the RAS and further decreases in cardiovascular morbidity and mortality.

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Competing interests statement
The authors declare competing financial interests: see web version for details.

DATABASES
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Angiotensinogen | angiotensin-converting enzyme | angiotensin II subtype 1 receptor | B-type natriuretic peptide | cathepsin D | cathepsin G | elastase | pepsin | renin

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