# Neprilysin Inhibition in the Time of Precision Medicine*

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The recent results of the PARADIGM-HF trial (Prospective Comparison of ARNI with ACEI to Determine Impact on Global Mortality and Morbidity in Heart Failure) showing that the combination of the neprilysin (NEP) inhibitor sacubitril (sac) and the angiotensin receptor antagonist (ARB) valsartan (sacubitril/valsartan [sac/val]; Entresto, Novartis) decreased the risk of death from cardiovascular cause or first hospitalization for heart failure (HF) while modestly reducing the risk of death from 19.8% to 17.0% (hazard ratio: 0.84) when compared with the angiotensin-converting enzyme inhibitor (ACEi) enalapril engendered considerable interest among cardiologist and HF specialists (1). In this issue of *JACC: Heart Failure*, Dr. Milton Packer posits that since "the PARADIGM-HF trial has demonstrated the need to inhibit NEP, we should do so as early as possible and not delay until we have achieved target doses of a conventional inhibitor of the renin-angiotensin system" (2). He uses the allegory of the novel *Love in the Time of Cholera* to make the point that physicians are having difficulty breaking from their long-standing comfort in using an ACEi as a pivotal therapy for patients with HF and reduced ejection fraction (HFrEF). Written by the Nobel laureate Gabriel García Márquez, *Love in the Time of Cholera* describes how Florentino Ariza meets and falls in love with Fermina Daza, only to have his advances spurned until fate brings them together over 50 years later, albeit with a less than happy ending. This is an interesting allegory to use because Márquez is universally recognized as one of the most preeminent members of a literary movement known as "magic realism" ("marvelous realism") and *Love in the*

*Time of Cholera* is a quintessential example of that genre (3). Magic realism mixes elements of fantasy into otherwise realistic or common settings. As physicians, we must however look at the results of PARADIGM-HF from a realistic and scientific perspective based on the elements that we have used to judge all HF therapies: 1) the pre-clinical data; 2) the design and results of all relevant clinical trials; and 3) associated risks - both observed and theoretical.

## PRE-CLINICAL CARDIAC DATA

NEP is a plasma membrane glycoprotein that is a member of the metalloendopeptidase family. Widely expressed in mammalian tissues, NEP is the principle mechanism for degradation of the natriuretic peptides. However, NEP is not precise in its actions—hydrolyzing numerous other peptides including angiotensin I, angiotensin II, endothelin-1, kinins, adrenomedullin, opiod peptides, enkephalin, gastrin, and amyloid beta (Aβ).

Most of what we know about the role of NEP inhibition in the heart and vasculature comes from classical pharmacologic studies begun 2 decades ago showing that NEP inhibition alone resulted in an increase in natriuretic peptides but also in peripheral vasoconstriction (4). When compared with placebo, the combination of a NEP inhibitor (NEPi) with an ACEi (omapatrilat) (5,6) or the combination of a NEPi with an ARB (valsartan) decreased maladaptive cardiac remodeling (7). Omapatrilat was more effective at preventing changes in left ventricular geometry and premature mortality in Syrian hamster cardiomyopathy then was captopril (8). By contrast, in rats with chronic HF, omapatrilat did not result in benefit as compared with captopril (9,10). Similarly, sac/val had no effect on left ventricular remodeling or hemodynamic indices including cardiac output, stroke work, or dP/dt when compared with val in the same model: there was however a significant increase in ejection fraction (11). Thus, the pre-clinical data does not consistently demonstrate robust beneficial effects of NEP inhibition when combined

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with an ACEi or an ARB in comparison with an ACE or ARB alone.

## CLINICAL EFFECTS OF NEP INHIBITION IN HF

Studies begun 2 decades ago also demonstrated that NEP inhibition alone did not have salutary effects in patients with HF (12). In addition, a phase II study comparing omapatrilat with lisinopril failed to show a difference in the primary endpoint of exercise performance (13). Omapatrilat also failed to meet its primary endpoint of death or hospitalization in the 5,770-patient phase III OVERTURE (Omapatrilat Versus Enalapril Randomized Trial of Utility in Reducing Events) trial and was associated with a 2-fold increase in angioedema—leading the sponsor to discontinue its development. Investigators posited that ARBs might be less likely than ACEis to interfere with bradykinin metabolism; thus, the combination of a NEPi and an ARB became a more attractive choice for further development.

The PARADIGM-HF trial was the first large phase III study of a NEPi/ARB to meet its primary endpoint; however, the design of the trial raises important questions. First, the PARADIGM-HF trial compared an optimal (titrated) dose of val/sac with a fixed dose of enalapril (10 mg twice a day): a dose of enalapril that is below the maximum dose recommended by the American College of Cardiology/American Heart Association Practice Guidelines (10 to 20 mg twice a day) (14). That this dose of enalapril may have been inadequate in the PARADIGM trial is demonstrated by the finding that blood pressure was significantly lower after treatment with sac/val than after treatment with enalapril (1).

Blood pressure is an important metric because "high-dose ACE" inhibitors proved more effective than "low-dose ACE" inhibitors when the high-dose ACEi lowered blood pressure more than did the low-dose ACEi but not when the 2 doses had the same blood pressure response. For example, the ATLAS (Assessment of Treatment with Lisinopril and Survival) trial showed only an 8% nonsignificant decrease in mortality with high dose ACEi compared with low-dose ACEi; however, there was a 12% lower risk of death or hospitalization for any reason (p = 0.002), 24% fewer hospitalizations for HF (p = 0.002) and systolic blood pressure decreased by 4.4 mm Hg more in the high-dose group (p < 0.001) (15). Konstam et al. (16) found similar outcomes in the HEAAL (Effects of high-dose versus low-dose losartan on clinical outcomes in patients with heart failure) trial: high-dose lisinopril as compared with low-dose lisinopril met the primary endpoint of death or admission for worsening HF (p < -0.027) and there was a 13% reduction in HF admissions (p < 0.025) and an 11% reduction in cardiovascular admissions (p < 0.023) (16). Furthermore, we demonstrated that in patients with HF, only a high dose of an ACEi diminished the negative impact of the presence of an ACE deletion allele (ACE-D or ACE-DD) that is associated with increased ACE activity and an increased risk of HF-related events (17). Studies that failed to show a decrease in blood pressure with a high dose of an ACEi or an ARB as compared with a low dose did not show any difference in outcomes between the 2 groups (18,19).

There were other factors in the design of the trial that make translation to patient care challenging. For example, a run-in with enalapril preceded the run-in with sac/val. The intent of the run-in period was to ensure that the maximum benefit from sac/val could be achieved by selecting for patients who would most likely tolerate the target doses of both medications; however, this design precludes physicians from understanding the true tolerance to sac/val. In particular, this selection bias may have resulted in an under-representation of angioedema. With only 2 doses evaluated in the trial physicians will face a second therapeutic conundrum: if patients do not reach their target dose of sac/val or if they require down-titration of sac/val because of hypotension, would a prudent approach be to switch patients to their prior dose of an ACEi or an ARB? In fact, 18% of sac/val patients developed symptomatic hypotension. Similarly, because pre-specified subgroup analysis suggested that sac/val was no better than enalapril in treating patients with New York Heart Association functional class III/IV symptoms, should patients who progress to worsening symptoms while on therapy be switched to an ACEi, an agent known to benefit patients with severe disease (20). Despite an overwhelming percentage of patients having an ejection fraction ≤35%, only 15% of patients enrolled in the trial had received an implantable cardioverter-defibrillator (a Class 1A recommendation) and only 7% of patients had received cardiac resynchronization therapy—raising the possibility that mortality rates might have been lower and the effect of drug therapy less evident had more patients been receiving what is considered appropriate therapy in the United States (14). Sac/val significantly increased the ratio of urine albumin to creatinine when compared with enalapril, a difference that could reflect worsening renovascular disease. Finally, it should be noted that treatment with sac/val can also impair monitoring of chronic HF patients with B-type natriuretic testing (21).

## OFF-TARGET EFFECTS

When NEPis were first developed 20 years ago, the off-target effects of a new drug could only be ascertained once that drug was synthesized and even then the experimental designs were often challenging. Today, in the era of proteomics, metabolomics, and pharmacogenomics, molecular genetics can be used to rapidly overexpress or knock down selected proteins in a tissue-dependent manner resulting in information being available from translational research well before the results of large multicenter clinical trials become available. These studies have raised the theoretical risk that inhibition of NEP could increase levels of Aβ in the brain and in the eye leading to the development of symptomatic Alzheimer's disease (AD) or age-related macular degeneration (AMD), respectively (22-25).

Aβ homeostasis is regulated by a balance between Aβ production through sequential cleavage of the Aβ precursor protein by secretases and either removal of Aβ from the central nervous system (CNS) by transport and perfusion mechanisms or by proteolytic degradation (23). That an increase in Aβ in the brain is associated with the development of AD is shown by the finding that: 1) accumulation of Aβ in genetically engineered mice results in a neurologic phenotype that mimics AD (26,27); 2) gene mutations in humans that increase Aβ production cause autosomal-dominant AD, whereas genetic variants that reduce Aβ production protect against the development of AD; and 3) Aβ oligomers are neurotoxic and are major precipitants of the Aβ cascade (26). Interestingly, HF itself has been identified as a risk factor for the development of cognitive dysfunction that may be attributable to decreased cerebral blood flow and/or alterations in molecules that are involved in Aβ metabolism (28-31).

There is also strong evidence that modulation of the activity of NEP in the brain plays a critical role in the pathogenesis of AD: 1) overexpression of NEP is sufficient to ameliorate AD in transgenic models of AD (32); 2) NEP levels are low in regions of the brain that are vulnerable to AD pathology but normal in areas not affected by AD (33,34); 3) disruption of the NEP gene causes an elevation in the levels of Aβ in the mouse brain (35); 4) NEP levels decline with age in areas of the brain most affected by AD; 5) genetic mutations in the NEP gene or in the APP gene have been associated with increased risk of AD (36,37); and 6) aging is associated with decreased levels of NEP and increased Aβ deposition and aggregation in those areas of the brain that are most affected by AD (38).

The brain is not the only organ in which altered Aβ homeostasis can cause disease (39). Aβ is present in drusen, extracellular deposits in the subretinal area of the eye that are associated with AMD, the most common cause of legal blindness in the United States in individuals older than 50 years of age (40). More recent studies have shown that: 1) mice lacking NEP develop degeneration of the retinal pigment epithelial cells and subretinal deposits that are similar to those in AMD in humans (39); 2) senescent mice have higher retinal levels of Aβ due to a decrease in the expression of NEP and an increase in Aβ synthesis (41,42); and 3) injection of a recombinant form of the NEP catalytic domain into the vitreous decreased ocular Aβ levels in models of retinal degeneration (43). Taken together, these studies have led investigators to suggest that overexpression of NEP via gene therapy is a promising therapeutic approach for both AD and AMD (32).

The PARADIGM-HF trial investigators addressed the potential adverse effects of NEP inhibition on the CNS (1). They pointed out that: there are redundant systems for NEP degradation and removal and therefore inhibition of NEP alone would not influence Aβ in the CNS; a study in cynomolgus monkeys showed increased levels of the Aβ oligomers in the cerebrospinal fluid without an increase in levels of Aβ in the brain; a 2-week study in healthy volunteers showed normal levels of Aβ1-42 and Aβ1-40 but an increase in Aβ1-38; and PARADIGM-HF trial investigators did not report adverse events associated with cognition, memory loss or dementia. These arguments are not convincing: NEP is generally regarded as playing a critical role in Aβ degradation (23); a characteristic feature of early (asymptomatic) AD is disruption of the blood brain barrier and increased permeability thus abrogating the value of studies in normal volunteers or young monkeys with normal blood-brain barriers; and older patients with HF are at high risk for alterations in blood-brain barrier permeability due to age, neurovascular disease, hypertension, diabetes, high cholesterol, or a family history of AD (44,45). Furthermore, patients with early AD have leakiness of the blood-brain barrier due to the vascular processes that are pathognomonic of AD (46). Vodovar et al. (47) posited that because most patients with HF are elderly, the risk of developing AD is limited by their shortened lifespan; however, the patients in whom sac/val appeared to be most effective in the PARADIGM-HF trial were those with the best prognosis.

Finally, the fact that neither patients nor the members of the study team reported changes in

cognitive function as an adverse event is also not reassuring. An early diagnostic finding in AD is a decrement in executive function that can only be diagnosed with specific cognitive testing and is often not noted by patients or their spouses. Similarly, patients can have early signs of AMD without noticing changes in their vision, particularly if drusen or fluid accumulation occurs outside of the macula or is too small to influence vision.

While the theoretical risks associated with val/sac have not been widely discussed the AD risk has not gone without recognition by regulatory authorities. The Office Director Decisional Memo (NDA 207620) noted that: "the unanswered questions are whether sacubitril causes subtle CNS toxicity in the short term, or more severe toxicity in the longer term. These are salient questions, given that approximately 50% of patients with HF will survive longer than 5 years" (48). Because of these concerns, the Food and Drug Administration is requiring the sponsor to conduct a "multicenter, randomized, double-blind, active-controlled trial to evaluate the effects of Entresto compared to valsartan on cognitive function as assessed by a comprehensive neurocognitive battery and [positron emission tomography] imaging in patients with chronic heart failure with preserved ejection fraction" (48). Unfortunately, the sponsor is not required to make the information from the trial available until 2022—a time period that could place a large number of patients at risk before definitive data regarding safety is available. Because patients with heart failure with preserved ejection fraction have a different phenotype, a lower incidence of coronary disease and a different prognosis, it is unclear whether evaluation of patients with HFpEF will approximate the potential effects in HFrEF. Unfortunately, the regulatory authorities have not recognized the theoretical risk of AMD with an NEPi and therefore there are no admonitions regarding evaluations of the potential effects of sac/val on vision.

It should be noted that there are also adverse economic consequences of treatment with sac/val for individuals who must pay for their own medicine. With a wholesale acquisition cost of $4,560 per year, at least 8 times that of enalapril, physicians must weigh the risk-benefit ratio for the use of sac/val in each patient since it is far better for a patient to fill their prescription for an ACEi then to defer therapy with a new agent because they cannot afford it (40). In fact, the California Technology Assessment Forum recommended that sac/val prescribing be restricted to cardiologists and its use be prioritized to younger patients who are more able to tolerate a change in medication, those with worsening disease on current therapy, and those in whom a pronounced decrease in blood pressure could be tolerated (49).

## CONCLUSIONS

The PARADIGM-HF trial was an ambitious study that has provided important information regarding the potential benefits of the combination of NEP inhibition and blockade of the angiotensin II receptor in patients with relatively mild HF due to reduced ejection fraction. Nonetheless, an important lesson that we have learned from drug development in HF over the past 4 decades is that the results from one trial provide information that is only informative about the specific group of patients enrolled in that trial. Important areas of equipoise still exist and provide opportunities for additional and important trials despite the mortality benefit demonstrated in the PARADIGM-HF trial. For example, is sac/val safe and effective in patients with New York Heart Association functional class III/IV symptoms, lower resting blood pressure, and a higher use of implantable cardioverter-defibrillators and resynchronization devices? A study in a population with a higher severity of disease will provide information as to whether sac/val is equally effective at lower doses since patients with lower blood pressures are far less likely to tolerate the doses of sac/val used in the PARADIGM-HF trial. Another as yet unanswered question is whether sac/val is beneficial in patients with left ventricular dysfunction post-myocardial infarction? It will also be important to know whether patients who can no longer tolerate the dose of sac/val used in the PARADIGM-HF trial should have their dose reduced or should they be returned to their pre-sac/val dose of an ACEi or an ARB? Only through additional and well-designed clinical trials will physicians be able to know how best to use sac/val in the treatment of the wide spectrum of phenotypes seen in patients with HF.

In view of the theoretical risks associated with NEP inhibition and the devastating effects that AD or AMD can have on HF patients and their families, any future studies in patients with HFrEF should include thorough, appropriate and serial assessment of cognitive and ophthalmic changes using diagnostic criteria defined by consensus panels of AD neurologists (cognitive testing including assessment of executive function and either cerebrospinal fluid chemistry or positron emission tomography scanning) (50) and retinal specialists (optical coherence tomography) (51). While recognizing that HF itself is a risk factor

for the development of AD, physicians should be prudent in using sac/val in patients at high risk for AD or AMD with particular care in patients with documented early forms of AD or AMD or with a family history of AD or AMD until more data is available. Finally, patients and their family members should be informed of the theoretical risks of cognitive and/or visual dysfunction associated with NEP inhibition so that any cognitive or visual changes can be recognized as early as possible. Sac/val may well provide a new paradigm for the treatment of HF patients; however, it can only reach that goal through additional studies that provide valid information about the precise use of the drug and its potential risks in real-world settings.

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