SCIENTIFIC SESSION SUMMARY

Basic Science and Clinical Trials: Accelerating the Future

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NOVEL THERAPIES TARGETING THE RIGHT VENTRICLE

Harm J. Bogaard, MD, PhD of Vrije Universiteit Medical Center, Amsterdam, The Netherlands, began the session with his presentation on clinical trials and preclinical studies of novel therapies targeting the right ventricle in pulmonary arterial hypertension (PAH).

Dr Bogaard began by explaining that despite recent advances in the treatment of PAH, survival is still poor, and significant room for improvement remains. Much of the mortality in PAH is explained by right ventricular (RV) response to pressure overload, and 2 PAH patients with otherwise similar characteristics may have very different functional capacity and prognosis on the basis of RV function. Furthermore, the response of the RV to current PAH treatment is poorly correlated with change in pulmonary vascular resistance (PVR). Patients with persistently low RV ejection fraction (RVEF) have poor outcomes, even if their PVR decreases after vasodilator therapy. The presentation focused on recent findings related to mechanisms of RV failure in PAH and potential targets for intervention, in particular chronic sympathetic neurohormonal activation. Chronic activation of the sympathetic nervous system in PAH has been known for decades. However, there has been controversy around trials with beta-blockers because cardiac output in PAH patients is dependent on heart rate, given known reduction in RV stroke volume. Historically, physicians have feared that slowing heart rate with beta-blockers would worsen exercise capacity in PAH patients. This concern, along with case reports of syncope associated with beta-blocker use in PAH patients has led to the widespread recommendation to avoid beta-blockers.

Challenging this long-held belief, Dr Bogaard explained that in SUGEN/hypoxia rat models of PAH, carvedilol (a nonselective alpha- and beta-blocker with some antioxidant properties) was shown to improve RV function, which led to a small clinical trial. A 6-patient proof-of-concept trial of carvedilol in PAH patients showed improvement in RVEF and less RV dilatation on cardiac MRI. A second study by his group in the Netherlands using bisoprolol, a selective beta-1-receptor blocker, did not show an improvement in RVEF, however, in a subgroup analysis, they reported that patients with poor RVEF prior to treatment may have improvement in RVEF with treatment, whereas patients with preserved RVEF and exercise capacity did not. Another trial of carvedilol in patients with PAH showed a decrease in heart rate and RV glycolysis, and proved to be safe in PAH patients, but did not show an increase in cardiac output or 6-minute walk distance (6MWD). In light of the present data Dr Bogaard emphasized that he does not advocate use of beta-blockers in PAH patients, and that further clinical trials are needed with careful patient selection to identify patients who are most likely to respond to therapy. An alternative for targeting chronically activated sympathetic neurohormonal signaling in PAH may be to increase parasympathetic tone. A recent paper by their research group showed that in the SUGEN/hypoxia rat model, blocking acetylcholinesterase improved survival and RV remodeling, and this may be a safer option than beta-blockers, which they hope to pursue in clinical trials.

A more controversial potential mechanism for RV failure in PAH is impaired microcirculation and ischemia. This is especially important because of ongoing trials using anti-angioproliferative agents targeting endothelial cell proliferation. Previous autopsy studies have shown preserved capillaries and microcirculation in PAH patients with...
RV hypertrophy and preserved RVEF, and loss of capillaries in PAH patients with decompensated RV failure and dilatation.\textsuperscript{16} If impaired microcirculation is causative in RV failure, then targeting this would make for a promising PAH treatment. This is supported by a recent study using an miRNA 126 mimic in experimental PAH that showed improved capillarization of the RV, which correlated with improved RV function.\textsuperscript{17}

Another novel therapy targeting the right ventricle in PAH is nintedanib, an antifibrotic drug currently approved for the treatment of idiopathic pulmonary fibrosis. A recent experiment in rats showed that nintedanib reduced fibrosis and improved RV function, despite having no significant effects on the pulmonary vasculature.\textsuperscript{18}

Lastly, for advanced PAH, novel interventional techniques such as RV assist devices, devices to improve pulmonary arterial compliance, and advances in balloon septostomy are being explored as options for bridging to transplantation.

**FINDING NEW THERAPIES FOR PULMONARY HYPERTENSION: HARNESSING NEW KNOWLEDGE IN BIOLOGY, BIOINFORMATICS, AND BIOENGINEERING**

Marlene Rabinovitch, MD, of Stanford University gave the second presentation of the Scientific Sessions. Like Dr Bogaard, she began by discussing the shortcomings of current PAH therapy, and specifically that none of the currently approved treatments address the underlying pathologic or genetic causes of PAH.

PAH is characterized by progressive loss of the distal pulmonary vasculature, endothelial cell apoptosis, occlusive proliferation of smooth muscle cells, and inflammation, none of which are currently targeted by the vasodilators in clinical use.\textsuperscript{19,20} Genetic studies have also led to the discovery of the key role of bone morphogenetic protein receptor II (BMPR2) signaling in PAH pathogenesis, with BMPR2 mutations found in approximately 70% of familial PAH patients, and 20% of “sporadic” idiopathic PAH patients.\textsuperscript{21-23} Furthermore, mutations in other genes that affect BMPR2 signaling such as ALK1, CAV1, and SMAD9 have also been associated with PAH, although their exact prevalence is unclear.\textsuperscript{24}

BMPR2 mutations have been shown to underlie endothelial cell dysfunction, impaired regeneration of small vessels and enhanced smooth muscle cell proliferation in response to injury, impaired assembly of elastic fibers causing vascular stiffness, and enhanced recruitment of inflammatory cells to injured pulmonary arteries.\textsuperscript{25-28} Given the centrality of BMPR2 to PAH pathogenesis, there are now several agents on the horizon aiming to improve the function of this receptor and signaling pathway in PAH (Figure 1). Elafin is an endogenous elastase inhibitor that enhances BMPR2 signaling by promoting interaction with CAV1, and has been shown induce neointimal apoptosis and improve endothelial cell function.\textsuperscript{29} FK506 is an immune suppressor that has been shown to improve BMPR2 receptor activity.
These cells have the advantage of shar-
ting the same genetic underpinning, and being readily accessible via minimally inva-
sive techniques. Dr Rabinovitch and her colleagues have developed induced pluripotent stem cell–derived endothelial cells (iPSC-EC) and smooth muscle cells (iPSC-SMC) from skin fibroblasts obtained from PAH patients at the time of lung transplant, and extensively compared these cells to PA endothelial and smooth muscle cells obtained from the same patients. They found that both iPSC and native cell types have low expression of BMPR2, impaired angiogen-
esis, and similar gene expression. Given these promising results, they went on to test the effects of FK506 and elafin on native cells vs iPSC cells and found that both cell types had similar response, with increased BMPR2 signaling.29,30

In further studies, they have used iPSC cells from unaffected family members of PAH patients with BMPR2 mutations and found that unaffected BMPR2 mutation carriers have different compensatory signaling mechanisms and gene expression, leading to improved overall BMPR2 signaling within cells compared to PAH patients.39

These iPSC cells present multiple avenues for development of PAH therapies, including the use of CRISPR gene editing, and high-throughput drug screening for the repurposing of drugs to treat PAH. Using this latter approach, they have discovered a TKI and potassium channel opener that show promise for PAH treatment, and also identified several drugs that have an adverse gene expression signature and should be avoided in PAH, including dasatinib, a drug that has previously been shown to cause PAH in cancer patients.40

**NOVEL CLINICAL TRIAL DESIGNS AND ENDPOINTS**

In the final presentation of this session, Steven Kawut, MD, MS of the University of Pennsylvania discussed recent developments in clinical trial design and novel endpoints. There has been remarkable progress in PAH treatment in the past 20 years, but until recently the approach to studying treatments in patients has remained unchanged.

There are several relatively new study design approaches being used in other fields of medicine, such as “enrichment” designs, where patients undergo some type of diagnostic screening and are then randomized on the basis of positive results.41 “Adaptive enrichment designs” use early results from an interim analysis of a trial to guide later recruitment targeting a long-term outcome, such as survival.42 “Umbrella” or “basket” designs are often biomarker-based and require fairly large numbers of patients. Unfortunately, none of these design approaches are easily applicable to future PAH trials because of the generally low number of eligible par-
ticipants, and lack of reliable diagnostics to identify potential “responders.”

In future PAH trials, rather than adopting one of these designs, it will be important to assess the underlying risk for a patient to have an event (eg, death, clinical worsening) and therefore select patients most likely to have detectable treatment benefit. Dr Kawut illustrated this point using meta-analyses of previ-
ous PAH trials. In a logistic regression model predicting clinical worsening based on pre-enrollment variables, he showed that in 11 clinical trials the pa-
tients with the lowest risk had very few events and did not derive benefit from treat-
ment, whereas the patients with the highest risk had many events and de-
rived significant benefit from therapy.43 By narrowing enrollment in trials to higher-risk patients that are more likely to have an event, sample sizes could be reduced, decreasing the time and cost of clinical trials, without affecting the power of these trials to detect treatment effect. This would be a valuable applica-
tion of the various risk prediction mod-
els recently published (REVEAL, ESC, French).1,7,44 He also showed preliminary data that machine learning algorithms may be able to identify “clusters” of patients more likely to have events and respond to therapies in general.

Other trial designs have been used with some success in PAH trials, such as crossover studies, in which each patient enrolled is treated with multiple interven-
tions (eg, active drug vs placebo, or 2 or more drugs) at different time points. This has the advantage of allowing for smaller sample sizes and reducing confounding, with each patient serving as their own control, but also has several limitations
such as longer study periods, and significant problems with patient dropout. Historically, crossover designs have been most effective for smaller trials with biomarker endpoints, as was seen in a small pilot study of aspirin and clopidogrel. 45

The use of factorial designs allows for the study of 2 drugs simultaneously in a single trial, which can be advantageous in terms of cost and feasibility, provided that the treatments can be safely coadministered. However, the risk for dropout due to side effects is higher, and side effects from one drug can impact the study of the other. 46

Another problem facing PAH clinical trials is the lack of reliable surrogate endpoints. The criteria for surrogate endpoints are that they should be reliable, integral to the disease causal pathway, targeted by treatment, correlated with the eventual outcome, and the effect of treatment on the surrogate endpoint must predict the effect on the clinical outcome. Most importantly, Dr Kawut emphasized, the effect on treatment on the surrogate endpoint should explain 50% to 65% of the treatment-outcome relationship. PVR has been used as a surrogate clinical outcome for several phase 2 trials; however, in a meta-analysis of 4 PAH drug trials only 14% of short-term clinical worsening can be explained by changes in PVR, and even less for other hemodynamic measurements such as cardiac index and PA pressure. 47 Similarly, using patient-level data of 10 randomized controlled trials (RCTs), 6MWD explained only 22% of the treatment effect on clinical worsening. 48 A study-level meta-analysis showed similar results: that while 6MWD outperformed hemodynamics, it is a poor surrogate outcome for short-term outcomes in PAH.

Because of these shortcomings, it has recently been proposed that multiparameter prediction rules and the “low-risk profile” should be used as endpoints for clinical trials. 49 This also has limitations, because none of these prediction rules have been validated as surrogates, and targeting a “low-risk” profile doesn’t necessarily translate to better outcomes, so further studies are needed to prove that targeting a prognostic rule results in better quality of life and long-term outcomes. 50

With the lack of validated surrogate endpoints, an alternative suggested by Dr Kawut is the use of intermediate endpoints. These are true clinical endpoints with direct benefit to the patient, such as improved quality of life, activity levels, or prevention of hospitalization, but are not the ultimate endpoint of survival. Such novel intermediate endpoints in current use are actigraphy and patient-reported outcomes/ questionnaires. 51-54 Use of these intermediate endpoints could make both phase 2 and 3 studies more feasible while also improving the validity of the results.

Lastly, composite endpoints offer the ability to increase power to detect differences between treatments by incorporating several clinically meaningful endpoints. However, they are also limited by mixing definitive endpoints (eg, death) with “softer” endpoints (eg, change in 6MWD) that risk diluting the overall impact of the findings.

References
High Points:

- Right ventricular function is a major determinant of functional capacity and survival in PAH. While there are currently no medications approved for PAH directly targeting the right ventricle, several new and repurposed drugs are in development.
- Current PAH therapies do not address the underlying pathology or genetics of PAH, but treatments targeting the BMPR2 pathway, altered cellular metabolism, and epigenetic changes are in development and clinical trials.
- Induced pluripotent stem cells offer potential for accelerating research in PAH drug development and treatment of PAH.
- Novel study designs incorporating modern PAH risk prediction scores, and the use of intermediate clinical endpoints offers a way to modernize and improve PAH clinical trials.