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MEETING HIGHLIGHTS

Proceedings From the 2019 Stanford Single Ventricle Scientific Summit: Advancing Science for Single Ventricle Patients: From Discovery to Clinical Applications

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ABSTRACTS: Because of remarkable advances in survival over the past 40 years, the worldwide population of individuals with single ventricle heart disease living with Fontan circulation has grown to ≈70 000, with nearly half aged >18 years. Survival to at least 30 years of age is now achievable for 75% of Fontan patients. On the other hand, single ventricle patients account for the largest group of the 6000 to 8000 children hospitalized with circulation failure, with or without heart failure annually in the United States, with the highest in-hospital mortality. Because there is little understanding of the underlying mechanisms of heart failure, arrhythmias, pulmonary and lymphatic vascular abnormalities, and other morbidities, there are no specific treatments to maintain long-term myocardial performance or to optimize overall patient outcomes.

Key Words: atrial tachycardia ■ congenital cardiac defect ■ pulmonary vascular changes ■ single ventricle ■ stroke in children

Aiming to accelerate discovery for single ventricle congenital heart disease, in April 2018 and again in April 2019, the Stanford Single Ventricle Scientific Summit convened a small group of international basic scientists, bioengineers, patients, and clinicians. These summits were a novel opportunity for synergy across disciplines. Because of its fundamental impact on survival, preservation of myocardial function was the main focus of the inaugural 2018 summit. To better understand the mechanisms of disease and to drive the development of new therapeutic targets, the 2019 summit had the following focus areas: developmental insights, arrhythmias, pulmonary vascular and lymphatic function and remodeling, and thrombosis. This synopsis summarizes identified gaps in knowledge and outlines research questions that multicenter and multidisciplinary collaborative efforts may begin to solve.

Landmark innovations in the management of single ventricle congenital heart defects have transformed survival over the past 40 years, with 75% of patients with Fontan circulation surviving to at least 30 years of age.[1] This dramatic improvement has led to an estimated 70 000 individuals living with Fontan circulation worldwide, nearly half of whom are adults. This population is expected to double in the next 20 to 25 years.[2] On the other hand, single ventricle patients account for the largest single group of the 6000 to 8000 children hospitalized with circulation failure, with or without heart failure annually in the United States, with a strikingly high in-hospital mortality ranging from 20% to 50% for those requiring intravenous inotropes.[3] Notably, most who require transplantation have preserved systolic ventricular function.[4] Teens and young adults progressively accrue morbidity, such as diastolic dysfunction, arrhythmias, liver fibrosis, increasing pulmonary vascular resistance, and lymphatic failure.[5] However, there is little understanding of the underlying mechanisms of heart failure, circulation failure, and other morbidities

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in single ventricle patients. With so many fundamental questions unanswered, it has not yet been possible to develop specific treatments to maintain long-term performance of the single ventricle or to optimize overall functional outcomes of individuals living with Fontan circulation.

With the goal of advancing discovery for single ventricle patients, the Stanford Single Ventricle Scientific Summits have brought together distinguished scientists, bioengineers, patients, and clinicians from around the world. Young adult patient speakers laid the foundation by showcasing their thriving lives and resilience while emphasizing the urgent need for research to improve longevity and quality of life. Clinicians framed the key issues, and scientists and engineers proposed how their approaches could inform the ability to better understand and investigate problems in this target population. These summits were a novel opportunity for synergy across disciplines. The inaugural Stanford Single Ventricle Scientific Summit in 2018 focused on preserving myocardial function in single ventricle patients. Bioengineering approaches for creating a pump to replace the missing ventricle and regenerative medicine strategies for augmenting or restoring systemic ventricular function were presented. Research objectives prioritized at the 2018 summit were included in the recently published American Heart Association Scientific Statement on Fontan care.[6]

The second annual summit, held on April 29 to 30, 2019, focused on the next most pressing challenges: arrhythmias, pulmonary vascular and lymphatic disease, and thromboembolism, as well as developmental insights into single ventricle heart disease. Among clinicians, many of these end-organ morbidities are currently considered a consequence of hemodynamics (ie, long-term manifestations of the physiological limitations of the Fontan circulation with the undesirable combination of systemic venous hypertension and relatively low cardiac output). The summit explored the potential role of developmental differences programmed into the ventricle and vasculature as an additive risk factor to the hemodynamics. Scientific exploration of alternative hypotheses may open up avenues for novel therapeutic approaches. This synopsis summarizes identified gaps in knowledge and outlines research questions that multidisciplinary and multicenter collaborative efforts may begin to solve.

ARRHYTHMIAS AND DEVELOPMENTAL INSIGHTS INTO THE SINGLE VENTRICLE

The development of atrial tachycardia in patients with Fontan circulation is associated with significant late morbidity and mortality. The cause of atrial arrhythmias is likely multifactorial. There are hemodynamic abnormalities, the effects of neonatal cyanosis and cardiopulmonary bypass with resultant injury to atrial connexins and the creation of reentrant circuits from suture lines, and the secondary consequences of atrial hypertrophy. At the cellular level, electrical remodeling of cardiac ion channels and disruption of transcriptional pathways predispose to arrhythmias.[7–10] Moreover, developmental programming of the cardiac conduction system has important implications for arrhythmogenesis. Embryonic perturbation of *Wnt* or *Notch* signaling results in chamber-specific transcriptional and epigenetic regulation of genes controlling cellular electrophysiological characteristics. These changes result in arrhythmia susceptibility in the adult heart and modulate chamber-specific ventricular responses to cardiac stressors.[11] Mutations in *Hand1* also affect conduction system development, leading to alterations in the His bundle, prolonged QRS duration, and decreased conduction velocity.[12] A better understanding of cardiac ion channel molecular regulation and arrhythmogenesis caused by chamber-specific, heritable or epigenetic factors may offer tailored therapeutic options.

Current treatment options include antiarrhythmic medications, catheter ablation, antitachycardia pacing, cardiac resynchronization therapy, Fontan conversion with arrhythmia surgery, or cardiac transplantation. Although atrial pacing for sinus node dysfunction may be beneficial, long-term ventricular pacing adversely affects transplant-free survival; an ongoing international multicenter study elucidating specific risk factors is in progress.[5,13]

Obesity has been identified as a significant risk factor for recurrence of atrial arrhythmias after ablation in adults with congenital heart disease.[14,15] In contrast, the elite minority of patients with Fontan circulation who achieved superior cardiopulmonary exercise capacity were characterized by body mass index of 23±3 kg/m^2 and participation in regular physical activity.[16] Thus, promotion of physical activity and attention to lean body mass beginning in childhood may be important interventions for arrhythmia prevention in this cohort of patients.[17] Several small studies have demonstrated that exercise training improves body composition and peak oxygen uptake as well as cardiac preload and stroke volume in children and adults living with Fontan physiology.[18] Fontan-associated myopenia and osteopenia are common, and preliminary studies suggest there may also be a predisposition to increased adiposity. For these reasons, Fontan patients may benefit from both aerobic conditioning and strengthening exercises.[19] A multicenter, randomized controlled trial is needed to build on existing evidence.

Single ventricle patients are at risk for ischemic insults, with resultant fibrosis, which may negatively impact ventricular and other end organ function and lead to arrhythmias. Diffuse myocardial fibrosis in the systemic ventricle of Fontan patients is in itself a model of heart failure with preserved ejection fraction. Early work suggests the extracellular volume burden is greater in Fontan patients than symptomatic heart failure with preserved ejection fraction patients with biventricular hearts.[20] Fibrosis not only affects ventricular performance but also leads to dyssynchrony.[21] Cardiac magnetic resonance imaging can now image perfusion defects and fibrosis and assess the effect of these lesions on cardiac function and blood flow.[22] Therapies to prevent or reverse fibrosis, including spironolactone, are currently being investigated in both animal models and adults with acquired heart failure.

Fibrosis, however, is not limited to the myocardium in single ventricle patients. Liver fibrosis is nearly universal, and although it is considered a consequence of elevated central venous pressure, this has never been proved. New data suggest an association with inferior vena caval flow rates, perhaps related to hepatic arterialization.[23] However, these hemodynamic changes do not explain the concomitant presence of fibrosis in the heart, liver, and pulmonary vessels. Nor do they explain arrhythmias, thrombosis, and early development of liver tumors. Defects in mitochondrial bioenergetics and increased apoptosis noted in the myocardium in a single ventricle mouse model support investigation of mitochondrial function and apoptosis in the liver, lung, and pulmonary vessels.[24]

Table 1. Arrhythmia and Developmental Insights Session Highlights

• Fibrosis, surgical scars, and hemodynamic burden are key determinants of arrhythmia. However, new basic science highlights the role of developmental programming and chamber-specific, heritable, or epigenetic factors in arrhythmogenesis.
• Cardiac and hepatic fibrosis are seen nearly universally in patients with Fontan circulation. Preliminary animal studies point to genetically programmed differences in metabolism and apoptosis in the development of fibrosis.
• Exercise improves body composition and functional capacity in Fontan patients, potentially decreasing the arrhythmia risk. Lifelong exercise should be considered a foundation of Fontan care.
• Arrhythmias are considered a sign of clinical deterioration in the Fontan patient. If they are the cause, options like ablation, pacing, and surgical Fontan revision may restore lost functionality. If they are a consequence, interventions should target reversing or preventing further hemodynamic deterioration
• Catheter-based ablation techniques for atrial tachycardia are less successful than in non-Fontan patients and may increase the risk of atrial fibrillation.
• Atrial pacing may be beneficial. Prolonged QRS and ventricular pacing are a hazard. Cardiac resynchronization may offset some harmful effects.

Table 2. Areas of Future Research Related to Arrhythmias and Developmental Biology Identified by the Summit

• Underlying cause: What determines and regulates chamber-specific developmental programming of ion channels and the cardiac conduction system? How strongly do these factors impact arrhythmogenesis?
• Risk reduction: What is the impact of body mass index and inflammation on atrial arrhythmias after Fontan? Can diet modify the risk?
• Early detection of clinical deterioration: Could routine use of extended arrhythmia monitoring or consumer wearable devices allow earlier detection of arrhythmias to prevent electrical remodeling and improve outcomes?
• Interventions: New ablation techniques, refinement of pacing strategies, and innovation in pacing technology are needed. When and how to pace? Is cardiac resynchronization beneficial? What are the effects of electromechanical dissociation of the atrium after Fontan?
• End-organ fibrosis: What are the determinants, nature, and extent of fibrosis?

Table 1 summarizes session highlights. Areas of suggested future research are shown in Table 2.

ABNORMAL PULMONARY VASCULAR AND LYMPHATIC DEVELOPMENT, FUNCTION, AND REMODELING

In single ventricle patients, disturbed fetal hemodynamics and surgical interventions in infancy may impair pulmonary vascular growth and development. In neonates with aortic atresia, for example, increased muscularity of both preacinar and intra-acinar arteries and veins has been noted, with muscle extending into smaller and more peripheral vessels.[25] Cavopulmonary anastomoses then result in reduced and nonpulsatile flow in the pulmonary vasculature, which can directly induce endothelial dysfunction and further adverse remodeling.[26–28] In addition, the altered genetic and epigenetic features of single ventricle heart disease, such as defects in *Notch* signaling, may be linked to abnormalities of pulmonary vascular maturation.[29] These abnormalities can be expected to be critical to Fontan function because the compliance and resistance of the pulmonary vascular bed has a major impact on systemic venous pressure and ventricular preload, as well as cardiac remodeling and function.[30,31]

Inflammation and the ensuing changes in the composition of the extracellular matrix contribute to the development and progression of pulmonary hypertension in animal models and in humans.[32,33] Perivascular activation of the complement cascade further contributes to extracellular matrix stiffness.[34] Investigating these mechanisms of pulmonary vascular remodeling is of importance in identifying the root cause of

elevated pulmonary vascular resistance in single ventricle patients.

Although the adverse impact of elevated pulmonary vascular resistance on the function of the Fontan circulation in single ventricle patients is known, it is yet unclear what subset of patients would benefit from pulmonary vasodilator medications. Some pulmonary vascular changes identified in Fontan patients (ie, increases in smooth muscle cells in normally nonmuscular arteries) are also observed in patients with left to right shunts as an early feature of pulmonary arterial hypertension.[35] However, in patients with long-standing Fontan circulation, a pattern of adverse pulmonary remodeling with reduced vascular smooth muscle in the medial layer of intra-acinar pulmonary vessels and intimal fibrosis has been noted. Several randomized studies comparing the effect of pulmonary vasodilator therapy on exercise capacity in patients with Fontan circulation have shown slight benefit.[36] There may be subgroups of symptomatic or high-risk patients who may benefit from pulmonary vasodilator therapy, including prostanoids. At this time, however, there is insufficient evidence to routinely recommend pulmonary vasodilator therapy for all Fontan patients. Beyond their pulmonary vasodilatory effects, phosphodiesterase type 5 inhibitors may mitigate adverse ventricular remodeling and improve ventricular contractility, which may provide additional benefit to Fontan patients.[37] New disease-modifying therapies that target inflammation, chromatin remodeling, and improving endothelial dysfunction may be of benefit.[38]

Computational approaches have recently been applied to better understand the mechanisms of abnormal pulmonary vasculature development and to identify novel therapeutic targets. Big data analytics have drawn attention to the molecular parallels between cancer and pulmonary hypertension, and this could facilitate selective repurposing of chemotherapies for pulmonary hypertension. For example, preliminary work using a computational algorithm called Evaluation of Differential Dependency 3, has established a pipeline to predict therapeutic benefit and toxicity of chemotherapies in pulmonary hypertension or other noncancerous diseases.[39] This type of analysis may shape discovery of agents better targeted to single ventricle patients.

In addition to abnormalities in the pulmonary vasculature, the lymphatic circulation may also be significantly impaired in single ventricle patients. Chylothorax, protein-losing enteropathy, and plastic bronchitis are serious and often fatal Fontan-associated morbidities attributable to disorders of the lymphatic circulation. State-of-the-art lymphatic imaging, including dynamic contrast magnetic resonance lymphangiography and T2-weighted magnetic resonance lymphatic mapping, now allows screening for pulmonary lymphatic abnormalities before Fontan operation. This has led to new avenues of treatment for symptomatic patients, including therapeutic embolization of abnormal lymphatic channels. However, the recurrence of new alternative lymphatic channels suggests only a short-term benefit.[40,41] Further research into the mechanisms causing abnormal development and regulation of the lymphatic circulation is warranted to reduce morbidity in the single ventricle population.

Table 3 summarizes session highlights. Areas of suggested future research are shown in Table 4.

THROMBOSIS IN SINGLE VENTRICLE CIRCULATIONS AND IN DEVICES FOR MECHANICAL SUPPORT OF THE FONTAN CIRCULATION

Infants, children, and adults with single ventricle heart disease are prone to thrombosis that can be life threatening or that can prevent progression to further surgical palliation. This may be explained by the Virchow triad with factors intrinsic to the anatomical and physiological characteristics that are further complicated by invasive procedures, cardiopulmonary bypass/mechanical circulatory support, infection, and inflammation.[42] The risk of thrombosis is not constant over time; it is greater in the immediate period after neonatal surgery, the first 6 months after the Fontan, and ≥10 years after the Fontan. In the neonatal period, prophylactic anticoagulation may be of benefit in the highest-risk groups.[43] There is no consensus on antithrombotic prophylaxis after Fontan. Stroke can be a devastating complication after Fontan, with an overall incidence of ≈2%, but silent stroke associated with neurocognitive impairment is higher, at 13%.[44,45] The incidence of stroke while on a ventricular assist device is particularly high at 19%.[46]

Single ventricle patients account for the largest cohort of the 6000 to 8000 children hospitalized with heart

Table 3. Pulmonary Vascular and Lymphatic Function Session Highlights

• The pulmonary vasculature may be a key target to improve the long-term outcome of Fontan patients. Some therapies primarily aimed at inflammation and pulmonary vascular remodeling may have synergistic benefits for ventricular remodeling, systemic vascular endothelial function, and lymphatic function.
• There is currently insufficient evidence to routinely recommend pulmonary vasodilator therapy for all Fontan patients, but early evidence suggests that it may prove efficacious in a subset.
• Computational drug discovery in pulmonary hypertension may inform development of future therapies that are better targeted to single ventricle patients.
• There is an urgent need for better understanding of lymphatic function, the effects of cardiovascular drugs on lymph production and lymphatic vessel function, and treatment of lymphatic complications. Lymphatic imaging may aid in risk stratification and management.

Table 4. Areas of Future Research Related to the Pulmonary Vasculature and Lymphatics Identified by the Summit

• Development of a biobank from single ventricle patients, ideally in combination with a large multicenter clinical registry, for deep phenotyping and genotyping crucial to elucidating underlying mechanisms of disease.
• What is the genetic and epigenetic architecture of the pulmonary vasculature in single ventricle patients? What molecular mechanisms initiate and perpetuate pulmonary vascular remodeling?
• What are the mechanisms of formation of abnormal lymphatic connections and altered flow? What are the effects of medications on the lymphatic system? What timing and type of interventions would be beneficial?
• What is the hemodynamic response to increased preload (eg, exercise or right-sided heart assist devices)? What is the role of occult diastolic dysfunction in the generation of secondary multiorgan dysfunction?
• Does exercise impact long-term clinical outcomes, including arrhythmias and end-organ and pulmonary vascular function? How can long-term behavior change for an active life best be implemented?

failure annually in the United States, and in-hospital mortality for those requiring intravenous inotropes is strikingly high, ranging from 20% to 50%.[3] This growing population has led to an increased need for mechanical support options and the urgent need for devices with lower thromboembolic complications in children. A new Fontan pump is in its early phase of preclinical testing. Computational fluid dynamic models that predict areas of thrombus formation within ventricular assist devices are currently being developed.[47] Emerging data using computational flow dynamics modeling suggest that CentriMag devices are the most favorable in reducing Fontan pressures and providing robust systemic output.[23] Computational modeling is expected to provide significant insights into flow dynamics and thrombosis.

Tissue-engineered vascular grafts for use as extracardiac conduits in children undergoing the Fontan operation are promising, and a trial with the next version of an engineered graft has started. A living-tissue graft that can grow with patients and has the potential to eliminate conduit restriction (associated with increased liver fibrosis) and eliminate in-graft thrombosis holds great promise.[48–50] Tissue engineering may yet provide a platform for developing a subpulmonary pump, which could be implanted early in life to augment venous return to the systemic ventricle and mitigate complications related to chronic venous congestion and thromboembolic risk.

Table 5 summarizes session highlights. Areas of suggested future research are shown in Table 6.

Table 5. Thrombosis in Fontan Circulation and Devices for Mechanical Support of Fontan Circulation Session Highlights

• Single ventricle patients have key periods of higher risk for thromboembolic events. The incidence of stroke while on a ventricular assist device is particularly high.
• Computational flow dynamic models have been developed to aid in the creation of pediatric ventricular assist devices and subpulmonary pumps and to reduce device thromboembolism risk.
• Personalized tissue-engineered grafts, applied as extracardiac conduits, have undergone improvement in scaffold composition to prevent stenosis and yield somatic growth.
• Advocacy for collaboration between academic centers within the ACTION network, industry, and the FDA will be crucial to overcome challenges in design, build, preclinical and clinical evaluation, and financial disincentives for pump innovation for single ventricle patients.

ACTION indicates Advanced Cardiac Therapies Improving Outcomes Network; and FDA, Food and Drug Administration.

Table 6. Areas of Future Research Related to Thrombosis Identified by the Summit

• Development of novel anticoagulants and development of devices with lower thrombotic potential, as well as techniques to detect clots earlier.
• Research into less thrombogenic surfaces and alterations in wall sheer stress.
• ACTION network partnership with the FDA in ventricular assist device assessment.
• Research on the role of left ventricular assist device support alone to improve Fontan hemodynamics as long-term destination therapy or bridge to transplant.
• Investigation of the role of right ventricular assist device in the Fontan circulation in patients with normal ventricular systolic function but abnormal Fontan venous pressures.
• Use of large animal model for Fontan pump testing.

ACTION indicates Advanced Cardiac Therapies Improving Outcomes Network; and FDA, Food and Drug Administration.

CONCLUSIONS

As we continue to make progressive improvements in clinical care and gain further understanding of the longitudinal trajectory of individuals living with Fontan circulation, a deeper understanding of the fundamental cellular and molecular mechanisms of disease will accelerate discovery toward prevention and treatment. This will require an integrative, interdisciplinary framework bridging genetics, development, disease modeling using stem cell technology, omics, imaging, pharmacology, and bioengineering. Reflecting on the "why" of single ventricle heart disease allows us to appreciate the patient's perspective, ignites our scientific passion, and inspires hope and innovation.

ARTICLE INFORMATION

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