Pulse my heart

By Amy Donner, Senior Editor

Despite the potential for VEGF-A to limit or even repair post-myocardial infarction heart damage, the molecule has stumbled in the clinic because of issues with its delivery and therapeutic window. Now, a multinational team thinks it has solved these problems by using synthetic RNA.¹ The compound is partnered with **Moderna Therapeutics Inc.** and **AstraZeneca plc.**

Myocardial infarction (MI) patients typically receive β -blockers and blood thinners to protect the heart from a second infarct and restore blood flow. Some also receive angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor blockers to limit damage to the heart.

Despite these treatments, “there is a compelling unmet need in repairing damage after heart attack,” said Kenneth Chien, a professor of cardiovascular research at the **Karolinska Institute**, a professor of stem cell and regenerative biology at **Harvard University** and a cofounder of Moderna.

An alternative approach using VEGF-A to promote neovascularization has also been tested to limit tissue damage after MI. However, multiple approaches for delivering VEGF-A, including injection of recombinant protein and gene therapy using naked DNA plasmids or engineered viruses, have been developed but then stumbled in the clinic.

Recombinant VEGF-A had limited stability in serum and was associated with hypotension and growth of atherosclerotic plaques.² DNA-mediated delivery of VEGF-A led to stable protein levels, but prolonged tissue exposure to the growth factor caused cases of excessive vascular permeability and edema.³

Chien and his team hypothesized that short, pulsed delivery of VEGF-A might circumvent these problems.

In addition, the group’s recent findings establishing that VEGF-A can mobilize cardiac progenitor cells to become endothelial cells and potentially help repair heart function provided one more reason to harness the therapeutic potential of the molecule by addressing the delivery challenges⁴ (see Figure 1, “VEGF-A promotes an endothelial cell fate in human cardiac progenitor cells”).

The researchers thus set out to develop an RNA-based platform with the ability to trigger a targeted but short-lived burst of protein expression in the heart.

The team chose to develop a modified RNA (modRNA) expressing VEGF-A. modRNAs are synthetic RNA molecules that contain chemical modifications at the 5’ guanine cap and incorporate 2-thiouridine (pseudouridine) in place of uridine and 5-methylcytosine in place of cytosine. The cap is essential to stabilize the RNA in cells and for recognition by the ribosome so the RNA is translated into protein.⁵

Figure 1. VEGF-A promotes an endothelial cell fate in human cardiac progenitor cells. Lui *et al.* identified VEGF-A as a paracrine factor that promotes endothelial cell differentiation from human cardiac progenitor cells. Endothelial cells contribute to the coronary artery and vein as well as endocardial compartments of the heart.

[I] Schematic representation of cardiac progenitor cell origins in the developing mouse embryo (pink). The first (red) and second (green) heart fields are shown at various stages of early embryonic development. The two fields make distinct regional and lineage contributions to the heart: right atrium (RA), left atrium (LA), right ventricle (RV) and left ventricle (LV). The second heart field makes major contributions to the outflow tract (OFT).

[II] ISL LIM homeobox 1 (ISL1)⁺ human cardiac progenitor cells can give rise to three cardiovascular cell lineages: cardiomyocytes, smooth muscle cells and endothelial cells. Lui *et al.* identified an intermediate population of endothelial cells, derived from the OFT of nine-week-old human fetal hearts (arrow), that resemble human embryonic stem cell (hESC)-derived ISL1⁺ endothelial cell progenitors. VEGF-A can promote *in vitro* differentiation of both types of multipotent progenitors (endogenous intermediate endothelial cells and ISL1⁺ cells derived from hESCs) toward an endothelial cell fate. In a mouse model, subcutaneous injection of human OFT-derived progenitor cells overexpressing VEGF-A from a synthetic modified RNA led to endothelial cell specification as well as the proliferation and survival of these cells *in vivo*.

(Figure based on Figure 3b in Buckingham, M. *et al.*, *Nat. Rev. Genet.* 6, 826–837; 2005.)

“Almost any cells can take up the modRNA—and immediately translate to the protein with an efficiency of over 90%,” said Chien.

In cultured primary cardiac cells from humans, mice and rats, VEGF-A modRNA transfection led to rapid, transient high levels of

gene expression, whereas *VEGF-A* DNA vectors led to slow, stable high levels of gene expression. Thus, modRNA delivered a rapid pulse of *VEGF-A* to cells.

In mice, a single injection of modRNA into the heart resulted in tenfold more efficient gene expression than injection of DNA. Because injection of *VEGF-A* modRNA led to pulse-like, high-level, localized gene expression, the team set out to test whether *VEGF-A* could function as a paracrine factor and promote tissue recovery after cardiac injury.

In a mouse model of MI, *VEGF-A* modRNA and *VEGF-A* DNA injected into the ischemic region of the heart decreased infarct size and cell death and increased capillary density compared with no treatment.

However, the DNA version decreased survival, whereas the RNA version increased survival. In addition, *VEGF-A* DNA-treated infarcted hearts had blood vessels with higher levels of permeability and edema than *VEGF-A* modRNA-treated hearts.

According to Chien, the group saw the same surprising expression

kinetics and efficiency with the modRNA delivery system *in vivo* as *in vitro*. “Over 50% of the ventricle will express almost any protein, making a nice mimic of a paracrine signal. There was also no persistence of expression. The RNA was taken up quickly, made into protein and then degraded.”

To determine whether

VEGF-A modRNA promoted regeneration after MI, the scientists tracked the fate of cardiac progenitor cells. In infarcted mice with epicardial progenitor cells and their descendants labeled with GFP, *VEGF-A* modRNA increased the number of progenitor cells fourfold and increased differentiation into endothelial cells compared with luciferase modRNA control.

Results were published in *Nature Biotechnology*.

The team on the *Cell Research* and *Nature Biotechnology* papers also included scientists from the **Boston Children's Hospital**, **Harvard Medical School**, **The University of Hong Kong**, **Massachusetts General Hospital** and the **Icahn School of Medicine at Mount Sinai**.

An RNA triangle

Moderna, AstraZeneca and Karolinska are collaborating through a pair of deals to take the modRNA-based *VEGF-A* strategy into the clinic.

In March, Moderna and AstraZeneca announced a deal to discover, develop and commercialize mRNA therapeutics to treat cardiovascular and metabolic diseases and cancer. AstraZeneca paid \$240 million up front for exclusive access to targets of choice in cardiometabolic disease and select cancer targets for a 5-year period and up to 40 products.

According to Stéphane Bancel, president and CEO of Moderna, “The chemical modifications used in the paper are older modifications. We have now improved many times on those nucleotide analogs.”

One of the programs that AstraZeneca is taking to the clinic through the partnership is the *VEGF-A* platform.

“Our initial interest is in heart failure as this is a major cause of mortality worldwide, with a steady increase in prevalence,” said Marcus Schindler, head of AstraZeneca's cardiovascular and metabolic disease innovative medicines unit.

In June, AstraZeneca and Karolinska formed a joint research center—the **Karolinska Institute/AstraZeneca Integrated Cardio Metabolic Centre**. According to Schindler, “The center's aim is to identify and validate novel targets within cardiometabolic diseases. It will focus mainly on our three strategic research themes: cardiac regeneration, islet health (diabetes) and diabetic nephropathy.”

AstraZeneca will provide up to \$20 million per year for the first 5 years of the agreement. The center will feature up to 6 research groups, and 20–30 scientists from AstraZeneca and Karolinska will be full-time employees. The ongoing collaboration with Chien is one of the research programs at the institute.

According to Chien, the collaborators will follow up their mouse studies in a larger animal model, but he plans to move the *VEGF-A* project ahead to the first human studies in 18–24 months in collaboration with AstraZeneca and Moderna onsite at Karolinska.

Moderna has filed patent applications covering its mRNA platform, including chemistry, formulation, composition and dosing.

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