Myocardial Transfection of Hypoxia Inducible Factor-1 α via an Adenoviral Vector During Coronary Artery Bypass Grafting

– A Multicenter Phase I and Safety Study –

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Background: Increasing numbers of patients with advanced coronary artery disease have limited options for percutaneous and/or surgical revascularization. A prospective, randomized, phase I clinical multicenter trial was performed to assess the feasibility and safety of delivering a pro-angiogenic transcription factor termed “hypoxia inducible factor-1 α ”, delivered to ischemic cardiac muscle via a type 2 adenoviral (Ad2HIF) vector.

Methods and Results: The 13 patients were included under the following criteria: 1 hypoperfused area of viable ventricular muscle without options for revascularization and left ventricular ejection fraction $\geq 30\%$. After coronary artery bypass grafting was completed, 10 injections of the study drug ($n=10$), in 3 escalating doses up to 1×10^{11} viral particles or saline ($n=3$) as a placebo control, were injected intramyocardially. After completion of the 1-year follow-up, all patients had uncomplicated postoperative courses, are alive and feeling well; 1 patient had a self-limited run of tachycardia postoperatively and at 6 months, 1 patient developed recurrent angina. Positron emission tomography perfusion analysis revealed improvement in the Ad2HIF injected areas in selected patients.

Conclusions: These data support the feasibility and preliminary safety of adenoviral transfection with Ad2HIF in regions of viable myocardium. Additional studies will be required to determine the efficacy and safety of Ad2HIF. (*Circ J* 2010; **74**: 916–924)

Key Words: Aortocoronary bypass; Coronary angiogenesis; HIF-1 α ; Viral transfection

Ischemic cardiovascular disease remains a predominant cause of death and disability in industrialized countries. In Europe, the mortality rates range widely, depending on sex and country of origin.¹ Current therapy for advanced coronary artery disease (CAD) includes percutaneous transluminal coronary intervention, with or without stent implantation and/or coronary artery bypass (CABG), in addition to pharmacologic therapy. However, these options may be limited or inadequate in patients with diffuse atherosclerotic disease, severe small vessel CAD, and in patients who have undergone multiple prior percutaneous and/or surgical procedures.

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Specifically, in those patients it has been estimated that

revascularization is often incomplete. In 1 study, 28% of 2,860 patients with multivessel disease could not be optimally revascularized,² because of the presence of diffuse disease in small caliber and/or extensively calcified target arteries.^{2,3} Therefore, in these “limited or no option patients”, viable but underperfused areas of the myocardium present a promising target for alternative technologies, such as gene therapy approaches designed to induce angiogenesis. Animal models of either peripheral vascular or cardiac ischemia have shown evidence of bioactivity in several pro-angiogenic genes, such as isoforms of vascular endothelial growth factor (VEGF),^{4,5} or basic fibroblast growth factor (bFGF),⁶ among other related angiogenic cytokines.⁷ Delivery of either recombinant peptide angiogenic cytokines or therapeutic transgenes that induce the transcription and synthesis of these cytokines (either as

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“naked DNA” (plasmids) or packaged in a viral vector) has been used extensively to date to deliver the therapeutic construct to the target tissue.^{8–10} Preliminary clinical results from safety and biological activity studies using recombinant angiogenic growth factors encoding either FGF or VEGF isoforms in patients with myocardial ischemia have not, however, shown superiority of any single growth factor over placebo,^{11–19} although the numbers of patients studied and reported to date in these early human trials have been relatively few.

Modified Hypoxia-Inducible Factor (HIF)-1 α Transcription Factor for Therapeutic Angiogenesis

HIF-1 α is an inducible transcriptional regulatory factor that, in combination with its constitutively expressed dimerization partner, HIF-1 β (also termed ARNT), plays a principal role in regulating the cellular response to changes in oxygen tension. Through control of a number of genes, including inducible nitric oxide synthase, multiple VEGF-A isoforms and enzymes involved in glycolysis, among others, HIF-1 regulates adaptation to hypoxia at the systemic, tissue, and cellular levels.²⁰ Selectively deleting the gene regions between amino acids 390 and 826 of HIF-1 α and replacing it with the VP16 transactivation domain of herpes simplex virus results in a synthetic transcription factor capable of sustained activation of genes involved in the cellular response to hypoxia (Genzyme Corporation, Cambridge, MA, USA).

Preclinical evidence for efficacy of this adenoviral HIF-1 α /VP16 vector (Ad2HIF) has been documented extensively both *in vitro* and *in vivo*, and was established in small and large animal model systems in several species, using, for example, both rat and murine models of hindlimb ischemia, as well as large animal models of coronary ischemia, utilizing canine and bovine preclinical models. In several experimental model systems that mimic occlusive vascular disease,

Table 1. Study Design

Cohort	Dose	Treatment patients	Control patients	Total patients
1	1 $\times$ 10 ¹⁰	3	1	4
2	3 $\times$ 10 ¹⁰	3	1	4
3	1 $\times$ 10 ¹¹	4	1	5
Total		10	3	13

we have demonstrated that the constitutively active HIF-1 α transgene (ie, Ad2/HIF-1 α /VP16) is at least as beneficial as recombinant proteins and pro-angiogenic transgenes under similar experimental conditions, at least within the time frame provided by the expression of the Ad2/HIF-1 α /VP16 construct, before the immune system could recognize and clear the construct.

Similarly, in a porcine model of chronic myocardial ischemia,²¹ Ad2HIF over a concentration range from 10⁸ to 10¹⁰ viral particles (VP), showed significantly improved perfusion and improved ventricular function after 4 weeks.

These preclinical safety and toxicity studies done in support of regulatory approval for this study have demonstrated safety to support the proposed highest dose of 1 $\times$ 10¹¹. Preclinical data from the 90-day intramyocardial toxicity study in rats with a dose of 1 $\times$ 10¹⁰ support dosing of 1 $\times$ 10¹¹ in humans.

In humans, the Ad2HIF vector–transgene combination has been used in patients suffering from critical limb ischemia. Those patients received injections containing Ad2HIF up to 2 $\times$ 10¹¹ VP in the lower limb, which was well tolerated.²²

Methods

Description of Ad2/HIF-1 α /VP16 Production

Ad2HIF is a replication-deficient adenovirus of serotype 2 (Ad2) in which the gene insert consists of the DNA-binding and dimerization domains of the HIF-1 α subunit combined with the VP16 transactivation domain of herpes simplex virus. Gene expression is controlled by a cytomegalovirus promoter and the polyA signal is from SV40. Production of the clinical material was initiated by infection of primary human embryonic kidney 293 cells with Ad2HIF. These cells were then harvested, followed by cell lysis by DNA digestion and filtration. Purification was achieved using column chromatography and filtration to concentrate vector concentration and final sterile filtration. The resulting formulated bulk subsequently underwent final dilution in phosphate-buffered saline in 10% sucrose.

Study Design

The trial was conducted as multicenter phase I, randomized, double-blind, placebo-controlled, dose-escalating study investigating the safety, bioactivity, and potential clinical outcomes of direct intramyocardial injection of Ad2HIF in phosphate-buffered saline with 10% sucrose or placebo (buffered saline). The use of buffered saline as placebo instead of an empty adenoviral vector as a control for studies in humans was not accepted by the regulatory agencies at the time this study was conducted, the rationale being that even the use of an empty adenoviral vector could have still posed some safety risks. Empty adenoviral vectors have been used as controls in all of the important preclinical studies (safety and efficacy) that justified moving into clinical trials with Ad2HIF. In those animal studies the use of empty adenoviral vectors as

Table 2. Cardiovascular History and Baseline Cardiovascular Characteristics

Parameter	Pooled HIF-treated (n=10)	Pooled placebo (n=3)	All (n=13)
Gender, male n (%)	8 (80)	2 (67)	10 (77)
Age (years) at operation, mean (range)	61 (48–76)	59 (58–65)	59 (58–76)
BMI, kg/m ² (median, range)	28.8 (20.8–32.3)	27.7 (25.3–28.1)	28.1 (20.8–32.3)
Arrhythmias (present or past), n (%)	4 (40)	1 (33)	5 (38)
Atrial fibrillation	0	0	0
Ventricular fibrillation	0	0	0
Bradycardia	0	0	0
Tachycardia	2 (20)	0	2 (15)
Other	4 (40)	1 (33)	5 (38)
Previous MI, n (%)			
Non-Q-wave	2 (20)	2 (67)	4 (31)
Q-wave	3 (30)	1 (33)	4 (31)
Previous CABG, n (%)	1 (10)	0	1 (8)
Previous PTCA/stent, n (%)	2 (20)	1 (33)	3 (23)
CCS angina class, n (%)			
I	1 (10)	0	1 (8)
II	2 (20)	2 (67)	4 (31)
III	7 (70)	1 (33)	8 (62)
LVEF (%) median (range)	63 (44–74)	65 (52–70)	64 (44–74)
Valvular heart disease (present or past), n (%)	1 (10)	0	1 (8)
PVD (present or past), n (%)	1 (10)	1 (33)	2 (15)
Hypertension (present or past), n (%)	8 (80)	3 (100)	11 (85)
Hyperlipidemia, n (%)	8 (80)	3 (100)	11 (85)
Diabetes, n (%)	4 (40)	0	4 (31)
Smoking status, n (%)			
Current	2 (20)	1 (33)	3 (23)
Former	3 (30)	2 (67)	5 (38)

Percentages are based on the total number of patients in each treatment group.

HIF, hypoxia inducible factor; BMI, body mass index; MI, myocardial infarction; CABG, coronary artery bypass graft; PTCA, percutaneous transluminal coronary angioplasty; CCS, Canadian Cardiovascular Society; LVEF, left ventricular ejection fraction; PVD, peripheral vascular disease.

controls added rigor and strengthened confidence in the positive Ad2HIF.

Patients were enrolled in 3 sites and 4 surgeons were appointed by the Principal Investigators at each site based on their proven experience. The test agent was administered to patients with multivessel CAD undergoing elective CABG who could not be completely revascularized during the surgical procedure. Patients received 1 or more bypass grafts during the procedure, 1 of which was to the left anterior descending artery (LAD) if flow to this vessel was compromised. Using SPECT radionuclide imaging of blood flow, an area of viable, but underperfused left ventricular myocardium had to be identified in either the right coronary artery (RCA) or the left circumflex (LCX) artery distributions that remained following placement of the bypass conduits. These underperfused areas received injections containing either Ad2HIF or placebo to promote therapeutic angiogenesis. Each patient received a single dose of the test article in 1 administration comprising 10 injections in a prespecified area of ischemic but viable myocardium (**Figure 1**). Patients were enrolled in 1 of 3 dosing or placebo groups as shown in **Table 1**. Additional screening assessments were done with a view to the possibility of side-effects caused by the study agent (ie, either Ad2HIF or placebo injection). Subjects were excluded if they had a history of cancer within the previous 5 years, proliferative retinopathy, or immune suppression (of

any cause). Lactating females were excluded and both men and women had to agree to use a barrier method of contraception for a minimum of 3 months after participating in the trial.

Follow-up

After surgery and administration of the test article, the patients underwent routine monitoring and postoperative care as indicated for a CABG procedure with periodic follow-up visits on days 7, 14, 21, 30, 45, 60, 90, 180 and 365 to assess the presence of adverse events (AE), as well as undergoing imaging studies to assess changes in ventricular function (ie, echocardiography and magnetic resonance imaging (MRI)) and myocardial perfusion (positron emission tomography: PET). To identify the injected areas all surgeons received study-specific training on how to record the injections given during surgery in a 16-segment model that was used for assessments such as echocardiography and PET. No specific markers were used.

In addition to routine clinical laboratory parameters, such as hematology, blood chemistry, coagulation, urinalysis and stool analysis, adenoviral titers and VEGF levels were evaluated.

PET

The quantification of myocardial blood flow was assessed

as the amount of flow reserve, defined as the ratio of stress retention over rest retention. Polar maps were used to calculate segmental LV values, which were analyzed by computer software to provide independent, unbiased data. Grafted as well as injected segments were analyzed and compared. Patients underwent rest and dipyridamole stress ^{13}N -ammonia dynamic PET imaging at baseline and after 60 and 180 days of follow-up. A blinded analysis of the data was performed by independent core laboratories.

Echocardiography

Transthoracic echocardiography at rest and under stress with dobutamine was performed at screening before the operation and postoperatively at days 7 or 14 and 60 and 180, respectively. Parasternal long- and short-axis, and apical 2- and 4-chamber views were acquired and recorded for subsequent analysis in a core lab. The LV was divided into 16 segments²³ and a regional wall motion score was used (normal=1, hypokinetic=2, akinetic=3, dyskinetic=4).

The study design, protocols and informed consent forms were approved by the local ethics committees, the German Arbeitsgemeinschaft Somatische Gentherapie and the Gene Therapy Advisory Committee in the UK, and Biosafety Committees according to national regulations. A Data Safety and Monitoring Board (DSMB) provided an ongoing, expert, independent review of safety data to assure that the risks to study patients were minimized during the conduct of the study. This protocol was conducted, recorded, and reported in compliance with the principles of good clinical practice regulations. These requirements are stated in the "Guidance for Good Clinical Practice", International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (available at: <http://www.fda.gov/cder/guidance/959fnl.pdf> [accessed January 29, 2007]).

Statistical Analysis

All patients receiving Ad2HIF or placebo injections were included in the safety analyses. No hypothesis testing between groups was planned or conducted in these phase I dose-escalation studies because of the small number of patients included in the study.

Results

Baseline Data

A total of 13 patients were screened, and all were enrolled; 10 patients were treated with Ad2HIF and 3 were given placebo as noted in **Table 1**.

All 13 patients received aortocoronary bypasses during the operation according to the inclusion criteria already noted and they completed the study and the required follow-up visits. Demographics and baseline cardiovascular characteristics and the distribution of bypassed coronary branches are listed in **Table 2**.

All patients received at least 1 bypass graft to the LAD. The target areas for study drug injections were exclusively in either the LCX and/or RCA distributions (**Table 3**).

Survival

No deaths occurred during the 1 year of follow-up.

Cardiac Disorders

AEs concerning the heart occurred in 9 patients (7 Ad2HIF-treated and 2 placebo patients). The 2 AE that occurred in the placebo patients were considered possibly related (sinus

Table 3. Grafted and Injected Areas

	HIF-1 α group (n=10)	Placebo group (n=3)
Grafted area		
LAD	10 $\times$ LIMA, 1 $\times$ SVG	2 $\times$ LIMA, 1 $\times$ SVG
LCX	4 $\times$ RIMA, 5 $\times$ SVG	1 $\times$ Rad., 1 $\times$ SVG
RCA	4 $\times$ SVG	2 $\times$ SVG
Injected area		
LAD	0	0
LCX	7	3
RCA	3	0

LAD, left anterior descending artery; LIMA, left internal mammary artery; SVG, saphenous vein graft; LCX, left circumflex artery; RIMA, right internal mammary artery; Rad., radial artery; RCA, right coronary artery. Other abbreviation see in Table 2.

bradycardia and ventricular extrasystoles) and were categorized as mild. Four Ad2HIF-treated patients experienced atrial fibrillation (all either unrelated or unlikely to be related events) while on the study. These events were consistent with the advanced disease state of the patient population in this study and their status as post-CABG patients. One additional patient from the 1 $\times$ 10¹¹ VP group, noted as having a serious AE (SAE; see below), had an asymptomatic, self-limited run of ventricular tachycardia on a Holter ECG recording. Follow-up electrophysiological investigation revealed no evidence of additional pro-arrhythmic events.

AEs

In total, 123 AEs occurred during the 1-year observation period, of which 2 were classified as serious and both of which occurred in the 1 $\times$ 10¹¹ VP/ml Ad2HIF-treated group.

One case of vasovagal syncope with onset at 11 days post surgery occurred in a 74-year-old male patient. As mentioned above, a 19-beat run of ventricular tachycardia occurred 1 month post surgery in a 75-year-old male patient who had had 1 previous episode of ventricular arrhythmia 12 days before receiving the study drug. Both events recovered without sequelae. The DSMB did not consider any of the SAEs as unexpected in this patient population. At no point did the DSMB request that the study data be unblinded nor did they express any safety concerns.

The majority of the 123 treatment-emergency AEs were mild to moderate in severity and were judged by the Investigators to be unrelated to study treatment (94 AEs for HIF-treated patients (n=10 patients) vs 29 AEs for placebo patients (n=3 patients)). These events could be categorized as typical postoperative events such as postoperative pain, pyrexia, and pleural effusions. One HIF-1 α treated patient presented with a postoperative wound infection. Patients also underwent retinal examinations to determine whether there was any acceleration of active proliferative diabetic retinopathy, a theoretical risk of using a constitutively active HIF-1 α transgene. Retinal examinations were performed for all subjects entered into the trial. No patient developed an active proliferative diabetic retinopathy or evidence of choroidal neovascularization.

Other events of interest regarding potential safety concerns, such as inflammation induced by the adenoviral vector, as well as direct effects of the several pro-angiogenic factors that are known to be induced by HIF-1 α (eg, local edema at the injection sites, flu-like symptoms, hepatobiliary disease, and precancerous changes or tumors) were not reported as treatment-emergency events.

Figure 2. Blood levels of selected biomarkers over time, before and after injections of Ad2HIF, including white blood count (WBC) and C-reactive protein (CRP), as well as the myocardial markers creatine kinase (CK) and troponin I, and brain natriuretic peptide (BNP) at preoperative screening (SCR), on the day of surgery and after 180 days of follow-up.

Clinical Laboratory Evaluation

As noted earlier, a potential concern for this study was the perceived theoretical safety risks of using a replication deficient adenoviral vector and a constitutively active HIF-1 α transgene. Nevertheless, there was no evidence of study-related changes in clinical laboratory testing parameters during the follow-up phase. White blood cell (WBC) counts, for example, showed inconsistent values over the entire follow-up period, with no differences between transfected and placebo patients. Another marker of active inflammation,

the plasma levels of the innate immunity protein C-reactive protein (CRP), peaked as expected early after operation and then decreased during the follow-up observation period. It appeared that in transfected patients this decrease took longer than in patients receiving placebo (Figure 2), although the data are too limited to draw any firm conclusions. The myocardial markers creatine kinase (CK) and troponin I both showed elevated levels early after the operation, and then fell to minimally elevated levels by 3 weeks after the procedure, with a slightly increasing tendency up to day 180 with

no preference for any patient group. Also, brain natriuretic peptide (BNP) levels rose as expected postoperatively, with the highest values in the early recovery phase after the operation, followed by a return to baseline, once again with no differences between treated and placebo patients.

Adenoviral Antibody Titer

Figure 3 presents Ad2 antibody detection using an ELISA assay for adenovirus-specific IgG in sera from the treated and the control subjects. Ad2 antibody titers were elevated over baseline in the HIF-treated patients, peaking between days 7 and 21 after the operation and decreasing during the follow-up period, although antibody levels remained above baseline through the remainder of the postoperative follow-

up. Patients who received placebo showed no elevation of Ad2 antibody titers, as expected.

VEGF Blood Levels

In most patients the VEGF-A blood levels did not change significantly during the observation period. The levels in 2 placebo and 1 treated patients peaked above all others, neither of which correlate with any specific clinical event.

Echocardiography

Echocardiographic evaluation at follow-up indicated an increase in the LV ejection fraction (EF) for the treated patients from 50% before operation to 55% after 6 months, whereas the placebo group exhibited a decline from 55% to

PET Data

Myocardial Blood Flow in $\text{ml} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ myocardial tissue

Figure 5. Myocardial blood flow detected by positron emission tomography (PET) was used to evaluate differences between treated (study agent) and placebo patients during follow-up. Changes in blood flow as an expression of the efficacy of the study drug are depicted at rest and under pharmacological stress for both groups. Perfusion imaging compared baseline data (ie, at day 7 or 14 after operation) with day 60 and day 180 after the surgical procedure. Evaluation of the data was done by independent core labs blinded to the treatment assignment.

50% until day 60, rising modestly to 53% at day 180 (statistically not significant; [Figure 4](#)).

MRI

MRI was performed in 6 of 13 patients, as this test was only done at selected hospitals that had MRI capability. The changes in LV function by MRI were similar to those observed during echocardiography, which showed no significant changes in LV function between treated and control subjects.

PET

PET for myocardial perfusion imaging was done at a baseline reference time point (on day 7 or 14) and on days 60 and

180 during follow-up, with analysis carried out by independent core laboratories blinded to treatment assignments ([Figure 5](#)). The flow reserve for the 23 observed grafted segments showed values between 1.39 and $3.82 \text{ ml} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ myocardial tissue, with no distinct changes during follow-up. Differences between the 17 grafted segments of the dose cohorts and the 6 grafted segments of the placebo patients did not reach clinical significance. In 36 injected segments that were not grafted, flow reserve ranged from 1.0 to $2.81 \text{ ml} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ tissue values during the follow-up. There were no significant differences between the 29 segments of the 3 treated cohorts and the 7 segments of the placebo group. It appeared that the 3 placebo patients showed a higher flow

reserve in both types of segments, but without clinical significance. **Figure 5** displays the mean values for coronary flow reserve of the 3 dosing cohorts (n=10) and the 3 placebo patients. The depiction was done separately for grafted and for injected segments, respectively.

Discussion

Therapeutic angiogenesis, as with any new and promising technology, must be sufficiently effective to provide sufficient reasonably durable benefit at an acceptable risk to patients. This is 1 of the first clinical studies to use a constitutively active formulation of the endogenous transcription factor HIF-1 α as a therapeutic transgene, a “master switch” gene that initiates and choreographs the expression of multiple pro-angiogenic genes involved in the response to tissue hypoxia. In this study, Ad2HIF was administered to regions of ischemic but viable myocardium in patients with no other therapeutic options for revascularization of a predetermined ischemic territory. As formation of new vessels is a complex procedure that is dependent on many coherent steps, we hypothesized that the approach described here should be superior to using any single pro-angiogenic factor.^{12–16} The most important finding of this study is that Ad2HIF appeared to be safe when delivered into myocardial muscle during CABG procedures at doses as high as 1×10^{11} VPs, using the dose escalation schedule as planned. All patients survived. No study-related problems had emerged within 1 year of follow-up, such as any evidence of acceleration of a malignancy or ocular neovascularization disorders. Additionally, no new safety concerns were identified. Focusing on the adenoviral vector itself, the data reported here are in concordance with earlier adenoviral studies for therapeutic angiogenesis, further emphasizing the safety of this approach for treatment of advanced vascular disease.^{11,16} With respect to pro-angiogenic transgenes, other clinical trials in cardiovascular disease using single vascular growth factors have also shown no indications of study agent related side-effects.^{11–16,24,25} Indeed, the safety profile in this clinical trial using Ad2HIF in advanced cardiac ischemia is consistent with results with the same vector and experimental transgene in patients suffering from peripheral arterial disease.²² The safety data in this trial reflect the relatively benign results to date regarding SAEs in angiogenesis trials.^{11–16,22,24–26}

Although Ad2HIF was administered to myocardial areas that were not amenable to conventional bypass, it was anticipated that this phase I safety study would not have sufficient power to be able to distinguish between clinical benefit related to the gene therapy and clinical benefit resulting from the restoration of myocardial perfusion to other areas of the myocardium by the CABG procedure. Trends toward modest improvements in ventricular function in the targeted areas of myocardium in selected patients who received Ad2HIF were apparent via echocardiography (**Figure 4**) and MRI. Similar results were apparent with PET myocardial perfusion data (**Figure 5**). Importantly, there was no evidence of worsening cardiac function in those segments targeted for Ad2HIF injections.

The numbers of both, treated and placebo patients were relatively small in this study, and consequently, very large differences in outcome parameters would have been necessary to provide statistical evidence of efficacy. Thus, the lack of statistical power obviously limits the analysis of differences in clinical outcomes between the treated and placebo patient groups, both at baseline and after treatment inter-

vention. All these factors could have been influenced by the high level of concomitant background morbidity as well in this high-risk patient population. Other trials using single vascular growth factors, which have included many more patients than reported here, have documented only sporadic positive results, rarely citing any statistical proof.^{13–15} There have been 2 recent exceptions, however, in a statistically adequately powered trial phase 2 clinical trial: the results of the TALISMAN trial in 125 critical limb ischemia patients treated with a plasmid formulation of hepatocyte growth factor,²⁵ and another cardiac study combining VEGF administration (ie, VEGF-A₁₆₅) with L-arginine, which documented an increased blood flow reserve as determined by PET scanning.²⁶

Regarding safety issues with intracardiac injections of Ad2HIF, the reported AEs include pericardial and pleural effusion, pulmonary problems, chest pain, pyrexia, atrial and ventricular arrhythmias and renal failure, all of which were considered to be consistent with the patients' underlying diseases and concomitant treatment regimens.

Regardless, longer-term studies in a larger number of patients will be needed, however, to establish both the safety and efficacy of the therapeutic transgene and viral vector.

Conclusions

Overall, there have been no major adverse consequences of Ad2HIF treatment identified during 1 year of follow-up, following 10 direct intramyocardial injections during CABG. The dose escalation schema was completed as planned. No new safety concerns were identified in addition to the theoretical concerns described previously. Although the small sample size and limitations in the comparator group, as well as the high level of background morbidity in this patient population, pose limits on the interpretation of the data, it appears that Ad2HIF can be safely administered as an adjunct to CABG surgery at doses of up to 1×10^{11} VP/ml.

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Competing Interests

Roland Hetzer, Bruno Reichart and Ravi Pillai were primary investigators for this Genzyme-sponsored clinical study. All other authors report no conflicts.

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