

## Effects of Ad5FGF-4 in Patients With Angina

### An Analysis of Pooled Data From the AGENT-3 and AGENT-4 Trials

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#### Objectives

The goal of this study was to explore the effects of angiogenic gene therapy.

#### Background

Preclinical studies with intracoronary administration of Ad5FGF-4 (alferminogene tadenovec, Generx, Berlex Biosciences, Richmond, California) suggested it could induce angiogenesis and provide a new clinical approach to the treatment of chronic angina pectoris. Two preliminary clinical trials provided evidence that it could improve exercise treadmill test (ETT) time and myocardial perfusion. The AGENT (Angiogenic GENE Therapy)-3 and -4 trials of a low and high dose of Ad5FGF-4 for chronic angina were initiated in the U.S. and other countries and enrolled 532 patients in a randomized, double-blind, placebo-controlled fashion. Both studies were halted when an interim analysis of the AGENT-3 trial indicated that the primary end point change from baseline in total ETT time at 12 weeks would not reach significance.

#### Methods

We performed a pooled data analysis from the 2 nearly identical trials to investigate possible treatment effects on primary and secondary end points in prespecified subgroups.

#### Results

The effect of placebo was large and not different than active treatment in men, but the placebo effect in women was negligible and the treatment effect was significantly greater than placebo. We found a significant, gender-specific beneficial effect of Ad5FGF-4 on total ETT time, time to 1 mm ST-segment depression, time to angina, and Canadian Cardiovascular Society class in women. This is the first clinical report of a gender difference in response to cardiac angiogenic therapy.

#### Conclusions

The potential importance of the observed gender-specific angiogenic response on the clinical treatment of refractory angina is substantial and deserves further investigation. (Efficacy and Safety of Intracoronary Ad5FGF-4 in Patients With Stable Angina; <http://www.clinicaltrials.gov/ct/show/NCT00346437>; NCT00346437) (Safety and Efficacy of Intracoronary Ad5FGF-4 in Patients With Stable Angina [AGENT-4]; <http://www.clinicaltrials.gov/ct/show/NCT00185263>; NCT00185263) (AWARE; <http://www.clinicaltrials.gov/ct/show/NCT000438867>; NCT000438867) (J Am Coll Cardiol 2007;50:1038-46) © 2007 by the American College of Cardiology Foundation

Angina pectoris is a disabling manifestation of coronary artery disease (CAD). In the U.S., 8.9 million people live with chronic angina, and an additional 400,000 people are newly diagnosed each year (1). There are 2 major mecha-

nistic approaches to the treatment of chronic angina. First, currently approved drug therapy includes beta-blockers, calcium-channel blockers, long-acting nitrates, and, most recently, ranolazine. Drug therapy alters the supply/demand relationship between the coronary arterial tree blood flow and cardiac muscle oxygen requirements. Second, revascularization by angioplasty, stent, or bypass surgery either reopens or bypasses blockages in the epicardial vessels. However, many patients continue to have recurrent angina with drug therapy and are not suitable for revascularization procedures (1). New mechanistic approaches are needed to improve blood flow to the myocardium (2).

In contrast to the mechanisms involved in approved therapeutic methods, the heart's natural responses to isch-

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emia include arteriogenesis and angiogenesis leading to the development of coronary collaterals. However, in patients with persistent angina, collateral formation is inadequate and/or the process stops before stress-induced ischemia is relieved (2). Enhancing angiogenesis and collateral formation represents a new mechanistic approach for which there are no approved products (2,3). Pioneering work by Dr. Jeffery Isner and colleagues showed the feasibility of augmenting angiogenesis (4–6). A series of promising phase 1 trials have led to a number of phase 2 randomized placebo-controlled trials with angiogenic proteins (7–10) and growth factor gene therapy (11–15).

The preclinical development of the angiogenic therapy product Ad5FGF-4 (replication deficient, E1A/E1B-deleted, human adenovirus serotype 5 with human FGF-4 gene insert: alferminogene tadenovec, Genex, Berlex Biosciences, Richmond, California) began with a pig model of coronary ameroid occlusion. In this model of chronic, stress-induced ischemia, intracoronary administration of a first-generation serotype 5 adenovector with either the FGF-5 gene (16) or FGF-4 gene (17) inserted increased angiogenesis evidenced by histology and relieved the stress-induced ischemia evidenced by increased left ventricular blood flow and function. Furthermore, there was remarkably high first pass uptake in the heart. High first pass uptake by the heart and preliminary evidence of efficacy were confirmed in an ascending-dose clinical trial in patients with refractory angina (AGENT [Angiogenic GENE Therapy]-1) (11), and a subsequent myocardial stress perfusion study (AGENT-2) confirmed increased flow (12). Based on these results, the phase 2b/3 AGENT-3 trial was initiated by Berlex in the U.S., and AGENT-4 was initiated by Schering AG in Europe, Latin America, and Canada (Berlex Canada). These trials enrolled a total of 532 patients under nearly identical protocols before enrollment in both trials was discontinued as described in the following text.

A preliminary cluster analysis of the AGENT-3 trial suggested a positive effect in some subgroups. Accordingly, we have performed an analysis of pooled original data from the AGENT-3 and -4 trials to investigate whether gender and older patients with severe angina subgroups would show possible treatment effects, and to ascertain if significant new hypotheses could be generated given the positive results of the AGENT-2 trial. We report here a positive response in clinical symptoms overall and a markedly positive effect in women, suggesting a gender-specific effect.

## Methods

**AGENT-3 and -4 protocols.** The AGENT-3 trial was initiated in October 2001 and the AGENT-4 trial in March 2002. Enrollment was stopped for both trials in January 2004, when a planned interim analysis of the AGENT-3 trial revealed the study was unlikely to yield a statistically significant result on the primary efficacy end point of change

from baseline in exercise treadmill time duration at 12 weeks. Both trials were designed as parallel group, double-blind, placebo-controlled, randomized studies using 2 active dose groups; a low dose of Ad5FGF-4,  $1 \times 10^9$  viral particles (vp), a high dose of  $1 \times 10^{10}$  vp, and placebo in a 1:1:1 randomization ratio with preplanned interim analyses. Protocols were approved by each institution's institutional review board for human subjects. Patients providing informed consent included patients 30 to 75 years of age with a history of recurrent stable angina (Canadian Cardiovascular Society [CCS]

class II to IV) who remained symptomatic despite antianginal medication and who did not require immediate percutaneous coronary intervention (PCI) or coronary artery bypass graft (CABG) surgery (AGENT-3) or were technically unsuitable for PCI or CABG (AGENT-4); the latter criteria was the only difference between the 2 studies. Patients had a left ventricular ejection fraction of  $\geq 30\%$  measured within 12 months of enrollment. Patients had 1-, 2-, or 3-vessel CAD, but patients with 3-vessel disease had to have at least 1 proximal major vessel or graft with  $< 70\%$  stenosis (to provide a conduit to collateral vessels).

To be randomized for treatment, patients had to be able to exercise for at least 3 min but no more than 10 min, and stop for moderately severe angina (grade 3 or 4 on a 1 to 4 scale) on the first qualifying baseline exercise tolerance test (ETT) using the modified Balke-Ware exercise protocol. Patients had to demonstrate  $\geq 1$  mm ST-segment depression (horizontal or down sloping) from baseline in the qualifying ETT. The variability in exercise duration (time to grade 3/4 angina or angina equivalent) had to be within 20% on 2 consecutive qualifying tests. The ETTs were performed in the morning with all antianginal medication withheld. The individual patient's antianginal medication had to be stable for 4 weeks before enrollment.

Exclusion criteria included patients with unstable angina, electrocardiograms unable to show myocardial ischemia (e.g., left bundle branch block, Wolff-Parkinson-White syndrome, atrial fibrillation), illness that might interfere with the patients' ability to perform the ETT, untreated life-threatening ventricular arrhythmias, left main coronary artery stenosis  $\geq 70\%$  unless bypassed by a patent graft, CABG surgery in the past 6 months unless all vessels were 100% occluded, myocardial infarction in the last 8 weeks, congestive heart failure (New York Heart Association [NYHA] functional class IV) despite treatment, PCI in the previous 6 months, prior transmyocardial laser revascularization, enhanced external counterpulsation within 12

## Abbreviations and Acronyms

<b>CABG</b>	= coronary artery bypass graft
<b>CAD</b>	= coronary artery disease
<b>CCS</b>	= Canadian Cardiovascular Society
<b>ETT</b>	= exercise tolerance test
<b>FGF</b>	= fibroblast growth factor
<b>NYHA</b>	= New York Heart Association
<b>PCI</b>	= percutaneous coronary intervention
<b>VEGF</b>	= vascular endothelial growth factor

weeks, moderate-to-severe nonproliferative or proliferative retinopathy from any cause (ETDRS [Early Treatment Diabetic Retinopathy Study] score >35), clinically significant macular edema or previous photocoagulation therapy, creatinine clearance <45 ml/min using the Cockcroft and Gault formula, immunosuppression therapy, and women of child-bearing potential (see NCT00346347 for further details). Patients were screened for cancer along the guidelines of the American Cancer Society. Patients were followed with clinic visits at weeks 2, 4, 8, and 12, and at months 6, 12, 18, and 24 with yearly telephone contact to continue through November 2008. Every effort was made to keep cardiovascular medication constant unless required by changes in clinical condition.

Treatment group allocation was randomized centrally and blinded to everyone except the data safety monitoring board statistician. After screening and randomization, using routine cardiac catheterization procedures, Ad5FGF-4 was delivered by intracoronary infusion over 90 s per injection followed by a 5-ml 90-s flush, with the dose divided among all major coronary arteries (right coronary artery, circumflex, left anterior descending coronary, and bypass grafts) targeting 60% of the total dose to the left coronary system and 40% to the right coronary system. A standard subselective catheter of the investigators' choice was used from among 4 catheters that had been shown to be biocompatible with Ad5FGF-4.

**Statistical analysis.** The primary end point in the AGENT-3 and -4 trials was change from baseline in total ETT time at 12 weeks, and at secondary time points of 4 weeks and 6 months. Prespecified secondary end points in both studies included time to 1 mm ST-segment depression, time to onset of angina, change in CCS class, percentage of patients with  $\geq 30\%$  increase in ETT, coronary events or death at 1 year, change in angina incidence and nitroglycerin use by patient diary, and change in quality of life. Subgroup analyses by gender, age, and angina severity were also prespecified in the AGENT-3 and -4 trials.

Based on the results of a cluster analysis of the AGENT-3 trial, we performed a pooled data analysis to identify whether there was an overall effect, and whether the subgroups of gender and older patients with more severe angina led to plausible hypotheses worthy of further clinical testing. The AGENT-3 and -4 trial databases were concatenated since the case report forms were identical, resulting in a pooled database. For safety data, all observations were included. For primary and secondary end points, a valid patient population was used. Thus, we censored at least 1 invalid ETT result in 15 patients (2 women) in whom the ETT protocol was not conducted according to the modified Balke-Ware protocol making the ETTs not comparable. We also censored ETT data from 22 patients (1 woman) obtained after an interim revascularization, angioplasty, or CABG, or who did not have an ETT at week 12. Only

available data were used; we did not carry forward last observations when data were missing.

A 2-way analysis of variance (ANOVA) was used to test for gender subgroup interaction with primary and secondary end points. The Wilcoxon rank sum test was used for differences in primary and secondary end points between patient groups. We report here only prespecified subgroup analyses and primary and secondary end points from the statistical analysis plan for the pooled analysis. Since this pooled analysis was hypothesis-generating, we report both uncorrected p values and note where the Bonferroni correction for the 3 subgroups (men, women, and older patients with more severe angina) remains significant ( $p < 0.0167$ ).

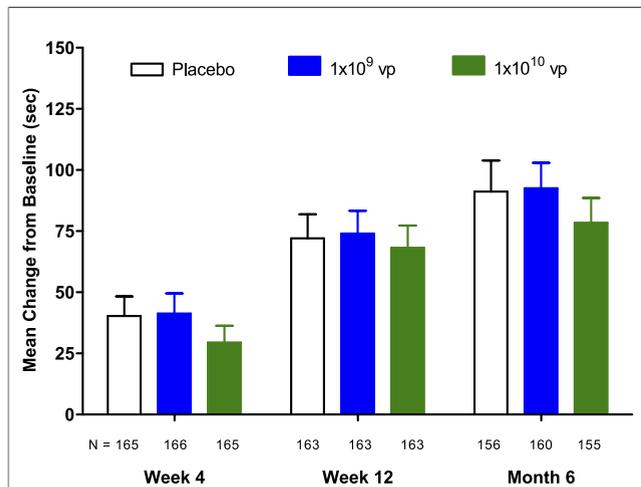
## Results

The AGENT-3 trial enrolled 139 placebo, 137 low-dose, and 140 high-dose patients, and the AGENT-4 trial enrolled 38 placebo, 43 low-dose, and 35 high-dose patients for a total of 532 patients in the 2 studies. There were 489 patients with evaluable ETT data at 12 weeks including 163 placebo, 163 low-dose, and 163 high-dose patients available for analysis. Separate analysis of the AGENT-3 and -4 trials failed to show any differences between placebo and treatment for any primary or secondary end points, with the exception of older patients with more severe angina and CCS class in the AGENT-3 trial, differences largely due to the female cohort. The results of the 2 trials were quite similar; therefore, we report here only the pooled analysis results.

**Primary and secondary end points.** In the valid case population (and the intent-to-treat population), using the pooled data from the 2 studies, there was no significant difference between the active groups and the placebo for the primary end point, change from baseline exercise time at 12 weeks (Fig. 1). There was a large and significant placebo effect compared with baseline, which persisted over 6 months. There were no differences between either dose group and placebo for any of the secondary end points except for CCS class. Subgroup analysis did show a significant effect on the primary end point in older patients with more severe angina. As discussed in the following text, these differences were biased by a large effect in women, and no effect in men.

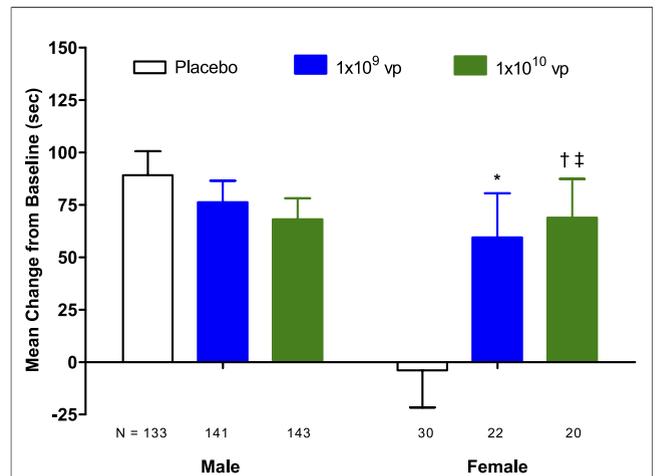
**Clinical effects.** The distribution of changes from baseline for CCS class for all patients was significantly improved over placebo only for the high-dose group at week 12, month 6, and month 12 ( $p < 0.05$ ). The latter effect was driven by the female population; in the male only subgroup, there was no significant change in CCS class. The changes in Seattle Angina Questionnaire, Medical Outcomes Study Short Form 36, angina frequency, or nitroglycerin use were not significant.

**Subgroup of older patients with more severe angina.** Subgroup analysis in all patients over age 55 years with more severe angina at baseline (CCS III or IV) also showed



**Figure 1** Change in ETT Time for All Patients

Change from baseline in total exercise time for all valid case patients (men and women) at 4 weeks, 12 weeks, and 6 months. Data are mean and standard error. ETT = exercise treadmill test; vp = viral particles.



**Figure 2** Change in ETT Time at Week 12 by Gender

Change from baseline in total exercise time at week 12 by gender. Data are mean and standard error. \* $p < 0.05$ , † $p < 0.01$ , †† $p < 0.0167$  (Bonferroni correction for subgroup analysis). Abbreviations as in Figure 1.

significant differences or trends in primary and secondary end points. Possible trends were not present in the male only population. None of these end points remains significant after Bonferroni correction.

**Subgroups by gender.** The baseline exercise times for men were not different across treatment groups, but for women baseline ETT time for the high dose was less than for the low dose (Table 1). Baseline CCS class was also similar across treatment groups for both gender subgroups, with mean values for women of 2.8 for placebo, 2.6 for low dose, and 3.0 for high dose.

Prespecified subgroup analysis by gender at 12 weeks of follow-up revealed a significant effect in women in the primary end point, total ETT time, for both dose groups (Fig. 2, Table 1) ( $n = 72$ ), but no effect in men ( $n = 417$ ). The gender treatment interaction by 2-way ANOVA for total ETT time was significant for the high-dose group at 12 weeks and 6 months. The difference between men and women was due to a negligible placebo effect in women compared with a large placebo effect in men, as the improvement in total ETT time over baseline in both active dose groups was similar in men and women. The gender-

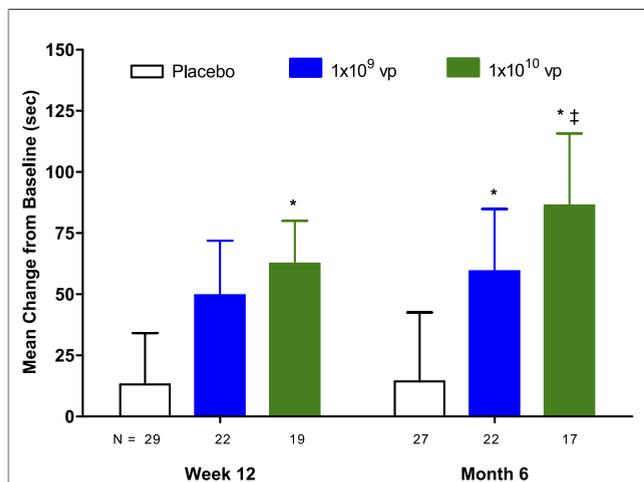
specific effect was also significant in women, or nearly so, for both active dose groups in the secondary end points of time to 1 mm ST-segment depression, time to angina, and CCS class (Figs. 3 to 5) at both 12 weeks and 6 months. The changes observed from baseline in the end points of total ETT time, time to 1 mm ST-segment depression, and CCS class and their  $p$  values suggested a dose-response effect. The primary end point (Table 1) and secondary end points (Figs. 3 to 5) remained significant after Bonferroni correction mostly for the high-dose group. There were no trends for efficacy in any of these end points in men. The durability of the effect was supported by a significant improvement in CCS class at 12 months in women (Fig. 5), but not in men (data not shown).

**Effect at 6 and 12 months in women.** Positive effects were seen only in the female subgroup and not in the population as a whole. In general, the positive effects on ETT duration, time to 1 mm ST-segment change, and time to angina in the female population were either significant or at least showed a nearly significant trend at 6 months (Figs. 3 and 4). The durability of the effect is confirmed by sustained improvement in CCS class at 12 months (Fig. 5).

**Table 1** Total Exercise Time Results (Seconds) in Female Patients

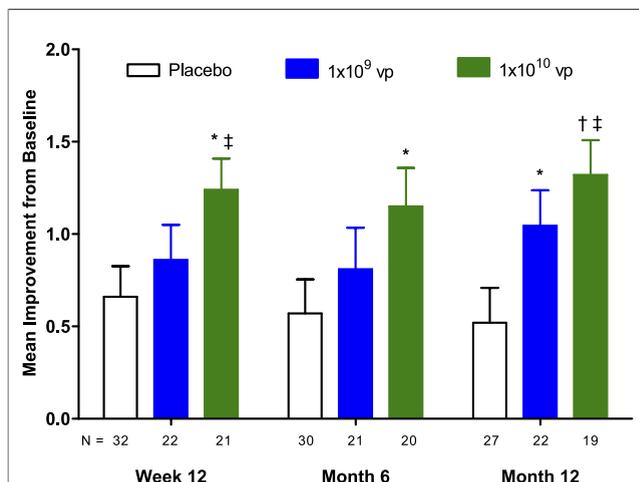
Study Visit	Ad5FGF-4					
	Placebo		1 × 10 <sup>9</sup> vp		1 × 10 <sup>10</sup> vp	
	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)
Baseline	31	345 (130)	22	372 (97)	20	300 (74)*
Week 4 change from baseline	31	-22 (73)	22	20 (116)	19	1 (69)
Week 12 change from baseline	30	-4 (97)	22	60 (99)†	20	69 (83)‡§
Month 6 change from baseline	28	6 (149)	22	75 (136)†	18	83 (113)‡§

\* $p < 0.05$  versus low dose; † $p < 0.05$  versus placebo; ‡ $p < 0.01$  versus placebo; § $p < 0.0167$  (Bonferroni correction).  
vp = viral particles.



**Figure 3** Change in Time to Onset of 1 mm ST-Segment Change in Female Patients

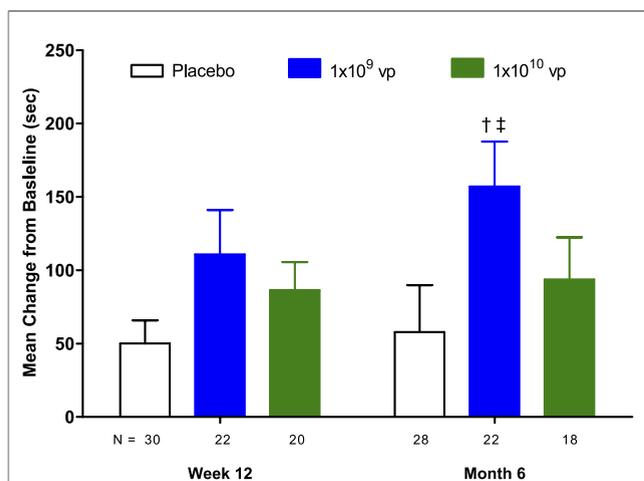
Change from baseline in time to onset of 1 mm ST-segment change in female patients. Data are mean and standard error. vp = viral particles. \* $p < 0.05$ , † $p < 0.0167$  (Bonferroni correction for subgroup analysis).



**Figure 5** CCS Class Improvement in Female Patients

Mean improvement over baseline in Canadian Cardiovascular Society (CCS) class (I to IV scale) in female patients. Data are mean and standard error. vp = viral particles. \* $p < 0.05$ , † $p < 0.01$ , ‡ $p < 0.0167$  (Bonferroni correction for subgroup analysis).

**Cardiovascular safety.** Follow-up data for death and severe adverse cardiovascular events (myocardial infarction and unplanned hospitalization or revascularization due to myocardial ischemia) occurring during the first 12 months after treatment were adjudicated by the clinical events committees for each trial. The incidence of the composite end point was not different across the groups: 22% placebo, 16% low dose, 19% high dose. In the gender subgroups, the incidences were 30% placebo, 9% low dose, and 19% high dose in women and 20%, 17%, and 19%, respectively, in men.



**Figure 4** Change in Time to Onset of Angina in Female Patients

Change from baseline in time to onset of angina in female patients. Data are mean and standard error. vp = viral particles. † $p < 0.01$ , ‡ $p < 0.0167$  (Bonferroni correction for subgroup analysis).

**Adverse events.** No significant safety concerns for Ad5FGF-4 were identified by the data and safety monitoring boards who reviewed the safety data for the AGENT-3 and -4 trials at the interim analyses. For this analysis, adverse event data were available for a minimum of 23 months and a maximum of 50 months of follow-up. The incidence of cardiovascular adverse events (death, myocardial infarction, worsening angina, unstable angina, chest pain, cardiac arrest, congestive heart failure, or cerebrovascular accident) (some patients had more than one)/total number of patients, and mean number of adverse events/patient for placebo, low- and high-dose in men were 91/145 (0.63), 81/158 (0.51), and 94/154 (0.61). The respective numbers in women were 31/33 (0.94), 7/22 (0.32), and 9/21 (0.43). Transient fever occurred in 3.4% of placebo and 7.6% of active treatment patients ( $p = 0.038$ ). This is an infrequent but expected side effect of adenoviral gene therapy. The majority of fevers that occurred within the first few days after study product administration resolved with no treatment or with antipyretic medication. Hemodynamics during and after product administration were stable. There was no evidence for pathological angiogenesis or ophthalmologic changes (neovascularization or progression of the ETDRS score). The incidence of angina/worsening angina was significantly lower ( $p = 0.04$ ) in active treatment patients (17.7%) versus placebo (25.4%). There were no differences in serious adverse events or other adverse events between the 3 groups. A Kaplan-Meier analysis of the incidence of cancer showed no significant difference between treatment and placebo. Other serious adverse events that have been observed during follow-up in single patients in the trials were pericarditis and torsades de pointes. Collection of long-term follow-up data to assess the risk of

delayed adverse events after intracoronary delivery of Ad5FGF-4 continues for the AGENT-3 and -4 trials until November 2008.

**Demographics.** There were no significant differences at baseline between placebo and treatment groups in age, CCS class, risk factors, or baseline medications. Baseline demographics between men and women were not different, but women had more severe angina (CCS class III and IV) than men (Table 2), and were more likely to be on beta-blockers, calcium-channel antagonists, long-acting nitrates, and triple therapy (Table 3) (all  $p < 0.05$ ). These differences suggest that the women had more significant symptoms of CAD, and were being treated with more medications.

## Discussion

**Safety.** The AGENT trials represent the largest number of patients treated with angiogenic gene therapy. In the AGENT-3 and -4 trials combined, 355 patients received active treatment. The fever observed in 7.6% of active treatment patients was expected and has been seen in many adenovirus vector gene transfection trials. In our pooled analysis, the incidence of angina/worsening angina was statistically less ( $p = 0.04$ ) in patients treated with Ad5FGF-4 (17.7%) compared with that in patients who received placebo (25.4%). No other adverse events were significantly associated with intracoronary administration of Ad5FGF-4 including cancer and undesirable angiogenesis. There were no hemodynamic, chemical, or hematologic changes. Adenovirus does adhere to platelets and has been occasionally seen to lower platelet count, an effect we did not observe (18). The safety findings in the AGENT-3 and -4 trials confirm results from the AGENT-1 and -2 trials (11,12).

**Table 2** Baseline Demographics

	Female Patients (n = 76)	Male Patients (n = 456)
Age, mean (min, max)	63 (44, 75)	62 (41, 75)
CCS angina class*		
II	30 (40%)	214 (47%)
III	33 (43%)	213 (47%)
IV	13 (17%)	29 (6%)
Heart failure NYHA functional class†		
None	51 (67%)	237 (52%)
I	7 (9%)	80 (18%)
II	15 (20%)	92 (20%)
III	3 (4%)	47 (10%)
Diabetes	34 (45%)	177 (39%)
Hypertension	60 (79%)	358 (79%)
Hyperlipidemia	74 (97%)	444 (97%)
Myocardial infarction	43 (57%)	300 (66%)
PCI	53 (70%)	277 (61%)
CABG surgery	58 (76%)	377 (83%)

\* $p < 0.01$  male versus female patients; † $p < 0.05$  male versus female patients.

CABG = coronary artery bypass grafting; CCS = Canadian Cardiovascular Society; max = maximum; min = minimum; NYHA = New York Heart Association; PCI = percutaneous coronary intervention.

**Table 3** Baseline Angina Medication Usage

	Female Patients (n = 76)	Male Patients (n = 456)
Beta-blockers*	74 (97%)	401 (88%)
Calcium-channel blockers*	40 (53%)	182 (40%)
Long-acting nitrates*	66 (87%)	342 (75%)
Monotherapy	8 (11%)	92 (20%)
Double therapy	30 (39%)	228 (50%)
Triple therapy or more*	38 (50%)	129 (28%)

\* $p < 0.05$  male versus female patients. Classification of patients to monotherapy and double and triple therapy groups does not total 100% in the male subset because 7 patients were not on any chronic antianginal medication.

**Efficacy.** In the valid case population (men and women) in the pooled database for the AGENT-3 and -4 trials, the change from baseline in the primary end point and most of the secondary end points showed no difference between placebo or either dose group. In fact, the only finding of statistical significance was improvement in CCS class, an overall finding attributable mostly to the female cohort.

Previous trials of intracoronary or intravenous protein therapy for refractory angina have not found a significant improvement in ETT time compared with placebo. In FIRST (FGF [fibroblast growth factor] Initiating Revascularization Trial), which investigated intracoronary FGF2 protein, both the placebo and active treatment groups achieved an increase in ETT time in the range of 45 to 75 s (7). This remarkably large placebo effect appears to be common and sustained in angiogenesis trials (19). In the VIVA (VEGF [vascular endothelial growth factor] in Ischemia for Vascular Angiogenesis) trial, which investigated 1 intracoronary infusion followed by 3 intravenous infusions of VEGF165, the improvement in ETT time in placebo and active was in the range of 18 to 48 s (8). Both of these trials used the modified Bruce protocol for ETT, a more rigorous protocol at longer exercise times than the Balke-Ware we employed in the AGENT trials; thus, physiologically equivalent changes would be expected to be represented by smaller ETT time changes in FIRST and VIVA than in the AGENT trials.

The largest other trial of gene therapy for angiogenesis used AdVEGF121 with epicardial injection at mini-thoracotomy (REVASC [Randomized Evaluation of VEGF for Angiogenesis]). The comparison group did not undergo surgery but continued medical therapy. There were some interesting positive results or trends for improvement in ETT time. Treated patients improved about 55 s at 26 weeks (14). Treated female patients in the AGENT-3 and -4 trial analysis, using the lower-intensity level ETT protocol, improved between 60 and 83 s, only slightly greater than in the FIRST, VIVA, and REVASC trials.

**Gender-specific effect.** The finding of significant treatment differences in women compared with men was surprising, especially since it appears to be largely due to differences in placebo effect. The consistency in improvement for the primary end point, total ETT time, and the

secondary end points of time to angina, time to 1 mm ST-segment depression, and CCS class provide evidence of a real treatment effect on the underlying disease in women. No trends for any differences were seen in the primary or any secondary end points in men. Total ETT time has been the traditional end point for most antiangina intervention trials (drugs or devices) as an objective indicator of clinical exercise activity improvement. In women, the concordance of improvement in CCS class confirms improvement in daily life activities. However, the questionnaires for lifestyle changes did not reach significance. The significant improvement in time to 1 mm ST-segment depression, as an objective measure of the time to onset of myocardial ischemia, further suggests a fundamental alteration in the supply/demand relationship for myocardial blood flow after Ad5FGF-4 in women.

We are aware of no prior reports of gender differences in angiogenesis trials. The number of women patients/total patients enrolled and percentage of women in the 3 largest trials were: FIRST: 54/337, 16% (5); VIVA: 21/178, 12% (6); and REVASC: 5/67, 8% (16). In the dataset for the AGENT-3 and -4 trials, the numbers were 76/532, 14%. If the treatment arm in any of the other 3 large trials had a gender-specific effect, the male population might completely dominate any overall population observations. Furthermore, the power to detect a female gender-specific subgroup effect would be minimal due to the low number of women. The AGENT trial pooled analysis would have the greatest power to detect gender-specific effects as substantially more female patients were enrolled.

The reasons for a significant treatment difference in women and men are unknown, but could involve hormonal or genetic differences between the genders. There are a large number of sexually dimorphic genes having tissue-specific expression and regulation differences (20). Sexual dimorphism of expression in various organs ranged from a few hundred to thousands of genes with little overlap between tissues in which gene families were dimorphic. Gender-specific differences in atherosclerotic lesions and lipid metabolism in animal models are well recognized and could be the result of differential gene expression. Gender-specific differences in vascular function have also been observed. Thus, the well-recognized phenomenon of sexually dimorphic gene expression and differences in vascular biology could affect the response to angiogenic agents or the physiologic effects of collateral development by angiogenic gene therapy.

The reasons for the highly significant difference in placebo effect between men and women are unknown. We could find no gender-specific data in the literature on placebo effect in CAD. There are, however, plausible reasons for the observed difference in placebo effect. One of the mechanisms of placebo response in high technology interventions is the psychological effect to inspire increased exercise training. Exercise training can improve ETT performance by both increasing peripheral efficiency (skeletal

muscle/vascular training) and stimulating collateral growth. In the AGENT trials, women tended to have more clinical symptoms (CCS class) and had significantly more medication usage, thus peripheral exercise training might have been symptomatically limited or limited by medications. This effect would also reduce another critical factor for collateral growth stimulation, namely exercise-induced increases in blood flow. Collateral growth is importantly stimulated by high shear flow through microvascular channels, which is both pressure- and downstream-resistance-dependent, and is increased by exercise (2). Furthermore, women are more likely to have microvascular dysfunction. The women in the AGENT trials could have had more intrinsic microvascular dysfunction, which reduced the placebo-induced exercise training effect (more limited by more symptoms) and the collateral growth effect (less shear dependent stimulation of collateral growth).

The results of the ACIP (Asymptomatic Cardiac Ischemia Pilot) study may be relevant to our results (21,22). The ACIP study found that women presenting for treatment with CAD tended to have more risk factors for CAD, a higher incidence of diabetes and hypertension, less severe CAD by angiography, shorter ETT time, but similar numbers and duration of ambulatory electrocardiogram abnormalities (ischemia). The demographics in the lower-powered AGENT trials trend in a similar direction. In the ACIP study results, women and men had equivalent ischemic burden during daily life on ambulatory monitoring, but women had more risk factors and *less* severe angiographic disease. One likely possibility to explain this paradox is that women had a greater component of microvascular disease not diagnosed by angiography. The observation that women have somewhat different symptoms and outcomes than men and that women less frequently receive invasive treatment has been discussed extensively in the medical and lay literature (23,24). One popular hypothesis is that women are smaller than men and have smaller hearts, thus have intrinsically smaller coronary arteries. The published angiographic data in the ACIP study (finding relatively less angiographic disease associated with relatively more severe clinical disease in women compared with men) would argue instead that women have more microvascular disease. In 2003 the American College of Cardiology reported that nearly 7 million angiograms were performed, with normal or <50% stenosis being reported in 20% of men and 60% of women (25). Furthermore, in the WISE (Women and Ischemia Syndrome Evaluation) study, 50% of women referred for angiograms for myocardial ischemia did not have significant flow-limiting lesions (26). Thus, there is sound evidence that women have a significantly larger component of microvascular disease than men contributing to CAD symptoms.

The angiogenic response is a microvascular phenomenon, and arteriogenesis leading to collaterals, which occurs in the coronary tree at the intermediate level between large vessels and the microcirculation, is dependent to some extent on

angiogenesis to develop new channels that can enlarge. The normal human arterial tree consists of end capillary loops with no native collaterals, so the formation of collateral vessels is a process that must start at the microvascular level. If women do have different microvascular physiology and pathophysiology as the literature suggests, one might expect them to have a different response to angiogenic stimuli. Thus, the hypothesis of more microvascular disease in women could explain both the lower placebo effect and an angiogenic effect of Ad5FGF-4 on the clinical manifestations of microvascular disease.

**Study limitations.** Meta-analysis is a technique that can potentially increase the power to detect an effect, but usually encounters the problems of nonidentical protocols, and using only statistical summary data. A pooled analysis overcomes some of these limitations by using original data and, in our case, identical protocols. The only difference between the 2 trials was the wording of 1 entry criteria, as noted in the preceding text, a difference not likely to effect the hypotheses being generated. Subgroup analyses, even when prespecified as in this case, can only be hypothesis-generating. The positive results in women were statistically significant after correction for subgroup comparisons mostly in the high-dose group. Thus, our conclusion regarding a gender-specific effect must be confirmed in subsequent trials.

## Conclusions

We conclude that neither a treatment effect on the change in ETT duration nor any other objective or subjective assessment of refractory angina was detectable in the male subgroup in the AGENT-3 and -4 trials pooled analysis. This is in contrast to the findings in the AGENT-2 trial demonstrating improvements in myocardial perfusion by quantitative single-photon emission computed tomography imaging (not measured in the AGENT-3 or -4 trials) in a population of predominately male patients (12). There was a remarkable difference in placebo response between men and women for reasons that are unclear. We found gender-specific female subgroup effects of Ad5FGF-4 treatment on a number of measures of the clinical manifestation of CAD. The hypothesis of a decreased placebo response in women and an increased angiogenic response to Ad5FGF-4 in women are not mutually exclusive and may both contribute to the current findings. These data support the hypothesis that there are gender different effects of angiogenic therapy, possibly based on differences in severity and/or location of coronary arterial dysfunction, and/or differences in gender-specific gene expression.

Treatment of women with angina presents a challenging problem considering the underutilization of invasive therapy and the nearly double standardized mortality ratios in women compared with men (27). Management of angina in these patients represents an unmet clinical need that is increasingly becoming one of the most pressing issues in

healthcare, particularly among the aging population (28). The potential importance of a gender-specific angiogenic response on the clinical treatment of CAD is substantial and deserves further investigation in adequately powered clinical trials.

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## REFERENCES

1. American Heart Association. Heart Disease and Stroke Statistics—2007 Update. Dallas, TX: American Heart Association, 2007.
2. Hoefler IE, Piek JJ, Pasterkamp G. Pharmaceutical interventions to influence arteriogenesis: new concepts to treat ischemic heart disease. *Curr Med Chem* 2006;13:979–87.
3. Yla Herttuala S, Alitalo K. Gene transfer as a tool to induce therapeutic vascular growth. *Nat Med* 2003;9:694–701.
4. Losordo DW, Vale PR, Symes JF, et al. Gene therapy for myocardial angiogenesis: initial clinical results with direct myocardial injection of phVEGF165 as sole therapy for myocardial ischemia. *Circulation* 1998;98:2800–4.
5. Vale PR, Losordo DW, Milliken CE, et al. Left ventricular electromechanical mapping to assess efficacy of phVEGF(165) gene transfer for therapeutic angiogenesis in chronic myocardial ischemia. *Circulation* 2000;102:965–74.
6. Vale PR, Losordo DW, Milliken CE, et al. Randomized, single-blind, placebo-controlled pilot study of catheter-based myocardial gene transfer for therapeutic angiogenesis using left ventricular electromechanical mapping in patients with chronic myocardial ischemia. *Circulation* 2001;103:2138–43.
7. Simons M, Annex BH, Laham RJ, et al. Pharmacological treatment of coronary artery disease with recombinant fibroblast growth factor-2: double-blind, randomized, controlled clinical trial. *Circulation* 2002;105:788–93.
8. Henry TD, Annex BH, McKendall GR, et al. The VIVA trial: Vascular endothelial growth factor in Ischemia for Vascular Angiogenesis. *Circulation* 2003;107:1359–65.
9. Laham RJ, Sellke FW, Edelman ER, et al. Local perivascular delivery of basic fibroblast growth factor in patients undergoing coronary bypass surgery: results of a phase 1 randomized, double-blind, placebo-controlled trial. *Circulation* 1999;100:1865–71.
10. Schumacher B, Pecher P, von Specht BU, Stegmann T. Induction of neovascularization in ischemic myocardium by human growth factors: first clinical results of a new treatment of coronary heart disease. *Circulation* 1998;97:645–50.
11. Grines CL, Watkins MW, Helmer G, et al. Angiogenic Gene Therapy (AGENT) trial in patients with stable angina pectoris. *Circulation* 2002;105:1291–7.
12. Grines CL, Watkins MW, Mahmarian JJ, et al. A randomized, double-blind, placebo-controlled trial of Ad5FGF-4 gene therapy and its effect on myocardial perfusion in patients with stable angina. *J Am Coll Cardiol* 2003;42:1339–47.
13. Rosengart TK, Lee LY, Patel SR, et al. Angiogenesis gene therapy: phase I assessment of direct intramyocardial administration of an adenovirus vector expressing VEGF121 cDNA to individuals with clinically significant severe coronary artery disease. *Circulation* 1999;100:468–74.
14. Stewart DJ, Hilton JD, Arnold JM, et al. Angiogenic gene therapy in patients with nonrevascularizable ischemic heart disease: a phase 2 randomized, controlled trial of AdVEGF<sub>121</sub> (AdVEGF121) versus maximum medical treatment. *Gene Ther* 2006;13:1503–11.
15. Gyongyosi M, Khorsand A, Zamini S, et al. NOGA-guided analysis of regional myocardial perfusion abnormalities treated with intramyocardial injections of plasmid encoding vascular endothelial growth

- factor A-165 in patients with chronic myocardial ischemia: subanalysis of the EUROINJECT-ONE multicenter double-blind randomized study. *Circulation* 2005;112 Suppl I:I157–65.
16. Giordano FJ, Ping P, McKirnan MD, et al. Intracoronary gene transfer of fibroblast growth factor-5 increases blood flow and contractile function in an ischemic region of the heart. *Nat Med* 1996;2:534–9.
  17. Gao MH, Lai NC, McKirnan MD, et al. Increased regional function and perfusion after intracoronary delivery of adenovirus encoding fibroblast growth factor 4: report of preclinical data. *Hum Gene Ther* 2004;15:574–87.
  18. Stone D, Shaoeng N, Li Z, Shayakmetov D, Lieber A. Intravascular delivery of adenovirus vectors rapidly targets platelets to the reticuloendothelial system. *Mol Ther* 2006;13 Suppl 1:S143.
  19. Rana JS, Mannam A, Donnel-Fink L, Gervino EV, Sellke FW, Laham RJ. Longevity of the placebo effect in the therapeutic angiogenesis and laser myocardial revascularization trials in patients with coronary heart disease. *Am J Cardiol* 2005;95:1456–9.
  20. Yang X, Schadt EE, Wang S, et al. Tissue-specific expression and regulation of sexually dimorphic genes in mice. *Genome Res* 2006;16:995–1004.
  21. Frishman WH, Gomberg-Maitland M, Hirsch H, et al. Differences between male and female patients with regard to baseline demographics and clinical outcomes in the Asymptomatic Cardiac Ischemia Pilot (ACIP) trial. *Clin Cardiol* 1998;21:184–90.
  22. Davies RF, Goldberg AD, Forman S, et al. Asymptomatic Cardiac Ischemia Pilot (ACIP) study two-year follow-up: outcomes of patients randomized to initial strategies of medical therapy versus revascularization. *Circulation* 1997;95:2037–43.
  23. Daly C, Clemens F, Lopez Sendon JL, et al., Euro Heart Survey Investigators. Gender differences in the management and clinical outcome of stable angina. *Circulation* 2006;113:490–8.
  24. Vacarino V. Angina and cardiac care. Are there gender differences, and if so, why? *Circulation* 2006;113:467–9.
  25. Gibbons RJ, Abrams J, Chatterjee K, et al. ACC/AHA 2002 guideline update for the management of patients with chronic stable angina: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee on the Management of Patients with Chronic Stable Angina). *Circulation* 2003;107:149–58.
  26. Shaw LJ, Bairey Merz N, Pepine CJ, et al. The economic burden of angina in women with suspected ischemic heart disease: results from the NHLBI-sponsored Women's Ischemia Syndrome Evaluation (WISE). *Circulation* 2006;114:894–904.
  27. Hemingway H, McCallum A, Shipley M, Manderbacka K, Martikainen P, Keskimaki I. Incidence and prognostic implication of stable angina pectoris among women and men. *JAMA* 2006;295:1404–11.
  28. Pepine CJ, Abrams J, Marks RG, Morris JJ, Scheidt SS, Handberg E. Characteristics of a contemporary population with angina pectoris. TIDES Investigators. *Am J Cardiol* 1994;74:226–31.

 APPENDIX

For a list of the clinical investigators and institutions who enrolled patients in the AGENT-3 and -4 trials, please see the online version of this article.