Long-term follow-up and safety assessment of angiogenic gene therapy trial VIF-CAD: Transcatheter intramyocardial administration of a bicistronic plasmid expressing VEGF-A165/bFGF cDNA for the treatment of refractory coronary artery disease

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Abstract There have been a number of angiogenic gene therapy trials, yielding mixed results as to efficacy, but demonstrating uniform short-term treatment safety. Data regarding long-term safety of angiogenic gene therapy are limited. Double-blind VIF-CAD trial (NCT00620217) assessed myocardial perfusion and clinical data in 52 refractory coronary artery disease (CAD) patients randomized into treatment (VIF; n = 33) and Placebo (n = 19) arms. VIF group received electromechanical system NOGA-guided intramyocardial injections of VEGF-A165/bFGF plasmid (VIF) into ischemic regions, while the Placebo group—placebo plasmid injections. Full 1-year follow-up data have been published. This study presents the results of over 10-year (median 133 months, range 95-149) safety follow-up of VIF-CAD patients. Overall, 12 (36.4%) patients died in VIF and 8 (42.1%) in Placebo group (P = .68). Cardiovascular mortality was 12/33 (36.4%) in the VIF group and 6/19 (31.6%) in Placebo group (P = .73). Two Placebo patients died due to malignancies, but no VIF patients (P = .17). The Kaplan-Meier curves of combined endpoint: cardiovascular mortality, myocardial infarction and stroke were similar for both patient groups (P = .71). Odds ratio of Placebo group increasing (reaching a worse) their CCS class versus VIF was non-significant (OR 1.28, 95% CI = 0.66-2.45; P = .47). However, CCS class improved in time irrespectively of treatment—OR of reaching a less favorable CCS class per each year of follow-up was 0.74 (95% CI 0.685-0.792; $P < .0001$, pooled data). There were no differences in readmission rates.

Intramyocardial VEGF-A165/bFGF plasmid administration appears safe, with no evidence of an increase in the incidence of death, malignancy, myocardial infarction or stroke during 10-year follow-up in this limited patient population. (Am Heart J 2019;215:78-82.)

Despite recent improvements in the treatment of atherosclerotic coronary artery disease, a significant percentage of patients with refractory angina remain in whom complete revascularization is not possible. This percentage has somewhat fallen and varies widely, but seems no less than 5% [1,2]. The annual mortality rate for these patients has been lately described as lower than formerly believed, but their morbidity remains very high [3]. Angiogenic gene therapy trials have been seen a few years ago as a promising emerging therapy for different types of ischemia. There have been quite a few studies in different clinical settings, testing different compounds and methods of administration [4-7]. They have yielded mixed results. In effect, the early hopes for clinical introduction of such therapy have somewhat withered. Currently, there are few such studies either underway or recently published—notably the KAT301, ASPIRE and AWARE trials [8,9]. It may just be, however, that more basic research is needed before we can embark on the next generation of angiogenic gene therapy clinical trials that will finally prove its efficacy. Apart from that, concerns have understandably been raised over the long-term safety of gene therapy in this and other

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settings. There is, however, striking paucity of long-term follow-up data from these trials. There are some safety data available, but published studies of this type are few and understandably—with some missing data and relatively small patient numbers [9,10]. Conscious of some similar imperfections of our data, we attempt to at least partly close this gap. In this study we present the results of a long term 10-year safety follow-up of patients participating in the placebo-controlled VIF-CAD angiogenesis trial (NCT00620217). To our knowledge, this is the first report of long term safety of a bicistronic VEGF-A165/bFGF plasmid cardiovascular gene therapy trial.

Methods

The design and results of the original VIF-CAD trial have been published [11,12]. The study was a double-blind placebo-controlled trial in which a plasmid encoding human VEGF-A165/bFGF (VIF plasmid) and a percutaneous catheter-based technique (NOGA electromechanical left-ventricular mapping with injections using the MyoStar catheter, Johnson & Johnson) were used (ClinicalTrials.gov identifier NCT00620217, acronym VIF-CAD). The VIF-CAD study was funded by grants from the Ministry of Scientific Research and Information Technology (PBZ-KBN-099/P05/01) and Polpharma Foundation for Development of Polish Pharmacy and Medicine (II/126/2003).

As the 10-year follow-up was not part of the original VIF-CAD trial protocol or consent form (the original protocol stated 1 year follow-up), we have initiated this long-term follow-up after obtaining new acceptance of the Institutional Review Board. The patients were contacted by telephone and/or mail for consent and filled out pre-prepared questionnaires either during an office visit (n = 24) or sent to them by mail (n = 7). Apart from that, electronic hospital records were accessed where possible and the hospital discharge forms analyzed either during an office visit or sent by mail (in Poland, discharge forms contain detailed medical description of every hospital admission). In case of deceased patients, every effort was made access full records and at least to obtain the medical documentation pertaining to the time and cause of death.

The study protocol required the collection of the following endpoint data for each patient:

1. Primary endpoints of the long-term follow-up were:
   a. cardiovascular and total mortality,
   b. occurrence of a malignancy,
   c. combined endpoint consisting of cardiovascular death, myocardial infarction, stroke.
2. Secondary endpoints were:
   a. CCS class,
   b. NYHA class
   c. hospital admissions for cardiovascular causes (angina exacerbation and heart failure).

The authors are solely responsible for the design and conduct of this study, all study analyses, the drafting and editing of the manuscript, and its final contents.

Statistics

Data are presented as means ± SD or, where appropriate, as median and interquartile range (Q1-Q3). Data were collected in a computerized case report form database. Simple comparisons were performed with Fisher exact test, Chi square, Wilcoxon test or t test, as appropriate. Time-to-event (including survival per se) were modeled with log-normal regression and proportional hazards methods and presented as Kaplan-Meier estimates with log-rank test results. Repeated events were analyzed using non-linear general mixed model. Multi-event analysis was performed according to the Prentice, Williams and Peterson model. Angina and heart failure clinical scales were analyzed using generalized estimated equations and Cochran-Mantel-Haenszel test. Differences were considered significant at $P < .05$. All comparisons were adjusted for multiplicity, if applicable. SAS software version 9.4 (SAS Institute, Cary, NC) was used.

Results

The baseline clinical characteristics of the study and placebo groups were presented in the original paper.

Out of 52 patients randomized into the original study in a 2:1 fashion (VIF:Placebo) we have collected complete mortality follow up data in all cases (100%), while full per-protocol follow-up details were obtained for 46 patients (88.5%). We were unable to collect full intended data for 6 patients. Five were from the VIF group: 4 died during the long-term follow-up period and we were not able to contact 1 patient who we know is alive. The single patient in the Placebo group with incomplete follow-up had also deceased.

The median follow-up period was 133 months (interquartile range, IQR 95-149 months). During this period 12 patients died in the active treatment (VIF) group (12 out of 33; 36.4%) and 8 in the Placebo group (8 out of 19; 42.1%; $P = .68$). Cardiovascular mortality was 12 out of 33 (36.4%) in the VIF group and 6 out of 19 (31.6%) in the Placebo group ($P = .73$). Two patients in the Placebo group died due to malignancies, while there were no deaths due to malignancies in the active treatment group ($P = .17$). The comparative incidence of the assessed clinical endpoints is presented in Table 1.

We also assessed cardiovascular mortality and total mortality in both groups using the Kaplan-Meier method (Figure 1A and C; $P = .96$ and $P = .57$, respectively).

The Kaplan-Meier curves of the combined endpoint consisting of cardiovascular mortality, myocardial

Table 1. The incidence of major clinical events assessed in both study groups of the over the whole follow-up period. Mortality data was collected for all patients, while other event data for 28 VIF and 18 Placebo patients.

Type of event	Active treatment group, VIF (n = 33)	Placebo group (n = 19)	*P*
Death	12	8	.68
Cardiovascular death	12	6	.73
	Active treatment group - VIF (n = 28)	Placebo group (n = 18)	*P*
Myocardial infarction (STEMI/NSTEMI)	4/6	4/3	.83
Intervention (PCI/CABG)	9/3	2/2	.3
Number of patients requiring hospital admission for angina or heart failure	21	12	.8
Number of hospital admissions for angina or heart failure	52	24	.68
Malignancy	0	3	.07
Death due to malignancy	0	2	.17

infarction and stroke were also similar for both groups (P = .71; Figure 1B).

Secondary endpoints

The odds ratio of Placebo patients reaching a worse CCS class than VIF was not significant (OR 1.28, 95% CI = 0.66-2.45; P = .47). A marked time effect was observed, however (Figure 2 A and B), as CCS class improved in time irrespectively of treatment—the OR of reaching a less favorable CCS class per each year of follow-up was 0.74 (95% CI 0.69-0.79; P < .001, pooled data).

The NYHA class of patients in both groups did not significantly differ between groups (P = .85).

The number of patients admitted for refractory angina did not differ between groups (10/18 for Placebo vs 19/28 for VIF, P = .53). The time to first hospital admission for refractory angina was similar in both groups of patients (median 64 vs 104 months, for Placebo and VIF, respectively, P = .52) as was the absolute number of hospital admissions (23 for Placebo vs 40 for VIF, P = .5). Also, the time to the first and the interval between any further hospital admission for angina exacerbation was similar for both groups (OR 0.98 for VIF vs Placebo group, P = .96).

The number of patients admitted for heart failure (2/18 for Placebo vs 5/28for VIF, P = .69), the time to first admission (median 125 vs 123 months, for Placebo and VIF, respectively, P = .77) and the absolute number of admissions (2 for Placebo vs 12 for VIF, P = .74) did not differ significantly between groups, either.

Discussion

This is one of few studies that assess long-term safety of angiogenic gene therapy in cardiovascular disease. We have not found any signal of harm for this type of gene therapy over a follow-up period exceeding 10 years.

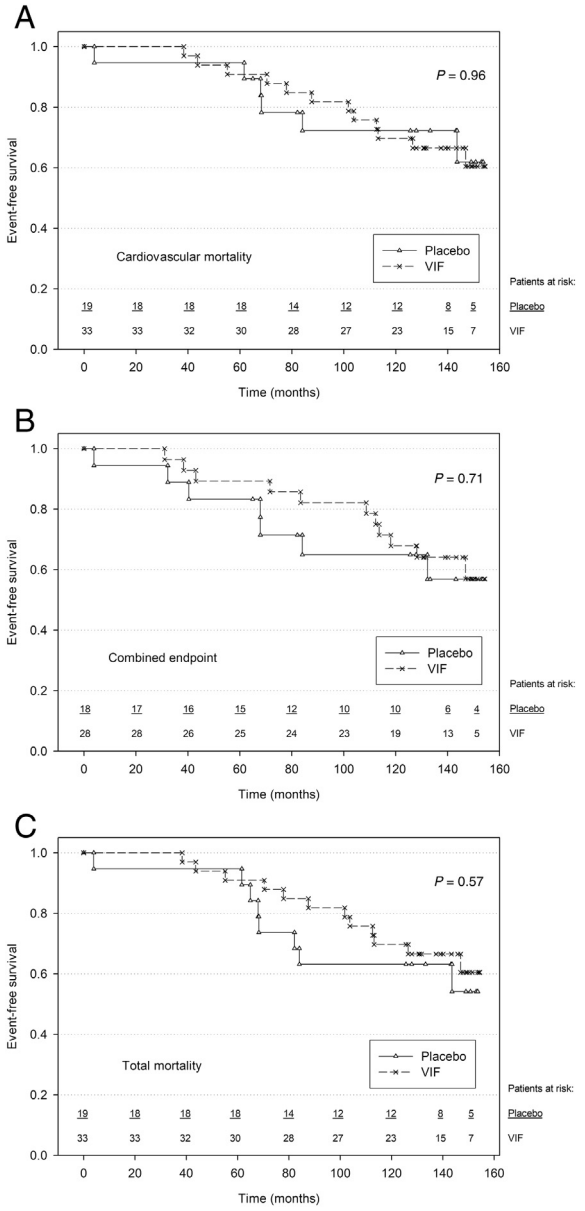
Numerous angiogenic gene therapy trials have been performed and published, but observations are usually limited to 1 year or even shorter periods [13]. Out of the previously published papers reporting long term observations, the most notable are those of the Finnish group from Kuopio. They reported 8-year results of the KAT study [14], confirming the safety of VEGF-A165 plasmid (n = 37) or adenoviral vector (n = 28) administration versus placebo (n = 38). There were no differences with respect to mortality, MACE, cancer, diabetes or arrhythmias.

There is just one other study demonstrating long term safety of gene therapy in coronary artery disease [15]—an 11.8 year follow-up of 31 patients who had an adenoviral vector encoding VEGF121 administered directly either during bypass surgery or during a minimally invasive procedure. There was no control group in this study. The authors found the mortality rate comparable to that of medically treated patients with similar comorbidities, described in the literature. Malignancy and retinopathy rates were also similar to those in age-matched cohorts.

We also have some data from a peripheral angiogenic gene therapy study [16], the authors of which performed a 10-year follow-up of a trial originally including 54 patients with symptomatic limb ischemia receiving VEGF-A either as a plasmid/liposome preparation or in the form of an adenoviral vector. The 10-year mortality in the study was very high, as was the mean age of the studied patients. Therefore, apart from mortality data, only 25 patients could be actually reviewed and the authors found no meaningful inter-group differences, demonstrating no deleterious effects of gene therapy in this setting.

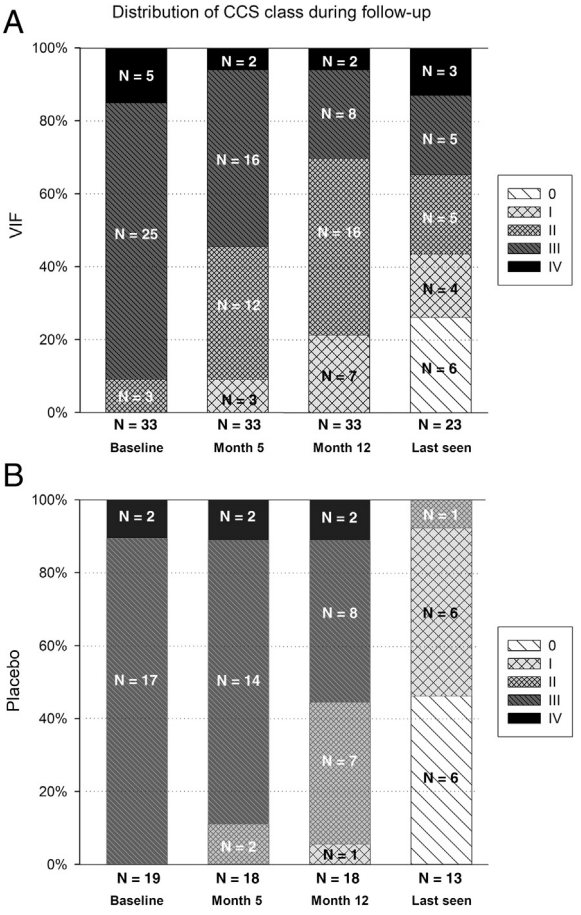
A few studies reporting a 2- to 3-year follow-up [17,18] in peripheral artery disease have also been published. The first one demonstrated 3-year safety of intramuscular FGF-1 plasmid administration into ischemic limb. The authors found that in 93 patients included in their registry, there was no increase in stroke, MI or death, retinopathy or renal dysfunction. The second study demonstrated both safety and efficacy of hepatocyte growth factor gene therapy in Buerger disease—there was reduction of ischemic limb symptoms and no patient developed severe adverse events.

Figure 1



Kaplan-Meier product-limit estimates of event-free survival (censored observations). *P* values shown in the Figures. A, cardiovascular mortality; B, combined endpoint consisting of cardiovascular mortality, myocardial infarction and stroke; C, total mortality.

Figure 2



Percentages of VIF (A) and Placebo (B) patients in each of the Canadian Cardiovascular Society (CCS) classes (0-IV) assessed over time. There was no treatment effect (OR for Placebo reaching a less favorable CCS class versus VIF was 1.28, 95% CI = 0.66–2.45; *P* = .47). There was only time effect seen.

There is also one particularly interesting study [19] as it tested the effects of a high dose of intramyocardial VEGF-A165 plasmid in refractory coronary artery disease, finding no major adverse events at 2-year follow-up. There was even a tendency toward some functional improvement over time, same as in our trial, but it is also hard to say whether that was a treatment or time effect.

Similarly to those few reports, we have found no deleterious long-term effects of intramyocardial VEGF-A165/bFGF plasmid administration. The original study was not powered for mortality data and expectedly we have found no statistically significant differences in mortality, either total or cardiovascular, between the treated (VIF) and the Placebo group. Overall, our study fully confirms the sparse but uniform data showing that angiogenic gene therapy does no harm, even if it fails to show clear benefit in terms of reduced ischemia or improved survival.

An interesting finding of this study is a tendency for functional improvement (CCS class) over time, irrespectively of the treatment arm. One may infer that long-term survivor patients with refractory CAD tend to stabilize over time. This may be due either to optimal medical therapy, in case of patients with sufficient functional reserve, or to progress in revascularization—some of

these patients do undergo subsequent revascularization procedures. The revascularization rates were similar in ours and in other studies [3,20]. However, due to a low number of patients in our study and a large observation gap between year 1 and final follow-up we cannot speculate on the causes this functional improvement and have to bear in mind this may just be a chance finding.

In conclusion, based on previous observations and the data obtained in this paper, there is at present no evidence for long-term deleterious effects of angiogenic gene therapy.

Our study shows clinical improvement in long term survivors over time, irrespectively of the treatment group —this makes proving the therapeutic effect even more difficult. There has been an interesting attempt at a meta-analysis addressing treatment efficacy of angiogenic gene therapy in refractory CAD, which generally seems to confirm a modest treatment effect [21]. Hence, it is perhaps time for a larger multicenter trial addressing this important clinical problem.

Conclusion

According to results of the few published papers, angiogenic gene therapy does not increase the risk of death, cardiovascular adverse events or malignancy. The current long-term follow-up results from the VIF-CAD trial complement and support these previous data.

References

1. Mannheimer C, Camici P, Chester MR, et al. The problem of chronic refractory angina: report from the ESC Joint Study Group on the Treatment of Refractory Angina. Eur Heart J 2002;23:355-70.
2. Henry TD, Satran D, Hodges JS, et al. Long-term survival in patients with refractory angina. Eur Heart J 2013;34:2683-8.
3. Povsic TJ, Broderick S, Anstrom KJ, Shaw LK, Ohman EM, Eisenstein EL, Smith PK, Alexander JHPredictors of long-term clinical endpoints in patients with refractory angina. J Am Heart Assoc 2015;4 pii: e001287.
4. Grines CL, Watkins MW, Mahmarian JJ, et al. A randomized, double-blind, placebo-controlled trial of Ad 5 FGF-4 gene therapy and its effect on myocardial perfusion in patients with stable angina. J Am Coll Cardiol 2003;42:1339-47.
5. Hedman M, Hartikainen J, Syvanne M, et al. Safety and feasibility of catheter-based local intracoronary vascular endothelial growth factor gene transfer in the prevention of postangioplasty and in-stent restenosis and in the treatment of chronic myocardial ischemia. Phase II results of the Kuopio angiogenesis trial (KAT). Circulation 2003;107:2677-83.
6. Kastrup J, Jorgensen E, Ruck A, et al. Direct intramyocardial plasmid vascular endothelial growth factor-A165 gene therapy in patients with stable severe angina A randomized double-blind placebo-controlled study: the Euroinject. J Am Coll Cardiol 2005;45:982-8.
7. Kaminsky SM, Rosengart TK, Rosenberg J, et al. Gene therapy to stimulate angiogenesis to treat diffuse coronary artery disease. Hum Gene Ther 2013;24:948-63.
8. Hartikainen J, Hassinen I, Hedman A, et al. Adenoviral intramyocardial VEGF-DΔNΔC gene transfer increases myocardial perfusion reserve in refractory angina patients: a phase I/IIa study with 1-year follow-up. Eur Heart J 2017;38:2547-55.
9. Ylä-Herttuala S, Bridges C, Katz MG, et al. Angiogenic gene therapy in cardiovascular diseases: dream or vision? Eur Heart J 2017;38: 1365-71.
10. Rosengart TK, Bishawi MM, Halbreiner MS, et al. Long-term follow-up assessment of a phase 1 trial of angiogenic gene therapy using direct intramyocardial administration of an adenoviral vector expressing the VEGF121 cDNA for the treatment of diffuse coronary artery disease. Hum Gene Ther 2013;24:203-8.
11. Kukuła K, Chojnowska L, Dąbrowski M, et al. Intramyocardial plasmid encoding human vascular endothelial growth factor A165/ basic fibroblast growth factor therapy using percutaneous transcatheter approach in patients with refractory coronary artery disease (VIF-CAD). Am Heart J 2011;161:581-9.
12. Kukuła K, Dąbrowski M, Chojnowska L, et al. Podstawy teoretyczne i plan badania VIFCAD – terapii genowej choroby wieńcowej u pacjentów bez możliwości zabiegowej rewaskularyzacji z zastosowaniem podawanego transendokardialnie plazmidu bicistronowego VEGF/FGF. (The theoretical background and plan of the VIF-CAD study). Post Kardiol Int 2006;2(1):116-23.
13. Johnson T, Zhao L, Manuel G, et al. Approaches to therapeutic angiogenesis for ischemic heart disease. J Mol Med (Berl) 2018 Dec 15, https://doi.org/10.1007/s00109-018-1729-3.
14. Hedman M, Muona K, Hedman A, et al. Eight-year safety follow-up of coronary artery disease patients after local intracoronary VEGF gene transfer. Gene Ther 2009;16:629-34.
15. Rosengart TK, Bishawi MM, Halbreiner MS, et al. Long-term follow-up assessment of a phase 1 trial of angiogenic gene therapy using direct intramyocardial administration of an adenoviral vector expressing the VEGF121 cDNA for the treatment of diffuse coronary artery disease. Hum Gene Ther 2013;24:203-8.
16. Muona K, Mäkinen K, Hedman M, et al. 10-year safety follow-up in patients with local VEGF gene transfer to ischemic lower limb. Gene Ther 2012;19:392-5.
17. Niebuhr A, Henry T, Goldman J, et al. Long-term safety of intramuscular gene transfer of non-viral FGF1 for peripheral artery disease. Gene Ther 2012;19:264-70.
18. Makino H, Aoki M, Hashiya N, et al. Long-term follow-up evaluation of results from clinical trial using hepatocyte growth factor gene to treat severe peripheral arterial disease. Arterioscler Thromb Vasc Biol 2012;32:2503-9.
19. Favaloro L, Diez M, Mendiz O, et al. High-dose plasmid-mediated VEGF gene transfer is safe in patients with severe ischemic heart disease (Genesis-I). A phase I, open-label, two-year follow-up trial. Catheter Cardiovasc Interv 2013;82:899-906.
20. Sharma R, Tradewell M, Kohl LP, et al. Revascularization in "no option" patients with refractory angina: Frequency, etiology and outcomes. Catheter Cardiovasc Interv 2018;92:1215-9.
21. Yuan R, Xin Q, Shi W, et al. Vascular endothelial growth factor gene transfer therapy for coronary artery disease: A systematic review and meta-analysis. Cardiovasc Ther 2018;36, e12461.