Effect of fibroblast growth factor NV1FGF on amputation and death: a randomised placebo-controlled trial of gene therapy in critical limb ischaemia

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Summary

Background Patients with critical limb ischaemia have a high rate of amputation and mortality. We tested the hypothesis that non-viral 1 fibroblast growth factor (NV1FGF) would improve amputation-free survival.

Methods In this phase 3 trial (EFC6145/TAMARIS), 525 patients with critical limb ischaemia unsuitable for revascularisation were enrolled from 171 sites in 30 countries. All had ischaemic ulcer in legs or minor skin gangrene and met haemodynamic criteria (ankle pressure <70 mm Hg or a toe pressure <50 mm Hg, or both, or a transcutaneous oxygen pressure <30 mm Hg on the treated leg). Patients were randomly assigned to either NV1FGF at 0·2 mg/mL or matching placebo (visually identical) in a 1:1 ratio. Randomisation was done with a central interactive voice response system by block size 4 and was stratified by diabetes status and country. Investigators, patients, and study teams were masked to treatment. Patients received eight intramuscular injections of their assigned treatment in the index leg on days 1, 15, 29, and 43. The primary endpoint was time to major amputation or death at 1 year analysed by intention to treat with a log-rank test using a multivariate Cox proportional hazard model. This trial is registered with ClinicalTrials.gov, number NCT00566657.

Findings 259 patients were assigned to NV1FGF and 266 to placebo. All 525 patients were analysed. The mean age was 70 years (range 50–92), 365 (70%) were men, 280 (53%) had diabetes, and 248 (47%) had a history of coronary artery disease. The primary endpoint or components of the primary did not differ between treatment groups, with major amputation or death in 86 patients (33%) in the placebo group, and 96 (36%) in the active group (hazard ratio 1·11, 95% CI 0·83–1·49; p=0·48). No significant safety issues were recorded.

Interpretation TAMARIS provided no evidence that NV1FGF is effective in reduction of amputation or death in patients with critical limb ischaemia. Thus, this group of patients remains a major therapeutic challenge for the clinician.

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Introduction

Peripheral artery disease[1,2] affects up to 20 million individuals in North America and Europe, with 2–5% developing the most severe form of the disease, critical limb ischaemia. This disorder is most often caused by atherosclerosis, but has a worse natural history than that of patients presenting with stable coronary or cerebrovascular disease. Although outcome is poorly documented, studies have supported the contention that prognosis is poor.[3–6] For example, 46% of patients unsuitable for revascularisation will have major amputation within 12 months,[3] with the substantial morbidity that this procedure entails. 2 years after a below-knee amputation, no more than 40% of the amputees will have reached full mobility, 15% will have had contralateral amputation and 15% an above-knee amputation, and 30% will have died.[4] Results from studies[5,6] have shown that the quality of life of a patient with critical limb disease equates to cancer patients with terminal disease.

In 1991, the European Working Group on Critical Limb Ischaemia concluded that no medical treatment had been shown to alter this natural history,[7] and little progress has been reported since then. Despite the success of limb salvage with leg bypass, this procedure remains associated with a substantial morbidity and mortality,[8] with the need for subsequent surgery and hospitalisation for wound complications as high as 50%. Furthermore, in a substantial proportion of patients, poor general health, or lack of a suitable vein for revascularisation precludes open surgery, and the multifocal distribution and extent of the arterial occlusive disease might also make both open surgical or endovascular revascularisation impossible. At present, no effective alternatives to either percutaneous or surgical revascularisation in patients with critical limb ischaemia exist.[9,10] Techniques to improve the perfusion of the ischaemic leg by less invasive means are needed. Substantial research has focused on development of therapeutic angiogenesis. In a meta-analysis[11] of trials that included gene and cell-based therapies in peripheral artery disease, the investigators concluded that these therapies had the potential for clinical benefit.

Fibroblast growth factor type 1 (FGF1) modulates and enhances new blood-vessel formation[12] and activates

migration, proliferation, and differentiation of endothelial cells, which result in sprouting of capillaries from pre-existing vessels. Non-viral 1 (NV1) FGF (riferminogene pecaplasmid), a naked DNA plasmid that includes the gene encoding for human FGF1, given intramuscularly into the calf and thigh leads to expression of human FGF1 protein. Results from studies in human beings of intramuscular administrations of NV1FGF showed expression of FGF1, and those from studies in animals[12–14] showed restoration of a functional vascular network near the site of administration.

In an open label phase 1 trial of 51 patients,[15] one intramuscular administration of NV1FGF in patients with critical limb ischaemia significantly improved symptoms (pain, ulcer size) and haemodynamic variables in the treated limb.[15] In the phase 2 TALISMAN trial,[16] administration of NV1FGF (4 mg every 2 weeks) showed a similar improvement in ulcer healing (the primary endpoint) to that of placebo, but a 62·9% reduction of the risk of major amputation (p=0·015) and a 56% reduction of the risk of major amputation or death (p=0·009) at 12 months as compared with placebo.

The main objective of this study was to show the clinical benefit of NV1FGF in delay of the time to major amputation or death in patients with clinical limb ischaemia with non-healing ischaemic skin lesions, in whom revascularisation was not possible.
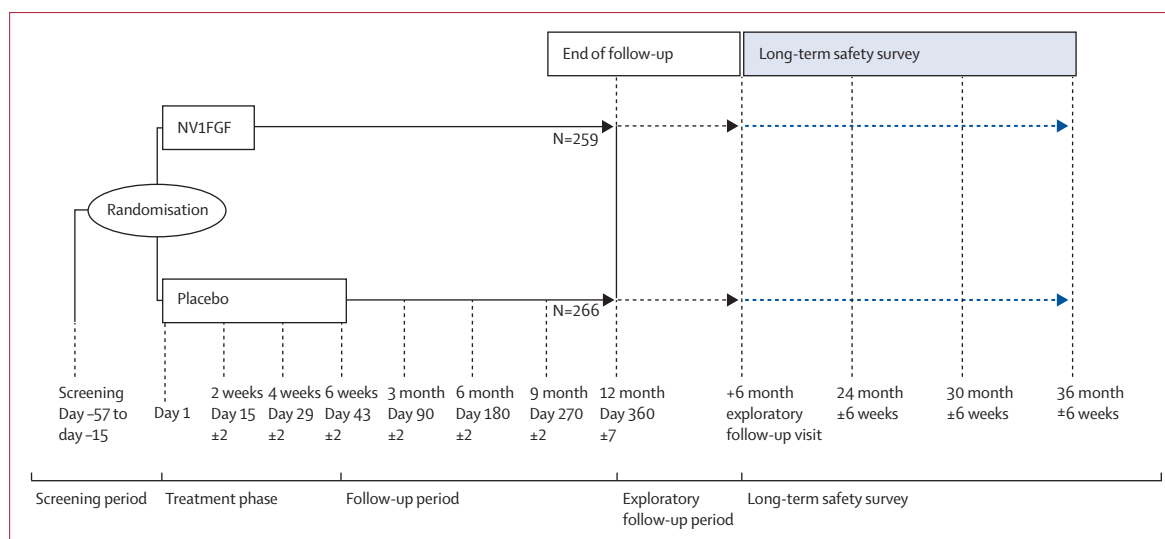
Methods

Patients

This TAMARIS study is a multinational, double-blind, placebo-controlled phase 3 randomised gene therapy trial (EFC6145), in critical limb ischaemia. Trial recruitment was from Dec 1, 2007, to July 31, 2009 in 171 hospitals in 30 countries. For enrolment, patients had to have critical limb ischaemia with ischaemic lesions (Fontaine stage IV), and the diagnosis confirmed by at least one haemodynamic measurement (ankle pressure <70 mm Hg or toe pressure <50 mm Hg, or transcutaneous oxygen pressure [TcPO$_2$] <30 mm Hg) and by one imaging technique (angiography or doppler examination). Vascular surgeons had to confirm that patients were unsuitable for revascularisation and to justify this decision to the independent adjudication panel (event adjudication committee). Panel 1 shows the major inclusion and exclusion criteria. The research protocol was approved by the relevant institutional review boards or ethics committees and all patients gave written informed consent.

Randomisation and masking

Eligible patients were randomly assigned to receive either NV1FGF or placebo (ratio 1:1). Randomisation was stratified by diabetes status and by country and managed centrally by a central interactive voice response system (IVRS). The randomisation code list, with a block size of four, was generated by the sponsor with an electronic technique. The investigator, patient, and sponsor study teams were masked to treatment. Both the active compound (NV1FGF) and the placebo (saline solution) were similar in their appearance (transparent solution) and could not be distinguished from one another.

Procedures

If the disease affected both legs and both were unsuitable for revascularisation, the leg with the lowest pressure index (ankle brachial index or toe brachial index) was identified as the leg to be treated (index leg). The data for the other leg were recorded in the case-report form but not

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Panel 1: Major inclusion and exclusion criteria

Inclusion criteria

- Age >50 years
- Critical limb ischaemia with skin lesions (ischaemic ulcer[s] or minor gangrene)
- Objective evidence of critical limb ischaemia, including ankle systolic pressure <70 mm Hg, or toe systolic pressure <50 mm Hg or TcPO$_2$ <30 mm Hg
- Patent femoral artery inflow assessed by digital angiography, magnetic resonance, or CT angiography (doppler ultrasonography if a previous angiography is available) <6 months before first administration of study treatment
- Unsuitable for standard revascularisation as assessed by a vascular surgeon
- Negative screening for cancer (including family history, a complete physical examination of every system organ including the skin, a haematological blood test, a chest radiography, a stool haemoccult test, and, in the 6 months before screening, a measurement of prostate-specific antigen for men and a mammography and a Papanicolaou test for women, and any investigation required by national guidelines for cancer screening)

Exclusion criteria

- Previous major amputation on the leg to be treated
- Planned major amputation within the first month after randomisation
- Infected gangrene affecting the forefoot evidenced by imaging (radiography)
- Critical limb ischaemia caused by Buerger's disease
- Ulcers from venous or neuropathic origin if not associated with at least one ulcer from arterial origin
- Successful revascularisation procedure of the lower leg, or any other successful treatment (eg, neurostimulation) of the leg to be treated <3 months before randomisation
- Uncontrolled blood pressure defined as systolic blood pressure ≥180 mm Hg or diastolic blood pressure ≥110 mm Hg
- Patient with a severe comorbid disorder, not expected to survive more than 12 months
- Acute cardiovascular events (eg, myocardial infarction, stroke, recent coronary intervention) within 3 months before randomisation
- Active or proliferative retinopathy and severe macular oedema
- Previous or present history of malignant disease, other than basal-cell carcinoma and cervical carcinoma in situ, within the past 5 years; previous malignant disease with relapse or therapy within the past 5 years
- Previous treatment with systemic growth angiogenic factors or with stem-cell therapy
- Women pregnant or breastfeeding, or of childbearing potential not protected by an effective contraceptive method of birth control; men not following effective contraceptive method with their partner of childbearing potential during the course of the study

TcPO$_2$=transcutaneous oxygen pressure.

***Figure 1:* Study design of the EFC6145/TAMARIS trial**
NV1FGF=non-viral 1 fibroblast growth factor.

analysed for efficacy. To aid standardisation, the methods used to assess skin lesions were clearly defined in the case-record form, which contained observer descriptions of ulcer sites, sizes, depths, texture (dry, moist, or gangrenous), and description of lesion management. Four treatment sessions were done 2 weeks apart. At every session, eight intramuscular injections of 0·5 mg NV1FGF each were given in the leg to be treated: four into the calf muscle covering the anterior and posterior region and four into the thigh muscle of the ischaemic leg. The injection sites were selected according to an accessible good striated muscle mass and as close as possible to areas of known collateral blood flow development. In patients with poor muscle mass, the investigator was allowed to identify the injection sites using B-mode ultrasound. Investigators were advised to use present guidelines for optimum medical management of patients.

Figure 1 shows the study design. All patients were reviewed at 2, 4, and 6 weeks during the treatment phase, and then at 3, 6, 9, and 12 months in the observation phase for assessment of both efficacy and safety. An exploratory extended observation phase to assess long-term safety is in progress, in which the patients will be reviewed at 18, 24, 30, and 36 months.

Endpoints
The primary combined endpoint of the trial was time to major amputation (above the ankle) of the treated leg or death from any cause in the study period of 12 months. All amputations were documented by anatomical level and indication (continuing ischaemia detrimental to the patient's health; presence of a lesion considered unlikely to heal; substantial infection, gangrene, or osteomyelitis such that the patient's life was endangered; and intolerable pain).

The main secondary endpoints were major amputation and death from any cause in the study period of 12 months analysed separately. Other secondary endpoints were: (1) all amputations, including all minor and major amputations on the treated leg; (2) skin lesion status (worsened, unchanged, improved, fully healed lesion); (3) pain intensity as assessed at rest (VAS Scale);[17] (4) functionality and general health assessment—ambulatory function and residential status for patients according to the Deneuville questionnaire,[15] and overall quality of life as assessed with the EuroQoL questionnaire;[16] (5) admittance to hospital for amputations because of critical limb ischaemia, prespecified events related to critical limb ischaemia (worsening or increase of ulcers, pain related to peripheral artery disease, necrosis or gangrene, complication caused by amputation of lower limb, infection of skin [ulcer or postamputation wound site], osteomyelitis, procedure related to the ischaemia [eg, revascularisation], wound care [debridement or dressing]), or further diagnostic procedures; and (6) ankle brachial index and toe brachial index.

Safety analysis
Safety assessments included all adverse and serious adverse events, subjective symptoms, vital signs, resting 12-lead electrocardiographs, ophthalmic examination, and blood tests. The potential safety concerns for growth-factor therapy were occurrence of cancer, cardiovascular ischaemic events, occurrence of active or proliferative retinopathy or neovascularisation in the retina, and renal failure. Occurrence of any of these was noted in the case report form. A history of cancer or an active tumour was an exclusion criterion (panel 1).

An ophthalmic examination (visual acuity, fundoscopy, and slit-lamp microscopy) was done during the
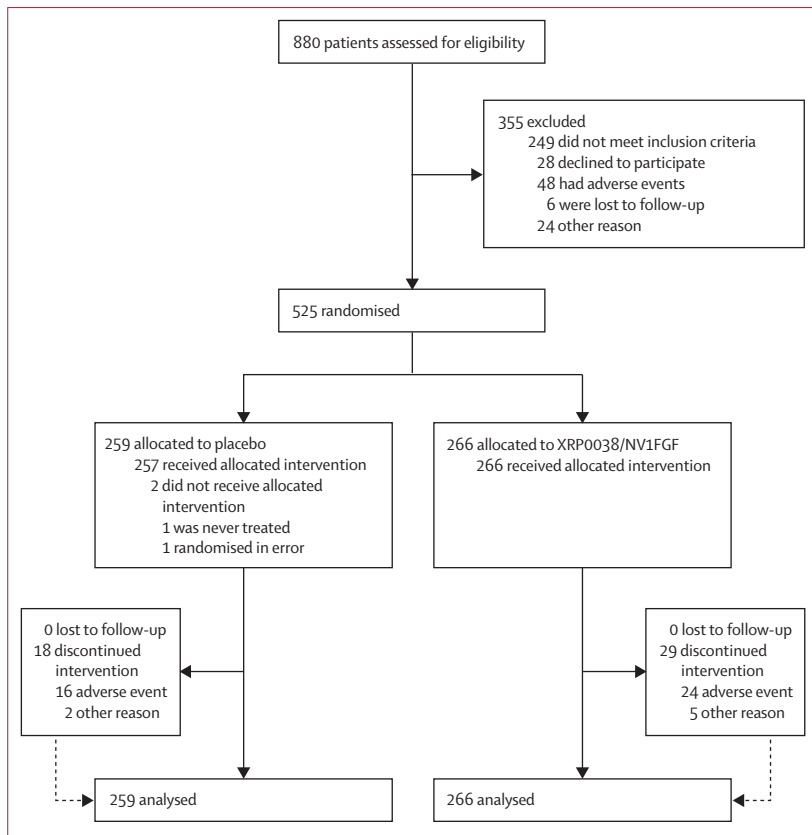
Figure 2: Trial profile
NV1FGF=non-viral 1 fibroblast growth factor.

screening period to exclude active or proliferative retinopathy, or severe macular oedema. The same ophthalmic examinations were repeated at the visits of 1, 3, 6, 9, and 12 months.

Myocardial infarction, unstable angina, ischaemic stroke, and acute ischaemia of the lower limbs were adjudicated by the events adjudication committee for confirmation of diagnosis. Treatment-emergent adverse events, defined as all adverse and serious adverse events occurring between randomisation and the patient's date of end of study were analysed. All adverse events were coded with the Medical Dictionary for Regulatory Activities.

Statistical analysis

The calculation of sample size for the combined event rate (amputation and death) was based on the TALISMAN PM201 study[16] in which a hazard ratio (HR) of 0·558 was recorded. With the assumption of a combined event rate (major amputation or death) at 1 year of at least 35% in the placebo group, a fixed HR of 0·56, a fixed patient follow-up of 12 months, and a drop-out rate of 5%, a sample size of 490 patients (245 patients in each treatment group) would give a minimum power of 90% to show a 44% reduction in events with NV1FGF compared with placebo, with a two-sided log-rank test at a 5% significance level.

All summaries and statistical analyses were generated with SAS version 8.2 on UNIX environment. All efficacy analyses were done by intention to treat. All safety analyses were based on patients who were treated, according to treatment actually received (treated population). We compared the primary efficacy variable (time to first major amputation of the treated leg above the ankle or to death from any cause) between the two treatment groups using a stratified log-rank test. To examine the relation between treatment effect and subgroups (age, sex, baseline creatinine, gangrene, and ankle brachial index), we analysed the primary efficacy variable using a Cox proportional hazards model in a univariate way with terms for treatment, subgroup, and treatment-by-subgroup interaction. We estimated HRs and 95% CIs for every subgroup.

We analysed the two secondary efficacy variables (time from randomisation to major amputation or time from randomisation to death) as described above, assessing each of the two parts of the composite primary endpoint separately. We also compared additional secondary efficacy variables (time from randomisation to any amputation, ulcer healing, pain intensity, functionality and health assessment, hospital admission because of critical limb ischaemia, index pressure) between the two groups. The incidence of adverse events was analysed with the Fisher's exact test; relative risks and associated 95% CIs were estimated.

This trial is registered with ClinicalTrials.gov, number NCT00566657.

Role of the funding source

A representative of the sponsor was a member of the trial steering committee and was present when decisions were made about trial design. The sponsor was responsible for data monitoring, data collection, and data analysis under the instruction of the trial steering committee. The sponsor of the study had no role in data interpretation or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

880 patients were screened during a period of 2–8 weeks to check the stability of the skin lesions and to screen for cancer and 525 were randomly assigned (figure 2). Most of these patients were at least 65 years old (364, 69%), men (365, 70%), white (481, 92%), were former or present smokers (321, 61%), and about 92 (18%) were obese (defined as a body-mass index >30 kg/m²). Key baseline patient characteristics were well balanced (table 1).[18]

Other manifestations of atherosclerosis were very frequent, including 234 (45%) patients with a history of coronary artery disease. Renal impairment, as estimated by a creatinine clearance lower than 50 mL/min was present in 152 (29%), including 44 (8%) patients needing haemodialysis. A history of cancer of more than 5 years before study entry (these patients were eligible) was

noted in 30 (6%) patients. Of note, those with a history of cancer within the past 5 years were excluded from the study.

258 (49%) of 525 patients had undergone previous revascularisation procedures of the index leg (either angioplasty or surgery) and 115 (22%) had a previous minor amputation. 190 (36%) patients had also undergone a previous revascularisation of the contralateral leg. Additionally, patients who were included had a major impairment in functional status, with only 40% of patients capable of a daily walking activity (n=212) or unassisted physical performance (n=220).

Except for a higher frequency of previous minor amputations in patients included in North America (21 [31% of the North American total]) than in the other regions (115 [22%]), the previous management and the status of the index leg to be treated was similar in all sites across the various regional areas.[18] Similarly, apart from a higher rate of previous bypass surgery in the non-diabetic population, no major difference in the previous management and status according to the diabetic status was noted.[18]

Unsuitability for revascularisation was retrospectively confirmed for 497 (95%) patients randomly assigned by the event adjudication committee (webappendix p 1) and was related to anatomical criteria (319 patients, 61%) and an expected low technical success rate of a revascularisation procedure (487, 98%) of all unsuitable patients. General safety concerns associated with the procedure in patients with a favourable anatomy were present in only eight (2%) patients and they did not differ according to geographical area or diabetes status (data not shown).

Almost all patients met the haemodynamic criterion defined at study entry, whereas only a few were enrolled on the basis of a TcPO$_2$ lower than 30 mm Hg as a single haemodynamic test to meet the inclusion criterion (table 1).

517 (98%) patients had infrainguinal arteries affected by stenotic lesions of more than 70%. Thigh arteries were affected in 354 patients (67%), and arteries below the knee were affected in almost all patients (493, 94%). Furthermore, multilevel locations of arterial lesions were common, and 495 (94%) patients had more than one diseased artery. Haemodynamic variables and diseased arterial territories did not differ according to regional area or diabetes status.[18]

Cardiovascular drugs were used in more than 80% of cases, including β blockers, which were given to 242 patients (46%) and angiotensin conversion enzyme inhibitors, given to 270 (51%; webappendix p 2). 341 (65%) patients took lipid lowering drugs (of whom 328 [62%] took statins), and 266 (51%) took antidiabetic drugs. 456 (87%) took antiplatelet drugs and 225 (43%) anticoagulants. Analgesics were given to 475 (90%) patients, of whom 272 (52%) needed opioids. Non-opioid non-analgesic pain relief drugs (eg, gabapentin, antidepressants) were given

	Placebo (n=259)	NV1FGF (n=266)
Mean age (years, range)	69 (50–92)	71 (50–95)
Sex		
Male	181 (70%)	184 (69%)
Female	78 (30%)	82 (31%)
Smoking status		
Never	94 (36%)	110 (41%)
Present	47 (18%)	39 (15%)
Former	118 (46%)	117 (44%)
Hypertension	213 (82%)	233 (88%)
Hyperlipidaemia	153 (59%)	164 (62%)
Diabetes	141 (54%)	139 (52%)
Concomitant cardiovascular disease		
Angina pectoris	73 (28%)	60 (23%)
Coronary intervention	67 (26%)	64 (24%)
Myocardial infarction	69 (27%)	61 (23%)
Atrial fibrillation	53 (21%)	46 (17%)
Unstable angina	18 (7%)	12 (5%)
Stroke	38 (15%)	40 (15%)
Congestive heart failure	39 (15%)	53 (20%)
Pulmonary embolism	7 (3%)	5 (2%)
Venous ulcer	11 (4%)	9 (3%)
Phlebothrombosis	15 (6%)	21 (8%)
Gangrene	55 (21%)	60 (23%)
Vascular measures		
Patients who met leg pressure criterion	249 (96%)	262 (99%)
Ankle systolic pressure <70 mm Hg	193 (75%)	209 (79%)
Toe systolic pressure <50 mm Hg	77 (30%)	82 (31%)
TcPO$_2$ <30 mm Hg	37 (14%)	42 (16%)
TcPO$_2$ only <30 mm Hg	13 (5%)	10 (4%)

Data are mean (range) or number (%). A patient can be counted in several categories. NV1FGF=non-viral 1 fibroblast growth factor. TcPO$_2$=transcutaneous pressure of oxygen.

Table 1: **Demographic details of the study population**

See **Online** for webappendix

	Placebo (n=259)	NV1FGF (n=266)	HR (95% CI)	RR (95% CI)*	p value
Efficacy					
Major amputation (above the ankle)/death	86 (33%)	96 (36%)	1·11 (0·83–1·49)	··	0·48
Major amputation	55 (21%)	67 (25%)	1·20 (0·84–1·72)	··	0·31
Death	39 (15%)	46 (17%)	1·15 (0·75–1·76)	··	0·53
Safety				··	
Ischaemic heart disease	26 (10%)	27 (10%)	··	1·00 (0·60–1·67)	1·00
Malignant neoplasms	4 (2%)	7 (3%)	··	1·69 (0·50–5·71)	0·55
Retinal disorders	15 (6%)	11 (4%)	··	0·71 (0·33–1·51)	0·42
Renal impairment	15 (6%)	20 (7%)	··	1·29 (0·67–2·46)	0·49

Efficacy was analysed by intention to treat. Safety was analysed in patients treated (257 patients treated with placebo and 266 treated with NV1FGF). NV1FGF=non-viral 1 fibroblast growth factor. HR=hazard ratio. RR=relative risk. *Estimated RR.

Table 2: **Efficacy and safety**
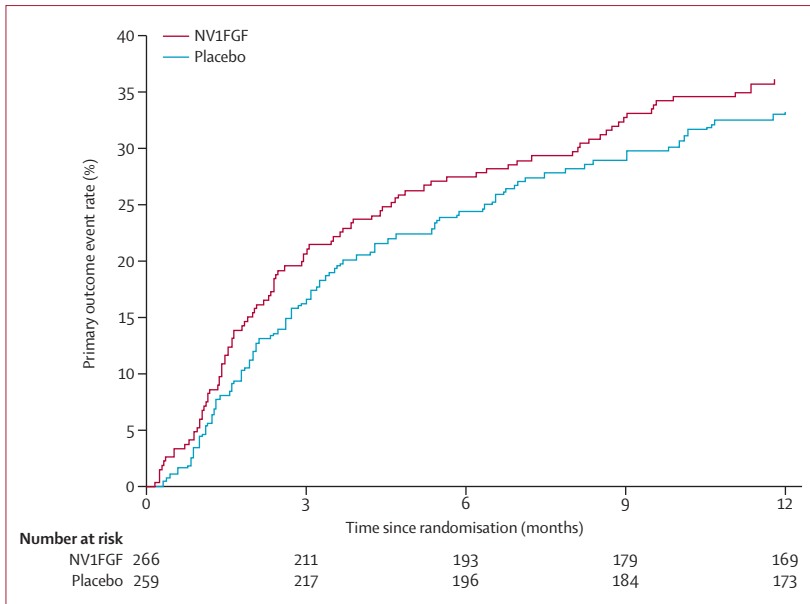
Figure 3: **Cumulative incidence curves over time of primary endpoint**
NV1FGF=non-viral 1 fibroblast growth factor.

	Placebo (n=259)	NV1FGF (n=266)	HR (95% CI)	p value for interaction
Age (years)				
<75	52/171 (30%)	57/173 (32%)	1·06 (0·73–1·55)	0·883
≥75	34/88 (39%)	39/93 (42%)	1·12 (0·70–1·79)	..
Sex				
Male	53/181 (29%)	66/184 (36%)	1·36 (0·94–1·95)	0·072
Female	33/78 (42%)	30/82 (37%)	0·78 (0·46–1·31)	..
Baseline creatinine clearance (mL/min)*				
<30	14/35 (40%)	20/36 (56%)	1·74 (0·84–3·63)	0·970
30–50	19/43 (44%)	15/44 (34%)	0·79 (0·39–1·60)	..
50–80	34/85 (40%)	32/90 (36%)	0·83 (0·50–1·37)	..
>80	18/85 (21%)	28/89 (31%)	1·51 (0·83–2·76)	..
Presence of gangrene*				
Yes	30/55 (55%)	33/60 (55%)	1·15 (0·69–1·93)	0·9469
No	56/202 (28%)	63/206 (31%)	1·11 (0·77–1·60)	..
Ankle brachial index*				
<0·4	46/125 (37%)	52/117 (44%)	1·23 (0·82–1·86)	0·287
0·4–0·79	27/80 (34%)	28/103 (27%)	0·74 (0·43–1·26)	..
0·80–0·99	0/9 (0%)	0/6 (0%)	..	..
1·0–1·3	2/7 (29%)	1/5 (20%)	0·71(0·04–1·79)	..
>1·3	2/6 (33%)	1/7 (14%)	..	..

NV1FGF=non-viral 1 fibroblast growth factor. HR=hazard ratio. *Full set of patients' data not available.

Table 3: **Primary endpoint by interaction demographic variables in all randomised patients**

to 201 (38%) patients. 14 (5%) of 259 patients given placebo received intravenous prostaglandin treatment, as did 29 (11%) of 266 patients in the active group (web-appendix p 3).

The duration of study treatment was 41 days (range 1–49) days in the placebo group and 40 days (1–52) in the active group. 228 (88%) of 259 patients in the placebo group and 228 (86%) of 266 in the NV1FGF group received four administrations of treatment.

The two groups did not differ significantly in the primary efficacy endpoint of time to major amputation on the treated leg or death, whichever came first (table 2 and figure 3). This endpoint did not vary significantly with the demographic variables (table 3). The time to first major amputation on the treated leg or death was similar between treatment and placebo groups (table 2 and figure 4). Post-hoc primary efficacy analysis on the subgroup of 444 patients (86%) who received the full course of treatment (four administrations of 4 mg) shows similar results (data not shown). Table 4 shows causes of death in the two groups.

The main secondary efficacy endpoints (time to first major amputation of the treated leg and time to death) were assessed separately and by the event adjudication committee. Because the primary efficacy endpoint was not significant, no significance could be claimed for the main secondary efficacy endpoints. However, time from randomisation to first major amputation on the treated leg was similar between the NV1FGF group and placebo, and the time from randomisation to any cause of death was similar between the NV1FGF group and placebo (table 2). In view of the large proportion of patients with diabetes, randomisation was stratified by diabetes status. In patients with diabetes, the HR for the primary endpoint was 1·18 (95% CI 0·79–1·77), whereas in patients without diabetes, the HR was 1·01 (0·66–1·56; p=0·6796 for interaction; data not shown).

To inform future trials we continued the analysis for an exploratory additional 6-month review period to see whether any difference in the primary event measures could be detected at this later time, if the primary endpoint was not achieved. This analysis gave no positive result.

Importantly, no patient was lost to follow-up in this trial. The number of patients having treatment-emergent adverse events that occurred after randomisation was similar in both groups: 214 (80%) in the NV1FGF group versus 205 (79%) in the placebo group. The number of patients with any treatment-emergent adverse event leading to permanent treatment discontinuation was higher in the NV1FGF group (24 of 257 patients who received treatement [9%]) than in the placebo group (15 of 266 [6%]). The reasons for discontinuation were infection in seven (44%) of 16 patients given placebo and 12 (50%) of 24 active patients who discontinued, and increasing ischaemia (five [33%] of 15 patients given placebo, five [21%] of 24 patients given NV1FGF). The safety analysis did not show any significant increase in any treatment-emergent adverse event, particularly for factors previously identified as potential risks associated with gene therapy and angiogenic growth factors, such as proliferative retinopathy or severe macular oedema, acute ischaemic events, and renal impairment (table 2).
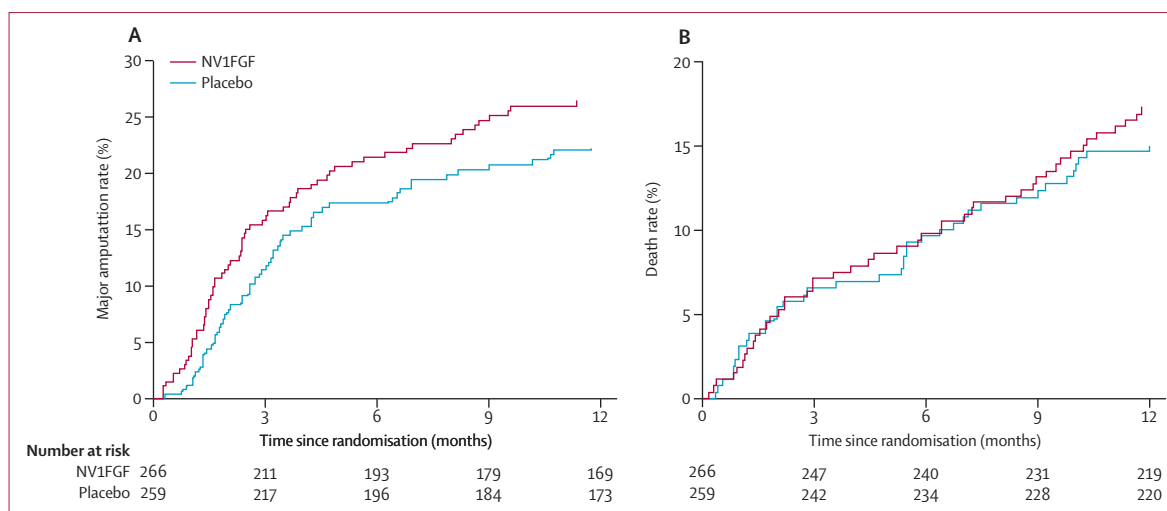
Figure 4: **Cumulative incidence curves over time of components of the primary endpoint**
(A) First major amputation of the treated leg. (B) Death rate over time. NV1FGF=non-viral 1 fibroblast growth factor.

A higher incidence of treatment-emergent adverse events in the NV1FGF group than in the placebo group was recorded for musculoskeletal and connective tissue disorders system organ class (SOC) (50 [19%] of 266 *vs* 25 [10%] of 257; p=0·004] mainly represented by pain and discomfort (34 [13%] of 266 *vs* 17 [7%] of 257; p=0·02) with predominant pain in leg or arm: 26 (10%) of 266 versus 13 (5%) of 257 (p=0·0057). A higher incidence of treatment-related adverse events was also recorded for the metabolism and nutrition disorders SOC (41 [15%] of 266 versus 19 [7%] of 257; p=0·0057). This difference was mainly represented by glucose metabolism disorders (including diabetes mellitus): 20 (8%) of 266 versus eight (3%) of 257; p=0·0316. No proteinuria was reported.

Discussion

The primary endpoint (fewer deaths or first major amputation of the treated leg whichever came first) was not achieved, nor were the secondary endpoints. This result contrasts with the phase 2 TALISMAN study in which significant benefit was recorded in the secondary endpoint (major amputation) and in the combined endpoint (major amputation or death, whichever came first).

Examination of the populations studied in the two trials showed no clear evidence of any baseline differences that could have caused the discrepancy in the results. Similar inclusion and exclusion criteria were required for both studies (in addition to the similar design, treatment dosing, and regimen used in TAMARIS to replicate phase 2 results). The phase 3 trial had about 10% more patients with diabetes than did the phase 2 trial, but a subgroup analysis of non-diabic patients alone did not change the results. Furthermore, TAMARIS included patients with end-stage renal disease (by contrast with

	Placebo (n=259)	NV1FGF (n=266)
All-cause mortality	39 (15%)	46 (17%)
Cardiovascular death	18 (7%)	20 (8%)
Sudden death	1 (<1%)	5 (2%)
Unknown cause	4 (2%)	9 (3%)
Non-cardiovascular death	16 (6%)	12 (4%)

Data analysed by intention to treat. NV1FGF=non-viral 1 fibroblast growth factor.

Table 4: **Description of all-cause mortality up to day 360**

TALISMAN). However, assessment of data with removal of these subgroups did not change the results. Nor did geographical distribution of study sites contribute to the differences recorded. When the analysis was restricted to the TALISMAN sites only, the results were still negative.

A key observation, however, was that the major amputation or death rate in the placebo group at 360 days in TALISMAN (more than 50%) differed from that in the placebo group in TAMARIS (33%). By contrast, the treated-group endpoint rates were similar in both studies, suggesting that the significance achieved in TALISMAN depended on a high placebo endpoint rate. It is unlikely that medical or background treatment improved in the 4 years between studies, since assessment of the baseline characteristics suggests similar use of statins and other vascular risk modifiers in the two studies. Geographical variation in event rates between studies could exist, although the low number of patients included in some regions might not support the conclusion that the observed placebo rates indicate the disease management in these countries. In the TAMARIS placebo group, the primary endpoint ranged from 20% in Latin America to 48% in North America. The TALISMAN study was done in Europe and had only 56 patients randomly assigned to

Panel 2: Research in context

Systematic review

We searched PubMed and Cochrane Library databases up to March 9, 2011, without language restrictions for articles reporting randomised trials, systematic reviews, and meta-analyses with the search terms "peripheral vascular disease", "peripheral artery disease", "gene therapy", and "angiogenesis". We excluded articles that included non-atherosclerotic symptoms of peripheral artery disease, such as thromboangiitis obliterans, and excluded trials of cell-based therapies. Our search identified two meta-analyses and three systematic reviews.[11,16,19–22] Additionally, we identified one report in press about the use of gene therapy in claudication.

Interpretation

The meta-analyses, reviews, and the report currently in press assessed several angiogenic methods, including proteins and genes delivered with a viral vector or as a plasmid. The routes of administration were intra-arterial and intramuscular. In the treatment of claudication, in which the primary endpoint was improvement in peak exercise performance, gene therapy had no benefit. In critical limb ischaemia, benefit was noted in some studies for several surrogate endpoints such as improved limb vascularity, transcutaneous oxygen content, ulcer healing, or relief of pain. These results were not consistent between studies. In one trial,[16] amputation-free survival was improved with NV1FGF.[16] However, overall, results from the meta-analyses and systematic reviews showed no benefit for gene therapy on risk of amputation or death.

NV1FGF=non-viral 1 fibroblast growth factor

This follow-up is in line with the European Medicinal Agency (EMEA) guidance for long-term safety assessment of patients treated with gene therapy products.

In conclusion, results from this large gene-therapy study in critical limb ischaemia to be completed to date (panel 2), portray the challenges faced by the development programmes of single genes, such as extrapolation from conclusive animal studies to define the optimum dose, vector, route, and duration of administration, as well as whether the administration of any one gene could result in therapeutic angiogenesis leading to the prevention of limb amputations.

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placebo. Moreover, infection-related amputations were more common in the placebo group of TALISMAN[16] than in the placebo group of TAMARIS. Thus, the ability to estimate a true untreated event rate in this population is very challenging.

Analyses of the data did not show any selection bias within the two groups since they were well matched for baseline characteristics. Because the event rate (major amputation or death) was lower than expected in the placebo group, it could be argued that the wrong patient population was selected, and that more severe disease should have been studied. However, no trend to efficacy was detected in the most ischaemic patients (table 3 shows results for presence of gangrene).

No clear adverse safety signals were noted with NV1FGF. Safety analyses showed no difference in cardiovascular events or death between active and placebo groups. The previously identified potential risks associated with gene therapy and angiogenic growth factors include proliferative retinopathy, malignancy, macular oedema, acute ischemic events, and renal impairment. Thus, a long-term safety follow-up of 36 months has been implemented and is still in progress.

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