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Insights on the Role of Diabetes and Geographic Variation in Patients with Criticial Limb Ischaemia

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Abstract *Background:* Patients with critical limb ischaemia (CLI) unsuitable for revascularisation have a high rate of amputation and mortality (30% and 25% at 1 year, respectively). Localised gene therapy using plasmid DNA encoding acidic fibroblast growth factor (NV1FGF, riferminogene pecaplasmid) has showed an increased amputation-free survival in a phase II trial. This article provides the rationale, design and baseline characteristics of CLI patients enrolled in the pivotal phase III trial (EFC6145/TAMARIS).
Methods: An international, double-blind, placebo-controlled, randomised study composed of 525 CLI patients recruited from 170 sites worldwide who were unsuitable for revascularisation and had non-healing skin lesions was carried out to evaluate the potential benefit of repeated intramuscular administration of NV1FGF. Randomisation was stratified by country and by diabetic status.
Results: The mean age of the study cohort was 70 ± 10 years, and included 70% males and 53% diabetic patients. Fifty-four percent of the patients had previous lower-extremity revascularisation and 22% had previous minor amputation of the index leg. In 94% of the patients, the index leg had distal occlusive disease affecting arteries below the knee. Statins were prescribed for 54% of the patients, and anti-platelet drugs for 80%. Variation in region of origin resulted in only minor demographic imbalance. Similarly, while diabetic status was associated with a frequent history of coronary artery disease, it had little impact on limb haemodynamics and vascular lesions.

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Conclusions: Clinical characteristics and vascular anatomy of CLI patients with ischaemic skin lesions who were unsuitable for revascularisation therapy show little variations by region of origin and diabetic status. The findings from this large CLI cohort will contribute to our understanding of this disease process.

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

Peripheral artery disease (PAD) is a progressive illness, and the most severe manifestation is critical limb ischaemia (CLI).[1] An estimated 500—1000 new cases of CLI per million individuals are diagnosed annually in the USA and Europe.[2] These patients experience chronic ischaemic rest pain, ulcers or gangrene in the lower limbs, and have a very poor prognosis, with an annual mortality rate of about 20%.[1—3]

Recommended treatment of the limb is primarily endovascular or open surgical revascularisation.[2] However, in a significant proportion of patients, revascularisation is not a suitable option or has previously failed. Of these end-stage patients, 35—50% will require a major amputation within 12 months,[3] since there is no effective pharmacological option available.[4]

Therapeutic angiogenesis is a concept based on the use of angiogenic factors or molecules to promote neovascularisation for the treatment of ischaemic tissues.[5] NV1FGF is a recombinant DNA plasmid constructed by inserting a gene encoding human sp.$FGF1_{21-154}$ into a pCOR plasmid backbone. The pCOR plasmid backbone ensures that the plasmids can only replicate in specially engineered *Escherichia coli* strains and does not replicate in wild *E. coli* strains. This represents a safety advantage, as it limits the plasmid dissemination to another patient or to the environment.[6,7]

Intramuscular administration of NV1FGF in animal models of hindlimb ischaemia is associated with local expression of FGF1 for up to several weeks, increased formation of capillaries and enlargement of collateral vessels, as well as increased tissue perfusion.[8,9] In a phase II double-blind, randomised, placebo-controlled, multicenter study (TALISMAN) including 125 CLI patients with non-healing ulcers or gangrene and without option for revascularisation, four administrations of NV1FGF (4 mg) were given at 2-week intervals. Although it failed to improve ulcer healing, treatment with NV1FGF demonstrated a 46.3% reduction of the risk of major amputation ($p = 0.01$) and a 53% reduction of the risk of major amputation or death at 12 months ($p = 0.001$), as compared with placebo.[10] Other studies based on the same concept but using different growth factors such as hepatocyte growth factor (HGF) or vascular endothelial growth factor (VEGF) failed to demonstrate major clinical benefit in patient with peripheral vascular disease.[11,12]

To confirm and extend the observations from the phase II trial, a larger international placebo-controlled, double-blind, randomised phase III trial (TAMARIS) is currently being performed. The primary objective of this study is to demonstrate the clinical benefit of NV1FGF (riferminogene pecaplasmid) in delaying the time to major amputation or death in CLI patients with non-healing ischaemic skin lesions who are not candidates for revascularisation. As diabetes mellitus and country of enrolment could interact with baseline characteristics of the disease and medical management,[6,13,14] randomisation was stratified according to these variables.

However, although diabetes mellitus is known to play a key role in the natural history of atherosclerosis and PAD[7] and while geographic differences have been associated with differences in patients' characteristics and management,[14] their impact in patients with CLI is unknown. The answer to these questions is important to understand the epidemiology of the disease and to improve the management of these patients. Analysis of the baseline data of the large cohort of CLI patients included in the TAMARIS trial provides an excellent opportunity to present current key patient characteristics, including risk factors, co-morbidities, previous history of revascularisation and amputation, wound care, the extent of arterial lesions and medications. The worldwide nature of the study, including 525 patients recruited from 170 sites, allows for description of these parameters by diabetic status and patient region of origin.

Methods

Patient selection

Recruitment was carried out from December 2007 to July 2009. To be considered as potential candidates for enrolment, patients were required to have CLI with ischaemic lesions (Fontaine stage IV). The diagnosis had to be confirmed by at least one haemodynamic measurement (ankle pressure < 70 mmHg, and/or toe pressure < 50 mmHg or $TcPO_2 < 30$ mmHg) and by one imaging technique (angiography and Doppler examination). Vascular surgeons had both to confirm that patients were unsuitable for revascularisation and to justify this decision. The detailed inclusion and exclusion criteria are described in Supplementary Tables 1 and 2.

Informed consent procedure and randomisation

Following the attainment of informed consent and a screening period of 2—8 weeks to allow for verification of eligibility criteria, patients were randomised using a central interactive voice response system. Randomisation was stratified by patient country of origin and by diabetes status. Separate lists were used for diabetic and non-diabetic patients for all centres in each country.

Treatment

Patients were randomly assigned to receive either NV1FGF (Sanofi-Aventis, Paris, France) or saline as placebo (Sanofi-Aventis, Paris, France) (Fig. 1). If the disease affected both

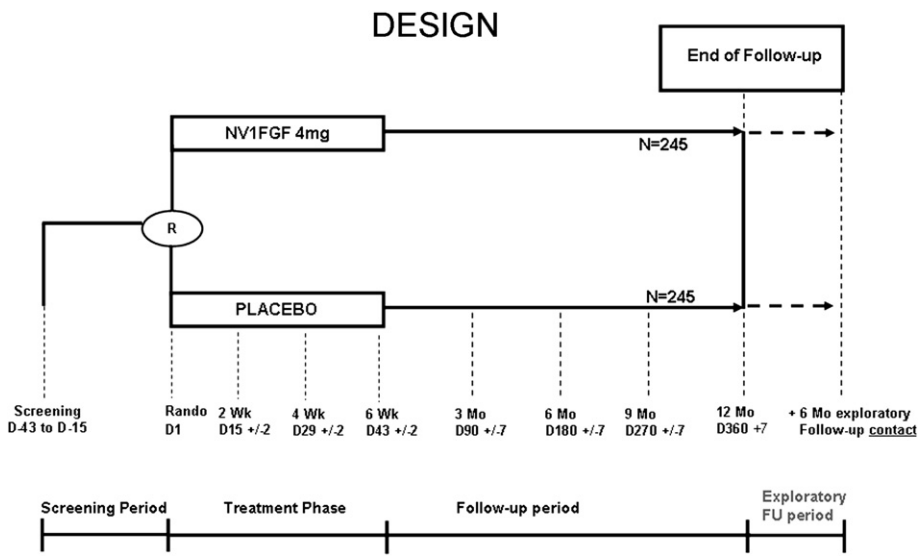

Figure 1 Study design of the EFC6145/TAMARIS trial.

legs and both were unsuitable for revascularisation, the leg with the lowest pressure index (ankle brachial index, ABI or toe brachial index, TBI) was designated as the index leg to be treated. The data of the other leg were recorded in the case report form (CRF), but not analysed for efficacy. Four treatment sessions were performed 2 weeks apart over a period of 6 weeks. At each session, eight intramuscular injections of 0.5 mg NV1FGF each were administered in the leg to be treated. Four injections were performed into the calf muscle and four into the thigh muscle.

Investigators were recommended to follow current guidelines for optimal medical care of patients. Prostanoids were to be avoided unless deemed necessary by the clinician during follow-up. Table 4 shows the type and distribution of drug therapy at baseline.

Follow-up

All subjects 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. All adverse events (AEs) and serious adverse events (SAEs) occurring between randomisation and the patient's study end date are being recorded for analysis.

Definition of end points

Primary end point

The primary combined end point of the trial is the first occurrence of either major amputation (above the ankle) of the treated leg or death from any cause over the 12-month study period. All amputations are documented by anatomic level and indication (continuing ischaemia detrimental to the patient's health, presence of a lesion considered unlikely to heal, significant infection/gangrene/osteomyelitis such that the patient's life is endangered, intolerable pain or other cause to be specified).

Secondary end point

The main secondary end points are major amputation and death from any cause over the 12-month study period, analysed separately. Other criteria include: (1) all amputations, including all minor and major amputations on the treated leg; (2) status of skin lesions (worsened, unchanged, improved or completely healed lesion); (3) pain intensity as evaluated at rest with a self-administrated visual analogue scale (VAS); (4) functionality and general health assessment, including ambulatory function and the residential status for patients with CLI as investigated by the Deneuville questionnaire[15] and the overall quality of life as evaluated by the EuroQol questionnaire;[16] (5) hospitalisations due to CLI for amputations, pre-specified main CLI-related events (worsening/increase of ulcers, pain related to PAD, necrosis or gangrene), complication due to amputation of lower limb, infection of skin, osteomyelitis, revascularisation, wound care or further diagnostic procedures; and (6) ABI and TBI measurements.

Safety

The potential safety concerns associated with growth factor therapy and systemic exposure include occurrence of cancer, cardiovascular ischaemic events, occurrence of active/proliferative retinopathy or neovascularisation and renal failure.

To exclude any proven or suspected cancer, an extensive cancer screening was performed for all the patients in this study. The screening included recording of family history of cancer, a complete medical examination, chest X-ray, stool haemocult, haematology blood sampling, PAP smear and mammography for women, prostrate specific antigen (PSA) for males, and any additional investigation required by national guidelines for cancer screening.

An ophthalmic was performed for all patients and repeated at each.

Myocardial infarction, unstable angina, ischaemic stroke and acute ischaemia of the lower limbs will be adjudicated

by the Events Adjudication Committee (EAC) for confirmation of diagnosis.

Sample size and strategy for statistical analysis

The sample size calculation for the assessment of efficacy in this study is based on the previous experience from the TALISMAN PM201 study where the major amputation event rate at 1 year was 43% in the placebo group and 22% in the NV1FGF group, and the death rate before amputation was equal to 15% in each group.[10] Assuming a hazard ratio of 0.558 with a combined event rate (amputation and death) of 51.5% in the placebo group vs. 33.2% in the NV1FGF group, a total sample size of 490 patients (245 patients in each treatment group) was sufficient to provide a power of 90% with a two-sided log rank test at a 5% alpha level, an expected dropout rate of 5% and a patient follow-up duration of 12 months.

All efficacy analyses will be based on all randomised patients, irrespective of whether the patient actually received the study drug (intention-to-treat population). All safety analysis will be based on randomised and treated patients according to treatment actually received (treated population, intention-to-treat analysis).

Statistical methods

In this baseline paper, data were summarised according to: diabetic status and region of origin (North America, Western Europe, Eastern Europe, Asia and Latin America). For a better understanding of concomitant medication use, data were also presented according to history of coronary artery disease (CAD), although this was not a stratification factor. Since no *a priori* hypotheses regarding baseline data were specified in the protocol, no statistical testing was performed; numerical differences between groups or regions are, thus, presented descriptively.

Results

Baseline characteristics of the study population

Upon completion of enrolment, 525 patients were randomised in the study. The CONSORT flow chart of the randomised population is presented in Fig. 2.

Baseline patient characteristics are presented in Tables 1–4. Data are presented for the whole population and according to the two stratification criteria used for randomisation in the study: country (grouped here by geographic area (North America, Western Europe, Eastern Europe and Latin America)) and diabetic status. Six patients recruited in South Africa and 28 recruited in Asia were omitted from the presentation by geographical area but are included in the total study population ($n = 525$).

Demographic characteristics

Demographic characteristics of the population are presented in Table 1. Patients enrolled included 70% males and the group had a mean age of 70 years. There was history of smoking (former or current) in 61% and diabetes in 53%, and 18% were currently obese, as defined by body mass index >30. Hypertension was observed in more than 80% of cases and hypercholesterolaemia in about 60%. Other manifestations of atherosclerosis were frequent and included 40% of patients with a history of CAD and 15% with a history of stroke. Renal impairment, as estimated by a creatinine clearance of <50 ml min^{-1}, was present in 31%, including 8% of patients requiring haemodialysis. A history of cancer of more than 5 years before study entry was found in 6% of the study patients. Of note, patients with a history of cancer within the past 5 years were excluded from study entry.

Among regions, a history of CAD was very common in patients from North America (69%) and relatively rare in patients from Latin America (16%). Diabetes mellitus was observed in more than 60% of patients from North America, Latin America and Asia. As expected, diabetics were more likely to have characteristics associated with the metabolic syndrome, including obesity, hyperlipidaemia or hypertension. A history of CAD (54%) or renal failure (30%) was also frequently observed in this high-risk population.

Previous management and clinical and functional status of the index leg

More than 50% of patients had undergone previous revascularisation procedures of the index leg by either angioplasty or surgery, and more than 20% had a previous minor amputation (Table 2). About one-third of the population had also undergone a previous revascularisation of the contralateral leg. Additionally, patients who were included in the study had a major impairment in functional status, with only 40% patients capable of a daily walking activity or unassisted physical performance.

Except for a high frequency of previous amputations in patients from North America (31% vs. the average of 21%), the previous management and the status of the index leg to be treated were relatively similar for each of the various regional areas. Similarly, except for a higher rate of previous bypass surgery in the non-diabetic population, there was no major difference in the previous management and status according to the diabetic status.

Unsuitability for revascularisation was retrospectively confirmed for 94.6% of patients randomised by the EAC, and was almost exclusively related to anatomic criteria and an expected low technical success rate of a revascularisation procedure (98%). General safety concerns associated with the procedure in patients with a favourable anatomy were present in only 2% of cases. No difference was observed according to geographical area or diabetic status. The high concurrence rate when confirming unsuitability for revascularisation suggests that the proposed study criteria used was able to appropriately identify adequate target patients.

Haemodynamic parameters and affected arterial territories

Almost all patients (96%) met the haemodynamic criteria defined for study entry (ankle pressure $= 70$ mmHg or toe pressure $= 50$ mmHg or $TcPO_2 < 30$ mmHg) (Table 3). A minority of patients (4%) were enrolled on the basis of

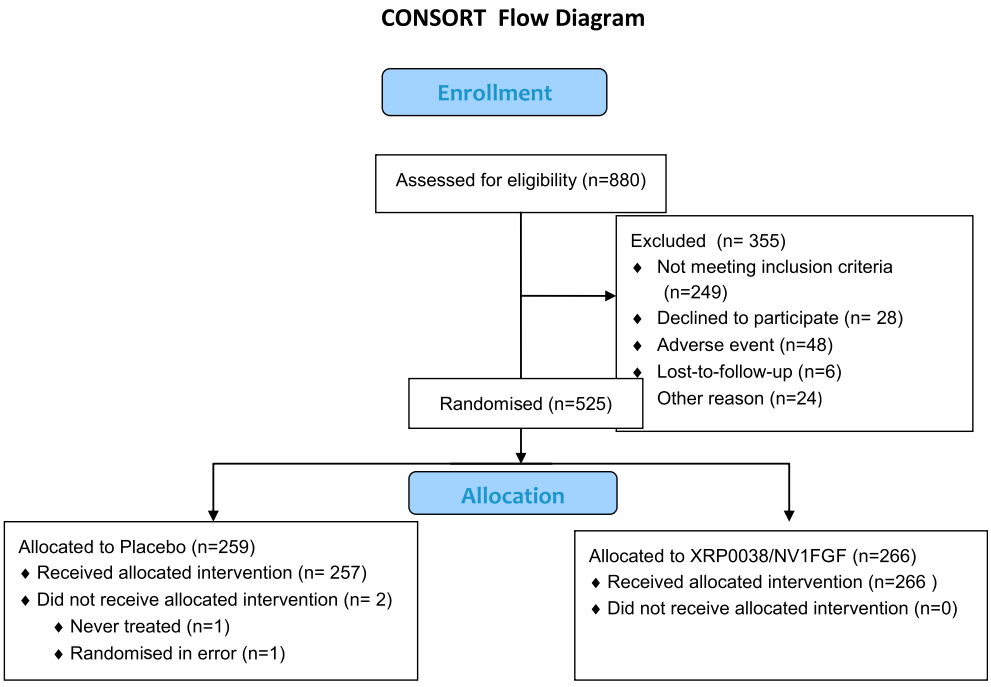

Figure 2 CONSORT flow chart of the study population.

Table 1 Demographic characteristics at study entry — all randomised patients.

	All (N = 525)	North America (N = 67)	Western Europe (N = 182)	Eastern Europe (N = 179)	Latin America (N = 63)	With diabetes mellitus (N = 277)	Without diabetes mellitus (N = 248)
Number of patients screened	860	150	308	245	110	NA	NA
Sex [*n* (%)]							
Male	365 (69.5%)	42 (62.7%)	136 (74.7%)	138 (77.1%)	31 (49.2%)	197 (71.1%)	168 (67.7%)
Race [*n* (%)]							
Caucasian/White	481 (91.6%)	58 (86.6%)	180 (98.9%)	179 (100%)	61 (96.8%)	249 (89.9%)	232 (93.5%)
Black	12 (2.3%)	8 (11.9%)	1 (0.5%)	0	1 (1.6%)	7 (2.5%)	5 (2.0%)
Asian/Oriental	30 (5.7%)	1 (1.5%)	1 (0.5%)	0	0	21 (7.6%)	9 (3.6%)
Other	2 (0.4%)	0	0	0	1 (1.6%)	0	2 (0.8%)
Age (years) — Mean (SD)	70(9.9)	71.5 (10.1)	72.6 (8.7)	66.6 (9.2)	68.7 (11.7)	70.8 (9.7)	69.1 (10.2)
Smoking status [*n* (%)]							
Never	204 (38.9%)	29 (43.3%)	59 (32.4%)	61 (34.1%)	37 (58.7%)	123 (44.4%)	81 (32.7%)
Former	235 (44.8%)	27 (40.3%)	99 (54.4%)	76 (42.5%)	21 (33.3%)	131 (47.3%)	104 (41.9%)
Current	86 (16.4%)	11 (16.4%)	24 (13.2%)	42 (23.5%)	5 (7.9%)	23 (8.3%)	63 (25.4%)
Hypertension	446 (85.0%)	65 (97.0%)	160 (87.9%)	146 (81.6%)	44 (69.8%)	248 (89.5%)	198 (79.8%)
Hyperlipidaemia	317 (60.4%)	55 (82.1%)	137 (75.3%)	87 (48.6%)	23 (36.5%)	185 (66.8%)	132 (53.2%)
Diabetes[a]	280 (53.3%)	43 (64.2%)	93 (51.1%)	81 (45.3%)	40 (63.5%)	273[a] (98.6%)	7[a] (2.8%)
Associated diseases							
Coronary artery disease	235 (44.8%)	47 (70.1%)	91 (50.0%)	71 (39.7%)	10 (15.9%)	151 (54.5%)	84 (33.9%)
Myocardial infarction	130 (24.8%)	27 (40.3%)	52 (28.6%)	37 (20.7%)	5 (7.9%)	87 (31.4%)	43 (17.3%)
Coronary intervention	131 (25.0%)	38 (56.7%)	64 (35.2%)	16 (8.9%)	3 (4.8%)	93 (33.6%)	38 (15.3%)
Congestive heart failure	92 (17.5%)	14 (20.9%)	43 (23.6%)	28 (15.6%)	2 (3.2%)	64 (23.1%)	28 (11.3%)
Stroke	78 (14.9%)	15 (22.4%)	24 (13.2%)	26 (14.5%)	4 (6.3%)	44 (15.9%)	34 (13.7%)
Creatinine clearance group [*n* (%)]							
<30	71 (14.0%)	17 (26.6%)	22 (12.4%)	9 (5.2%)	14 (51.9%)	49 (18.4%)	22 (9.2%)
[30—50]	87 (17.2%)	11 (17.2%)	38 (21.3%)	25 (14.5%)	7 (25.9%)	55 (20.7%)	32 (13.3%)
Chronic renal failure undergoing haemodialysis	44 (8.4%)	13 (19.4%)	15 (8.2%)	3 (1.7%)	10 (35.7%)	32 (11.6%)	12 (4.8%)

[a] Diabetes was a stratification factor. Four non-diabetic patients and five diabetic patients were randomised in the wrong stratum in error.

Table 2 Previous history of revascularization, amputation, and local wound therapy of the Index leg — functional status of the treated leg. Number (%) — all randomised patients.

	All ($N = 525$)	North America ($N = 67$)	Western Europe ($N = 182$)	Eastern Europe ($N = 179$)	Latin America ($N = 63$)	With diabetes mellitus ($N = 277$)	Without diabetes mellitus ($N = 248$)
Status of the index leg							
Patients with at least one previous revascularisation	285 (54.3%)	43 (64.2%)	134 (73.6%)	69 (38.5%)	21 (33.3%)	141 (50.9%)	144 (58.1%)
Peripheral angioplasty	160 (30.5%)	30 (44.8%)	88 (48.4%)	18 (10.1%)	10 (15.9%)	89 (32.1%)	71 (28.6%)
Bypass surgery	191 (36.4%)	23 (34.3%)	88 (48.4%)	57 (31.8%)	16 (25.4%)	82 (29.6%)	109 (44.0%)
Patients with at least one amputation (minor)	115(21.9%)	21 (31.3%)	42 (23.1%)	33 (18.4%)	14 (22.2%)	68 (24.5%)	47 (19.0%)
Patients with at least one ischemic/mixed ulcer or gangrene	524 (99.8%)	67 (100%)	181 (99.5%)	179 (100%)	63 (100%)	276 (99.6%)	248 (100%)
Nb of ulcers/patient [*n* (%)]							
[1—2] ulcers	394 (75.0%)	50 (74.6%)	136 (74.7%)	129 (72.1%)	54 (85.7%)	214 (77.3%)	180 (72.6%)
[3—4] ulcers	64 (12.2%)	8 (11.9%)	27 (14.8%)	19 (10.6%)	3 (4.8%)	36 (13.0%)	28 (11.3%)
$\geq$5 ulcers	17 (3.2%)	4 (6.0%)	9 (4.9%)	2 (1.1%)	1 (1.6%)	7 (2.5%)	10 (4.0%)
Local wound therapy:							
Debridement	131 (25.0%)	12 (17.9%)	32 (17.6%)	78 (43.6%)	5 (7.9%)	77 (27.8%)	54 (21.8%)
Skin graft	1 (0.2%)	1 (1.5%)	0	0	0	1 (0.4%)	0
Other local therapy	318 (60.6%)	33 (49.3%)	116 (63.7%)	106 (59.2%)	42 (66.7%)	163 (58.8%)	155 (62.5%)
Pain VAS (mm)							
Mean (SD)	47.81 (25.95)	40.33 (28.48)	45.02 (25.83)	51.60 (24.33)	49.11 (23.72)	45.32 (26.33)	50.59 (25.28)
Median	48.00	44.00	48.00	50.00	50.00	47.00	50.00
Ambulatory function n (%)							
Daily walking activity	213 (40.6%)	22 (32.8%)	93 (51.1%)	66 (36.9%)	23 (36.5%)	106 (38.3%)	107 (43.1%)
Walking only inside	210 (40.0%)	32 (47.8%)	56 (30.8%)	91 (50.8%)	21 (33.3%)	108 (39.0%)	102 (41.1%)
Patient confined to a chair	89 (17.0%)	12 (17.9%)	28 (15.4%)	20 (11.2%)	14 (22.2%)	56 (20.2%)	33 (13.3%)
Bedridden	12 (2.3%)	1 (1.5%)	5 (2.7%)	1 (0.6%)	5 (7.9%)	7 (2.5%)	5 (2.0%)
Residential status *n* (%)							
Capable of unassisted performance	220 (41.9%)	25 (37.3%)	83 (45.6%)	75 (41.9%)	28 (44.4%)	102 (36.8%)	118 (47.6%)
Able to perform personal hygiene, family	200 (38.1%)	30 (44.8%)	53 (29.1%)	86 (48.0%)	19 (30.2%)	108 (39.0%)	92 (37.1%)
Dependance on family	77 (14.7%)	9 (13.4%)	28 (15.4%)	18 (10.1%)	13 (20.6%)	49 (17.7%)	28 (11.3%)
Dependance on family or paramedical care	28 (5.3%)	3 (4.5%)	18 (9.9%)	0	3 (4.8%)	18 (6.5%)	10 (4.0%)

a $TcPO_2$ <30 mmHg as single positive haemodynamic test to meet the inclusion criteria.

Almost all patients (>95%) had infrainguinal arteries affected by stenosis >70%. Thigh arteries were affected in two-thirds of the patients, and arteries below the knee were involved in almost all patients (94%). Furthermore, multilevel locations of arterial lesions were common and 70% of patients had more than one diseased artery.

These haemodynamic and angiographic patterns were typically observed in all regional areas, as well as in diabetic and non-diabetic patients (Table 3).

Concomitant medications

Cardiovascular drugs were used in more than 80% of patients, and included β-blockers in 41% and angiotensin-converting enzyme (ACE) inhibitors in 46%. Lipid-lowering drugs were used in 60% (56% of those were statins), and anti-diabetic drugs were used in half of the study population. Anti-platelet agents and anticoagulants were used in 80% and 35%, respectively. Analgesics were used in about 80% of patients, among which 50% were opioids.

Besides the use of anti-diabetic drugs and of systemic antibiotics (23%), the diabetic status had very little impact on the use of concomitant medications (Table 4). There were, however, variations in the use of some of the medications according to the presence of CAD and geographical areas. The presence of CAD was driving the use of some medications, in particular β-blockers, statins, diuretics and vitamin K antagonists. The frequent use of these medications in North America and in Western Europe mirrored the high prevalence of CAD in these two areas — 69% and 50%, respectively. Despite a high prevalence of CAD (54%), the use of these medications in Asia was low.

Table 3 Haemodynamic and angiographic parameters on the treated leg at screening visit — all randomised patients.

	All ($N = 525$)	North America ($N = 67$)	Western Europe ($N = 182$)	Eastern Europe ($N = 179$)	Latin America ($N = 63$)	With diabetes mellitus[a] ($N = 277$)	Without diabetes mellitus[a] ($N = 248$)
Patients who met haemodynamic criterion[b]	511 (97.3%)	66 (98.5%)	173 (95.1%)	178 (99.4%)	61 (96.8%)	266 (96.0%)	245 (98.8%)
Patients with only $TcPO_2<30$ mmHg:[c]	23 (4.4%)	8 (11.9%)	13 (7.1%)	1 (0.6%)	0	12 (4.3%)	11 (4.4%)
Affected arteries							
Suprainguinal arteries	21 (4.0%)	3 (4.5%)	10 (5.5%)	5 (2.8%)	1 (1.6%)	9 (3.2%)	12 (4.8%)
Abdominal aorta	3 (0.6%)	0	1 (0.6%)	0	0	0	3 (1.2%)
Common iliac artery	8 (1.5%)	0	5 (2.8%)	1 (0.6%)	0	3 (1.1%)	5 (2.0%)
External iliac artery	16 (3.1%)	1 (1.5%)	8 (4.4%)	4 (2.2%)	1 (1.6%)	6 (2.2%)	10 (4.0%)
Common femoral artery	18 (3.4%)	3 (4.5%)	7 (3.8%)	5 (2.8%)	1 (1.6%)	8 (2.9%)	10 (4.0%)
Infrainguinal arteries	517 (98.5%)	64 (95.5%)	182 (100%)	177 (98.9%)	61 (96.8%)	272 (98.2%)	245 (98.8%)
Above the knee arteries	354 (67.4%)	42 (62.7%)	117 (64.3%)	132 (73.7%)	39 (61.9%)	174 (62.8%)	180 (72.6%)
Profunda femoris	40 (7.6%)	7 (10.8%)	18 (9.9%)	12 (6.7%)	2 (3.2%)	26 (9.5%)	14 (5.6%)
Superficial femoral artery	286 (54.5%)	30 (44.8%)	103 (56.6%)	112 (62.6%)	25 (39.7%)	129 (46.6%)	157 (63.3%)
Poplitaeal artery	269 (51.3%)	29 (43.9%)	91 (50.0%)	104 (58.1%)	27 (42.9%)	124 (44.9%)	145 (58.5%)
Below the knee	493 (94.1%)	60 (89.6%)	176 (96.7%)	172 (96.1%)	55 (87.3%)	259 (93.8%)	234 (94.4%)
Anterior tibial artery	413 (78.8%)	51 (76.1%)	141 (77.5%)	150 (83.8%)	42 (66.7%)	222 (80.4%)	191 (77.0%)
Posterior tibial artery	442 (84.4%)	52 (77.6%)	157 (86.3%)	158 (88.3%)	45 (71.4%)	230 (83.3%)	212 (85.5%)
Peroneal artery	368 (71.3%)	42 (62.7%)	126 (71.2%)	133 (75.6%)	41 (65.1%)	191 (70.0%)	177 (72.8%)
Dorsalis pedis artery	381 (75.0%)	42 (62.7%)	134 (77.0%)	136 (79.5%)	47 (74.6%)	196 (72.9%)	185 (77.4%)

[a] Diabetes and country were stratification factors.
[b] Ankle pressure<70 mmHg and/or Toe pressure<50 mmHg and/or $TcPO_2 < 30$ mmHg.
[c] Patients in whom $TcPO_2 <30$ mmHg was the only haemodynamic parameter matching the inclusion criteria.

Anti-platelet agents were frequently used (80%) and were mostly not impacted by diabetic status, presence of CAD or regional area; the only exception being Latin America, where the use of anti-platelet drugs was relatively low (63%).

No major variation was observed in the use of the other medications.

Discussion

Patients with CLI have a poor natural history and yet no treatment option beyond revascularisation has shown a relevant impact on the prevention of amputation and death. Based on preliminary data,[10] therapeutic angiogenesis using plasmid DNA encoding for the angiogenic growth factor FGF1 might provide a new treatment strategy that is associated with a reduced risk of major amputation or mortality. The current report on the rationale, design and baseline characteristics of this pivotal trial on therapeutic angiogenesis in patients with CLI is important for two main reasons. First, it demonstrates the feasibility of enrolling a homogeneous worldwide cohort of patients with end-stage CLI and no option for revascularisation into a large clinical trial of gene therapy-based angiogenesis. Second, it provides important epidemiological insights from a global perspective and, thus, a unique description of a CLI population that is unsuitable for revascularisation.

Patients enrolled were primarily elderly, white males with a high burden of cardiovascular risk factors and concomitant cardiovascular diseases. About half of the participants had diabetes mellitus as a major risk factor. This presented a challenge to recruit suitable patients, as diabetes may lead to chronic and non-healing skin lesions caused by dysfunctions other than ischaemia, such as distal symmetric neuropathy and neuro-ischaemic combinatorial lesions, hence the need for objective haemodynamic criteria.

The haemodynamic criteria used for patient selection with ischaemic ulcers were an ankle pressure of = 70 mmHg or a toe pressure of = 50 mmHg; these parameters correspond to recommended levels for inclusion in trials and other scientific purposes according to TASC II.[2] We also included patients with CLI based on toe pressure or $TcPO_2$ in the event of non-compressible ankle pressures.

As expected, variations between diabetic and non-diabetic patients were found to be related to the presence of certain co-morbidities, including a higher rate of CAD and heart and renal failure.

The control of risk factors remains suboptimal in patients with CLI, as illustrated by the general under-utilisation of statins given the systemic nature of atherosclerosis in this population. This was especially true in patients without established CAD, in whom statins were used in only 49% as compared to 64% when CAD was known to be present. These results confirm and extend to the CLI population the recent observation of the Reduction of Atherothrombosis for Continued Health (REACH) registry in which patients with peripheral vascular disease achieved a suboptimal control of risk factors, including a use of statins in only 58% of the patients.[14] The poor natural

Table 4 Medications at study entry. Number (%) of patients with at least one specific medication at study entry — all randomised patients.

	Total ($N = 525$)	North America ($N = 67$)	Western Europe ($N = 182$)	Eastern Europe ($N = 179$)	Latin America ($N = 63$)	With diabetes mellitus ($N = 277$)	Without diabetes mellitus ($N = 248$)
Concomitant medications at study entry	521 (99.2%)	67 (100%)	182 (100%)	175 (97.8%)	63 (100%)	277 (100%)	244 (98.4%)
Cardiovascular	428 (81.5%)	65 (97.0%)	167 (91.8%)	128 (71.5%)	42 (66.7%)	239 (86.3%)	189 (76.2%)
Beta blockers	217 (41.3%)	44 (65.7%)	87 (47.8%)	62 (34.6%)	15 (23.8%)	130 (46.9%)	85 (34.3%)
ACE-inhibitors	240 (45.7%)	36 (53.7%)	88 (48.4%)	90 (50.3%)	20 (31.7%)	128 (46.2%)	111 (44.8%)
Diuretics	225 (42.9%)	34 (50.7%)	102 (56.0%)	59 (33.0%)	21 (33.3%)	134 (48.4%)	89 (35.9%)
CA antagonists	148 (28.2%)	25 (37.3%)	58 (31.9%)	38 (21.2%)	13 (20.6%)	81 (29.2%)	67 (27.0%)
Angiotensin II antagonists	90 (17.1%)	13 (19.4%)	43 (23.6%)	15 (8.4%)	11 (17.5%)	58 (20.9%)	32 (12.9%)
Other anti-hypertensives	45 (8.6%)	12 (17.9%)	16 (8.8%)	9 (5.0%)	7 (11.1%)	28 (10.1%)	17 (6.9%)
Lipid lowering drugs	310 (59.0%)	50 (74.6%)	140 (76.9%)	89 (49.7%)	20 (31.7%)	169 (61.0%)	142 (57.3%)
Statins	295 (56.2%)	45 (67.2%)	134 (73.6%)	85 (47.5%)	20 (31.7%)	160 (57.8%)	135 (54.4%)
Other lipid lowering drugs	36 (6.9%)	10 (14.9%)	15 (8.2%)	11 (6.1%)	0	24 (8.7%)	13 (5.2%)
Hypoglycemic drugs	266 (50.7%)	40 (59.7%)	86 (47.3%)	79 (44.1%)	39 (61.9%)	261 (94.2%)	5 (2.0%)
Insulin	154 (29.3%)	22 (32.8%)	54 (29.7%)	49 (27.4%)	21 (33.3%)	151 (54.5%)	3 (1.2%)
Oral hypoglycemics	147 (28.0%)	25 (37.3%)	44 (24.2%)	39 (21.8%)	25 (39.7%)	145 (52.3%)	2 (0.8%)
Antiplatelets	423 (80.6%)	56 (83.6%)	150 (82.4%)	148 (82.7%)	40 (63.5%)	222 (80.1%)	201 (81.0%)
Anticoagulants	183 (34.9%)	15 (22.4%)	95 (52.2%)	41 (22.9%)	14 (22.2%)	98 (35.4%)	84 (33.9%)
Systemic antibiotherapy	122 (23.2%)	11 (16.4%)	55 (30.2%)	30 (16.8%)	17 (27.0%)	78 (28.2%)	43 (17.3%)
Analgesics	436 (83.0%)	58 (86.6%)	155 (85.2%)	143 (79.9%)	50 (79.4%)	222 (80.1%)	211 (85.1%)
Non opioids	370 (70.5%)	47 (70.1%)	124 (68.1%)	129 (72.1%)	47 (74.6%)	188 (67.9%)	178 (71.8%)
Weak opioids	187 (35.6%)	31 (46.3%)	65 (35.7%)	49 (27.4%)	20 (31.7%)	92 (33.2%)	93 (37.5%)
Strong opioids	88 (16.8%)	17 (25.4%)	49 (26.9%)	14 (7.8%)	3 (4.8%)	41 (14.8%)	46 (18.5%)

history of CLI at this stage may be further worsened by under-treatment with anti-platelet drugs and other risk modifying therapies. However, while it has been suggested that secondary prevention may be less frequently applied in diabetic than in non-diabetic patients,[17] our data show that this is not the case at that relatively late stage of the disease with a use of β-blockers in 47% of the cases (vs. 34% in non-diabetic patients), statins in 58% of the cases (vs. 54% in patients without diabetes) and of anti-platelet drugs in 80% of the cases (vs. 81% in patients without diabetes).

Regional variation in patient demographics was rare. They were mainly related to the prevalence of some risk factors, as illustrated by a high rate of diabetes mellitus in North America and South America, of CAD in Western countries and Asia. These variations were responsible for most of the observed variation in the use of medication among regions. The most noticeable were the low use of previous revascularisation of the index leg in Eastern Europe and Latin America (<40%) compared to other regions (>60%) and the low use of statins (26%) in Latin America as compared to other countries with a high prevalence of CAD, such as North America (52%) or Western Europe (68%).

These findings suggest that in non-reconstructable CLI, the clinical, haemodynamic and anatomical presentation are homogeneous and independent of a major risk factor, such as diabetes, and patients are basically treated with only small variation between the countries.

The little variations among the studied population that was recruited based on simple haemodynamic parameters is important in the light of the interpretation of the present trial and demonstrates that the CLI population can be considered globally. This observation is also important for the design of future phase III trials to test the benefit of gene-based or cell-based angiogenic therapies in patients with CLI.

Limitations

The fact that patients were selected as part of a randomised trial may have impacted the results and could limit the ability to generalise the observations to patients with CLI in general. Similarly, the absence of formal statistical comparison decreases our ability to detect small differences among groups.

Conclusions

Diabetic status and regional origin are important factors of variations in patients with PAD. However, their impact in patients with CLI is unknown. TAMARIS is the largest trial in patients with non-reconstructable CLI with skin lesions (Fontaine stage IV). The analysis of the baseline characteristics of the population included in the present study showed that at this stage of the disease, the population can be considered

with little variations in terms of arterial lesions and management according to regional origin and diabetic status. This observation is important for the overall understanding of this disease. It will also have to be taken into account for the design of future trials in this patient population.

Conflict of Interest

All authors are members of the steering committee of the TAMARIS trial and receive an honorarium from Sanofi -Aventis, Paris, France. In the case of WH the funds were sent to 'Colorado Prevention Center' Clinical Research.

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Appendix
Supplementary data

Supplementary data related to this article can be found online at doi:10.1016/j.ejvs.2011.04.030.

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