A randomized phase II study of Xilonix, a targeted therapy against interleukin 1α, for the prevention of superficial femoral artery restenosis after percutaneous revascularization

Hosam El Sayed, MD, PhD,[a] **Richard Kerensky, MD,**[b] **Michael Stecher, MD,**[c] **Prasant Mohanty, MBBS,**[c] and **Mark Davies, MD, PhD,**[d] *Columbus, Ohio; Thomasville, Ga; and Austin and San Antonio, Tex*

Objective: **The purpose of this study was to evaluate an anti-interleukin 1α antibody for its ability to reduce acute postprocedural inflammation, thereby reducing neointimal hyperplasia and restenosis after superficial femoral artery (SFA) angioplasty. Restenosis of the SFA after endovascular intervention is a common problem leading to 1-year primary patency as low as 40%. These failures are primarily due to the development of neointimal hyperplasia, resulting from arterial wall inflammation.**

Methods: **This was a randomized, phase II trial examining SFA restenosis in patients after percutaneous revascularization. Randomization occurred after successful revascularization, and patients were assigned to either the standard of care arm or the Xilonix (XBiotech USA, Inc, Austin, Tex) plus standard of care arm (N = 43). Xilonix was administered immediately after revascularization, every 2 weeks intravenously for four doses, and monthly subcutaneously until month 12. The major efficacy end points were target vessel event-free survival and incidence of major adverse cardiovascular events (MACEs).**

Results: **At 12 months of follow-up, MACE (43% vs 36%; *P* = .76) and target vessel restenosis (24% vs 27%; log-rank, *P* = .79) rates were not significantly different between the groups. At 3-month follow-up, which covers the intravenous dosing period, a trend toward lower incidence of restenosis (0 of 22 [0%] vs 2 of 21 [10%]; *P* = .14) and MACE (2 of 22 [9%] vs 5 of 21 [24%]; *P* = .22) was observed in the Xilonix cohort. Adverse events were equally distributed in both arms.**

Conclusions: **Xilonix was well tolerated. Observed tendency to improved vessel patency with intravenous dosing suggests Xilonix could potentially represent a safe and effective therapeutic approach to preserving vessel patency. (J Vasc Surg 2016;63:133-41.)**

Peripheral arterial disease is a serious condition that has a negative impact on the health and well-being of millions of Americans every year.[1] Occlusive disease of arteries in the lower extremities, such as the superficial femoral artery (SFA), results in diminished mobility because of pain on exertion, pain at rest, nonhealing infections, gangrene, and amputation.[2] In addition, atherosclerotic disease in these arteries is a herald for progressive arterial disease in the coronary and cerebral vasculature, with some studies showing a relative risk for cardiovascular mortality six times higher than that for those without peripheral arterial disease.[3]

The SFA is a particularly frequent site of atherosclerotic disease occlusion, and interventions in this area are common. The endovascular modalities available for treatment are angioplasty, atherectomy, and stenting. Although there are reports of high success rates for the initial intervention, the long-term success of these procedures is limited by the high prevalence of restenosis. Angioplasty and stenting of the SFA have reported restenosis rates of 40% to 60% at 1 year.[4-6] Initial reports of newer stent technology have reported lower rates of SFA restenosis at 1 year; nonetheless, the natural history of these lesions after intervention appears to be progression to restenosis.[7] Therefore, a pharmacologic approach to prolong the restenosis-free duration would be a major step forward for the treatment of these patients.

Currently, there is only one approved drug therapy for SFA restenosis (ie, paclitaxel) after endovascular intervention using Zilver PTX, the drug-eluting stent from Cook Medical (Bloomington, Ind). Drug-eluting balloons are currently in clinical trials.[8] Drug-eluting stents have shown promising results; however, long-term safety and efficacy with these therapies vs standard of care (SOC) are yet to be established. In addition to hindering the process of

From the Division of Vascular Surgery and Diseases, The Ohio State University, Columbus[a]; the Department of Cardiology, Archbold Medical Center, Thomasville[b]; Clinical Regulatory, XBiotech, Austin[c]; and the Division of Vascular Surgery, University of Texas, San Antonio.[d]

This study was funded by XBiotech USA, Inc.

Clinical Trial registration: NCT01270945.

Author conflict of interest: P.M. and M.S. are employees of XBiotech and have stock options.

Additional material for this article may be found online at www.jvascsurg.org.

Correspondence: Hosam El Sayed, MD, PhD, Division of Vascular Surgery, The Ohio State University, 376 W 10th Ave, 701 Prior Hall, Columbus, OH 43210 (e-mail: hosam.elsayed@osumc.edu).

The editors and reviewers of this article have no relevant financial relationships to disclose per the JVS policy that requires reviewers to decline review of any manuscript for which they may have a conflict of interest.

0741-5214

http://dx.doi.org/10.1016/j.jvs.2015.08.069

neointimal hyperplasia fundamental to restenosis, drug-eluting stents have an unwanted propensity to restrict re-endothelialization of the stent luminal surface. The resulting perpetually exposed stent surface appears to predispose patients to later thrombus formation coinciding with the cessation of dual antiplatelet therapy.[9,10] Because ongoing antiplatelet therapy is not without significant morbidity in itself, other mechanisms to reduce neointimal hyperplasia induced by stent deployment are of great interest.

Restenosis after intervention, whether it occurs in the SFA or in the coronary arteries, appears to be the result of an inflammatory response to the revascularization procedure. Interleukin 1α (IL-1α) is a potent, proinflammatory cytokine that plays a major role in atherosclerosis, the acute injury response to revascularization, and the ongoing process of neointimal hyperplasia. Inhibition of IL-1α-mediated inflammatory responses initiated within peripheral vessel walls after intervention, including angioplasty and stent deployment, represents a potentially safe and powerful therapeutic approach to preserving vessel patency after intervention.

Multiple human and animal studies have demonstrated an association between IL-1α and the progression of atherosclerotic plaques. Knocking out IL-1α in mouse models prone to the development of atherosclerosis (apo $E^{-/-}$) results in a significant decrease in the size of atherosclerotic lesions.[11] IL-1α expression is also higher in the peripheral blood mononuclear cells of patients with both stable and unstable angina compared with healthy controls.[12] Foam cells, vascular smooth muscle cells, and endothelial cells from atherosclerotic arteries in animal models have demonstrated elevated expression of IL-1α.[13] Examination on autopsy of atherosclerotic plaques involving arteries within the circle of Willis of human subjects has demonstrated IL-1α expression on infiltrating macrophages, with increasingly high numbers of IL-$1\alpha^{+}$ macrophages corresponding with the degree of plaque severity.[14] In vitro and in vivo models have also established the significance of IL-1α signaling as the potential driving force behind vascular smooth muscle cell proliferation—the key pathologic feature of neointimal hyperplasia.[15-19] In vivo models have also been used to illustrate the importance of IL-1 in response to vascular injury.

METHODS

Xilonix (XBiotech USA, Inc, Austin, Tex) is a true human monoclonal antibody that specifically targets IL-1α. This trial is intended to assess the safety and tolerability of Xilonix therapy in patients with vascular disease as well as to assess the ability of IL-1α blockade to reduce SFA restenosis after intervention and to reduce systemic complications of vascular inflammation. The initial phase of the trial consisted of a higher intravenous dose during the immediate preoperative period, followed by a long-term follow-up and maintenance dosing regimen. The hypothesis being tested is that IL-1α blockade will safely reduce the rate of reintervention after endovascular intervention on the SFA.

Study design. This was a phase II open-label, randomized (1:1), parallel-group, multicenter study evaluating the safety and efficacy of Xilonix in patients undergoing percutaneous SFA revascularization including angioplasty, atherectomy, and stenting. A total of 43 patients were enrolled between July 2011 and August 2012 from nine participating centers.

This study was designed to enroll a total of 80 patients, which would allow 80% power to detect 20% reduction of restenosis rate from 35% null proportion with 10% oversampling. The subcutaneous dosing of 200 mg Xilonix every month starting from month 2 was exploratory in nature and was intended to be administered as a maintenance dose that could potentially improve the durability of the revascularization procedure. However, pharmacokinetic data obtained from this trial as well as from other ongoing studies using Xilonix demonstrated that the once-monthly subcutaneous dosing used in the long-term follow-up portion of the study did not provide adequate serum levels of the antibody. This observation as well as evidence of efficacy in the short-term follow-up period led to the decision to stop the trial at approximately 50% enrollment.

Eligible subjects were randomly assigned (1:1 ratio) to control or treatment arm. Patients in the control arm received SOC postprocedure treatment for 1 year. Patients randomized to the experimental arm received 3.75 mg/kg intravenous infusion of Xilonix diluted in 100 mL of normal saline within 1 hour of completion of the revascularization procedure (week 0) and again on weeks 2, 4, and 6. These patients continued Xilonix therapy for a year, with subcutaneous administration of 200 mg every month starting on month 2, with the last dose occurring on month 11. Patients randomized to SOC did not receive placebo infusions but were observed for a year and underwent the same routine assessments as the Xilonix arm. It was recommended that all subjects be prescribed dual antiplatelet therapy for the first month of treatment and then low-dose aspirin for the duration of the trial, unless there were any contraindications.

Patients. Eligible patients had symptomatic peripheral vascular disease, characterized by claudication, rest pain, or limited tissue loss (ulcers or gangrene limited to toes or distal forefoot) and were undergoing endovascular intervention as a part of their standard treatment.

Further eligibility was determined during the revascularization procedure, and patients were included only if they had a "qualifying lesion," defined as follows:

- Treatment with angioplasty, atherectomy, or bare-metal stent and
- At least one-vessel infrapopliteal tibial vessel runoff and
- Single or multiple lesions of occlusion or stenosis at least 5 cm in length and
- Hemodynamically significant lesions, with ≥50% stenosis as determined by angiography

Additional lesions, such as tibial, iliac, and popliteal, could be treated as well. Other than failing to meet the

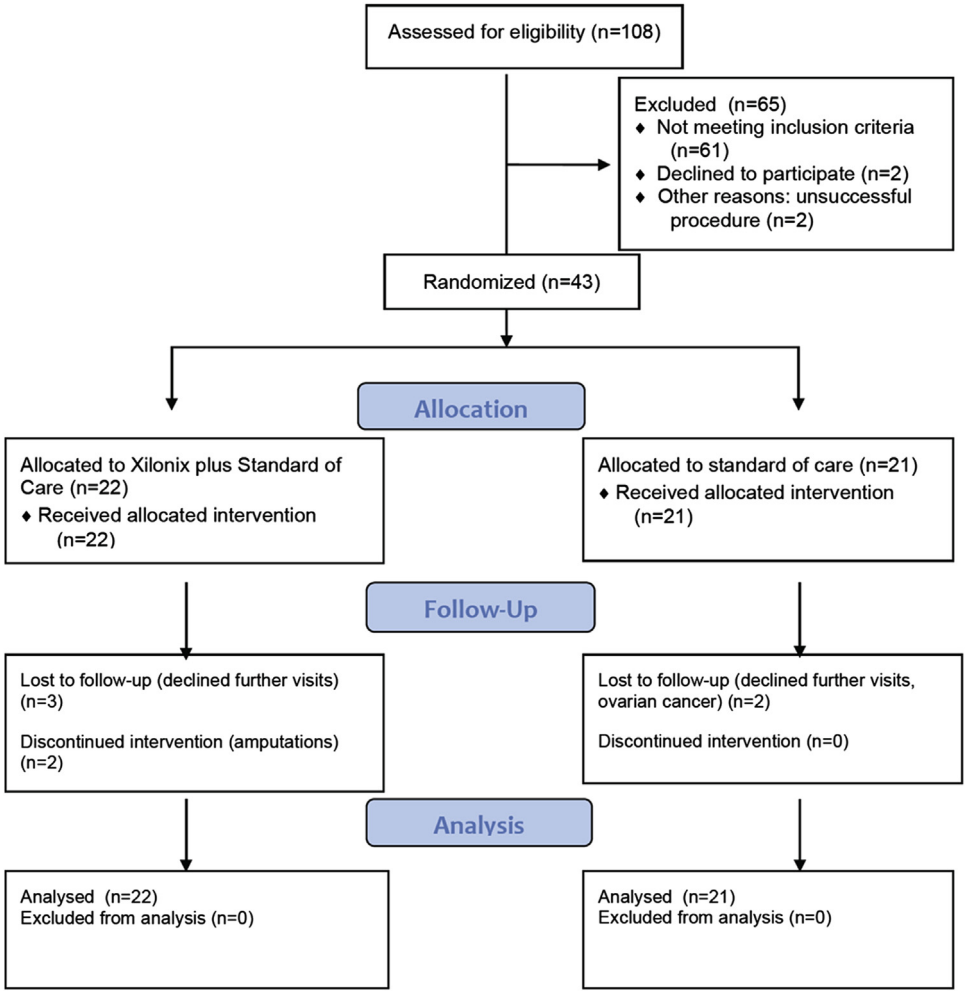

Fig 1. Patient flow diagram.

definition of a qualifying lesion, patients were excluded for the following reasons: acute critical limb ischemia, history of allergic reactions to monoclonal antibodies, female subjects who are pregnant, any surgical or medical condition that in the opinion of the investigator may place the patient at higher risk from his or her participation in the study or is likely to prevent the patient from complying with the requirements of the study or completing the study, concomitant treatment with any agent that blocks the IL-1 or tumor necrosis factor α signaling pathway, history of active or latent tuberculosis, history of human immunodeficiency virus infection or hepatitis C, and active infection requiring treatment with antibiotics. A central Institutional Review Board approved the study, and informed consent was obtained from each patient before revascularization. Individual center Institutional Review Boards also approved the study as required.

Follow-up. Patients in the study were observed for 1 year after the revascularization procedure with postoperative visits at 2, 4, and 6 weeks and once a month thereafter. At each visit, patients were screened for symptoms consistent with clinically significant restenosis as well as for ankle-brachial index (ABI) decreases of ≥0.15. If it was not possible to obtain ABI because of calcified or noncompressible arteries, toe-brachial index was collected. Patients who developed lifestyle-limiting claudication, claudication with an ABI or toe-brachial index decrease of ≥0.15, critical limb ischemia, or gangrene underwent repeated angiography to confirm the presence or absence of target vessel restenosis. Occlusions as a result of thrombosis that occurred within 1 month of the initial procedure were considered technical failures rather than restenosis.

Study outcomes. Primary efficacy end points were clinically significant target vessel restenosis, time to restenosis, and incidence of major adverse cardiovascular events (MACEs).

MACE was defined as 30-day death, stroke, myocardial infarction/unstable angina, emergent surgical revascularization, significant embolization of the target limb,

thrombosis of the target vessel, worsening of symptoms of chronic limb ischemia, or restenosis.

Xilonix. Xilonix is an immunoglobulin G1κ monoclonal antibody specific for human IL-1α. Two formulations were used in this trial. The 15 mg/mL formulation was used for intravenous infusions at a dose 3.75 mg/kg. The 100 mg/mL formulation was used for subcutaneous administration at a dose of 2 mL every month.

The Xilonix antibody is a next-generation, "true human" monoclonal antibody that was cloned directly from human B cells as described by Garrone et al.[20] In contrast to previous generations of fully human antibodies, Xilonix has not undergone in vitro manipulation to improve binding affinity.

Pharmacokinetics and immunogenicity. Plasma Xilonix concentration and antidrug antibody responses were determined by a proprietary enzyme-linked immunosorbent assay.

Randomization. Randomization occurred after angiographic confirmation of qualifying lesions, and patients were assigned (1:1 allocation) to either arm. Block randomization, using a computer algorithm written in SAS (SAS Institute, Cary, NC), was used to create a balanced allocation between the arms at the participating sites. The participating investigators were given the treatment allocations within sealed opaque envelopes. After the patient's eligibility was confirmed, the envelope was opened.

Statistical considerations. Baseline, demographic, and safety variables were summarized by descriptive statistics; continuous variables were reported as mean ± standard deviation or median and interquartile range if non-normal. Categorical variables were reported as number of cases and percentage. Mann-Whitney *U* test and χ^2 tests were used to compare groups. A paired *t*-test (Wilcoxon signed rank test for non-normal data) was used to compare significance of differences between baseline and follow-up measurements. The log-rank test was used to compare cumulative freedom from restenosis across groups. Univariate and multivariate Cox proportional hazards models were used for identifying significant predictors of restenosis. Hazard ratios (HRs) along with 95% confidence intervals (CIs) from the Cox model are reported in the results. All tests were two sided, and a P value $< .05$ was considered statistically significant. Analyses were performed with SAS 9.2 (SAS Institute).

RESULTS

Forty-three patients were enrolled and randomized to either the Xilonix arm (n = 22) or the SOC arm (n = 21; Fig 1). The baseline demographic and clinical characteristics and laboratory values are presented in Tables I-III. Fifteen patients (68%) and 12 patients (57%) reported rest pain in the lower extremity at baseline in the Xilonix group and SOC group, respectively ($P = .75$). The disease severity of the target limb, assessed by Rutherford criteria, was available for 34 patients (17 in each arm); 16 of 17 patients (94%) in the Xilonix group and 15 of 17 patients (88%) in the SOC group presented with moderate to severe claudication ($P = 1.0$).

Table I. Patient demographic and clinical characteristics

	Xilonix (n = 22)	*SOC (n = 21)*	P
Demographics			
Age, years, mean ± SD	63 ± 9	64 ± 11	.68
Gender, male	13 (59)	16 (76)	.23
Medication			
Statins	14 (64)	8 (38)	.13
ASA	16 (73)	11 (52)	.18
Clopidogrel	16 (73)	14 (67)	.67
Symptoms			
Moderate-severe claudication	16 (94)[a]	15 (88)[a]	1.00
Rest pain	15 (68)	12 (57)	.74
Tissue loss (ulcer or gangrene)	3 (14)	2 (10)	1.00
Comorbidities			
History of CAD	7 (32)	8 (38)	.75
Diabetes	13 (59)	6 (29)	.07
Diabetes patients requiring insulin	11 (50)	3 (14)	.02
Hypertension	22 (100)	21 (100)	1.00
History of stroke or TIA	5 (23)	2 (10)	.41
Dyslipidemia	18 (82)	8 (38)	.01
Renal insufficiency	2 (9)	2 (10)	1.00
Current smoker	10 (45)	9 (43)	.86
Quit smoking <10 years	5 (23)	4 (19)	1.00
RCRI[b]			.39
0	5 (23)	7 (33)	
1	4 (18)	5 (24)	
2	6 (27)	7 (33)	
≥3	7 (32)	2 (10)	

ASA, Acetylsalicylic acid; *CAD,* coronary artery disease; *RCRI,* Revised Cardiac Risk Index; *SD,* standard deviation; *SOC,* standard of care; *TIA,* transient ischemic attack.

Categorical variables are presented as number (%).

[a]Claudication status was assessed according to Rutherford criteria and was available for 34 patients (17 in the Xilonix arm and 17 in the SOC arm). Therefore, the denominator used for percentage calculation is 17 for each group.

[b]Lee Revised Cardiac Risk Index.

Baseline medication use was similar between Xilonix and SOC populations; 16 (73%) and 11 (52%) received acetylsalicylic acid and 16 (73%) and 14 (67%) were taking clopidogrel, respectively. Whereas most of the comorbidities were equally distributed between the groups, diabetes (59% vs 29%; $P = .07$) and dyslipidemia (82% vs 38%; $P = .005$) were more prevalent in the Xilonix group. This imbalance in baseline risk factors contributed to differences in statin use, with 14 patients (64%) in the Xilonix arm and 8 patients (38%) in the SOC arm receiving statin therapy. In addition, half (50%) of the Xilonix patients required insulin to control diabetes compared with only 3 patients (14%) in the SOC group ($P = .02$).

The distributions of Project of Ex-Vivo vein graft Engineering via Transfection III (PREVENT III) critical limb ischemia risk score and TransAtlantic Inter-Society Consensus (TASC) II lesions were not different between the study groups. Nine (41%) of Xilonix and 5 (24%) of SOC patients presented with TASC II C and D lesions ($P = .33$). The majority of the patients (86%) presented

Table II. Characteristics of lesions and intervention procedures

	Xilonix (n = 22)	*SOC (n = 21)*	P
Hemodynamic measures			
Baseline ABI	0.71 ± 0.21 (0.70)	0.65 ± 0.18 (0.66)	.33
Anatomic measures			
Lesion length, cm	16.0 ± 10.8 (12.5)	17.1 ± 16.1 (9)	.46
TASC categories			.26
A	3 (14)	6 (29)	
B	10 (45)	8 (38)	
C	6 (27)	2 (10)	
D	3 (14)	3 (14)	
PREVENT III risk category			1.00
Low (≤3 points)	19 (86)	18 (86)	
Medium (4-7 points)	3 (14)	3 (14)	
Interventions[a]			
Angioplasty	20 (91)	18 (86)	.59
Stent placement	9 (41)	10 (48)	.76
Atherectomy	9 (41)	6 (29)	.52

ABI, Ankle-brachial index; *PREVENT III* (Project of Ex-Vivo vein graft Engineering via Transfection III), risk score for critical limb ischemia; *SOC,* standard of care; *TASC,* TransAtlantic Inter-Society Consensus.
Continuous data are presented as mean ± standard deviation (median), and categorical data are presented as number (%).
[a]The intervention procedures were performed alone or in combinations. The counts are not mutually exclusive.

Table III. Baseline laboratory values according to study group

Laboratory test	*Xilonix + SOC (n = 22)*	*SOC (n = 21)*	P
Hematologic parameters			
Hematocrit, %	36.82 ± 5.15	37.06 ± 5.19	.88
Hemoglobin, g/dL	12.35 ± 1.94	12.44 ± 1.98	.88
Platelets, $10^3/\mu L$	246.32 ± 84.10	220.48 ± 66.84	.27
WBCs, $10^3/\mu L$	8.24 ± 2.22	7.30 ± 1.88	.15
Absolute neutrophil count, $10^3/\mu L$)	4.96 ± 2.00	4.47 ± 1.52	.40
Biochemical parameters			
Albumin, g/dL	3.68 ± 0.69	3.82 ± 0.53	.46
Alkaline phosphatase, IU/L	85.27 ± 29.25	89.19 ± 28.14	.66
ALT, IU/L	21.95 ± 14.44	35.45 ± 18.88	.01
AST, IU/L	19.68 ± 10.12	33.55 ± 24.06	.03
BUN, mg/dL	20.77 ± 12.88	20.05 ± 7.85	.82
Calcium, mg/dL	9.37 ± 0.60	9.36 ± 0.81	.98
Creatinine, mg/dL	1.58 ± 2.08	1.17 ± 0.68	.39
Glucose, mg/dL	126.95 ± 65.52	104.52 ± 28.49	.15
Magnesium, mg/dL	1.93 ± 0.18	1.94 ± 0.20	.83
Potassium, mEq/L	4.22 ± 0.55	4.34 ± 0.47	.47
Sodium, mEq/L	139.18 ± 2.56	139.29 ± 3.29	.91
Total bilirubin, mg/dL	0.44 ± 0.25	0.55 ± 0.38	.26
Ultrasensitive CRP, mg/dL	16.03 ± 37.07	4.20 ± 5.35	.19

ALT, Alanine aminotransferase; *AST,* aspartate aminotransferase; *BUN,* blood urea nitrogen; *CRP,* C-reactive protein; *SOC,* standard of care; *WBCs,* white blood cells.
Data are presented as mean ± standard deviation.

with low PREVENT III risk score (≤3 points); only 14% patients (3 in each group; $P = 1.0$) were in medium-risk (4-7 points) category. The overall distribution of the Revised Cardiac Risk Index (RCRI) score, computed from the six predictors of major cardiac complications, was not different between the groups; however, in considering RCRI score ≥3, a trend toward higher prevalence was noted in the Xilonix group compared with the SOC group (7 [32%] vs 2 [10%]; $P = .13$). The RCRI scores are presented in Table I.

Intervention and immediate outcomes

The mean lesion treatment length was 16.0 ± 10.8 cm (median, 12.5 cm) and 17.1 ± 16.1 cm (median, 9 cm) in the Xilonix and SOC groups, respectively ($P = .46$). The average preintervention and postintervention ABIs were 0.71 ± 0.21 (median, 0.70) and 0.81 ± 0.24 (median, 0.89) in the Xilonix group (paired test, $P = .01$) and 0.65 ± 0.18 (median, 0.66) and 0.91 ± 0.13 (median, 0.87) in the SOC group (paired test, $P < .001$). However, after the revascularization procedure, larger hemodynamic improvement was documented in SOC patients compared with Xilonix patients (ABI change, 0.24 ± 0.13 vs 0.14 ± 0.21; $P = .08$), indicating relatively worse vascular patency and possible baseline disease severity among the treated population. The distribution of intervention modalities (angioplasty, atherectomy, stent placement) is presented in Table II. Angioplasty was performed in the majority of cases.

There were no periprocedural or 90-day mortalities observed in the study population. Technical failure was reported in four patients (9.3%), two in each arm. Procedure-related complication rate was 7.0% (3 groin hematomas, 2 in the Xilonix group, and 1 in the SOC group; $P = 1.0$).

Immediate outcomes (up to 4 months). Two of 21 patients (10%) in the SOC cohort and none in the Xilonix group (0.0%) experienced target vessel restenosis in the first 4 months after intervention (log-rank, $P = .14$). During this time, MACEs were reported in five (24%) of the SOC

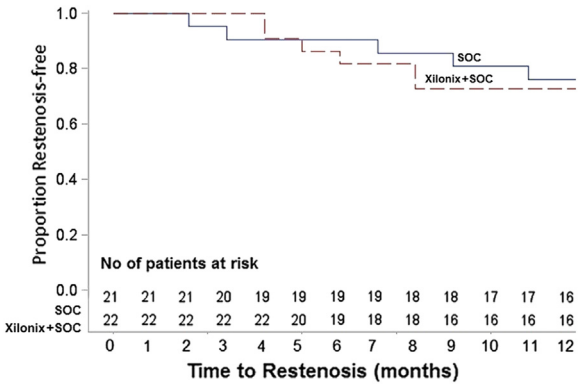

Fig 2. The Kaplan-Meier curve compares the cumulative freedom from restenosis at long-term follow-up. The trend in the treatment benefit observed at 4 months after the procedure (restenosis rates of 9% in the standard of care [*SOC*] group and 0% in the Xilonix group) diminished during the course of the study. At 12 months, the restenosis rates were not different between the Xilonix and SOC groups; 27% (6 of 22) Xilonix patients and 24% (5 of 21) SOC patients experienced target vessel restenosis (log-rank, $P = .79$).

patients and two (9.1%) of the Xilonix patients ($P = .22$). The impact of relatively higher dose intensity tended to be associated with the beneficial effect observed at this time point. The peak serum level during the first 6 weeks of biweekly intravenous Xilonix administrations was 85.9 ± 3.6 μg/mL; during monthly subcutaneous injections, it was <10 μg/mL.

Long-term outcomes. The median follow-up duration was 12 (interquartile range, 7-12) months. The follow-up duration was not different between the groups ($P = .39$). There was no mortality reported during the study period.

Freedom from restenosis. The restenosis rates at 12 months were not different between the groups; 27% (6 of 22) Xilonix patients and 24% (5 of 21) SOC patients experienced target vessels restenosis (log-rank, $P = .79$; Fig 2).

Freedom from MACE. Eight of 22 patients (36%) in the Xilonix arm and 9 of 21 patients (43%) in the SOC arm reported MACEs ($P = .76$). The total number of events was 12 and 14 in each group, respectively ($P = .69$).

Risk factors for restenosis

At the univariate analysis, atherectomy (alone or in combination) was significantly associated with postprocedure restenosis (unadjusted HR, 4.4; 95% CI, 1.3-15.2; $P = .02$). After adjusting for risk factors (age, gender, TASC II score, target lesion length) in a multivariate Cox model, diabetes mellitus (HR, 7.43; 95% CI, 1.07-51.58; $P = .04$) and atherectomy (HR, 17.54; 95% CI, 1.95-157.97; $P = .01$) showed strong association with higher restenosis rate. Postprocedure ABI change demonstrated a trend toward reducing restenosis risk by 93% for every unit increase (HR, 0.07; 95% CI, 0.003-1.38; $P = .08$).

Drug pharmacokinetics and safety profile

The average peak plasma levels during the first 6 weeks of intravenous administration were 86.6 ± 21.7, 94.6 ± 44.3, 90.2 ± 21.7, and 85.6 ± 18.7 μg/mL on week 0, week 2, week 4, and week 6, respectively. Postdose samples for subcutaneous administration were not collected in this trial. However, as observed in a comparable population from a different study, the mean peak concentration after subcutaneous injection of 200 mg is predicted to be approximately 12 μg/mL.[21]

The mean trough plasma level, measured before the next dose, was maintained at 5.6 ± 0.6 μg/mL in the first 6-week period of intravenous infusion, whereas during the subcutaneous dosing (month 2 to month 11), the trough levels reached 1.1 ± 0.8 μg/mL. Pharmacokinetic parameters are presented in Supplementary Table I (online only).

Laboratory findings. Serum creatinine and high-sensitivity C-reactive protein levels were both higher in the Xilonix group at baseline compared with the SOC group. Although these differences were not statistically significant ($P = .38$ and $P = .18$, respectively), these differences are potentially clinically significant as elevations in both measures are known prognosticators for future cardiovascular events. The changes in laboratory parameters from baseline to 6 weeks and 12 months were equally distributed across both groups (Table IV).

Adverse events (AEs). The most common AEs in the Xilonix group, classified according to *Medical Dictionary for Regulatory Activities* version 15.1 system organ class, were as follows: musculoskeletal and connective tissue disorders (15%); infections and infestations (11%); injury, poisoning, and procedural complications (10%); and general disorders and administration site conditions (9%). The AEs most prevalent in the SOC arm were as follows: injury, poisoning, and procedural complications (20%); vascular disorders (15%); musculoskeletal and connective tissue disorders (11%); cardiac disorders (9%); and general disorders and administration site conditions (9%). Grade 3 or grade 4 AEs were equally distributed in both the study arms (Xilonix + SOC, 26%; SOC, 21%; $P = .46$). Only three grade 3 or grade 4 AEs were reported as possibly related to Xilonix therapy (lower extremity claudication, gastrointestinal hemorrhage, groin hematoma). Twenty-three percent of AEs were recorded as serious AEs in the Xilonix group and 15% in the SOC group ($P = .19$). As expected in this population of patients, most of the serious AEs were cardiovascular in nature. Listings of all grade 3 and grade 4 AEs by study arm are presented in Supplementary Tables II, *A* and *B* (online only).

DISCUSSION

In this proof-of-concept trial, a novel true human monoclonal antibody targeting a mediator of sterile

Table IV. Laboratory parameters: change from baseline to 6 weeks (n = 16, Xilonix group; n = 18, standard of care [*SOC*] group) and 12 months (n = 12, Xilonix group; n = 15, SOC group)

Variable	*Change at week 6 (Xilonix + SOC)*	*Change at week 6 (SOC)*	P *value (week 6 change)*	*Change at month 12 (Xilonix + SOC)*	*Change at month 12 (SOC)*	P *value (month 12 change)*
Hematologic parameters						
Hemoglobin, g/dL	−0.84 ± 1.5	−0.21 ± 1.0	.18	0.21 ± 1.4	−0.56 ± 1.11	.19
Platelets, $10^3/\mu L$	18.8 ± 84.9	11.1 ± 25.5	.42	−3.3 ± 54.9	6.8 ± 17.5	.67
WBCs, $10^3/\mu L$	0.51 ± 3.1	0.36 ± 1.8	.87	−0.53 ± 1.62	−0.21 ± 2.3	.25
Absolute neutrophil count, $10^3/\mu L$	0.48±2.9	0.82 ± 2.3	.72	−0.59 ± 1.2	−0.04 ± 2.1	.45
Biochemical parameters						
Albumin, g/dL	−0.15 ± 0.53	0.16 ± 0.34	.06	−0.1 ± 0.39	0.05 ± 0.40	.35
Alkaline phosphatase, IU/L	0.13 ± 14.7	1.7 ± 19.9	.97	5.1 ± 15.1	3.7 ± 21.9	.61
ALT, IU/L	1.9 ± 10.3	−4.6 ± 18.3	.17	0.36 ± 10.2	−8.2 ± 23.8	.1
AST, IU/L	0.43 ± 6.9	−0.7 ± 18.9	.39	−1.7 ± 6.9	2.5 ± 28.9	.87
Total bilirubin, mg/dL	−0.06 ± 0.17	−0.09 ± 0.17	.68	−0.09 ± 0.24	−0.04 ± 0.14	.49
Glucose, mg/dL	1.3 ± 73.6	22.0 ± 108.0	.09	5.5 ± 42.6	32.4 ± 122.8	.71
Calcium, mg/dL	−0.14 ± 0.67	−0.12 ± 0.55	.94	−0.16 ± 0.45	−0.17 ± 0.49	.96
Creatinine, mg/dL	0.07 ± 0.16	0.09 ± 0.12	.58	0.49 ± 1.15	0.07 ± 0.22	.23
Ultrasensitive CRP, mg/dL	−1.2 ± 2.5	0.08 ± 4.4	.63	−14.6 ± 47.4	1.6 ± 8.5	.75
Magnesium, mg/dL	0.06 ± 0.16	0.09 ± 0.11	.66	−0.03 ± 0.17	0.08 ± 0.31	.35
Sodium, mEq/L	0.62 ± 2.7	−1.2 ± 3.2	.1	0.416 ± 2.02	0.533 ± 3.02	.57

ALT, Alanine aminotransferase; *AST,* aspartate aminotransferase; *CRP,* C-reactive protein;*WBCs,* white blood cells.
The *P* values presented in the table are from Mann-Whitney *U* test, which compares the magnitude of change between the two groups.
Data are presented as mean ± standard deviation.

inflammation was used in the hope of improving patient outcomes after peripheral vascular intervention. The aim of this trial was to demonstrate that IL-1α blockade is safe in this population and, more important, to interrupt the very early triggering events in the inflammatory cascade that drive leukocyte recruitment and extravasation into the arterial wall after intervention with the intent of modulating the neointimal hyperplasia response.

Xilonix therapy was well tolerated, with no apparent adverse safety signals. There were no infusion reactions, discontinuations for drug toxicity, or death of any patients in the trial.

Rates of target vessel restenosis and MACE were not different between the groups at the 12-month follow-up time point. After careful analysis, however, a considerable imbalance of several clinically significant factors was noted between the two groups. The Xilonix group had higher prevalence of diabetics and insulin-dependent diabetics. A history of dyslipidemia was more common in the Xilonix group, as were higher baseline high-sensitivity C-reactive protein and serum creatinine scores. Compared with controls, Xilonix patients had less ABI improvement postoperatively on day 0 and more frequent atherectomy procedures. These characteristics should have led to a worse outcome compared with the control group on the basis of previous reports in the literature. There was also an imbalance in the type of procedure and improvement of blood flow immediately after the procedure between the two groups. This imbalance in baseline characteristics may be an explanation for the narrowing of beneficial effect seen in the treatment arm over time.

There was a difference in restenosis rates between the two groups at the 4-month postprocedure time point. This apparent early treatment benefit, which diminishes during the course of the study, could have been the result of the dosing regimen inherent in the study design. In the study, 3.75 mg/kg was administered every 2 weeks commencing immediately after the revascularization procedure. In total, intravenous doses were administered four times during a 6-week period. The average dose was approximately 320 mg of Xilonix every 2 weeks. The patients then began receiving their once-monthly 200-mg subcutaneous injections for the duration of the study. Thus, patients went from receiving about 640 mg of drug per month with peak serum levels of >80 μg/mL to receiving 200 mg per month with peak serum levels of about 12 μg/mL. The total amount of Xilonix administered was less by about one third during the subcutaneous phase of the study, and peak serum levels were reduced by more than sevenfold.

The concept of the study to provide significant dosing immediately around the time of surgical intervention, followed by maintenance dosing after the acute arterial injury, still appears to be a good strategy. In light of these data indicating a critical dose-dependent effect at doses of 3.75 mg/kg intravenously every 2 weeks, biweekly or weekly maintenance subcutaneous dosing may provide more durable clinical benefit.

The use of a medical therapy for treating restenosis must have the potential to reduce activation of inflammatory cascades where there is an indolent process or, in the case of restenosis, where there is heightened interaction between circulating leukocytes, the complex milieu of a disrupted plaque, and an acutely injured artery. The last situation has been considered a very high bar from which to assess the potential for Xilonix therapy to reduce inflammatory processes within the vasculature. Furthermore, Xilonix antibody therapy was evaluated for restenosis and MACEs in a peripheral vascular disease setting in which intervention is associated with a relatively rapid and high rate of restenosis. The peripheral vascular disease population typically receives multiple medications and has frequent comorbidities, such as diabetes or cardiovascular disease. Therefore, it was considered critically important that in a proof-of-concept study, Xilonix must exhibit an exceptionally high degree of safety and tolerability with little evidence of drug-drug interactions.

Clinical findings with Xilonix in these 43 patients undergoing percutaneous revascularization of the SFA did show a trend toward reduced rates of restenosis and an overall reduction in MACEs at the end of a 16-week treatment phase. Xilonix, the first true human monoclonal in this setting, demonstrated a high degree of tolerability and safety. Taken together, these findings suggest further clinical evaluation of the treatment approach in a larger randomized study, with a more frequent maintenance dosing regimen. Larger numbers should reduce the potential for imbalance in baseline factors between treatment arms, and more frequent subcutaneous dosing will allow a sustained therapeutic dose. Ultimately, Xilonix therapy for patients with advanced peripheral vascular disease is a doorway to addressing a broader set of vascular pathologic processes relating to atherosclerosis, ischemia-reperfusion injury, and restenosis.

CONCLUSIONS

Xilonix therapy was safe and well tolerated. There was an observed tendency toward fewer target vessel reinterventions in the group treated with Xilonix at the 4-month time point, suggesting that this agent could potentially represent a safe and effective therapeutic approach to preserving vessel patency.

AUTHOR CONTRIBUTIONS

Conception and design: HES, RK, MD
Analysis and interpretation: HES, RK, MS, PM, MD
Data collection: HES, RK, PM
Writing the article: HES, RK, MS, MD
Critical revision of the article: HES, RK, MD
Final approval of the article: HES, RK, MD
Statistical analysis: PM
Obtained funding: HES, MS
Overall responsibility: HES

REFERENCES

1. Shammas N. Epidemiology, classification, and modifiable risk factors of peripheral arterial disease. Vasc Health Risk Manag 2007;3:229-34.
2. Hirsch AT, Haskal ZJ, Hertzer NR. ACC/AHA guidelines for the management of peripheral arterial disease (lower extremity, renal, mesenteric, and abdominal aortic): executive summary a collaborative report from the American Association for Vascular Surgery/Society for Vascular Surgery, Society for Cardiovascular Angiography and Interventions, Society for Vascular Medicine and Biology, Society of Interventional Radiology, and the ACC/AHA Task Force on Practice Guidelines (Writing Committee to Develop Guidelines for the Management of Patients With Peripheral Arterial Disease) endorsed by the American Association of Cardiovascular and Pulmonary Rehabilitation; National Heart, Lung, and Blood Institute; Society for Vascular Nursing; TransAtlantic Inter-Society Consensus; and Vascular Disease Foundation. J Am Coll Cardiol 2006;47:1239-312.
3. Aronow WS. Peripheral arterial disease of the lower extremities. Arch Med Sci 2012;8:375-88.
4. Dormandy JA, Rutherford B. Management of peripheral arterial disease (PAD). TASC Working Group. TransAtlantic Inter-Society Consensus (TASC). J Vasc Surg 2000;31:S1-296.
5. Johnston KW. Femoral and popliteal arteries: reanalysis of results of balloon angioplasty. Radiology 1992;183:767-71.
6. Minar E, Pokrajac B, Maca T, Ahmadi R, Fellner C, Mittlböck M, et al. Endovascular brachytherapy for prophylaxis of restenosis after femoropopliteal angioplasty: results of a prospective randomized study. Circulation 2000;102:2694-9.
7. Schlager O, Dick P, Sabeti S, Amighi J, Mlekusch W, Minar E, et al. Long-segment SFA stenting—the dark sides: in-stent restenosis, clinical deterioration, and stent fractures. J Endovasc Ther 2005;12:676-84.
8. Scheinert D, Duda S, Zeller T, Krankenberg H, Ricke J, Bosiers M, et al. The LEVANT I (Lutonix paclitaxel-coated balloon for the prevention of femoropopliteal restenosis) trial for femoropopliteal revascularization: first-in-human randomized trial of low-dose drug-coated balloon versus uncoated balloon angioplasty. JACC Cardiovasc Interv 2014;7:10-9.
9. Stone GW, Moses JW, Ellis SG, Schofer J, Dawkins KD, Morice MC, et al. Safety and efficacy of sirolimus- and paclitaxel-eluting coronary stents. N Engl J Med 2007;356:998-1008.
10. Lagerqvist B, James SK, Stenestrand U, Lindbäck J, Nilsson T, Wallentin L. Long-term outcomes with drug-eluting stents versus bare-metal stents in Sweden. N Engl J Med 2007;356:1009-19.
11. Kamari Y, Shaish A, Shemesh S, Vax E, Grosskopf I, Dotan S, et al. Reduced atherosclerosis and inflammatory cytokines in apolipoprotein-E-deficient mice lacking bone marrow-derived interleukin-1α. Biochem Biophys Res Commun 2011;405:197-203.
12. Waehre T, Yndestad A, Smith C, Haug T, Tunheim SH, Gullestad L, et al. Increased expression of interleukin-1 in coronary artery disease with downregulatory effects of HMG-CoA reductase inhibitors. Circulation 2004;109:1966-72.
13. Moyer CF, Sajuthi D, Tulli H, Williams JK. Synthesis of IL-1 alpha and IL-1 beta by arterial cells in atherosclerosis. Am J Pathol 1991;138:951-60.
14. Larionov S, Dedeck O, Birkenmeier G, Thal DR. Expression of α_2-macroglobulin, neutrophil elastase, and interleukin-1α differs in early-stage and late-stage atherosclerotic lesions in the arteries of the circle of Willis. Acta Neuropathol 2007;113:33-43.
15. Schultz K, Murthy V, Tatro JB, Beasley D. Endogenous interleukin-1α promotes a proliferative and proinflammatory phenotype in human vascular smooth muscle cells. Am J Physiol Heart Circ Physiol 2007;292:H2927-34.
16. Beasley D, Cooper A. Constitutive expression of interleukin-1α precursor promotes human vascular smooth muscle cell proliferation. Am J Physiol Heart Circ Physiol 1999;276:H901-12.
17. Chamberlain J, Wheatcroft M, Arnold N, Lupton H, Crossman DC, Gunn J, et al. A novel mouse model of in situ stenting. Cardiovasc Res 2010;85:38-44.

18. Isoda K, Shiigai M, Ishigami N, Matsuki T, Horai R, Nishikawa K, et al. Deficiency of interleukin-1 receptor antagonist promotes neointimal formation after injury. Circulation 2003;108:516-8.
19. Morton AC, Arnold ND, Gunn J, Varcoe R, Francis SE, Dower SK, et al. Interleukin-1 receptor antagonist alters the response to vessel wall injury in a porcine coronary artery model. Cardiovasc Res 2005;68:493-501.
20. Garrone P, Djossou O, Fossiez F, Reyes J, Ait-Yahia S, Maat C, et al. Generation and characterization of a human monoclonal autoantibody that acts as a high affinity interleukin-1α specific inhibitor. Mol Immunol 1996;33:649-58.
21. Coleman KM, Gudjonsson JE, Stecher M. Open-label trial of MABp1, a true human monoclonal antibody targeting interleukin 1α, for the treatment of psoriasis. JAMA Dermatol 2015;151:555-6.

Submitted Mar 31, 2015; accepted Aug 17, 2015.

Additional material for this article may be found online at www.jvascsurg.org.

Supplementary Table I (online only). Pharmacokinetics of Xilonix

	Peak concentration		*Lowest concentration*	
	Average, μg/mL	*CV, %*	*Average, μg/mL*	*CV, %*
Week 0	86.6	25.1	5.0	67.5
Week 2	94.6	46.9	5.6	81.8
Week 4	90.2	24.0	6.3	62.8
Week 6	85.6	21.8	5.5	74.3
Month 2	—	—	1.5	68.8
Month 3	—	—	1.1	73.8
Month 4	—	—	0.9	72.5
Month 5	—	—	1.0	99.6
Month 6	—	—	0.9	75.6
Month 7	—	—	1.2	76.2
Month 8	—	—	0.9	83.0
Month 9	—	—	1.1	50.1
Month 10	—	—	1.0	80.0

CV, Coefficient of variation.
Blood samples were collected for pharmacokinetic analysis before each intravenous infusion and 60 to 90 minutes after infusion. For subcutaneous administrations (month 2 and later), only preinjection samples were collected.

Supplementary Table II. A, (online only). Listing of adverse events (*AEs*) in the Xilonix + standard of care (SOC) arm

MedDRA preferred term (2010-PT017; Xilonix arm)	*No. of AEs by CTCAE grade (% of total subjects, n = 22; no grade 5 AE reported)*		
	Grade 1-2	*Grade 3*	*Grade 4*
Pain in extremity	6 (27.3)	0 (0.0)	0 (0.0)
Diarrhea	3 (13.6)	0 (0.0)	0 (0.0)
Fever	3 (13.6)	0 (0.0)	0 (0.0)
Hypertension	2 (9.1)	0 (0.0)	0 (0.0)
Arterial restenosis	1 (4.5)	5 (22.7)	0 (0.0)
Gangrene	1 (4.5)	2 (9.1)	1 (4.5)
Anemia	1 (4.5)	1 (4.5)	0 (0.0)
Hypotension	1 (4.5)	1 (4.5)	0 (0.0)
Skin ulcer	1 (4.5)	1 (4.5)	0 (0.0)
Osteomyelitis	0 (0.0)	3 (13.6)	0 (0.0)
Arterial insufficiency	0 (0.0)	1 (4.5)	0 (0.0)
Arteriosclerosis	0 (0.0)	1 (4.5)	0 (0.0)
Cardiac failure aggravated	0 (0.0)	1 (4.5)	0 (0.0)
Cardiac failure congestive	0 (0.0)	1 (4.5)	0 (0.0)
Cellulitis	0 (0.0)	1 (4.5)	0 (0.0)
Convulsion	0 (0.0)	1 (4.5)	0 (0.0)
Coronary artery disease	0 (0.0)	1 (4.5)	0 (0.0)
Gastrointestinal hemorrhage	0 (0.0)	1 (4.5)	0 (0.0)
Groin hematoma	0 (0.0)	1 (4.5)	0 (0.0)
Large intestinal ulcer	0 (0.0)	1 (4.5)	0 (0.0)
Localized infection	0 (0.0)	1 (4.5)	0 (0.0)
Peptic ulcer	0 (0.0)	1 (4.5)	0 (0.0)
Pneumonia	0 (0.0)	1 (4.5)	0 (0.0)
Pulmonary edema	0 (0.0)	1 (4.5)	0 (0.0)
Acute kidney injury	0 (0.0)	0 (0.0)	1 (4.5)
Thrombus of access site	0 (0.0)	0 (0.0)	1 (4.5)
Dysarthria	0 (0.0)	0 (0.0)	1 (4.5)

CTCAE, Common Terminology Criteria for Adverse Events; MedDRA, Medical Dictionary for Regulatory Activities.

Supplementary Table II. B, (online only). Listing of adverse events (*AEs*) in the standard of care (SOC) arm

MedDRA preferred term (2010-PT017; control arm)	*No. of AEs by CTCAE grade (% of total subjects, n = 21; no grade 5 AE reported)*		
	Grade 1-2	*Grade 3*	*Grade 4*
Intermittent claudication	3 (13.0)	1 (4.3)	0 (0.0)
Cataract	2 (8.7)	0 (0.0)	0 (0.0)
Hypertension	2 (8.7)	0 (0.0)	0 (0.0)
Edema peripheral	2 (8.7)	0 (0.0)	0 (0.0)
Respiratory tract infection	2 (8.7)	0 (0.0)	0 (0.0)
Shortness of breath	2 (8.7)	0 (0.0)	0 (0.0)
Arterial restenosis	0 (0.0)	7 (30.4)	0 (0.0)
Cardiac failure congestive	0 (0.0)	1 (4.3)	1 (4.3)
Arteriovenous fistula	0 (0.0)	1 (4.3)	0 (0.0)
Angina pectoris	0 (0.0)	1 (4.3)	0 (0.0)
Dehydration	0 (0.0)	1 (4.3)	0 (0.0)
Hyperglycemic hyperosmolar nonketotic syndrome	0 (0.0)	1 (4.3)	0 (0.0)
Osteomyelitis	0 (0.0)	1 (4.3)	0 (0.0)
Ovarian mass	0 (0.0)	1 (4.3)	0 (0.0)
Pulmonary embolism	0 (0.0)	1 (4.3)	0 (0.0)
Syncope	0 (0.0)	1 (4.3)	0 (0.0)
Thrombosis in device	0 (0.0)	1 (4.3)	0 (0.0)
Cerebrovascular accident	0 (0.0)	0 (0.0)	1 (4.3)
Small cell lung cancer metastatic	0 (0.0)	0 (0.0)	1 (4.3)

CTCAE, Common Terminology Criteria for Adverse Events; MedDRA, Medical Dictionary for Regulatory Activities.