Local Intracoronary Administration of Antisense Oligonucleotide Against c-myc for the Prevention of In-Stent Restenosis

Results of the Randomized Investigation by the Thoraxcenter of Antisense DNA Using Local Delivery and IVUS After Coronary Stenting (ITALICS) Trial

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OBJECTIVES
This study was designed to determine whether antisense oligodeoxynucleotides (ODN) directed against the nuclear proto-oncogene c-myc could inhibit restenosis when given by local delivery immediately after coronary stent implantation.

BACKGROUND
Failure of conventional pharmacologic therapies to reduce the incidence of coronary restenosis after percutaneous revascularization techniques has prompted interest in the use of agents that target intracellular central regulatory mechanisms.

METHODS
Eighty-five patients were randomly assigned to receive either 10 mg of phosphorothioate-modified 15-mer antisense ODN or saline vehicle by intracoronary local delivery after coronary stent implantation. The primary end point was percent neointimal volume obstruction measured by computerized analysis of electrocardiogram-gated intravascular ultrasound (IVUS) at six-month follow-up. Secondary end points included clinical outcome and quantitative coronary angiography analysis.

RESULTS
Analysis of follow-up IVUS data was performed on 77 patients. In-stent volume obstruction was similar between groups (44 ± 16% and 46 ± 14%, placebo vs. ODN; p = 0.57; 95% confidence interval: 1.13 to 0.85). Minimum luminal diameter increased from 0.84 ± 0.36 and 0.90 ± 0.45 (p = 0.55) to 2.70 ± 0.37 and 2.80 ± 0.37 (p = 0.28) after stent implantation, which decreased to 1.50 ± 0.61 and 1.50 ± 0.53 (p = 0.98) by six months, yielding similar loss indexes (placebo vs. ODN, respectively). There were no differences in angiographic restenosis rates (38.5 and 34.2%; p = 0.81; placebo vs. ODN) or clinical outcome.

CONCLUSIONS
Treatment with 10 mg of phosphorothioate-modified ODN directed against c-myc does not reduce neointimal volume obstruction or the angiographic restenosis rate in this patient population. (J Am Coll Cardiol 2002;39:281–7) © 2002 by the American College of Cardiology

The superiority of coronary stent implantation over conventional percutaneous transluminal coronary angioplasty for long-term outcome demonstrated by several randomized trials has led to the widespread use of coronary stents for the treatment of coronary artery disease (1–3). However, coronary stenting procedures are plagued by unacceptably high rates of restenosis, primarily as a result of neointimal accumulation. A potential approach for the prevention of the in-stent neointimal proliferative process involves the use of antisense oligodeoxynucleotides (AS-ODN) therapeutically targeted to genes believed to be critical for the pathogenesis of restenosis. Binding of the AS-ODN to its target messenger RNA (mRNA) results in an interference with its stability and translation, thereby preventing the expression of the targeted gene. c-myc, an immediate-early proto-oncogene that encodes c-Myc has been successfully targeted with AS-ODNs. c-Myc is a short-lived sequence-specific DNA binding nuclear phosphoprotein, which is involved in the transcriptional modulation of a number of genes involved in the cell cycle.

The purpose of this study was to determine whether AS-ODN directed against the nuclear proto-oncogene c-myc could inhibit the development of restenosis in humans when given by local delivery techniques immediately after coronary stent implantation.

METHODS

Patient selection. The trial was approved by the Medical Ethics Committee of the Academic Hospital Dijkzigt Rotterdam. Informed consent was obtained from each patient before enrollment.

The criteria for patient enrollment included the following: a single symptomatic or ischemia provoking de novo or restenotic lesion, suitable for stent implantation in a native...
coronary artery. Patients were excluded if they had experienced a myocardial infarction within 15 days of the procedure, impaired left ventricular function (left ventricular ejection fraction <35%), renal impairment (serum creatinine >2.0 mg/dl [>180 μmol/l]), hepatic dysfunction (total bilirubin >2.0 mg/dl [>35 μmol/l], alanine aminotransferase or aspartate aminotransferase >2× the upper limit of the reference range), an ostial lesion, a total occlusion, a target artery <2.5 mm or >4.0 mm in diameter or a target lesion >40 mm in length.

End points. The primary end point was percent in-stent volume obstruction as measured by intravascular ultrasound (IVUS) at six months follow-up catheterization. Secondary angiographic end points included minimum luminal diameter and loss index of the stented segment as measured by off-line quantitative coronary analysis (QCA) at six-months follow-up angiography. Secondary clinical end points were the composite outcome (and each component) of death (cardiac and noncardiac), myocardial infarction (at least two of: a history of chest discomfort of at least 30 min duration, twice the upper limit of normal of creatine kinase [CK] together with a pathological increase in the MB iso-enzyme of CK and the development of new and abnormal Q waves) (4), emergency or elective bypass surgery involving the previously treated segment and percutaneous revascularization of the treated vessel within six months of follow-up. The feasibility, safety and tolerability of the administration of LR-3280 within a stented segment using a local delivery device were also assessed.

Stent implantation. All patients received intra-arterial aspirin (250 mg) and heparin (10,000 IU) at the beginning of the procedure. Intraprocedural supplemental doses of heparin were given in order to maintain an activated clotting time of more than 300 s. Randomization took place after successful balloon predilation and the implantation of a self-expanding coronary Wallstent (Schneider AG, Bülach, Switzerland). After successful stent deployment, balloon postdilation to ≥12 atm was performed using semi- or noncompliant balloons of sufficient diameter to achieve a diameter stenosis of <20% as measured by on-line QCA. The use of IVUS for guidance of stent deployment was left to the discretion of the treating physician. Although the intention was to use a single Wallstent for each target lesion, second stents could be used when clinically indicated.

Antisense oligonucleotides. LR-3280 (Lynx Therapeutics, Hayward, California) is a 15-mer phosphorothioate oligonucleotide antisense to the 5′ translation initiation region of c-myc mRNA with the sequence 5′-AACGTGGAGGGGCAT-3′. It was supplied as a lyophilized powder and reconstituted with physiologic saline just before administration. Patients randomized to placebo treatment received the saline vehicle only. Blinding was achieved by the use of tamper-proof, foil-covered vials.

Local delivery. All LR-3280 and placebo treatments were administered in 2.0 ml using the Transport (Scimed, Boston Scientific Corporation, Minneapolis, Minnesota) local delivery catheter, which consists of a dual balloon system with an inner support balloon and an outer delivery balloon (1 cm in length). The inner support balloon was inflated to 3 atm. A total of 10 mg of LR-3280 was delivered to the stented segment using one, two or three deliveries, depending on stent length. The duration of each delivery was 1 min and controlled with a programmable infusion pump.

QCA analysis. Angiographic analysis was performed offline at the angiographic core lab (Cardialysis, Rotterdam, the Netherlands) using the Cardiovascular Angiographic Analysis System (CASS II, Pie Medical Imaging, Maastricht, The Netherlands) as described elsewhere (1,5–8). For each patient, three angiograms in multiple projections were obtained after the intracoronary administration of 0.2 mg of isosorbide dinitrate, pre- and postprocedure and at six-month follow-up.

IVUS image acquisition and analysis. At six month follow-up, the stented vessel segments were examined with a mechanical IVUS system (ClearView console, Cardiovascular Imaging System [CVIS], Sunnyvale, California) using a sheath-based IVUS catheter incorporating a transducer rotating at 1,800 rpm (MicroView, CVIS) after the intracoronary administration of isosorbide dinitrate (0.2 mg to 0.4 mg). The 30 MHz single-element IVUS transducer was withdrawn through the sonolucent imaging sheath by an automated electrocardiogram-triggered pullback device with a stepping motor (EchoScan, Tomtec, Munich, Germany) at 0.2 mm per step (9). A computer-based contour detection program, developed at our institute, was used for the automated three-dimensional analysis of up to 200 IVUS images (10). The feasibility and reproducibility of this system have been previously validated in vitro and in vivo (11–13), and the inter- and intraobserver differences in volumetric measurement (nonstented segments) have been reported (n = 30; r = 0.99) (11).

Plasma LR-3280 concentrations. Blood serum concentrations of LR-3280 were measured as part of the safety assessment of the antisense compound. Blood samples were collected from the arterial sheath before, immediately after and at 10 min and 30 min after delivery. Samples were collected in evacuated tubes containing lithium-heparin and placed immediately on ice for transport to the analytical laboratory where the plasma component was separated. The plasma samples were then stored at −20°C until batch assayed in a blinded manner at a central laboratory (Schwarz Pharma, AG, Monheim, Germany) by a competitive hybridization immunoassay (14).


**Figure 1.** Patient flow in the ITALICS trial.

**Power calculation and statistical analysis.** The size of the patient group required to test the hypothesis was calculated based on an expected in-stent neointimal volume as measured by IVUS of 129 mm$^3$, a value that was determined from earlier observations at six-month follow-up of patients who had received the Wallstent (15). To detect a reduction in neointimal volume of 30% with LR-3280 treatment with 81% power at a two-sided type 1 error rate (alpha) of 0.05, it was calculated that a total of 70 evaluable patients were necessary. This assumes that the SD is 50% of the actual plaque volume in the control group.

Continuous variables are expressed as means ± SD and were compared by the unpaired Student t test. The chi-square test with Yates’ correction was used to compare proportions. Discrete variables are expressed as counts and percentages and were compared in terms of relative risks with 95% confidence intervals calculated by the formula of Greenland and Robins (16). All statistical tests were two-tailed.

**RESULTS**

**Baseline characteristics.** Patient flow through the study is shown in Figure 1. The baseline characteristics of the 85 randomized patients included in the intention-to-treat analysis are shown in Table 1. Four placebo- and three antisense-treated patients had the IIb/IIa platelet receptor antagonist ReoPro (Eli Lilly Nederland BV, Nieuwegein, The Netherlands) administered after stent implantation.

**Plasma AS-ODN concentrations.** All pretreatment blood samples showed values below the quantification limits for the assay (<10 ng/ml). In addition, random analyses of all timed blood samples from three patients in the placebo group were analyzed and were also below the limit of quantification. The serum concentrations of antisense oligonucleotide in the study patients are shown in Figure 2. At all time points the serum concentrations were <5 μM (25 μg/ml), which is below the level at which nonspecific effects of phosphorothioate AS-ODNs have been suggested to occur (17).

**Outcome of IVUS analysis.** Intravascular ultrasound analysis data at six-month follow-up were available in 34 (87%) of the placebo-treated patients and 34 (89%) of the LR-3280-treated patients. In the remaining patients, IVUS interrogation was not possible because the degree of intimal proliferation prevented passage of the IVUS catheter. For these patients, prospectively defined imputation rules were employed to estimate the neointimal volume (see Appendix). The in-stent volume obstruction was similar between the two groups (44 ± 16% and 46 ± 14%; mean ± SD; placebo vs. LR-3280, p = 0.57) (Table 2). In addition, there were no significant differences in lumen area, stent length, stent volume, lumen volume or in-stent neointimal volume.

**Outcome of angiographic follow-up.** Complete six-month angiographic follow-up data were available for 77 patients. There were no significant differences in the baseline or follow-up angiographic variables between the two treatment groups. Minimum luminal diameter increased from 0.84 ± 0.36 and 0.90 ± 0.45 mm to 2.70 ± 0.37 mm and 2.80 ± 0.37 mm (mean ± SD) immediately after stent implantation (placebo vs. control, respectively), but by six months follow-up it had decreased to 1.50 ± 0.61 and 1.50 ± 0.53 (p = 0.98), yielding loss indexes that were not significantly different. The binary restenosis rate (>50% lumen obstruction) at six-month follow-up was likewise

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**Table 1.** Baseline Clinical and Angiographic Characteristics of 85 Patients Included in the Intention-to-Treat Analysis According to Treatment Group

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Placebo (n = 43)</th>
<th>LR-3280 (n = 42)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yrs*</td>
<td>63.0 ± 9.4</td>
<td>58.0 ± 10.0</td>
</tr>
<tr>
<td>Male gender, no. (%)</td>
<td>25 (58)</td>
<td>33 (79)</td>
</tr>
<tr>
<td>Risk factors, no. (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current smoking</td>
<td>8 (19)</td>
<td>10 (24)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>4 (9)</td>
<td>4 (10)</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>21 (49)</td>
<td>16 (38)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>13 (30)</td>
<td>15 (36)</td>
</tr>
<tr>
<td>Family history of ischemic heart disease</td>
<td>16 (37)</td>
<td>18 (43)</td>
</tr>
<tr>
<td>Previous myocardial infarction, no. (%)</td>
<td>16 (37)</td>
<td>15 (36)</td>
</tr>
<tr>
<td>Exertional angina, no. (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Class I</td>
<td>0 (0)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Class II</td>
<td>6 (14)</td>
<td>7 (17)</td>
</tr>
<tr>
<td>Class III</td>
<td>7 (16)</td>
<td>8 (19)</td>
</tr>
<tr>
<td>Class IV</td>
<td>4 (9)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Unstable angina, no. (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Class IIB</td>
<td>4 (9)</td>
<td>6 (14)</td>
</tr>
<tr>
<td>Class IIC</td>
<td>16 (37)</td>
<td>16 (38)</td>
</tr>
<tr>
<td>Class IC</td>
<td>2 (5)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Class IIC</td>
<td>3 (7)</td>
<td>4 (10)</td>
</tr>
<tr>
<td>Vessel treated, no. (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LAD</td>
<td>18 (42)</td>
<td>18 (43)</td>
</tr>
<tr>
<td>LCx</td>
<td>6 (14)</td>
<td>6 (14)</td>
</tr>
<tr>
<td>RCA</td>
<td>19 (44)</td>
<td>18 (43)</td>
</tr>
</tbody>
</table>

*Expressed as mean ± SD.

1. LAD = left anterior descending coronary artery; LCx = left circumflex coronary artery; RCA = right coronary artery.
similar in the two groups (38.5% and 34.2%, placebo versus LR-3280; \(p = 0.81\)).

**Clinical events.** The incidence of major cardiac events presented as single worst event per patient is shown in Table 3. There was no significant difference in the event rate between patients treated with placebo and those treated with LR-3280. At 210 days, event-free survival by Kaplan-Meier analysis was 72.1% in the placebo group as compared with 71.4% in the LR-3280-treated group (Fig. 3, \(p = 0.91\)). The single death in the placebo-treated group occurred five months after the local delivery procedure and resulted from a complication of coronary artery bypass grafting.

**DISCUSSION**

The AS-ODNs directed against c-myc have been the most extensively studied of the antisense DNA sequences considered for the prevention of restenosis after coronary interventions and have been shown to decrease c-Myc protein expression and inhibit the growth of both rat and
human vascular smooth muscle cells in vitro (18,19) and prevent restenosis in animal models (20–23). In the present trial, 10 mg of LR-3280 administered using a local delivery device immediately after coronary stenting did not lead to a reduction in the neointimal volume obstruction as measured by IVUS at six-month follow-up, nor were there any differences in the angiographic parameters of minimum luminal diameter, late loss, loss index or binary restenosis rate at six-month follow-up. The frequency of clinical events was also similar between the two groups.

**Study limitations.** There may be several reasons for the observed lack of effect of the antisense compound. First, the local concentration of antisense compound achieved may not have been high enough or maintained long enough to show a significant effect. The dose of antisense compound used in this study was chosen based on existing safety and efficacy data. Using the same antisense compound and a porous balloon delivery catheter, Shi et al. (23) have shown that the efficiency of delivery is <1% in a swine coronary model of vessel injury. In diseased human vessels, the tissue retention of compound delivered via local intracoronary administration using a coil balloon has been shown to be between 1% and 8% (24). It is possible that the use of a delivery device that better targets the media could have resulted in higher tissue concentrations of AS-ODNs.

Second, the single administration of AS-ODN employed in the present trial may not have been effective. It is known that after acute vessel injury, c-myc expression shows a biphasic response with early and late expression peaks (19,25,26). The delivered antisense compound may have been effective in suppressing early c-Myc protein production, but washout and degradation may have resulted in tissue concentrations too low to suppress expression by the later peak. Very little is known about the expression of c-myc expression in stented vessels, and the self-expanding nature of the stent chosen for use in the present trial with its persistent radial strain on the vessel wall may have further confounded the results (15).

Third, it has been shown that the intraluminal delivery of saline using a local delivery device can exaggerate the intimal proliferative response (27). It is possible that the use of a saline vehicle in this trial aggravated the neoproliferative

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**Table 3.** Major Adverse Cardiac Events to 210 Days: Hierarchical Ranking of Worst Event per Patient (Intention-to-Treat) According to Treatment Group

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n = 43)</th>
<th>LR-3280 (n = 42)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Death, no. (%)</td>
<td>1 (2)</td>
<td>0</td>
</tr>
<tr>
<td>MI, no. (%)</td>
<td>3 (7)</td>
<td>5 (12)</td>
</tr>
<tr>
<td>Q-wave, no. (%)</td>
<td>2 (5)</td>
<td>2 (5)</td>
</tr>
<tr>
<td>Non-Q-wave, no. (%)</td>
<td>1 (2)</td>
<td>0</td>
</tr>
<tr>
<td>CABG, no. (%)</td>
<td>7 (16)</td>
<td>7 (17)</td>
</tr>
<tr>
<td>re-PTCA, no. (%)</td>
<td>31 (72)</td>
<td>30 (71)</td>
</tr>
<tr>
<td>No adverse event, no. (%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CABG = coronary artery bypass grafting; MI = myocardial infarction; PTCA = percutaneous transluminal coronary angioplasty.

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**Figure 3.** Kaplan-Meier survival curves for major cardiac events (death, myocardial infarction, coronary artery bypass surgery and repeat percutaneous revascularization of the target lesion). *p* = 0.91 determined by the log-rank test. The cluster of events just after 180 days reflects the scheduled follow-up. Dashed line = placebo group; solid line = LR-3280 group.
response and masked any beneficial effect of the antisense compound.

Finally, it must be considered that the target chosen was not appropriate. Although the c-myc proto-oncogene is believed to be one of the members of the final common pathway of cell cycle regulation, it is not the sole controlling mechanism for neointimalization. It is possible that, with a combination of antisense ODNs targeting different genes, inhibition of neointimal formation might be achieved. Proof of this concept has been shown in an animal model of vascular injury (28).

Conclusions. The results of this trial indicated that the administration of 10 mg of AS-ODN directed against c-myc using the transport local delivery catheter immediately after implantation of a self-expanding intracoronary stent was ineffective for the prevention of restenosis measured at six-month follow-up. There were no adverse effects to the trial medication or from the method of delivery so that a safe platform for future studies has been established.

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REFERENCES


APPENDIX

Imputation Rules

In situations of total vessel occlusion, the assumption was that the entire length and volume of the stent was filled with neointimal tissue. In these cases, a contrast free frame was analyzed by quantitative coronary analysis (QCA), and the length and mean area of the stent were determined.
This analysis was performed in the angiographic view with the longest stent length. The following regression equations were generated from the unblinded data comparing intravascular ultrasound (IVUS) and QCA determined variables:

1. IVUS empty stent length = 2.06 + 1.02 (QCA determined stent length). \( r^2 = -0.93 \)
2. IVUS determined lumen area = 1.90 + 0.85 (QCA determined lumen area); QCA area was calculated using \( \pi \) (one-half mean diameter)^2. \( r^2 = 0.66 \)
3. Preliminary predicted IVUS lumen volume = equation 1 multiplied by equation 2.
4. IVUS lumen volume = 4.41 + 0.96 (preliminary predicted IVUS lumen volume). \( r^2 = 0.78 \)

These equations were used to correct for the systematic difference between IVUS and QCA-determined measurements.

In the case of severely stenotic but nonocclusive lesions, not only was the volume of the stent calculated, but the volume of the lumen opacified by contrast was also analyzed. Stent volumes were determined using regression equations 1 to 4, and the neointimal volume was calculated as the difference between the stent and the luminal volume.