Alternatively, direct interference of the critical steps in the smooth deposition of extracellular matrix material, which often lead to proliferation of smooth muscle cells around the vessel intima and cell migration [8]. This cascade of events results in an uncontrolled remodeling of the extracellular matrix, and initiating smooth muscle cell growth has been attempted [10,11]. In a previously performed trial (ITALICS) using antisense DNA using local delivery of phosphorothioate modified 15-mer antisense oligonucleotides showed no reduction of neointimal volume or restenosis rate [12]. With local intramural delivery and intracoronary administration of Resten-NG [7] a dramatic reduction of neointimal formation was shown.

Perfluorobutane gas microbubbles (PGMC) with a coating of dextrose and albumin efficiently bind antisense oligomers [13]. These 0.3 to 10 μm particles bind to sites of vascular injury. Further, the perfluorobutane gas is an effective cell membrane fluidizer. The potential advantages of micro bubble carrier delivery include minimal addition to vessel injury from delivery, no resident polymer to degrade leading to eventual inflammation, rapid bolus delivery, and repeated delivery is highly feasible. Further, the potential of PGMC to deliver to vessel regions both proximal and distal to stents in vessels.

This first in man (FIM) clinical study will evaluate the safety and potential effectiveness of RESTEN-MP™ to reduce in-stent restenosis.

Keywords: c-myc; Restenosis; Clinical trial; First-in-men

Introduction

Over the past decade, the use of endoluminal metallic stents has become common practice during percutaneous coronary intervention (PCI). Clinical trials showed a reduced restenosis rates when compared with balloon angioplasty alone [1]. Although stents significantly reduce restenosis when compared with balloon angioplasty, restenosis rates in stented patients still reached 20% to 40% at 6 months [2]. Recently, the concept of drug-eluting stents has emerged. The overall restenosis rate of <10% has been reported depending on the lesion treated. But still concept of drug-eluting stents has emerged. The overall restenosis when compared with balloon angioplasty, restenosis rates in stented patients still reached 20% to 40% at 6 months [2]. Recently, the concept of drug-eluting stents has emerged. The overall restenosis rate of <10% has been reported depending on the lesion treated. But still concept of drug-eluting stents has emerged. The overall restenosis when compared with balloon angioplasty, restenosis rates in stented patients still reached 20% to 40% at 6 months [2].

The Appraisal-Trial: Evaluating RESTEN-MP™ in Patients with Bare Metal Stent De Novo Native Coronary Artery Lesions

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Abstract

Neointimal hyperplasia is one of the key components of the restenotic process. The aim of this study was to evaluate the efficacy and safety of a microbubble delivery of c-myc antisense peptide in reducing restenosis after coronary stenting in de novo stenosis with intravascular ultrasound. A Multi-Link Zeta bare metal stent was implanted in de novo coronary artery lesions (RD ≥ 2.5–4.0 mm; TL ≥ 15–30 mm in length). Serial intravascular ultrasound analyses were performed in 25 lesions. A dose of 16mg RESTEN-MP™ (AVI BioPharma supplied by Global Therapeutics LLC) was intravenously administered after stenting and again 24 hours later. In three centres in Germany a total of 50 patients (51 lesions) were enrolled, 34 in Essen, 13 in Coburg and 3 in Heidelberg. Before stenting, the minimal lumen diameter (MLD) and length of stenosis were determined. 84% (43/51) of the lesions were either Type B2 or C lesions. At six-month follow-up, angiography was performed. Generally the neointimal proliferation was minor. Major adverse cardiac events (MACE) were 10.0% after 6 months and 21.9% after 12 months. The target lesion revascularization was 15.6%, the target vessel revascularization 18.8% after 12 months. Of the 34 patients studied at six-months in the IVUS sub-study, six patients required target lesion revascularization (TLR). Binary restenosis rate by intravascular ultrasound was 26 ± 4%. Generally the neointima proliferation was minor and open vessel lumen could be demonstrated during follow-up. Microbubble delivery of c-myc antisense seems to be effective in reducing neointimal tissue proliferation without the problem of late stent thrombosis. Neointima proliferation seems to be attenuated but not eliminated. MACE was not increased in this study population.

Keywords: c-myc; Restenosis; Clinical trial; First-in-men

In-stent restenosis is primarily due to neointimal hyperplasia [3]. Vessel injury by an angioplasty balloon or stent struts leads to the activation of platelets and mural thrombus formation [4]. The presence of vascular injury, mural thrombus, and a metallic foreign body activates circulating neutrophils and tissue macrophages [5]. These cellular elements release cytokines and growth factors that activate smooth muscle cells [6]. Upregulation and expression of genes such as c-myc that regulate cell division ensues, leading to cell proliferation [7]. Production of matrix metalloproteinases is also upregulated, leading to remodeling of the extracellular matrix, and initiating smooth muscle cell migration [8]. This cascade of events results in an uncontrolled proliferation of smooth muscle cells around the vessel intima and the deposition of extracellular matrix material, which often lead to significant luminal narrowing 3 to 6 months after PCI.

A potential application for therapy with antisense oligonucleotides is the prevention of restenosis after coronary interventions [9]. Alternatively, direct interference of the critical steps in the smooth muscle cell growth has been attempted [10,11]. In a previously performed trial (ITALICS) using antisense DNA using local delivery of phosphorothioate modified 15-mer antisense oligonucleotides showed no reduction of neointimal volume or restenosis rate [12]. With local intramural delivery and intracoronary administration of Resten-NG [7] a dramatic reduction of neointimal formation was shown.

Phosphorothioate modification of antisense peptide in reducing restenosis c-myc

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following balloon angioplasty. In order to objectively assess the therapeutic value of RESTEN-MPTM compared to other drugs used in combination with coronary artery stents and to utilize a sensitive method to assess the effectiveness of RESTEN-MPTM as a neointimal hyperplasia inhibitor, late loss between the time of stent placement and 6 months later is the therapeutic endpoint in this study.

Methods

Study design and eligibility

This first-in-man study using RESTEN-MPTM after successful stent-placement is a prospective, multi-center, nonrandomized, single-arm study of 50 patients with symptomatic ischemic heart disease with de novo lesions of native coronary arteries. The study complied with the provisions of the Declaration of Helsinki regarding investigations in humans, and was approved by local ethic committees for all investigational sites, and written informed consent was obtained for all patients. The study was financially supported by COOK MEDICAL INC., Bloomington, IN, USA.

Eligible patients had a history of stable or unstable angina and/ or signs of myocardial ischemia. A single newly diagnosed lesion in a native coronary artery resulting in stenosis of ≥50 and <100% of the luminal diameter, given a reference diameter between ≥2.5mm and ≤4.0mm and a lesion length between ≥15mm and ≤30mm (as estimated visually on angiography) was targeted for treatment. The major criteria for exclusion were recent myocardial infarction (within the previous 72 hours), a Left Ventricular Ejection Fraction (LVEF) ≤40%, an ejection fraction of <30%; a target lesion located in an ostium, at a bifurcation with a side branch over 2.0mm or an intervention in the target lesion. Late luminal loss was defined as stenosis of at least 50% of the luminal diameter in the target lesion. Late luminal loss was defined as the difference between the minimal luminal diameter (MLD) at the completion of the stenting procedure and that measured during follow-up.

Study endpoints

The primary end point of this study was safety and potential effectiveness of RESTEN-MPTM to reduce in-stent restenosis following balloon angioplasty and stent placement. The post-dose follow-up period is up to six-months. The secondary clinical end points included major adverse cardiac events (MACE; defined as cardiac death, MI (Q wave and non-Q wave), emergent cardiac bypass surgery, and clinically-driven target lesion revascularization (TLR) at days 14 and 30, and month 6, 9 and 12. All clinical end points were adjudicated by an independent clinical-events committee. A separate independent data and safety monitoring board reviewed all data periodically to identify potential safety issues.

Data collection, follow up, and core laboratory analyses

All data were submitted to a third-party data centre (Harvard clinical research institute – HCRI) independent of the sponsor, and the investigators had full access to the data. Clinical follow-up information was obtained for all patients by the research coordinators at each site at days 14 and 30 and the months 6, 9, and 12. All clinical end points were adjudicated by an independent clinical-events committee. A separate independent data and safety monitoring board reviewed all data periodically to identify potential safety issues.

Coronary angiograms

Obtained at baseline, at the completion of the stenting procedure, and at 6 months follow-up, were submitted to HCRI. "Binary" restenosis was defined as stenosis of at least 50% of the luminal diameter in the target lesion. Late luminal loss was defined as the difference between the minimal luminal diameter (MLD) at the completion of the stenting procedure and that measured during follow-up.

Results

Characteristics of the patients and the lesions

Between December 2005 and December 2006, 51 patients were
enrolled in three centres in Germany, 35 in Essen, 13 in Coburg and 3 in Heidelberg. A total of 53 lesions were treated (table 1). Overall, 77 percent of the patients were men, and the mean age was 63.9 years, with the expected prevalence of diabetes, smoking and known coronary artery disease.

**Procedural factors**

The targeted balloon-inflation pressure after stenting was 16 atm. A total of 33 stents were implanted. In one patient a second stent was necessary due to a demarcated distal stenosis discovered after the placement of the first stent. In another patient a distal type a dissection had to be covered. Both patients received their first treatment with slow-push intravenous administration of RESTEN-MP™ after placement of the second stent. There were no complaints of side effects after the administration of RESTEN-MP™.

**Baseline angiographic characteristics**

According to the ACC/ AHA lesion class [17], 3.9% had a lesion type A (adjudicated from HCRI, since type A was excluded), 11.8% had type B1, 51.0% type B2 and 33.3% type C. Table 2 shows the baseline angiographic characteristics. The patients enrolled represent a typical selection comparable to diverse interventional studies.

**Quantitative coronary angiography**

Before stenting, the minimal lumen diameter (MLD) and length of stenosis were determined by QCA (Quantitative coronary analysis). Table 3 shows the baseline data. At six month, follow-up angiography with a QCA was performed. Table 4 shows the 6-month data. Generally the neointimal proliferation was 22% and open vessel lumen could be demonstrated during follow-up.

**IVUS sub study**

Of the 35 patients currently studied at six-months in the IVUS sub-study, six patients required TLR. Late lumen loss by QCA in that subgroup was 0.47 ± 0.09mm compared to 0.32 ± 0.07mm with IVUS. Overall percent NIHV was 27.9% (Figures 2 and 3). Notably, no lesions (Table 5) patients with a type B1 stenosis had no TLR and a less percent NIHV was 27.9% (Figures 2 and 3). Notably, no lesions (Table 5) patients with a type B1 stenosis had no TLR and a less increase of volume stenosis compared to type B2 lesions.

**Clinical outcomes**

Major adverse cardiac events are listed in table 6. After 30 days there was one target vessel revascularization necessary. After 6 months one non Q-Wave MI needs to be reported due to stent thrombosis of the study stent. Furthermore one death of unknown cause needs to be reported. Of the necessary TLR and TVR one patient needs CAGB due to a massive progression of his coronary artery disease. The total MACE was 26% after 1 year, which was mainly due to TLR/ TVR.

**Discussion**

One of the potential clinical applications of antisense therapy is the prevention or treatment of restenosis following coronary interventions. Inhibition of several cellular proto-oncogenes has been

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**Table 1: Demographics and Baseline Characteristics.**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>RESTEN-MP™ (n=51)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>63.9 ± 10.2 (50)</td>
</tr>
<tr>
<td>Male</td>
<td>78% (39/50)</td>
</tr>
<tr>
<td>Revascularization for Angina or MI*</td>
<td>60% (30/50)</td>
</tr>
<tr>
<td>History of Previous Revascularization</td>
<td>8% (4/50)</td>
</tr>
<tr>
<td>History of Prior MI*</td>
<td>33% (16/50)</td>
</tr>
<tr>
<td>History of CABG*</td>
<td>2% (1/50)</td>
</tr>
<tr>
<td>History of Diabetes</td>
<td>34% (17/50)</td>
</tr>
<tr>
<td>History of Smoking</td>
<td>38% (19/50)</td>
</tr>
</tbody>
</table>

* MI = myocardial infarction, CABG = coronary artery bypass graft

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**Table 2: Baseline Angiographic Characteristics.**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>RESTEN-MP™ (n=51)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-procedure RVD</td>
<td>2.7 ± 0.6 mm</td>
</tr>
<tr>
<td>MLD (mm)</td>
<td>1.1 ± 0.4 mm</td>
</tr>
<tr>
<td>% Stenosis</td>
<td>57.7 ± 10.8</td>
</tr>
<tr>
<td>Post-stent (In-segment) RVD</td>
<td>2.7 ± 0.5 mm</td>
</tr>
<tr>
<td>MLD (mm)</td>
<td>2.2 ± 0.5 mm</td>
</tr>
<tr>
<td>% Stenosis</td>
<td>20.5 ± 8.4</td>
</tr>
<tr>
<td>Post-stent (In-stent) MLD</td>
<td>2.6 ± 0.5 mm</td>
</tr>
<tr>
<td>% Stenosis</td>
<td>4.5 ± 9.5</td>
</tr>
</tbody>
</table>

* RVD = reference vessel diameter; MLD = minimal lumen diameter

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**Table 3: Quantitative Angiographic Characteristics at baseline.**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>RESTEN-MP™ (n=28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RVD* (mm)</td>
<td>2.56 ± 0.46</td>
</tr>
<tr>
<td>In-segment (mm) MLD*</td>
<td>1.52 ± 0.61</td>
</tr>
<tr>
<td>% Stenosis</td>
<td>40.67 ± 21.79</td>
</tr>
<tr>
<td>In-stent (mm) MLD*</td>
<td>1.68 ± 0.54</td>
</tr>
<tr>
<td>% Stenosis</td>
<td>34.67 ± 18.21</td>
</tr>
</tbody>
</table>

* RVD = reference vessel diameter; MLD = minimal lumen diameter

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**Table 4: Quantitative Angiographic Characteristics (6-month Follow-up).**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>RESTEN-MP™ (n=28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>In-lesion (mm) MLD*</td>
<td>32.1% (9/28)</td>
</tr>
<tr>
<td>% Stenosis</td>
<td>22.2% (6/27)</td>
</tr>
<tr>
<td>Proximal edge</td>
<td>3.7% (1/27)</td>
</tr>
<tr>
<td>Distal edge</td>
<td>3.7% (1/27)</td>
</tr>
</tbody>
</table>

* RVD = reference vessel diameter; MLD = minimal lumen diameter
In the AVAIL trial, a prospective, evaluator – blinded, randomized study of 44 patients with de novo lesions or restenosis were evaluated [30]. AVI-4126 was delivered locally via Infiltrator catheter after percutaneous intervention. In that study three different dosages were evaluated, low dose (3mg), high dose (10mg) and control. Angiographic follow up was only performed in 25 out of the 44 patients. Binary restenosis was 38% in the control group, 29% in the low dose group and 0% in the high dose group. Interestingly TVR (target vessel revascularization) was done in 50% of the control group, 100% of the low dose group and 10% in the high dose group. It was concluded that local delivery of antisense is feasible. But there are clear disadvantages. Therefore in this trial Perfluorocarbon Microbubble Carriers (PGMC) were used for site-specific drug delivery. Using intravenous administration of PGMC bound AVI-4126 the vascular injury site proximal and distal of the stent could be reached during the procedure and after 24hours.

The present "first in man" (FIM) multicenter study assessed the safety and efficacy of a novel treatment. Microbubble delivery of c-myc antisense seems to be effective in reducing neointimal tissue proliferation without any systemic side effects. Comprehensive clinical follow-up was achieved in all patients at 30 days, with one TLR. At 6-month clinical follow-up the total TLR per patient was 7.5% (3 of 40 patients), with one non-Q-wave MI due to a stent thrombosis after 3 months. Further a non-cardiac death needs to be reported. After 12months the TLR/TVR per patient was less than 18.8 %.The lesion characteristics as well as the high rate of diabetic patients would under the current guidelines be recommended for the usage of DES in these patients. The risk of TLR in patients with type B2 and C lesions as well as the risk factor diabetes with bare metal stents is according to literature between 30 and 45% with bare metal stents and between 8 and 15% with drug eluting stents.

12-month administration of RESTEN-MP™ seems to be efficient in preventing in-stent-stenosis with a TLR of less than 15%. Compared with bare metal stents the TLR rate was lower without reaching the high standards of novel drug eluting stents (i.e. SES 8.8% Scorpius study). There was no difference in the neointimal proliferation between diabetics and non-diabetics. The intravascular ultrasound at follow-up showed a 25% volume stenosis demonstrating a percentage of neointimal proliferation that compares well with other IVUS studies on BMS. Interestingly, we observed that diabetes seems not to influence volumetric IVUS assessment of patients treated with RESTEN-MP™.

**Study limitations**

One of the major pitfalls of most "first-in-man studies" is the small number of patients and in our case the lack of a control-group. An open question of course is the local concentration of the antisense compound achieved. The dose of the antisense compound used in this study was chosen based on existing safety and efficacy data. In the AVAIL trial using the Infiltrator technique for local drug delivery the high dose (10mg) showed an improved binary restenosis rate compared to the APPRAISAL trial, whereas the low dose (3mg) seems to be inferior.

However, the present data provide important information about the safety and feasibility of this technology to prevent restenosis. The next step will be an even more sophisticated drug delivery via stent.

**Conclusions**

In summary, the Appraisal trial showed a favorable early and midterm safety profile of microbubble delivery of c-myc antisense. The use of RESTEN-MP™ might be an alternate treatment for patients with de novo coronary lesions to BMS and DES, with enough reduction in the safety profile of microbubble delivery of c-myc antisense.
of unfavourable neointimal tissue proliferation to prevent TLR but without altering the endothelialization of the stent struts to prevent late stent thrombosis.

### References


