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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 50patients (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

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 major adverse events are late stent malapposition, subacute and late thrombosis and aneurysm formations.

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.

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

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following balloon angioplasty. In order to objectively assess the therapeutic value of RESTEN-MP™ compared to other drugs used in combination with coronary artery stents and to utilize a sensitive method to assess the effectiveness of RESTEN-MP™ 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-MP™ after successful stent-placement is a prospective, multi-center, nonrandomized, single-arm study of 50patients 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 h); 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 same vessel before the study.

At the study site in Essen 34 patients were enrolled in an IVUS (intravascular ultrasound) sub study. In that sub study the MLD and lumen volume were determined after stenting with IVUS.

Preparation of RESTEN-MP™

Oligonucleotide synthesis and purification: Phosphorodiamidate morpholine oligomers (PMOs) were synthesized at AVI Bio Pharma as described previously [14,15]. The AVI-4126 sequence is complementary to the *c-myc* messenger RNA at the translation initiation start site, 5'-ACGTTGAGGGGCATCGTCGC-3'. The PMOs were purified by ion exchange chromatography. Purity was greater than 90% full-length 20-mer as determined by reverse-phase high-performance liquid chromatography and matrix-assisted laser desorption/ ionization time-of-flight mass spectroscopy [16]. Perfluorocarbon microbuble delivery delivers the active ingredient preferentially to the site of vascular injury (Figure 1). AVI-4126, the active ingredient of RESTEN-MP™, is a proprietary antisense drug designed to interrupt the translation of the human *c-myc* gene by mRNA.

Coronary stent procedure and drug adminstration: All patients received oral ASA (at least 100mg) before the procedure. In case patients were treated with clopidogrel before the index procedure they received a loading dose of 300mg clopidogrel, all other patients received a loading dose of 600mg. Clopidogrel was continued for 6months. During the procedure intravenous heparine boluses were administered (ACT goal > 250s). Lesions were treated with standard interventional techniques, including in most cases balloon dilatation before placement of the stent. All lesions were treated with a MULTI-LINK ZETA bare metal stent. A dose of 16mg RESTEN-MP™ (AVI BioPharma supplied by Global Therapeutics LLC) was intravenously administered at the time a stent is successfully placed in a coronary artery, and again 24 hours later, via slow-push intravenous administration.

IVUS procedure and analysis

IVUS interrogation was planned for all subjects at post-procedure and at 4 months or 9 months after stent implantation. The IVUS procedure was performed in a standard fashion using automated motorized pullback (0.5 mm/s) with commercially available imaging systems (20 MHz IVUS catheter, Volcano Corp, Rancho Cordova, CA, USA). The same IVUS system was used for serial (baseline and follow up) IVUS procedures. The IVUS analysis was performed with dedicated software (pcVH2.2, Volcano Corp., USA) by a single operator, who was experienced in the analysis of IVUS. The following measurements were obtained: mean stent volume, lumen volume, neointimal hyperplasia volume (NIHV), and percentage of NIHV.

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.

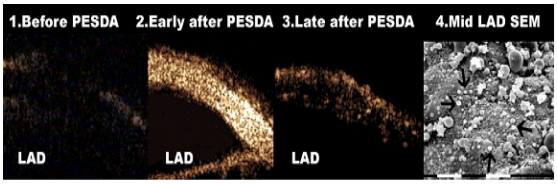
Study endpoints

The primary end point of this study was safety and potential effectiveness of RESTEN-MP™ 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 post-stent placement. Further target vessel failure (TVF), angiographic binary restenosis (≥50% diameter stenosis), in-stent MLD, late loss as well as in-stent and in-segment percent (%) diameter stenosis at month 6 post-stent placement were included.

Results

Characteristics of the patients and the lesions

Between December 2005 and December 2006, 51 patients were



Ultrasound Imaging of Perfluorocarbon Gas Microparticle Carriers (PGMC also referred to as PESDA) preferentially bind to sites of vascular injury (Data by AVI BioPharma). Black arrow show the PGMC.

Figure 1: Perfluorocarbon Microbubble Delivery: Coronary Artery localization.

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 53 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 was 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 differences were observed between diabetics and non diabetics based upon all IVUS measurements. Generally the neointimal proliferation was minor and open vessel lumen could be demonstrated during follow-up. In a sub analysis focusing on patients with type B1 and B2 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 CABG 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.

Characteristic	RESTEN-MP™
Age (years)	63.9 ± 10.2 (50)
Male	78% (39/50)
Revascularization for Angina or MI*	60 % (30/50)
History of Previous Revascularization	8 % (4/50)
History of Prior MI*	33 % (16/50)
History of CABG*	2 % (1/50)
History of Diabetes	34 % (17/50)
History of Smoking	38% (10/26)

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

Table 1: Demographics and Baseline Characteristics.

Characteristic	RESTEN-MP™
Eccentric	52.9% (27/51)
Thrombus	2.0% (1/51)
Tortuosity – None-Mid	82.4% (42/51)
Calcification – None-Mod	72.5% (37/51)
Total Occlusion	0.0% (0/51)
Side branch Stenosis	15.0% (18/51)
ACC/AHA Lesion Class A B1 B2 C	 3.9% (2/51) 11.8% (6/51) 51.0% (26/51) 33.3% (17/51)
Vessel RCA LAD LCX	 23.5% (12/51) 35.3% (18/51) 41.2% (21/51)
Lesion – Location Proximal Mid Distal Other	 41.2% (21/51) 35.3% (18/51) 17.6% (9/51) 5.8% (3/51)
Discrete Tubular Diffuse	19.6% (10/51) 52.9% (27/51) 27.5% (14/51)

Table 2: Baseline Angiographic Characteristics.

Characteristic	RESTEN-MP™ (n=51)
Pre-procedure RVD MLD % Stenosis	 2.7 ± 0.6 mm 1.1 ± 0.4 mm 57.7 ± 10.8
Post-stent (In-segment) RVD MLD % Stenosis	 2.7 ± 0.5 mm 2.2 ± 0.5 mm 20.5 ± 8.4
Post-stent (In-stent) MLD % Stenosis	 2.6 ± 0.5 mm 4.5 ± 9.5

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

Table 3: Quantitative Angiographic Characteristics at baseline.

Characteristic	RESTEN-MP™ (n=28)
RVD* (mm)	2.56 ± 0.46
In-segment (mm) MLD* % Stenosis	 1.52 ± 0.61 40.67 ± 21.79
In-stent (mm) MLD* % Stenosis	 1.68 ± 0.54 34.67 ± 18.21
Binary Restenosis In-lesion Within the stent Proximal edge Distal edge	 32.1% (9/28) 22.2% (6/27) 3.7% (1/27) 3.7% (1/27)

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

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

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

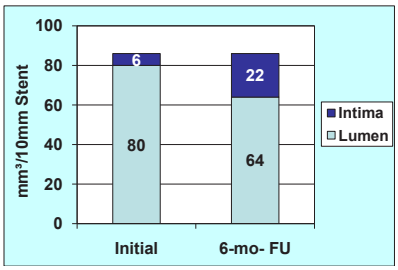
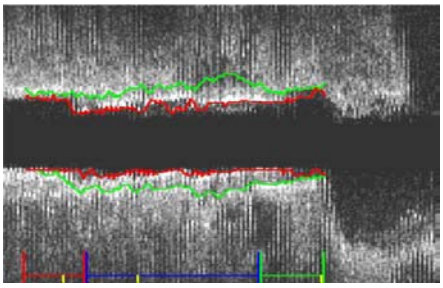


Figure 2: IVUS – substudy: Vessel and intimal volume after 6 months measured with IVUS.



The green line represents the analyzed vessel; the red line the luminal border

Figure 3: IVUS pullback 6 month after Stenting.

shown to inhibit smooth muscle cell proliferation in vitro and to reduce neointimal thickening in vivo [18]. Antisense RNA technology is emerging as an effective strategy for lowering the levels of specific gene products. It is based on the findings that these "antisense", sequences hybridize to specific RNA transcripts, disrupting normal RNA processing, stability, and translation, thereby preventing the expression of a targeted gene [19]. Administration of antisense oligonucleotides or transfer of expression constructs capable of producing intracellular antisense sequences complementary to the mRNA of interest have been shown to block the translation of specific genes in vitro and in vivo [20]. The potential impact of this technology on the treatment of vascular disease was illustrated recently by reports demonstrating that antisense oligonucleotides can be used to inhibit VSMC accumulation both in vitro and in vivo [21]. The persistent problem of restenosis after angioplasty might therefore be addressed with antisense oligonucleotides [22].

c-myc antisense oligomers reduced *c-myc* expression, which was associated with a significant growth inhibition of human SMCs. This demonstrates that *c-myc* gene activation plays an important role in the process of human SMC proliferation. A potent growth-inhibitory effect and pharmacokinetic profile of *c-myc* antisense oligomers provide the basis for studies assessing the therapeutic role of this approach in vascular restenosis [23]. AVI-4126 is an antisense Phosphorodiamidate Morpholino oligomer (PMO) with a sequence complementary to the translation initiation start site of the c-myc mRNA. The mechanism of action of AVI-4126 involves the interference with ribosomal assembly, preventing translation of c-myc, and the interference with intron 1-exon 2 splicing of the c-myc pre-mRNA, preventing appropriate translation of the c-myc mRNA [24-26]. The cellular response to AVI-4126 is diminished cell growth associated with arrest of cells in the G0/G1 phase of the cell cycle [27,28]. Slow-push intravenous administration of RESTEN-MP™ in pharmacological doses in the restenosis porcine model prevented subsequent in-stent stenosis [29].

In the AVAIL trial, a prospective, evaluator – blinded, randomized study 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 Perflourocarbon 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 mid-term 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

	Type B 1 (n =8)		Type B 2 (n = 14)	
	Post Stent	6 month FU	Post Stent	6 month FU
Stent volume (/10mm Stent)	95 ± 8 mm³	94 ± 9 mm³	92 ± 10mm³	93 ± 12 mm³
Lumen volume (/10mm Stent)	89 ± 7 mm³	71 ± 6 mm³	85 ± 6 mm³	65 ± 8 mm³
Intima hyperplasia (/10mm Stent)	7 ± 1 mm³	23 ± 5 mm³	6 ± 1 mm³	22 ± 5 mm³
Volume Stenosis	6 ± 1 %	22 ± 4 %	7 ± 1 %	30 ± 5 %
Late loss (mm)	0.04 ± 0.01	0.19 ± 0.03	0.09 ± 0.02	0.26 ± 0.05
TLR*		0		3

*TLR = target lesion revascularization

Table 5: IVUS – substudy: Subgroup Analyses.

Event	30 days	180 days	360 days
Death	0.0% (0/50)	2.5% (1/40)	3.1% (1/32)
Q-Wave MI	0.0% (0/50)	0.0% (0/40)	0.0 % (0/32)
Non Q-Wave MI	0.0% (0/50)	2.5% (1/40)	6.3% (2/32)
Target Lesion CABG	0.0% (0/50)	0.0% (0/40)	0.0% (0/32)
Target Vessel CABG	0.0% (0/50)	2.5% (1/40)	9.4% (3/32)
Target Lesion Revascularization	0.0% (0/50)	7.5% (3/40)	15.6% (5/32)
Target Vessel Revascularization	2.0% (1/50)	10.00% (4/40)	18.8% (6/32)
Acute Stent Thrombosis	0.0% (0/50)	0.0% (0/40)	0.0% (0/32)
Subacute Stent Thrombosis	0.0% (0/50)	0.0% (0/40)	0.0% (0/32)
Late Stent Thrombosis	0.0% (0/50)	2.5% (1/40)	3.1% (1/32)
MACE (Death, MI, CABG, TLR)	0.0% (0/50)	10.00% (4/40)	21.9% (7/32)

Table 6: Major Adverse Events.

of unfavourable neointimal tissue proliferation to prevent TLR but without altering the endothelialization of the stent struts to prevent late stent thrombosis.

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