Regenerative cell therapy and pharmacotherapeutic intervention in heart failure

Part 2: Pharmacological targets, agents and intervention perspectives

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Regenerative medicine represents a promising perspective on therapeutic angiogenesis in patients with cardiovascular disease, including heart failure. However, previous or ongoing clinical trials show ambiguous outcomes with respect to the benefit of regenerative therapy by means of bone marrow stem cell infusion in myocardial infarction patients. Therefore, it is necessary to set up a rational therapeutic strategy in the treatment of congestive heart failure. Chemokines, cytokines and growth factors, as well as pharmaceutical agents, may have an impact on endothelial progenitor cell (EPC) physiology and thus can provide targets for pharmacological intervention. Indeed, EPCs and stem cell niches both in bone marrow and myocardial tissue can be treated as an integral target for recruitment of EPCs from the bone marrow to the cardiac ischaemic niche. In this article, we individually place the signalling factors in their specified context, and explain their roles in the

various phases of neovascularisation (see Part 1). (*Neth Heart J* 2008;16:337-43.)

Keywords: angiogenesis, bone marrow derived stem cells, endothelial progenitor cells, heart failure, pharmacotherapy

Current pharmacological therapy in patients with heart failure is focused on the relief of symptoms and prevention of ventricular remodelling. In contrast, stem cell therapy aims at repair, in among other ways by angiogenesis and/or neovascularisation. Combining both strategies, by pharmacologically interfering with processes or factors regulating homing and function of these stem cells, may provide optimal therapeutic potential. Several factors involved in the process of bone marrow cell mediated repair could well provide targets for pharmacological therapy.

Hypoxia-inducible factor-1

Hypoxia-inducible factor-1 (HIF-1), a central transcriptional regulator of hypoxia-specific gene expression, induces signalling factor expression such as stromal-derived factor (SDF-1) and vascular endothelial growth factor (VEGF) in endothelial progenitor cells (EPCs) or endothelium and facilitates adhesion of progenitor cells to ischaemic endothelium.¹ Transcriptional activity of HIF-1 α occurs through dimerisation with the constitutively expressed HIF-1 β and subsequently binds to enhancer regions termed hypoxia-response elements. The recruitment of EPC in the ischaemic tissues is predominantly regulated by gradient levels of hypoxia.² The dependence of progenitor cell recruitment and function on a hypoxic microenvironment suggests that targeting the hypoxic response mediated by the transcription factor HIF-1 α may be a more rational strategy for achieving therapeutic angiogenesis in patients with congestive heart failure (CHF).

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Recently, Rajagopalan and colleagues reported the first clinical results of HIF-1 α gene therapy predicated on transcriptional activation of a patient's own gene. The high dose of HIF-1 α gene therapy improved therapeutic outcomes in critical limb ischaemic patients. More importantly, it appears to be safe for clinical application.³ In fact, HIF-1 α overexpression on EPCs promotes hypoxia-induced EPC differentiation, proliferation and migration,⁴ establishing HIF-1 as a putative pharmacological target.

Stromal-derived factor-1

Stromal-derived factor-1 (SDF-1, also known as CXCL12), released under the control of HIF-1, through interaction with its receptor CXCR4 (also known as CD184), modulates angiogenesis as well as haematopoiesis. SDF-1 regulates angiogenesis in part by recruiting EPCs from the bone marrow niches to ischaemic tissues.^{2,5} Specific stimulation of osteoclasts with receptor activator of nuclear factor-kappa B (NF- κ B) ligand (RANKL) in bone marrow niches recruited primarily immature progenitor cells to the circulation in a CXCR4 and matrix metalloproteinases (MMP)-9 dependent manner.⁶ The release of SDF-1 is modulated by different cytokines, such as soluble kit ligand (sKitL) and VEGF-A.⁷ These cytokines were demonstrated to induce the release of SDF-1 from platelets, thereby facilitating the mobilisation of CXCR4⁺ stem/progenitor cells including EPCs.⁸ The mobilising effect initiated by reversing the SDF-1 gradients between the bone marrow niches and peripheral ischaemic niches was demonstrated by the direct elevation of plasma SDF-1.⁷

However, Sano et al. recently reported that, although increasing levels of both HIF-1 and SDF-1 were observed in ischaemic myocardial tissue, HIF-1 activity was inhibited by accumulation of p53, which paradoxically counterregulates cardiac angiogenesis.⁹ Therefore, it is envisioned that solely promoting recruitment of EPCs may be insufficient for cardiac angiogenesis, since there is a disruption in the link between HIF-1 and SDF-1 by accumulation of p53.

Taken together, SDF-1 that increases in the vascular niches in bone marrow is a prerequisite to initiate stem/progenitor cell mobilisation. Strikingly, interactions between the chemokine SDF-1 and its receptor CXCR4 are also of importance in the development and/or homeostasis of heart and blood vessels. Thus, the SDF-1/CXCR4 axis in both cardiac and bone marrow niches may be considered a pharmacological target to improve stem cell mobilisation after MI, taking into consideration, though, its dependency on cell cycle kinases such as p53.

Stem cell factor

Stem cell factor (SCF), a soluble kit ligand or steel factor, binds to c-kit (also known as CD117), a receptor

that is expressed on the surface of stem/progenitor cells and has chemoattractant effects on these cells. The release of SCF is related to the activity of MMP-9. MMP-9 is induced in bone marrow cells, such as osteoclasts, initiating the migration of c-kit⁺ BM-EPCs and HPCs from quiescent endosteal niches to proliferative vascular niches.¹⁰ SCF has a proven pharmacotherapeutic effect; the combination of SCF and granulocyte-colony-stimulating factor (G-CSF) improved cardiac output and ameliorated arrhythmia in a murine MI model, followed by an enhanced G-CSF receptor expression and arteriogenesis in cardiac tissue.¹¹ However, a recent study revealed that beneficial effects of G-CSF/SCF on post-MI cardiac function and remodelling are dramatically diminished with age. Interestingly, in the older patients, the amount of circulating CD34⁺ cells was similar to that in young patients, suggesting that the reduced efficacy in this population can be attributed to a local defect in cardiac ischaemic niches in response to G-CSF/SCF therapy.¹²

Granulocyte-colony-stimulating factor

Cytokines that promote granulocytes, such as G-CSF and granulocyte macrophage-colony stimulating factor (GM-CSF), also affect EPC mobilisation from bone marrow vascular niches to peripheral circulation. Combined administration of G-CSF with basic fibroblast growth factor (bFGF) may further increase capillary density in a hind limb ischaemia model, compared with either treatment alone.¹³ Furthermore, combined treatments of GM-CSF/SDF-1 were also found to increase proangiogenic cells such as angiogenic macrophages and EPCs, which may eventually contribute to neovascularisation.¹⁴ Indeed, G-CSF is one of the most important cytokines under intensive clinical investigations. Abundant evidence showed that administration of G-CSF significantly stimulated stem cell mobilisation, and short-period stimulation using G-CSF appeared to be safe and had no adverse effects on restenosis.¹⁵

Not surprisingly, the effect of G-CSF after MI has been studied. Unexpectedly, administration of G-CSF alone failed to reduce infarct size and improve cardiac function in a clinical trial.¹⁶ However, combined administration of GM-CSF and SDF-1 increases neovascularisation,¹⁴ suggesting that the therapeutic effect of GM-CSF is enhanced when it is combined with other medicines. As G-CSF is also a pro-inflammatory factor, the use of G-CSF should be cautiously considered.

Fibroblast growth factors

Basic fibroblast growth factor (bFGF)-2 is one of the FGF families of heparin-binding growth factors. FGF-2 exerts its proangiogenic activity through interactions with various endothelial cell surface receptors, including tyrosine kinase receptors and integrins. Their activity is modulated by a variety of free and extracellular matrix-associated molecules.¹⁷ FGFs, in cross-talk with VEGF and inflammatory cytokines/chemokines,

play a role in the modulation of blood vessel growth in different pathological conditions, including cardiovascular disease.¹⁸ Binding of FGF-2 to its receptor initiates protein kinase C-mediated activation of MAPK pathways. In an *in-vitro* cell culture study, FGF-2 suppressed long-term culture-induced cellular senescence in human mesenchymal stem cells by reducing mRNA expression of tumour growth factor- β 2 and suppression of cyclin-dependent kinases such as p21 and p53.¹⁹ In addition, FGF-2 plays an essential role in oestrogen-induced re-endothelialisation and EPC mobilisation from bone marrow niches.²⁰ Unlike VEGF, FGF enhances angiogenesis via protein kinase C-mediated activation of MAPK pathways, in an NO-independent manner, thus providing an additional target for pharmacotherapy in cardiac tissue.

Vascular endothelial growth factor and its signalling factor nitric oxide

The vascular endothelial growth factor (VEGF) was first isolated as a tumourigenic factor responsible for angiogenesis in neoplasms.²¹ VEGF production is regulated through HIF-1 in ischaemic tissues,¹ and in turn is a regulator of SDF-1, as noted above. Most importantly, VEGF-A, the most potent member of VEGF family, has been identified as one of the major growth factors involved in neovascularisation. VEGF binds to VEGFR1 or VEGFR2 on the endothelial as well as the EPC surface. Most of the angiogenic effects attributed to VEGF are related to VEGFR2. VEGF, through VEGFR2, increases proliferation and mobilisation of BM-EPCs and augments corneal neovascularisation *in vivo*.²² Cultured human EPCs also express VEGFR1, which plays a critical role in prevascular retention in a prevascular niche in ischaemic tissue.^{23,24} Interestingly, VEGFR1 can also be stimulated by placental growth factor (PlGF), leading to increased tumour vasculogenesis.²⁵ It has been suggested that PlGF activates EPC *via* VEGFR1 and enhances mobilisation of VEGFR1+ bone marrow progenitor cells by up-regulation of MMP-9 and release of soluble kit ligand.⁷ It is therefore tempting to speculate that future therapeutic strategies based on PlGF administration may improve neovascularisation in CHF patients.

Apart from the VEGF/VEGFR axis, VEGF-A may also stimulate platelet-derived growth factor receptors (PDGF) and thus regulate endothelial tip cell and pericyte recruitment to ischaemic niches, which plays a critical role in vascular permeability and stabilisation.^{22,26}

It is evident that the VEGF family and its receptors are an important target for future pharmacotherapy to support EPC-mediated cardiac neovascularisation. Downstream signalling pathways of VEGF could also be relevant, and are particularly attractive targets as they might be shared by more angiogenic hormone-receptor systems. One such important VEGF-activated signalling pathway is nitric oxide (NO) release.

Apart from its well-known vasodilatory property, NO has been considered to be an important mediator

of angiogenesis. NO is a downstream signalling pathway of VEGF-activated endothelial proliferation. Long-term VEGF stimulation induces an increase in the level of endothelial NO synthase (eNOS) expression, while short-term stimulation promotes subsequent NO production via activation of the mitogen-activated protein kinase (MAPK) cascade, which plays a critical role in angiogenesis.²⁷ Bone marrow-derived NO is correlated with enhanced EPC mobilisation to the circulation post-MI, as well as enhanced adhesiveness via increased integrin expression, which favours the homing capacity of EPCs to ischaemic myocardial tissue.²⁸ In eNOS-deficient mice VEGF-induced BM-EPC mobilisation is inhibited, leading to impaired neovascularisation in a hind-limb ischaemia model.²⁹ NO is a ubiquitously employed signalling factor, which, apart from VEGF, might also serve other angiogenic growth factors. However, NO is not the only signalling compound at play on these premises. The further search for downstream signalling factors is warranted.

Oestrogen

Animal experiments as well as clinical studies show that oestrogen has beneficial effects on the cardiovascular system. Important for regenerative therapy, oestrogen treatment accelerates the incorporation of BM-EPCs at the site of re-endothelialisation in a Matrigel assay and stimulates EPC mitogenic and migration activity.³⁰ Apart from being an attractive hormone to find targets for pharmacological intervention, EPC-mediated vascular repair might also play a role in the protective effects of oestrogen observed in females.³¹

Oestrogen has been shown to reduce angiotensin II-induced accelerated senescence of EPCs by down-regulation of angiotensin II type I receptor (AT₁).³² Further evidence shows that both oestrogen receptor (ER) α and β contribute to oestrogen-mediated EPC activity after myocardial infarction, which is correlated with upregulation of VEGF in EPCs,³³ implying a role for NO yet again. The importance of NO is underscored by observing that oestrogen increases vascular production of NO, which in turn improves endothelial progenitor cell function.

In conclusion, several growth factors, signalling factors, and signal transduction pathways are at play when it comes to recruitment of EPC. Table 1 summarises the most important of these factors and the niches and phases in which they exert their favourable effects on cardiac neovascularisation. It is, however, clear that a large array of pharmacological targets, larger than described herein, is available for the optimisation of regenerative therapy based on neovascularisation. In fact, some of the medications used in the clinic today exert their beneficial cardiovascular effects at least partly through this mechanism. We will now review these medications.

Table 1. Physiological and pharmacological factors involved in EPC-induced neovascularisation.

Factors	Effects on EPCs					Relative phase(s) of neovascularisation	References
	Mobilisation	Proliferation	Differentiation	Angiogenesis	Senescence		
Physiological factors							
- HIF-1 α	↑	ND	ND	↑	ND	Phase I, II	1,2,4
- SDF-1	↑↑	ND	ND	↑	ND	Phase I, II, V	5,8
- SCF	↑	↑	↑	↑	↓	Phase I, II, IV	10,12
- G-CSF	↑↑	ND	ND	↑/--	ND	Phase I, II	15,16
- FGF	↑	↑	↑	↑	↓	Phase I-IV	19,20
- VEGF	↑↑	↑↑	↑↑	↑↑	↓	Phase I-V	7,22,24,25,28
- Oestrogen	↑	↑	↑	↑	↓	Phase I-IV	30,31
Pharmacological factors							
- Statins	↑	↑	↑	↑	↓	Phase I-IV	35-39
- Epo	↑	↑	↑	↑	↓	Phase I-IV	42,44-46
- Heptapeptide	↑	↑	↑	ND	ND	Phase I	59,60
- ACE inhibitor	↑	ND	ND	↑	ND	Phase I	28,61
- ATR antagonist	↑	↑	↑	↑	↓	Phase I/II	52,63,64

ND=no description, ↑=increased, -=no change.

ND=no description, ↑=increased, --=no change.

Potential pharmacotherapeutic agents for improvement of stem cell therapy

Statins

In the last five years, 3-hydroxy-3-methylglutaryl co-enzyme A (HMG-CoA) reductase inhibitors (statins) have been widely used for the prevention of CHF in clinical treatment. With the increasing prescription rate of statins from 38 to 80% of the patients suffering from coronary artery disease, significant reductions in cardiac mortality and hospitalisation have been observed.³⁴ One of the earliest observed favourable effects of statins was improvement in endothelial function.³⁵ Subsequently, it was demonstrated that statins improve the angiogenic capacity of endogenous EPCs in patients with stable coronary disease.³⁶ The actual mechanism behind this favourable effect may be attributed to increased levels of VEGF and its antiapoptotic effects on EPC.

Furthermore, statins stimulate EPC proliferation and reduce senescence via cell cycle regulatory genes.³⁷ The prevention of senescence probably involves upregulation of the telomere repeat-binding factor TRF2,³⁸ which protects against damage from telomeres.³⁹

Apart from cell cycle effects and antisenesence effects on EPC, statins reduce C-reactive protein (CRP) levels in patients. In clinical situations, patients with lower CRP after statin treatment have the lowest rate of recurrent events after AMI, regardless of the level of LDL cholesterol.⁴⁰ Interestingly, statin treatment did not display its beneficial effects in patients

with systolic heart failure (NYHA class III) and failed to reduce the CRP level.⁴¹ Therefore, statins might favourably influence EPC function through an anti-inflammatory mechanism. In patients with class III systolic heart failure the lack of a positive outcome of statin treatment may be associated with EPC dysfunction, since EPC function is damaged as heart failure progresses.⁴¹ Taken together, statins have pleiotropic effects on the cardiovascular system that might be mediated by effects on EPC during mild heart failure, involving various signalling pathways that are therefore targets for improvement of regenerative therapy. Because of the versatility of the pleiotropic effects of statins, it might be a daunting but rewarding task to sort out the most relevant pathways.

Erythropoietin

Erythropoietin (Epo), a stimulator of haematopoiesis, has been shown to stimulate angiogenesis irrespective of its haematopoietic properties.⁴² The production of Epo is upregulated by hypoxia and is exclusively mediated via HIF-2 α .⁴³ In bone marrow, Epo acts on the Epo receptor (EpoR) and activates various signal pathways including MAPK and PI-3k/Akt, which probably prevent apoptosis.⁴⁴

Epo has been shown to stimulate angiogenesis or even vasculogenesis by mobilising EPCs from bone marrow.^{42,45} The effects of Epo on EPC biology are both time- and dose-dependent.⁴⁶ Chronic Epo treatment in both healthy and CHF patients enhanced functional activity of EPC by improving proliferative

potential and adhesive capacity under *in vitro* conditions. Nevertheless, the issue that needs to be addressed is that Epo stimulates neovascularisation via direct or indirect effects on bone marrow stem cells (BMSCs) or circulating EPCs. Recent studies demonstrate that Epo/EpoR plays a pivotal role in enhancing the number of BMSCs or circulating EPCs accompanied by elevated serum levels of VEGF.^{42,47} A more recent study from our group has indicated that the cardiac protective effects of Epo positively correlated with the recruitment of BM-EPCs and VEGF levels into the cardiac ischaemic niche post-MI.⁴⁵ Hence, Epo likely acts on the vascular EpoR system and thereby activates or upregulates the VEGF/VEGF receptor system, which may further contribute to recruitment of BM-EPCs to ischaemic niches and enhance neovascularisation. Another possible parallel with statins is that Epo reduces the inflammatory reaction after stroke.⁴⁸ It is unknown whether this contributes to the observed stimulation of EPCs, and needs to be addressed in future studies.

In summary, Epo is a potent factor for EPC-mediated neovascularisation and in this sense an attractive pharmacotherapeutic agent to be further evaluated. In spite of the positive effects of Epo on neovasclogenesis, some concerns may arise because of elevated blood viscosity and hypertension induced by Epo.⁴⁹ In this regard, the doses and administration frequencies of Epo should be investigated thoroughly.

Role of renin-angiotensin system modulation in progenitor cell function

The renin-angiotensin system (RAS) contributes significantly to the development and progression of vascular disease and CHF, and is one of the best-studied hormone systems in the cardiovascular field. Angiotensin-converting enzyme (ACE) inhibitors and angiotensin II type 1 receptor (AT₁R) blockers are currently prescribed in the clinic for various cardiovascular indications. The production of bioactive angiotensins starts with the cleavage of angiotensinogen by renin to form the inactive peptide angiotensin I. ACE is responsible for conversion of Ang I to its active metabolite Ang II, which through its action on AT₁R increases vascular tone and acts as an antidiuretic, resulting in an increase in blood pressure. Ang-(1-7) is an active metabolite that can be formed from both Ang I or Ang II, involving various enzymes such as ACE, ACE2 and neutral endopeptidase. Ang-(1-7) is considered to be the endogenous counterregulator of Ang II, and displays protective properties.⁵⁰ Importantly, local angiotensin expression may influence haematopoietic or mesenchymal stem cell differentiation into an unexpected, unfavourable direction, thus exerting a possible negative effect on the cardiac niches.⁵¹

Effects of Ang II and Ang-(1-7) on stem cells

Angiotensin II (Ang II) is the best-studied peptide in the renin-angiotensin system (RAS). The main physio-

logical effects of Ang II are mediated by AT₁R, leading to paradoxical effects on progenitor cells. On the one hand, Ang II potentiates VEGF-induced capillary tube formation of EPCs in a transient manner.⁵² On the other hand, local cardiac Ang II regulates mesenchymal stem cell differentiation into adipocytes.⁵³ Furthermore, Ang II accelerates senescence of EPCs in sustained cultures.⁵⁴ Therefore, long-term exposure to Ang II may contribute to EPC dysfunction and loss of neovascularisation capacity.

Ang-(1-7) is attracting increasing interest in the cardiovascular community as a hormone that is augmented by ACE inhibitors and that counteracts the deleterious effects of Ang II. Ang-(1-7) effects can either take place through angiotensin receptors, ACE inhibitors or Mas receptors.⁵⁵ Studies from our group have demonstrated that Ang-(1-7) significantly attenuated post-MI remodeling and ameliorated cardiac and systemic endothelial function.⁵⁶ In addition, we also demonstrated that Ang-(1-7) administration markedly reduced neointimal formation and endothelial dysfunction in a rat abdominal aortic in-stent implantation model.⁵⁷ These effects might be related to reduction in cardiac myocyte and vascular smooth muscle cell growth,⁵⁸ but a possible involvement of progenitor cells has not been investigated yet, although this possibility is supported by several studies.

The studies performed by Rodgers and Zerega et al. show that Ang-(1-7) administration significantly improved bone marrow recovery after radiochemotherapy in cancer patients,^{59,60} suggesting a role of the Ang-(1-7)/Mas axis in bone marrow progenitor cells proliferation and differentiation. In very recent pilot studies from our group we explored the effects of Ang-(1-7) on cardiovascular progenitor cells and observed that Ang-(1-7) promotes proliferation and differentiation of BM-EPCs. Ang-(1-7) would therefore again identify itself as a counterregulator of Ang II, this time through its favourable effect on EPC.

Angiotensin-converting enzyme inhibitors

Intervention with RAS by inhibition of ACE is currently a primary routine treatment for CHF. ACE inhibitors block the formation of Ang II and increase Ang-(1-7) levels, thus preventing the most detrimental effects of Ang II on the development of CHF. Apart from direct therapeutic effects on adult cardiovascular cells, ACE inhibitors have recently been shown to promote mobilisation of BM-EPCs after AMI,⁶¹ or even to augment EPC cell therapy after AMI.⁶² The molecular mechanism of beneficial effects of ACE inhibitors on EPC is still far from clear; however, it has already in part been elucidated by Thum et al.²⁸ ACE inhibitors enhance bone marrow stem cell migration mainly by increasing MMP-9 activity, thus stimulating the release of soluble c-kit ligand (SCF).²⁸ Considering the difference in mechanism and signalling pathways that are activated, statins and ACE inhibitors could complement each other during stem cell therapy for CHF, an interaction that has not been studied yet.

Angiotensin II type 1 receptor antagonists

The effects of AT₁R antagonists on EPC were first addressed in a clinical study by Bahlmann et al.⁶³ In this study, treatment with AT₁R antagonists selectively increases the EPC subpopulation but not haematopoietic cells. A possible explanation could be prevention of Ang II-induced senescence of EPCs by up-regulation of telomerase activity.⁵⁴ Importantly, Yu et al. recently detected mRNA expression of AT1 and AT2 receptors on EPC colonies.⁶⁴ Moreover, angiotension II receptor blockers improve EPC function and cardiac c-kit expression.⁶⁴ Although favourable actions of AT1 antagonists on EPCs have emerged, further clinical and preclinical studies are needed to confirm these data.

In summary, RAS modulation has evident therapeutic potential when it comes to beneficial modulation of progenitor cell function in cardiovascular disease. Various angiotensins, notably Ang II and Ang-(1-7), are tools for further pharmacological research to reveal signalling systems for enhancement of progenitor cell function and cardiovascular repair. Moreover, a supportive role of RAS modulation during cell therapy could be indicated.

Therapeutic perspectives

The therapeutic approach of using bone marrow stem cells (BMSCs) in the treatment of CHF represents a milestone in regenerative medicine. In the past five years, however, BMSC therapy for myocardial disease has yielded inconsistent outcomes in clinical trials. The crucial question is why the reported successes in scientific or preclinical studies cannot be transferred to clinical practice. Obviously, a variety of factors, ranging from standard cell preparation protocol,⁶⁵ cell sub-type(s)^{66,67} and transplantation time point, to the criteria of patient selection in CHF⁶⁸ may be responsible for the discrepant results.

Understanding the cell-cell interactions and molecular mechanisms that govern vascular growth and development during pathophysiological situations would facilitate the success of stem cell-based therapy for CHF. However, the potential impact of appropriate concomitant pharmacological treatment on myocardial disease progression and on cardiac response to stem cell therapy is largely underestimated with regard to success of angiogenic cell therapy in heart failure. Moreover, an additional critically important prerequisite for successful stem cell therapy in CHF is provided by an ameliorated endogenous cardiac microenvironment; the cardiac stem cell niche.

In conclusion, a combination of stem cell therapy and pharmacological treatment that, in addition to a direct effect on cardiac function, stimulates the diverse regulatory mechanisms involved in the various niches and phases of neovascularisation through BM-EPCs, will provide optimal angiogenic therapy in CHF. ■

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