

# The Value of Angiogenic Therapy with Intramyocardial Administration of Basic Fibroblast Growth Factor to Treat Severe Coronary Artery Disease

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**Background:** Basic fibroblast growth factor (bFGF) was administered intramyocardially together with CABG to induce myocardial neovascularization and collateral growth in patients with ungraftable coronary arteries. Coronary angiographic and myocardial scintigraphic findings revealed that the effects of CABG were potentially confounding.

**Methods and results:** Patients in the bFGF group (n = 16) underwent angiogenic therapy using bFGF for ungraftable territory, and incomplete revascularization (IR) patients (n = 22) underwent only CABG. The magnitude of collateral development was assessed by the Rentrop score and collateral connection (CC) grade. Rentrop scores tended to increase among patients in the bFGF group (before vs. after surgery,  $1.9 \pm 1.2$  vs.  $2.3 \pm 1.2$ ,  $p = 0.05$ ), but not in the IR group. The CC grade significantly increased among patients in the bFGF group (before vs. after surgery,  $1.0 \pm 0.9$  vs.  $1.4 \pm 0.5$ ,  $p < 0.05$ ), but not in the IR group. Myocardial perfusion in territories injected with bFGF improved in 13 patients (81%) of the bFGF group, and also in the nonbypassed territory in 4 IR patients (25%) ( $p < 0.05$ ).

**Conclusion:** Angiogenic therapy with bFGF induced collateral development and improved myocardial perfusion in territories injected with bFGF. (*Ann Thorac Cardiovasc Surg* 2010; 16: 174–180)

**Key words:** angiogenic therapy, coronary artery disease, angiography, bFGF

## Introduction

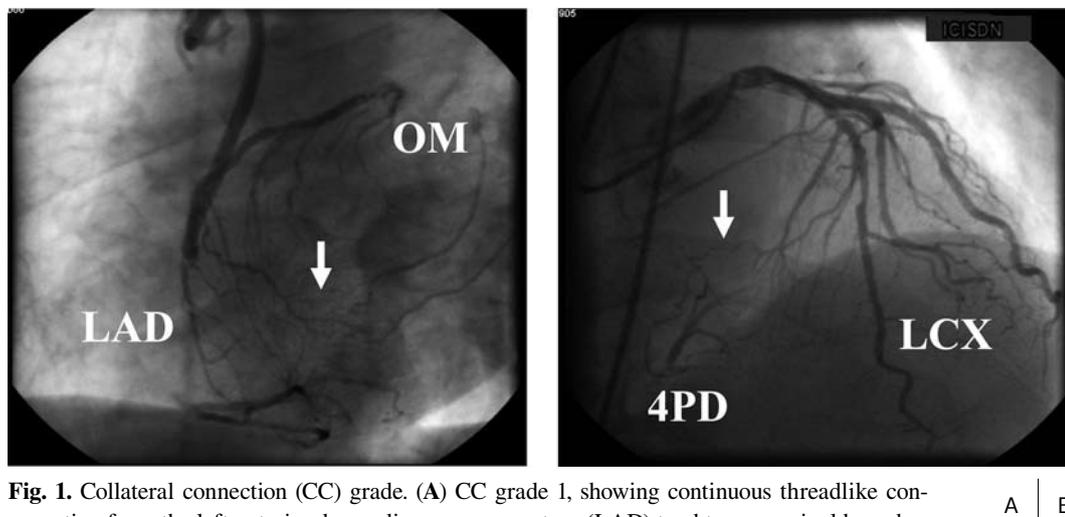
Conventional methods of myocardial revascularization are often limited because of diffuse atherosclerotic lesions or small-caliber vessels. Angiogenic therapy to induce myocardial neovascularization is not dependent

on vessel caliber and provides an alternative treatment alone or in combination with conventional revascularization therapy. Basic fibroblast growth factor (bFGF) is a potent angiogenic protein that induces endothelial and smooth muscle cell proliferation in vivo and elicits angiogenesis that induces the migration and proliferation of endothelial cells, vascular tube formation, and linkage to the extant vascular network.<sup>1)</sup> We previously demonstrated that the intramyocardial administration of bFGF increases the number of capillaries and arterioles in the peri-infarct region, increases regional myocardial blood flow, and consequently improves ventricular function in a canine infarction model. Based on the preclinical results,<sup>2,3)</sup> and with approval of our institutional review board, we started intramyocardial administration of

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**Fig. 1.** Collateral connection (CC) grade. (A) CC grade 1, showing continuous threadlike connection from the left anterior descending coronary artery (LAD) to obtuse marginal branches of the left circumflex coronary artery (OM). (B) CC grade 2, showing small-side branchlike collateral through its course from the left circumflex coronary artery (LCX) to the posterior descending branch of the right coronary artery (4 PD).

bFGF as angiogenic therapy in 2002 to patients undergoing coronary artery bypass grafting (CABG) who had an ischemic and viable, but ungraftable, myocardial territory.

Angiogenic therapies with angiogenic growth factor proteins, gene transfection of DNA encoding angiogenic growth factors, or bone marrow derived progenitor cells have been advocated to promote the growth of new vessels and to induce collateral development for ischemic heart disease.<sup>4-8</sup> The most popular method of assessing the effect of angiogenic therapies is myocardial scintigraphy representing myocardial perfusion. Therapeutic approaches to induce collateral development require refined methods to assess collaterals *in vivo*.<sup>9</sup> Moreover, CABG exerts potential confounding effects on perfusion of the ungraftable myocardial territory through extant or de novo collaterals. To evaluate the effects of angiogenic therapy, we compared coronary angiographic and myocardial scintigraphic findings from patients who underwent angiogenic therapy as an adjunct to CABG with those from patients who underwent CABG, but not angiogenic therapy (incomplete revascularization).

## Methods

### Patients

The ethics review board of Kumamoto University Medical Center approved the application of angiogenic therapy with intramyocardial bFGF for coronary artery disease. Angiogenic therapy was indicated for patients with at

least one graftable obstructed coronary artery and at least one ischemic, viable myocardial territory supplied by a major coronary branch, which was not amenable to complete revascularization because of severe diffuse atherosclerotic disease, calcifications, or small size. Patients with a history of malignancy, diabetic retinopathy, or renal insufficiency with serum creatinine values of > 2.0 mg/dL, left ventricular ejection fraction < 25%, or significant valvular disease requiring concomitant valve surgery were excluded from angiogenic therapy. All of the 18 patients provided written, informed consent to undergo angiogenic therapy with bFGF from February 2002 to April 2007. Sixteen (bFGF group) of them were examined with selective coronary angiography before and one month after treatment. Two patients elected not to undergo postoperative coronary angiography. Selective coronary angiography was retrospectively evaluated in 22 patients (IR group) who had undergone only CABG during the same period for graftable obstructed coronary arteries and who had at least one major arterial distribution that was not amenable to complete revascularization.

Table 1 summarizes the clinical characteristics of the patients, and none of age, male gender, extant hypertension and hyperlipidemia, number of grafts, rate of CABG without cardiopulmonary bypass, or early graft patency significantly differed between the bFGF and IR groups. The incidence of diabetes mellitus in the bFGF group was significantly lower than that in the IR group, resulting from diabetic retinopathy and nephropathy being contraindicated for angiogenic therapy with bFGF

**Table 1. Clinical characteristics of patients**

	IR group	bFGF group	p value
Age (y)	67 ± 9	68 ± 8	N.S.
Sex (male/female)	19:3	16:0	N.S.
Diabetes mellitus	14 (64%)	5 (31%)	p <0.05
Hypertension	11 (50%)	5 (31%)	N.S.
Hyperlipidemia	9 (41%)	9 (56%)	N.S.
Pre-CCS	1.9 ± 1.3	1.6 ± 0.7	N.S.
Post-CCS	0.5 ± 0.7	0.2 ± 0.4	N.S.
Pre-LVEF (%)	53 ± 17	50 ± 17	N.S.
Post-LVEF (%)	54 ± 14	48 ± 16	N.S.
Previous CABG	2 (10%)	6 (38%)	<0.05
Numbers of grafts	2.2 ± 0.6	2.0 ± 0.8	N.S.
Rate of off-pump CABG	14 (65%)	12 (75%)	N.S.
Graft patency	95%	97%	N.S.

Data are means ± SD. bFGF, basic fibroblast growth factor; CCS, Canadian Cardiovascular Society classification; LVEF, left ventricular ejection fraction; CABG, coronary artery bypass grafting; N.S., not significant.

because of potential exacerbation. Six patients in the bFGF group and 2 in the IR group had undergone previous CABG. Five in the bFGF group and 7 in the IR group had poor left ventricular function with ejection fraction ≤ 40%.

#### Angiogenic therapy using bFGF

We initially performed CABG in the standard manner, either with or without cardiopulmonary bypass. After all distal and proximal anastomoses were completed, 500 µg of human recombinant bFGF protein (Kaken Pharmaceutical, Tokyo, Japan) diluted in 5 ml of saline was injected into the myocardium at approximately 15 sites of targeted myocardial territory using a 27-gauge needle. Epicardial stabs were immediately sealed with fibrin glue for hemostasis. Basic FGF was injected both in the ischemic but ungraftable territory and in the border territory of a grafted or patent coronary artery. The territory injected with bFGF corresponded to the inferior wall (right coronary artery distribution) in 7 patients, the antero-lateral wall (diagonal artery distribution) in 5, the lateral wall (intermediate artery distribution) in 2, and the posterior wall (left circumflex artery distribution) in 5. The nonbypassed myocardial territory corresponded to distribution of the right coronary artery in 12 control patients and to that of the circumflex coronary artery in 10 of them. None of the patients required intra-aortic balloon-pump support after the operation.

#### Assessment of coronary angiography

Angiographic studies were performed using a Siemens

biplane cine system (BICOR), with a 7-inch field. To evaluate the effect of angiogenic therapy, we compared preoperative coronary angiographic findings with those at one month after CABG in both the bFGF and IR groups. Postoperative coronary angiographic findings demonstrated collateral development and myocardial perfusion in myocardial territories injected with bFGF. The magnitude of collateral development was assessed using the Rentrop score,<sup>10)</sup> collateral connection (CC) grade 11, and myocardial blush (MB) grade 12. The Rentrop score is defined as grades of collateral filling from the contralateral vessel and comprises Rentrop grade 0, none; Rentrop 1, filling of side branches of the artery via collateral channels without visualization of the epicardial segment; Rentrop 2, partial filling of the epicardial segment via collateral channels; and Rentrop 3, complete filling of the epicardial segment of the artery being dilated via collateral channels. The size of the collateral connection was further assessed in patients with Rentrop scores of 2 or 3. Collateral connection grade is defined as that assessed from the collateral connection diameter and comprises CC 0, no continuous connection between donor and recipient artery; CC 1, continuous, threadlike connection; CC 2, continuous, small-side branchlike size of the collateral throughout its course. The magnitude of myocardial perfusion was angiographically assessed using myocardial blush (MB), the grade of which was also determined based on visually assessed contrast density in the myocardium. The MB grades were defined as follows: MB 0, no myocardial blush or contrast density; MB 1, minimal myocardial blush or contrast density; MB

**Table 2. Angiographic changes in collateral flow**

		IR group	bFGF group	p value
Rentrop score	Pre-	1.3 ± 1.0	1.9 ± 1.2	N.S.
	Post-	1.4 ± 1.1	2.3 ± 1.2*	N.S.
CC grade	Pre-	0.9 ± 0.3	1.0 ± 0.9	N.S.
	Post-	1.1 ± 0.6	1.4 ± 0.5†	N.S.
MB grade	Pre-	0	0.2 ± 0.4	< 0.03
	Post-	0	0.8 ± 1.0†	<0.001
Angiographic Improvement		14% (3/22)	53% (9/16)	0.05

Data are means ± SD. \*p = 0.05 vs. preoperative value; †p < 0.05 vs. preoperative value; bFGF, basic fibroblast growth factor; CC, collateral connection; MB, myocardial blush; N.S., not significant.

2, moderate myocardial blush or contrast density, but less than that obtained during angiography of a contralateral or ipsilateral noninfarct-related coronary artery; MB 3, normal myocardial blush or contrast density, comparable with that obtained during angiography of a contralateral or ipsilateral coronary artery. Angiographic flow improvement was defined as an increase in one of the Rentrop score, CC grade, or MB grade at the territory that was injected with bFGF or nonbypassed arterial distribution.

#### Assessment of myocardial scintigraphy

Myocardial scintigraphy was performed with thallium 201 at rest or during either exercise or pharmacological (dipyridamole) stress, using a Philips SKYLIGHT system and a gated SPECT protocol to ensure viability and ischemia in the myocardial territory of interest. Myocardial images were subdivided into 5 regions (septal, anterior, lateral, inferior, and apex). To evaluate improvements in regional myocardial perfusion at the territory of interest, we examined myocardial scintigraphic findings before and one month after treatment. An improvement in myocardial perfusion was postoperatively defined on myocardial scintigrams as either an increase in uptake at the stress phase or a decrease in the size of a fixed defect during the rest phase at the myocardial territory of interest compared with preoperative findings.

#### Statistical analysis

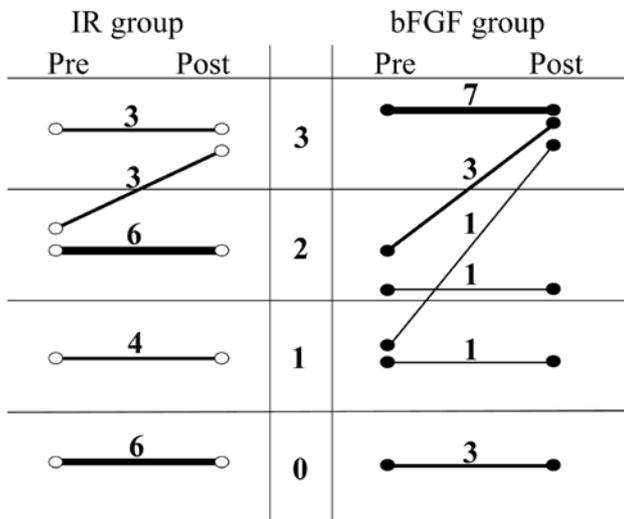
All values are expressed as means ± SD. Data were statistically evaluated by the analysis of variance (StatView 5.0, SAS Institute, Berkeley, Calif.). Dichotomous variables were compared with Fisher's exact test. Differences were considered statistically significant at p < 0.05.

## Results

All patients underwent successful CABG with or without concomitant angiogenic therapy. All patients in the bFGF group were discharged free of angina and with no significant adverse effects. All patients in the IR group were discharged free of angina, but one had a non-Q wave myocardial infarction in the ungraftable myocardial distribution.

#### Coronary angiography

Table 2 shows the development of collateral flow in the bFGF and IR groups. Eleven patients in the bFGF group and 12 in the IR group had preoperative Rentrop scores of 2 or 3 (Fig. 2). Rentrop scores increased in 4 patients in the bFGF group and in 3 in the IR group, but tended to increase in patients in the bFGF group (before vs. after surgery, 1.9 ± 1.2 vs. 2.3 ± 1.2, p = 0.05), whereas they did not differ between before and after surgery in the remainder of the IR group patients. Seven patients in the bFGF group and 11 in the IR group had preoperative CC grade 1 or 2 (Fig. 3). The CC grades increased in 5 patients in the bFGF group and in 2 in the IR group. They significantly increased in patients in the bFGF group (before vs. after surgery, 1.0 ± 0.9 vs. 1.4 ± 0.5, p < 0.05), but did not significantly change in patients in the IR group after surgery. The preoperative MB grade was 1 in 2 patients in the bFGF group, but in none of the IR group (p < 0.03). The MB grades were increased in 5 patients in the bFGF group (before vs. after surgery, 0.2 ± 0.4 vs. 0.8 ± 1.0, p < 0.05), but in none of the IR group. The postoperative MB grade in the bFGF group was higher than that in the IR group (p < 0.001). Angiographic improvement was achieved in 9 patients (53%) in the

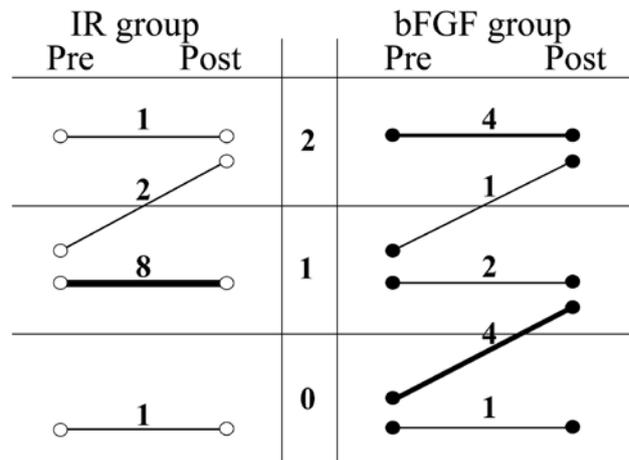


**Fig. 2.** Changes in Rentrop scores. IR, incomplete revascularization; bFGF, basic fibroblast growth factor.

bFGF group according to one of the Rentrop scores, CC grades, or MB grades, and in 3 patients in the IR group (14%) ( $p < 0.05$ ).

### Myocardial scintigraphy

Preoperative and postoperative myocardial scintigraphy was performed on 16 patients each in the bFGF and IR groups. Myocardial perfusion at the bFGF-injected territory improved in 13 patients (81%) of the bFGF group, whereas myocardial perfusion in the nonbypassed territory improved in only 4 patients in the IR group (25%) ( $p < 0.05$ ). We compared the results of coronary angiography representing collateral development with those of myocardial scintigraphy representing myocardial perfusion. Postoperative scintigraphic perfusion at the bFGF-injected territory improved in all 11 patients in the bFGF group with preoperative Rentrop scores of 2 or 3, but in only 2 of 5 patients with preoperative Rentrop scores of 0 or 1. Scintigraphic perfusion at the bFGF-injected territory was improved after surgery in all of the 11 patients with postoperative CC grades 1 or 2. Scintigraphic perfusion at the nonbypassed territory improved in only 3 of 12 patients in the IR group with preoperative Rentrop scores of 2 or 3 ( $p < 0.005$ , compared with the bFGF group). Scintigraphic perfusion at the nonbypassed territory improved after surgery in only 3 of 8 patients in the IR group with postoperative CC grade 1 or 2 ( $p < 0.01$ , compared with the bFGF group). None of the 3 patients in the IR group with angiographic improvement in the



**Fig. 3.** Changes in collateral connection (CC) grade. IR, incomplete revascularization; bFGF, basic fibroblast growth factor.

Rentrop score and/or CC grade had postoperative scintigraphic improvement in the nonbypassed territory. Scintigraphic perfusion improvement in the bFGF-injected territory depended on the status of preexisting collaterals (Rentrop scores of 2 or 3) and on postoperative collateral development (increased Rentrop score or CC grade). Scintigraphic perfusion improvement at the nonbypassed territory did not correlate with the status of preexisting collaterals or with postoperative collateral development, if any.

### Discussion

Angiographic findings representing collateral development were more significantly improved among patients in whom an ungraftable myocardial territory was treated with intramyocardial bFGF compared with the IR group. Myocardial scintigraphy showed that myocardial perfusion had improved in most patients having territories injected with bFGF and in a few patients in the IR group in the nonbypassed territory. Scintigraphic perfusion improvement at the bFGF-injected territory depended on the magnitude of preexisting collaterals as well as postoperative collateral growth. Our results suggested that angiogenic therapy with intramyocardial bFGF induces collateral development and improves perfusion at the injected myocardial territory. These results also suggest that the collaterals at the bFGF-injected territory helped to improve regional myocardial perfusion, probably

because the vascular bed increased at this site.

Angiogenic therapies with recombinant angiogenic growth factors or their genes have been advocated to promote the growth of new vessels in patients undergoing CABG if complete revascularization is unattainable. Schumacher et al. injected FGF-1 (acidic FGF, 0.01 mg/kg body weight) near the vessels after completing internal mammary artery/left anterior descending coronary artery anastomosis in 20 patients with three-vessel coronary disease.<sup>4)</sup> All patients had further stenosis in the distal third of the LAD or at the origin of one of its branches in addition to severe proximal stenosis. Selective visualization of the IMA bypasses by intra-arterial digital subtraction angiography 12 weeks later revealed that pronounced contrast medium accumulation had extended peripherally around the artery at injection sites. Angiograms of control patients who had received only heat-denatured FGF-1 showed absent accumulation of contrast medium. Schumacher et al. concluded that FGF-1 might be especially suitable for myocardial revascularization among patients with additional peripheral stenoses that cannot be surgically revascularized. A dense new capillary network was angiographically demonstrated in the region of FGF-1 administration 3 years later.<sup>13)</sup>

Sellke et al. reported early results and technical considerations of bFGF administration to induce collateral growth using heparin-alginate slow-release devices in patients undergoing CABG.<sup>5)</sup> Reul et al. reported long-term effects of angiogenic therapy with bFGF.<sup>14)</sup> Patients in whom an ungraftable territory was concomitantly treated with CABG and perivascular administration of sustained-release FGF-2 (bFGF) capsules experienced significantly greater freedom from angina recurrence than did control patients at a mean follow-up approaching 3 years.<sup>14)</sup> Late nuclear imaging showed that all but one patient in the control group had either a persistent reversible perfusion defect or evidence of a new fixed defect in the ungrafted myocardial territory, whereas this occurred in only 1 of 9 patients treated with FGF-2.

Well-developed coronary collaterals should ameliorate myocardial ischemia in the incidence of an occlusion. We previously reported that myocardial blood flow in the myocardial territory supplied by collateral vessels and not by a totally occluded coronary artery was comparable with that at the territory supplied by the coronary artery with 90% stenosis.<sup>15)</sup> Schaper noted that capillaries, regardless of numbers, cannot replace an occluded epi-

cardial coronary artery and that only arterial collaterals come close to replacing the conductance of a coronary artery.<sup>9)</sup> Attempts to induce collateral development aim at vascular territories requiring sustained collateral perfusion distal to total occlusions not amenable to revascularization. The most accurate way to assess coronary collaterals is by quantitative angiography. The Rentrop grade is only weakly correlated with the invasive parameters of collateral function. Angiographic grading of CC in total chronic occlusions is closely associated with invasively determined parameters of collateral hemodynamics.<sup>11)</sup> The CC grade might be suitable for assessing functional collaterals compared with the Rentrop grade. Collaterals of CC grade 0 represent early stages of collateral development, and collaterals of CC grade 2 represent mature collaterals. However, the potential confounding effects of CABG on perfusion of the ungraftable territory are inherent in the collateral study. Bypass grafting might significantly increase perfusion to a distant myocardial territory through preexisting collaterals or those formed *de novo*, regardless of the effects of angiogenic therapy. We evaluated the effects of angiogenic therapy by comparing angiographic and scintigraphic findings of patients in whom the nonbypassed territory was treated with angiogenic therapy and the graftable territory was treated with CABG, with those of IR group patients who underwent only CABG, leaving the ungraftable territory untreated. A previous study reported that coronary collateral development is poorer in patients with diabetes mellitus than in patients without it.<sup>16)</sup> Moreover, patients in the present study with previous CABG had more preexisting collaterals. Differences in the incidence of diabetes mellitus and previous CABG between bFGF and IR groups may affect the results. The present study is not a randomized, controlled study, but it may somewhat overcome these limitations.

In conclusion, angiographic findings representing collateral development were significantly more improved among patients in whom an ischemic but ungraftable myocardial territory was treated with intramyocardial bFGF than in IR group patients. Scintigraphic myocardial perfusion was more frequently improved in territories injected with bFGF than in the nonbypassed territories of IR group patients. These results suggest that angiogenic therapy with intramyocardial bFGF induces collateral development and improves myocardial perfusion at the site of bFGF delivery.

## References

1. Carmeliet P. Mechanisms of angiogenesis and arteriogenesis. *Nat Med* 2000; **6**: 389–95.
2. Kawasuji M, Nagamine H, Ikeda M, Sakakibara N, Fujii S, et al. Therapeutic angiogenesis with intramyocardial administration of basic fibroblast growth factor. *Ann Thorac Surg* 2000; **69**: 1155–61.
3. Harada K, Grossman W, Friedman M, Edelman ER, Prasad PV, et al. Basic fibroblast growth factor improves myocardial function in chronically ischemic porcine hearts. *J Clin Invest* 1994; **94**: 623–30.
4. Schumacher B, Pecher P, von Specht BU, Stegman Th. Induction of neoangiogenesis in ischemic myocardium by human growth factors. *Circulation* 1998; **97**: 645–50.
5. Sellke FW, Laham RJ, Edelman ER, Pearlman JD, Simons M. Therapeutic angiogenesis with basic fibroblast growth factor: Technique and early results. *Ann Thorac Surg* 1998; **65**: 1540–4.
6. Rosengart TK, Lee LY, Patel SR, Sanborn TA, Parikh M, et al. Angiogenesis gene therapy: phase 1 assessment of direct intramyocardial administration of an adenovirus vector expressing VEGF121 cDNA to individuals with clinically significant severe coronary artery disease. *Circulation* 1999; **100**: 468–74.
7. Simons M, Bonow RO, Chronos NA, Cohen DJ, Giordano FJ, et al. Clinical trials in coronary angiogenesis: issues, problems, consensus: An expert panel summary. *Circulation* 2000; **102**: E73–86.
8. Hamano K, Nishida M, Hirata K, Mikamo A, Li TS, et al. Local implantation of autologous bone marrow cells for therapeutic angiogenesis in patients with ischemic heart disease: clinical trial and preliminary results. *Circ J* 2001; **65**: 845–7.
9. Schaper W. Therapeutic arteriogenesis has arrived. *Circulation* 2001; **104**: 1994.
10. Rentrop KP, Cohen M, Blanke H, Phillips RA. Changes collateral channel filling immediately after controlled coronary artery occlusion by an angioplasty balloon in human subjects. *J Am Coll Cardiol* 1985; **5**: 587–92.
11. Werner GS, Ferrari M, Heinke S, Kueth F, Surber R, et al. Angiographic assessment of collateral connections in comparison with invasively determined collateral function in chronic coronary occlusions. *Circulation* 2003; **107**: 1972–77.
12. Van't Hof AWJ, Liem A, Suryapranata H, Hoorntje JCA, de Boer M-J, et al. Angiographic assessment of myocardial reperfusion in patients treated with primary angioplasty for acute myocardial infarction; myocardial blush grade. *Circulation* 1998; **97**: 2302–6.
13. Pecher P, Schumacher A. Angiogenesis in ischemic human myocardium. Clinical results after 3 years. *Ann Thorac Surg* 2000; **69**: 1414–9.
14. Reul M, Laham RJ, Parker JA, Parker JA, Post MJ, et al. Long-term effects of surgical angiogenic therapy with fibroblast growth factor 2 protein. *J Thorac Cardiovasc Surg* 2002; **124**: 28–34.
15. Kawasuji M, Kawajiri F, Iwa T. Measurement of regional myocardial blood flow: Application of the electrolytic hydrogen clearance method in man. *J Thorac Cardiovasc Surg* 1988; **96**: 775–81.
16. Abaci A, Oguzhan A, Kahraman S, Eryol NK, Unal S, et al. Effect of diabetes mellitus on formation of coronary collateral vessels. *Circulation* 1999; **99**: 2239–42.