# Repair of old myocardial infarction by intracoronary transplantation of autologous bone marrow mesenchymal stem cells: A pilot clinical trial

**Amir Farhang Zand Parsa[1*], Mandana Mohyeddin Bonab[2], Kamran Alimoghaddam[2]**

*[1]Department of Cardiology, Imam Khomeini Medical Center, Keshavarz Blvd. P.O. Box 14197-33141, Tehran, I.R. Iran [2]Shariati Hematology-Oncology and Bone Marrow Transplantation (BMT) Research Center, P.O. Box 14114 Tehran, I.R. Iran*

***Abstract***

Experimental and clinical studies have shown that intracoronary transplantation of autologous bone marrow mesenchymal stem cells (BMSCs) has resulted in regenerated infarcted myocardium and improved left ventricular (LV) function. The aim of this pilot study was to assess the benefical effects of intracoronary transplantation of BMSC in patients with old myocardial infarction (OMI). Autologous BMSCs were transplanted by the intracoronary method *via* percutaneous transluminal coronary balloon angioplasty (PTCA) in five patients with old myocardial infarction. Time from myocardial infarction (MI) to cell therapy was 5.2 ± 3.11 months (mean ± SD). All patients were <70 years old (32-61 years) and had significant LV dysfunction (LV ejection fraction, mean ± SD, 34% ± 10.83%), and severe wall motion abnormality (akinesia and / dyskinesia) at the location of infarcted area. Follow up angiography was performed 6-9 months (mean ± SD,7 ± 1.4 months) after BMSC transplantation, which revealed an increased trend in the LV ejection fraction (LVEF) of patients after treatment (LVEF: Mean ± SD from 34% ± 10.83% to 46.25% ± 9.46%, P= 0.051 and median from 35% to 42.5%). Clinical follow up (for 12-18 months) also revealed appreciable improvement in their symptoms or functional class [dyspnea from New York Heart Association(NYHA)-Class Ш-IV to I–II and Chest discomfort from Canadian Cardiovascular Society (CCS) Class II-IV to I-II]. Intracoronary transplantation of autologous BMSC in patients with old myocardial infarction appears to be feasible, safe and effective .The therapeutic effect could be attributed to BMSCs ability to regenerate myocardium.

***Keywords*:** Myocardial infarction; Bone marrow mesenchymal stem cell; Percutaneous coronary intervention; Left ventricular function; Autologus Transplantation.

**Correspondence to:* **Amir Farhang Zand Parsa,** M.D.
*Tel: +98 21 66931999, Fax: +98 21 66939537,*
*E-mail: zandparsa@tums.ac.ir*

## INTRODUCTION

Myocardial infarction is the leading cause of congestive heart failure and death in developed countries. World Health Organization (WHO) predicts that the disease will be the main cause of death world wide in the near future (Lee and Makkar, 2004; Lee *et al.,* 2004).

Several studies have documented the ability of the human heart to regenerate, albeit to a degree that is without clinical benefit, in patients with end stage heart failure, and after myocardial infarction. Therefore stem cell transplantation offers a simple and cost-effective way of repairing the infarcted heart (Lee and Makkar, 2004; Lee *et al.,* 2004; Mathur and Martin, 2004).

Both animal and human studies suggest that stem cells capable of improving cardiac function do exist in adults. Stem cells such as mesenchymal stem cells have the potential to differentiate into cardiomyocytes (Mohyeddin Bonab *et al.,* 2006 and 2005). Preliminary data from animal models and human studies also indicate that infarcted myocardium can be regenerated by implanting embryonic or/adult stem cells (Lee and Makkar, 2004; Lee *et al.,* 2004; Liu *et al.,* 2004; Hodgson *et al*., 2004; Mangi *et al*., 2003; Mathur and Martin, 2004; Soukiasian *et al*., 2004).

Different cell types have been used in cell transplantation. Meanwhile, autologous bone marrow stem cells and skeletal myoblasts have generated the most data (Liu *et al.,* 2004; Mathur and Martin, 2004; Soukiasian *et al.,* 2004; Chierchia and Deferrari, 2004; Stamm *et al.*, 2003b). In this study, separated mesenchymal stem cells (MSCs) from the bone marrow of patients who had suffered from old myocardial infarction were grown (expanded) *in vitro* and then injected

into the infarcted region *via* the infarct related artery by the balloon angioplasty method (mean ± SD, 5.2 ± 3.11 months, time from MI to cell therapy).

## MATERIALS AND METHODS

From May 2004 to March 2005, implantation of autologous bone marrow mesenchymal stem cells (BMSC) was performed in five patients (4 males and one female, mean ± SD, age = 48.4 ± 11.28 years) *via* percutaneous transluminal coronary angioplasty (PTCA). All patients were suffering from old anterior myocardial infarction. Time from acute myocardial infarction (AMI) to stem cell transplantation was 5.2 ± 3.11 months. Majority of patients had moderate to severe left ventricular dysfunction. Their LVEF were 34% ± 10.83% (mean ± SD). Their functional class were III-IV (NYHA class).

**Inclusion criteria**: 1- Patients with a history of old myocardial infarction, 2- Age <70 years old, 3- Presence of regional wall motion abnormality (WMA), severe akinesia or dyskinesia, documented by left ventriculography and echocardiography, 4- Absence of viable myocardium in ≥ 2 segments documented by radionuclide scintigraphy.

**Exclusion criteria**: 1- Age >70 years old, 2- Coronary anatomy unsuitable for percutaneous coronary intervention (PCI), 3- Severe co-morbidity, 4- Presence of viable myocardium at the territory of the infarcted zone, 5- Presence of cardiogenic shock or life threatening cardiac dysrhythmias, 6- Unwilling to do stem cell transplantation.

Written informed consent has been signed by each participant in the study. The study gained the approval of the ethical committee of the Tehran University of Medical Sciences under the supervision of the diagnostic digestive disease research center (FWA00001331).

Before making a decision for BMSC transplantation, diagnostic coronary angiography was performed in all patients. After evaluation of their coronary angiography and making sure that the infarct related artery (IRA) is suitable for PCI, they were referred for bone marrow (BM) aspiration and BMSC expansion.

**Sample collection and MSC expansion:** Under local anesthesia, 30 ml of BM was obtained from the iliac crest of each patient in a sterile and standard condition.

The BM mononuclear cells (MNCs) were separated by the ficoll density gradient method. Eight average vented flasks (75 $cm^2$) with 21 ml of MSC medium, consisting of Dulbecco's Modified Eagle's Medium (DMEM) with 10% (v/v) Fetal Bovine Serum (FBS)* and 1% penicillin and streptomycin (all from Gibco, Sigma, Germany), were seeded with $1\times10^6$ MNC/ml for the purpose of primary culture. Flasks were incubated at 37ºC in a humidified atmosphere containing 5% $CO_2$ and were fed by complete medium replacement every 4 days, until the fibroblast-like cells at the base of the flask reached confluence.

On reaching confluence, the adherent cells were resuspended using 0.025% trypsin and reseeded at $1\times10^4$ cells/ml ($1^{st}$ passage). These were incubated again until confluence, and were once again trypsinized and reseeded at $1\times10^4$ cells /ml. The number of passage of cells depending on the required amount of cells can be repeated, (normally 1-3 passages).

At the end of the last passage, when the cells reached confluence, they were washed with tyrode salt and incubated with MI99 medium for an hour. Cells were detached by trypsinization and washed with normal saline supplemented with 1% human serum albumin and heparin and then resuspended at 1-1.5× $10^6$/ml density. This washing process eliminates trace amounts of FBS as well.

**Immunophenotyping:** At the end of the last passage, expression of CD166, CD105, CD44 and CD13-which are MSC surface markers, and CD34 and CD45-which are Hematopoietic Stem Cell (HSC) surface markers were determined in culture-expanded MSCs. The monoclonal antibodies used were anti-CD44, CD45, CD34 fluorescein isothiocyanate (FITC) and anti-CD13 phycoerythrin (PE) (all purchased from Dako, Denmark), anti-CD166 FITC and anti CD105 RPE (from Serotec, Germany). Relevant isotope control antibodies were also used. Flowcytometry was performed on a FACS calibur system (Becton Dickinson) and data were analyzed with Cellquest software.

**Safety assessment:** To make sure that the cells are not contaminated, bacteriological tests were performed on the samples for every passage and at the time of injection. Viability of the cells was assessed by the Methylene Blue dye exclusion test just before injection.

We used high grade FBS from Gibco Co. which has been checked for virus, micoplasma, sterility, bacteriophage and endotoxin contamination.

**Intracoronary injection of BMSC:** Patients were

treated with antiplatelets (325 mg of Aspirin on the day before transplantation followed by 300 mg of a loading dose of clopidogrel, at least 6 hours before the procedure). In addition 75-100 U/kg of heparin was given during the procedure to maintain activated clotting time (ACT) between 250-300 seconds.

The procedure was initiated with diagnostic coronary and left ventricular (LV) angiography to re-evaluate coronary anatomy and LV function. Then, a diagnostic catheter was replaced with a guiding catheter.

After crossing the lesion with a long (300 cm), 0.014 inch diameter guide wire, an over-the-wire (OTW) balloon was introduced into the infarct related artery and positioned at the occlusion site. Then, the guide wire was drawn out from the central lumen of the OTW balloon. The procedure of BMSC injection into the infarcted area began with sequential inflation and deflation of OTW balloon every 2-3 minutes. During inflation of the balloon, injection of 1-2 ml of the prepared suspension of bone marrow MSC in to the infarcted region was performed through the central lumen of the balloon. The balloon remained inflated for at least 2-3 minutes in order to prevent backflow of BMSCs suspension, and thus facilitate distribution of the suspension into the infarcted zone. After each inflation, the balloon was deflated and remained so for 2-3 minutes in order to permit perfusion of the area. This cycle was repeated for 5-6 times. The mean number of $8 \times 10^6$ (ranging $5\text{-}12 \times 10^6$) bone marrow mesenchymal cells was injected into the infarcted area (Table 2). After the cell injection process, the procedure was completed with stenting the lesion with an appropriate sized bare metal stent (BMS). Procedures were tolerated well by all patients without any hemodynamic or rhythm disturbances.

**Follow up:** Patients have been visited every month for three months, and then every 3 months during follow up. In each visit, clinical and paraclinical evaluation in terms of physical examination and 12 leads of electrocardiography were carried out.

Selective coronary angiography and left ventriculography were performed between 6 to 9 months after BMSC transplantation in order to evaluate regional

**Table 1.** Baseline and clinical characteristics of patients.

| Characteristics \ Patients | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Age | 61 | 54 | 43 | 42 | 32 |
| Sex | female | male | male | male | male |
| Time from MI to cell therapy | 4 months | 3 months | 9 months | 8 months | 2 months |
| **Coronary artery disease** | | | | | |
| Single vessel | − | + | − | + | + |
| Multi vessel | + | − | + | − | − |
| Angiographic LVEF (%) | 30 | 50 | 35 | 20 | 35 |
| **Symptoms** | | | | | |
| chest discomfort (CCS FC) | III | III -II | IV | III | IV |
| Dyspnea (NYHAFS) | III | III | IV | III | III |
| **Risk factors** | | | | | |
| Hypertension | + | + | − | + | − |
| Diabetes | − | − | − | − | − |
| Smoking | − | − | + | + | + |
| Hyperlipidemia | − | − | − | + | − |

LVEF: Left ventricular ejection fraction. CCS FC: Canadian Cardiovascular Society functional class. MI: myocardial infarction NYHAFC: New York Heart Association functional class.

and global left ventricular ejection fraction (LVEF) and binary instent restenosis (binary restenosis defined as > 50% luminal loss).

**Statistical analysis:** Statistical analysis was performed with SPSS program (version 11.5). A comparison of pre and post-procedure data were carried out by using the paired t-test and nonparametric test of wilcoxon. Discrete variables were compared as rates. All data were presented as mean ± SD and median, with $p \leq 0.05$ considered as significant.

## RESULTS

From the beginning of May 2004 to the end of March 2005, intracoronary injection of BMSC was performed in five patients, with old anterior myocardial infarction. Baseline and clinical characteristics of patients are presented in Table 1.

At the end of the processes of immunophenotyping and safety assessment, the mean number of $8 \times 10^6$ (ranging $5\text{-}12 \times 10^6$) cells representing the prepared suspension of BM mesenchymal cells was utilized for transplantation (Table 2). The results of flowcytometry analyses of CD13 (78%), CD44 (79%), CD166 (51%), and CD 105 (56%) were positive and for the hematopoetic cell markers, CD34 and CD45 they were negative.

The results of bacteriological analyses were negative for all samples. The viability of injected cells was over 95%.

Procedural success rate was 100% (there were no failures and no major complications in terms of death, nonfatal myocardial infarction or urgent CABG).Three patients had single vessel disease, left anterior descending (LAD) and two patients had multivessel disease LAD, LCX (left circumflex) and LAD,OM (left anterior descending,obtuse marginal). Angiographic characteristics of LV and coronary arteries as well as procedural results are shown in Table 2.

Follow up LV and coronary angiography are carried out 6 to 9 months (mean ± SD, 7 ± 1.4 months) after BMSC transplantation in four patients (80%). One patient has been lost regarding follow up (couldn't be accessed during the follow up). Therefore, follow up coronary angiography was carried out in four

**Table 2.** Pre- procedural LV and coronary angiographic characteristics with procedural results.

| Characteristics \ Patients | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| **Severity of stenosis** | | | | | |
| LAD | 100% | 90% | 75% | 99% | 70% |
| LCX | 80% | – | 90% | – | – |
| RCA | – | – | 70% | – | – |
| LVEF (%) | 30 | 50 | 35 | 20 | 35 |
| LVWMA | Anterior hypokinesia, apical dyskinesia | Apical akinesia | Antero apical akinesia | Anterior akinesia, apical dyskinesia | Anterior hypokinesia, apical akinesia |
| **Number of vessels dilated** | | | | | |
| One | + | + | + | + | + |
| Two | – | – | – | – | – |
| Size of stents used | 3 × 22 mm | 23 × 3 mm | 15 × 3 mm | 18 × 3 mm | 12 × 3 mm |
| Procedural success rate (%) | 100 | 100 | 100 | 100 | 100 |
| Number of BMSCS | $5 \times 10^6$ | $7.8 \times 10^6$ | $11.7 \times 10^6$ | $11.9 \times 10^6$ | $4.75 \times 10^6$ |
| Successful cell Transplantation | Yes | Yes | Yes | Yes | Yes |
| in-hospital major complication (death, MI or urgent CABG) | No | No | No | No | No |

LAD: Left anterior descending; LCX: Left circumflex; RCA: Right coronary artery; LVEF: Left ventricular ejection fraction; LVWMA: Left ventricular wall motion abnormality; BMSCS: Bone marrow mesenchymal stem cells; CABG: Coronary artery bypass grafting, LV: left ventricule.

**Table 3.** Baseline versus follow up angiographic and clinical characteristics and major adverse cardiac event (MACE).

| Characteristics \ Patients | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Time from cell therapy To follow up angiography | 9 months | 6 months | 7 months | 6 months | Loss of follow up |
| Restenosis | No | Yes | No | No | - |
| **MACE** | | | | | |
| TLR | No | Yes | No | No | - |
| Nonfatal MI | No | No | No | No | No |
| Death | No | No | No | No | No |
| Cardiac arrhythmia | No | No | No | No | No |
| LVEF (baseline vs follow up) | 30% vs 40% | 50% vs 60% | 35% vs 45% | 20% vs 40% | 35%vs lost follow up |

MI: Myocardial infarction. LVEF: Left ventricular ejection fraction.

patients, during wich there was one target lesion revascularization (TLR) (patient number 2). Therefore, the major adverse cardiac event (MACE) i.e. death, nonfatal MI and TLR) during follow up in four patients was identified only as TLR. In four patients, follow up angiography revealed improvement in their LV ejection fraction (EF) (mean ± SD from 34 ± 10.8% to 46.25 ± 9.46%, p= 0.051 and median from 35% to 42.5%). Baseline and follow up angiographic characteristics are presented in Table 3.

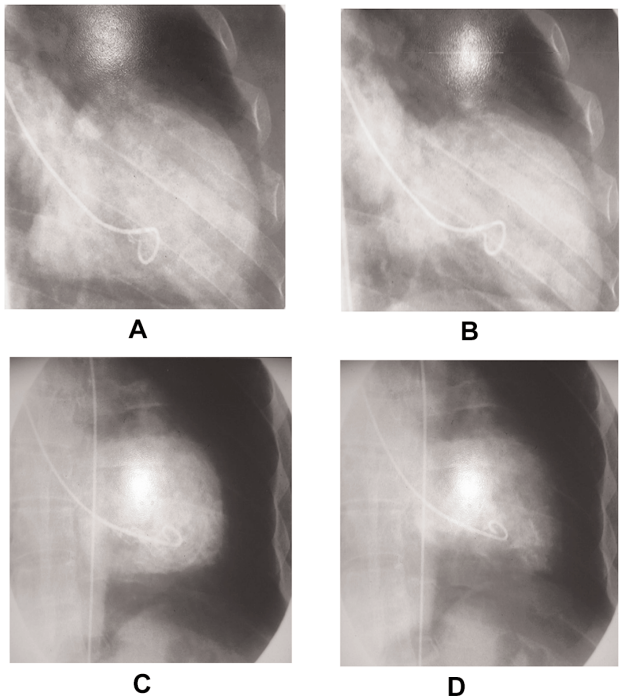


**Figure 1.** Left ventriculography of patient number 4 reveals significant improvement in LVEF, and wall motion abnormalities . **A)** systole; **B)** diastole, before BMSC therapy; **C)** systole; **D)** diastole, 6 months after BMSC therapy,

Figure 1 shows baseline and follow up LV, wall motion abnormalities (WMA), and ejection fraction of patient number 4.

Clinical follow up was carried out for 12 to 18 months. During clinical follow up, no cardiac arrhythmia, death or nonfatal myocardial infarction occurred. There was only one TLR due to in-stant restenosis, revascularization was carried out by repeat percutaneous coronary intervention (rePCI) and implanting a drug eluting stent (DES).

The symptoms (dyspnea and chest discomfort) of all patients improved and their functional class changed from NYHA and /CCS class Ш-IV to class I-П, during clinical follow up. Baseline and follow up clinical characteristics are presented in Table 4.

## DISCUSSION

The possibility of stem cell therapy for repairing myocardial infarction has created a new situation, quite unlike any previous therapeutic development process. Open collaboration amongst basic scientists and clinicians around the world are crucial for this superior procedure to be carried out successfully.

The traditional concept implies that the heart muscle itself has no house keeping mechanism to repair

**Table 4.** Clinical characteristics, baseline versus follow up (12-18 months).

| Characteristics \ Patients | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| **Dyspnea (NYHA functional class)** | | | | | |
| Baseline | III | III | IV | III | III |
| Follow up | I | I | I-II | I | Loss of follow up |
| **Chest discomfort (CCS functional class)** | | | | | |
| Baseline | III | III-II | IV | III | IV |
| Follow up | I | I-II | I-II | I | Loss of follow up |

NYHA: New York Heart Association; CCS: Canadian Cardiovascular Society.

any minor damage, but recent investigations suggest that large numbers of mitotic figures are present in the adult heart. The source of dividing cells in the myocardium is unclear. Two sources have been suggested, first, bone marrow stem cells, second, cardiac stem cells (Lee and Makkar, 2004; Lee *et al.,* 2004; Mathur and Martin, 2004; Wu *et al.,* 2003; Caplice and Gersh, 2003; Forrester *et al*., 2003; 15- Barbash *et al.*, 2003). Studies in animals and patients with heart transplant, have revealed that, donor-derived cardiomyocytes were present in the recipient heart. Investigators believe that these cells are donor MSCs which have differentiated into cardiomyocytes (Bayes-Genis *et al.,* 2002; Laflamme *et al*., 2002; Muller *et al*., 2002; 20- Sauer *et al.*, 2002; Toma *et al.,* 2002; ).

The most consistent improvement in myocardial function combined with safety has come from studies using autologous bone marrow stem cell transplantation in myocardial infarction. Compared to other current methods that have been used for cell transplantation in patients with myocardial infarction, the intracoronary approach is one of the safest, the most feasible and minimally invasive method for cell transplantation. In most studies of intracoronary injection of autologous bone marrow stem cells (Siminiak *et al.,* 2004, Chen *et al*., 2004; Wollert *et al*., 2004; Assmus *et al.*, 2002; Strauer *et al.,* 2002) no adverse effect was reported. Only in one study (Bartunek *et al.,* 2005) increased incidences of coronary events following intracoronary administration of enriched CD133 cells were reported.

The genetic and cellular mechanisms that initiate transdifferentiation of stem cells are very complex and poorly understood, nevertheless it has been shown that transplanted stem cells undergo a “homing” process in which they are attached to the site of injury (Lee *et al.,*2004; Mangi *et al.,* 2003; Wu *et al.,* 2003) .

Strauer and his colleagues for the first time in 2002 reported cases of intracoronary injection of autologous bone marrow derived progenitor cells for repairing myocardial infarction. According to their study an improvement in the left ventricular function including a significant reduction in the infarct size has occurred (from 30 ± 13% to 12 ± 7%, P = 0.005) (Strauer *et al.*, 2002).

To our knowledge phase-1 clinical studies of stem cell transplantation (before 2004) included 13 studies. These attempts have been undertaken in humans for repairing injured myocardium in patients with acute myocardial infarction. In these studies, different protocols such as PTCA, thoracotomy (during CABG) and transcatheter intramyocardial injection have been implicated, which in four of them PTCA was the prefered method (Chen *et al*., 2004; Lee and Makkar, 2004; Lee *et al.,* 2004; Siminiak *et al*., 2004; Stamm *et al*., 2004a; Wollert *et al*., 2004; Menasche *et al.,* 2003; Assmus *et al*., 2002; Strauer *et al.,* 2002).

In those studies that BMSC were used *via* the PTCA technique. Although their methods were different but they reached the same results. Strauer and his colleagues (2002) reported a significant decrease in the infarct region within the cell therapy group compared to the control group (p= 0.04) after 3 months.

In BOOST (intracoronary autologous BOne marrOw cell tranSfer afTer and myocardial infarction) and TOPCARE-AMI (Transplantation Of Progenitor Cells And REgeneration in Acute Myocardial Infarction) randomized trials, improvements in global LVEF after 4-6 months follow up were significant (from 50 ± 10% to 56.7 ± 12.5%, p= 0.0026 and from

51.6 ± 9.6% to 60.1 ± 8.6%, p= 0.003 respectively), (Wollert *et al*., 2004; Assmus *et al*., 2002).

Chen and his colleagues (2004) also reported significant improvement in the LVEF of their cases 3 months after cell therapy (from 49 ± 9% to 67 ± 11%, p = 0.01).

There are few investigations regarding stem cell therapy in patients with old myocardial infarction. Similar to attempts carried out in AMI; results of these studies were surprising as well.

Improvement of coronary endothelial function and enhanced reserve flow due to intracoronary application of BMSC after successful PCI (post-recanalization) in patients with chronic coronary total occlusion has been reported by lenk and his colleagues (2004).

Manginas and his colleagues (2004) for the first time in human study documented that intracoronary administered radio-labeled BMSC were clearly seen adhered to the infracted zone in patients with old myocardial infarction, In another study they observed reduction in the left ventricular dimensions and improvement in perfusion of the previously infracted region (Manginas *et al.,* 2004a; Manginas *et al.,* 2004b).

The results of this study were comparable with those that have been performed in patients with AMI as well as with those studies in patients with old myocardial infarction.

In our patients, as in other studies, improvement in the left ventricular function and functional capacity were consistent during the follow up period (12-18 months). In this period, no cardiac arrhythmia, death or nonfatal MI has occurred. Also safety and feasibility of the application of BMSC for myocardial regeneration in patients with old myocardial infarction and moderate to severe LV dysfunction have been elucidated in our, albeit small, study.

**Limitation:** The absence of a matched control group and follow up positron emission tomography with foulorodeoxyglucose (FDG-PET) study to quantitate the magnitude of regional viability after cell therapy and the small number of patients were the limitations of our pilot study. For reaching a stronger conclusion, a large randomized controlled clinical trial is necessary.

## CONCLUSION

Application of autologous BMSC transplantation for repairing myocardium in patients with old myocardial infarction is safe, feasible and effective. Catheter based percutaneous transluminal coronary approach is one of the safest and minimally invasive techniques for this novel method of myocardial regeneration in such patients.

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