Received: 30 December 2017 | Revised: 19 June 2018 | Accepted: 21 June 2018
DOI: 10.1111/joic.12539

ACUTE CORONARY SYNDROME

WILEY Journal Of Interventional Cardiology

Adjunctive laser-stimulated stem-cells therapy to primary reperfusion in acute myocardial infarction in humans: Safety and feasibility study

Gabby Elbaz-Greener MD, MHA[1,2,3] | Maneesh Sud MD[3] | Oran Tzuman MD[1,2] | Marina Leitman MD[1,2] | Zvi Vered MD[1,2] | Nissan Ben-Dov MD[4] | Uri Oron PhD[5] | Alex Blatt MD, BSc[1,2]

[1] Cardiology Division, Assaf Harofeh Medical Center, Zerifin, Israel

[2] Sackler School of Medicine, Tel Aviv University, Tel-Aviv, Israel

[3] Schulich Heart Centre, Division of Cardiology, Sunnybrook Health Sciences Centre, University of Toronto, Toronto, Ontario, Canada

[4] Cardiology Department, Lady Davis Carmel Medical Centre, Haifa, Israel

[5] Faculty of Life Sciences, Department of Zoology, Tel-Aviv University, Tel-Aviv, Israel

Correspondence
Uri Oron, Faculty of Life Sciences, Department of Zoology, Tel Aviv University, Israel.
Email: oronu@post.tau.ac.il

Funding information
Shlezak Internal Fund of Tel-Aviv University

Background: Low-level laser therapy (LLLT) has photobiostimulatory effects on stem cells and may offer cardioprotection. This cell-based therapy may compliment primary percutaneous coronary intervention (PPCI) in patients with ST-segment elevation myocardial infarction (STEMI).

Objective: In this randomized control trial, our primary objective was to determine the safety and feasibility of LLLT application to the bone marrow in patients with STEMI undergoing PPCI.

Methods: We randomly assigned patients undergoing PPCI to LLLT or non-laser therapy (NLT). In the LLLT group, 100 s of laser therapy was applied to the tibia bone prior to PPCI, as well as 24 and 72 h post-PPCI. In the control group, the power source was turned off. The primary outcome was the difference in door-to-balloon (D2B) time, and additional outcomes included differences in circulating cell counts, cardiac enzymes, and left-ventricular ejection fraction (LVEF) at pre-specified intervals post-PPCI.

Results: Twenty-four patients were randomized to LLLT ($N = 12$) or NLT ($N = 12$). No adverse effects of the treatment were detected. The D2B time was not significantly different between the groups (41 ± 8 vs 48 ± 1 min; $P = 0.73$). Creatinine Phosphokinase area under the curve, was lower after LLLT (22 ± 10) compared to NLT (49 ± 12), but this was not statistically significant ($P = 0.08$). Troponin-T was significantly lower after LLLT (2.7 ± 1.4 ng/mL) in comparison to NLT (5.2 ± 1.8 ng/mL. $P < 0.05$). At 9 months, LVEF improved in both groups without a significant difference between LLLT (55 ± 9%) and NLT (52 ± 9%; $P = 0.90$).

Conclusion: LLLT is a safe and feasible adjunctive cell-based therapy to PPCI that may benefit ischemic myocardium.

KEYWORDS
acute myocardial infarction, bone marrow, humans, low level laser therapy, safety, ST segment myocardial infarction

All authors listed meet the authorship criteria according to the latest guidelines of the International Committee of Medical Journals Editors. All authors approve the manuscript.

1 | INTRODUCTION

Coronary heart disease is the leading cause of death worldwide. Almost 4 million men and 3.5 million women die of the disease each year.[1] The new field of cell-based therapy offers a complementary mode of treatment, in addition to coronary reperfusion, in patients with acute myocardial infarction (AMI) by replacing dysfunctional myocytes and improving heart function.[2,3]

Cell-based therapies promote tissue repair through the introduction of exogenous cells, such as bone-marrow (BM)–derived mesenchymal stem-cells, that may secrete paracrine factors, stimulate neovascularization, and activate cardiac regeneration. These exogenous cells were traditionally derived from the BM, but recently, cells originating from adipose tissue, cardiospheres, and CD34+ cells have been utilized. Clinical trials, however, have revealed either no improvement or only modest improvement in heart function with stem-cell-based therapies applied to patients with AMI or an underlying cardiomyopathy.[4–7] Finding the optimal type of stem-cell for cardiac cytoprotection and regeneration and introducing stem-cells at multiple time points after cardiac injury may improve clinical outcomes and optimize regenerative gains in heart function.[8]

The cardioprotective effects of low-level laser therapy (LLLT) have been demonstrated in the ischemic heart in vivo and in vitro.[9,10] In rat and dog models, LLLT application directly to ischemic myocardium at optimal power parameters significantly reduced myocardial scar tissue formation.[11–14] Furthermore, in a rat model, LLLT application to autologous BM induced proliferation and recruitment of mesenchymal stem cells to the infarcted myocardium and markedly reduced scarring, while simultaneously inducing cardiogenesis along the border of the infarcted area.[15–18] In a recent study using a porcine model of AMI, applying LLLT to the BM caused a significant reduction in myocardial scarring relative to non-laser treated pigs.[19] Furthermore, in the same study, the left ventricular ejection fraction (LVEF) in the laser treated pigs was found to be significantly higher than in the non-laser treated ones.[19] Despite these promising results in animal models, to the best of our knowledge, application of LLLT in human subjects has not yet been studied.

In patients presenting with ST-elevation myocardial infarction (STEMI), acute occlusion of an epicardial artery results in myocardial ischemia and infarction in the area subtended by the occluded artery. Early reperfusion salvages viable myocardium, but despite this, a large proportion of patients develop left-ventricular dysfunction from irreversible cell death, reperfusion injury, and remodeling. LLLT has not yet been applied to patients with STEMI but recruitment of BM-derived stem-cells may benefit these patients by limiting scarring and left ventricular dysfunction. In this single-center randomized controlled trial, we tested the feasibility and safety of LLLT applied to the tibia bones of patients with STEMI undergoing primary percutaneous coronary intervention (PPCI) and evaluated its impact on circulating blood cells, cardiac biomarkers and clinical parameters such as LVEF.

2 | METHODS

2.1 | Patients selection

This clinical study was approved by the ethical committee of Assaf-Harofeh Medical Centre.

All patients gave their informed consent prior to their inclusion in the study. We included patients without a prior documentation of coronary artery disease that presented with STEMI in the first 6-hour after chest pain onset and were scheduled for PPCI as the reperfusion strategy (Figure 1). Patients under 18 years of age, pregnant females, patients with severe underlying illness, patients presenting with cardiogenic shock, those requiring cardiopulmonary resuscitation and patients that could not provide informed consent were excluded. All patients received mechanically reperfusion by PPCI. Patients were randomly assigned to two groups: low-level laser treatment group (LLLT) or controls; non-laser treated group (NLT). Randomization was stratified by infarct location as revealed by the ECG [left anterior descending (LAD), left circumflex artery (LCX), and right coronary artery (RCA)] in order to minimize variability between the groups (Figure 2).

2.2 | Chemical analysis

Blood samples were collected for leukocytes and platelet counts, as well as Troponin-T and Creatinphosphokinase (CPK) levels. The blood samples were collected at admission and 12-hour after admission and then daily for the next 5 days. After discharge, blood samples collected at 30 days and 9 months.

Serum was prepared from fresh blood and stored immediately at −70°C in small aliquots for determination of Troponin-T and CPK. For

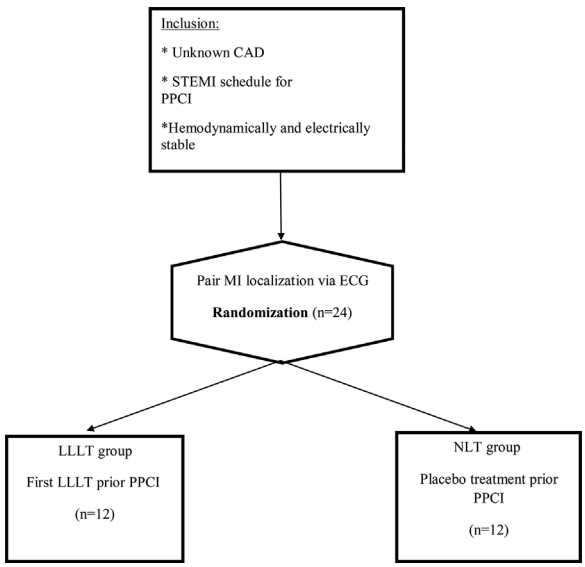

FIGURE 1 Baseline clinical characteristics, procedural characteristics, and outcomes. CAD, coronary artery disease; STEMI, ST elevation Myocardial Infarction (MI); PPCI, primary percutaneous coronary intervention; LLLT, low level laser therapy; NLT, non-laser therapy

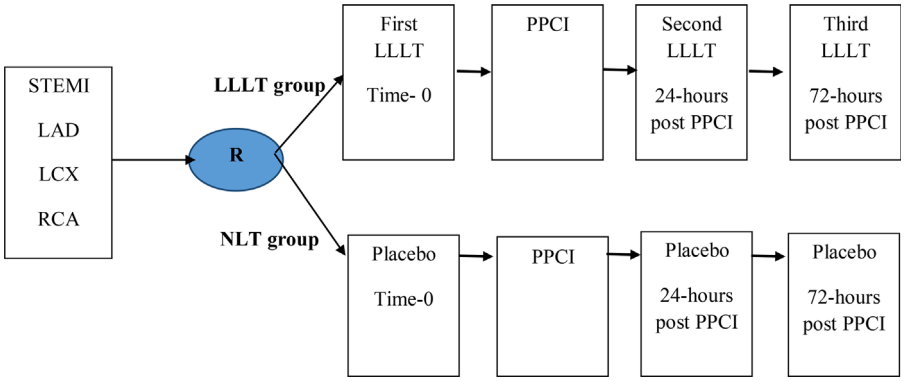

FIGURE 2 Patients treatment LLLT vs Placebo. STEMI, ST elevation MI; R, Randomization; PPCI, primary percutaneous coronary intervention; LLLT, low level laser therapy

CPK, the results were expressed as CPK accumulation in the blood, which was calculated from the area under curve (AUC) of CPK activity daily from admission until 5-days post STEMI. Leukocytes and platelet analysis was performed using standard hematological methods.

2.3 | Echocardiography

All patients were evaluated by two-dimensional echocardiography (Vivid E90, GE Healthcare) during the first 12 h, 1 month, and 9 months after STEMI. Regional left-ventricular function was assessed semi-quantitatively according to the recommendations of the American Society of Echocardiography scoring scheme.[20]

2.4 | Laser treatment

We utilized the Tunable Ga-Al-As 808 nm wavelength diode laser with a 900 mW power output (Thore Photomedicine Ltd., England) in this study. The optimal laser power, frequency and timing for transmission through bone was ascertained from our previous experiments including our recent BM histological analysis after laser application to the tibia of the infarcted porcine model.[19] A rigid glass (metal backed) fiber optic (8 mm diameter) connected to a flexible glass fiber optic was used to deliver the energy from the laser unit to the patient's tibia. The laser was applied by contact of the distal tip of the rigid fiber optic with the external side of the tibia bones (non-invasive to the bone or the skin) to ensure optimal power density delivery (10 mW/cm^2) to the BM tissue (cells) within the bones. The first laser treatment was applied as soon as possible after admission and usually in the coronary angiography laboratory (CAT- lab) during preparation for the procedure. Second and third laser treatment were given 24 h and 3-days post admission (Figure 2). Laser therapy was delivered for 100 s (1J/cm^2) to the tibia in one leg and then to the tibia in the contralateral leg. In the NLT control groups, patients were treated in a similar fashion but the laser was not turned on. Due to the dispersion of the laser beam after transmission through the tibia bone, the beam covers the entire BM underneath the laser probe.[21] Taking into consideration the anatomy of the BM space, it is estimated that about 30% of the BM volume of the tibia bone is irradiated by the laser beam.

2.5 | Statistical analysis

Discrete variables were compared using Pearson's Chi-squared test, or Fisher's exact test, as appropriate. Continuous variables were compared between groups using One-Way Analysis of Variance (ANOVA) with repeated measures to assess changes over time. Correlations between certain variables were estimated using Spearman's Correlations, since most of the variables did not have Gaussian distributions. A two-sided P <0.05 was considered statistically significant. The data were analyzed using BMDP Statistical Software.

3 | RESULTS

3.1 | Patient characteristics

The baselines characteristics are summarized in Table 1. Twenty-four patients (21 males and 3 females) were recruited. The mean age was 60 and 54 in the LLLT and NLT groups respectively. A higher proportion of patients in the NLT (41.6%) group had a previous history of hypertension compared to the LLLT group (33.3%, P = 0.02). There were no other significant differences in baseline risk factors between the LLLT and NLT groups. A sudden presentation without a prodromal of chest pain was similar in the LLLT (58.3%) and NLT (41.6%) groups. On admission, all the patients had normal myocardial biomarker values. No significant differences were observed in the ischemic time (chest pain onset to wire crossing the lesion) and the door to balloon time (D2B: time from admission to the hospital to wire crossing the lesion). The ischemic time was 196 ± 3 vs 188 ± 7 min (P = 0.93) and the D2B was 41 ± 8 and 48 ± 1 min (P = 0.73) in the LLLT group and in the NLT group, respectively.

3.2 | Laboratory analysis

At day one and at each consecutive day thereafter during the first 5-days post STEMI, leukocyte and platelet densities in the circulating blood were not statistical different from admission levels in both the LLLT or NLT groups.

The peak level of Troponin-T in the blood at 8–10 h after STEMI was 52% lower in the LLLT group compared to the NLT group (P < 0.05;

TABLE 1 Baseline characteristics

Variable	LLLT (*n* = 12)	No-LLLT (*n* = 12)	*P*
Age (years) (mean ± SD)	60.25 ± 11.6	54.4 ± 9.6	0.81
Women	8.3%	16.6%	0.58
T2DM	16.6%	25	0.68
Hypertension	33.3%	41.6%	0.77
Dyslipidemia	41.6%	41.6%	1
Current smoker	58.3%	50%	0.82
Statins	25%	25%	1
Aspirin	16.6%	8.3%	0.58
Oral hypoglycemic	16.6%	25%	0.68
Beta blockers	25%	16.6%	0.68
ACE/ARBS	16.6%	25%	0.68
STEMI	100%	100%	1
Prodromal UAP	41.6%	58.3%	0.63
Ischemic time (minutes)	196.3	188.7	0.93
Door to balloon (minutes)	41.8	48.1	0.73
Number of disease vessel	1.4 ± 2	1.7 ± 2	0.46
Culprit artery:			
LAD	33.3%	33.3%	1
LCX	33.3%	33.3%	1
RCA	33.3%	33.3%	1
TIMI flow pre-PCI			
0-I	33.3%	41.6%	0.77
I-II	50%	25%	0.39
II-III	16.6%	33.3%	0.68
Final TIMI flow			
III	83.3%	91.6%	0.87
I-II	16.6%	8.3%	0.58
Number of stents	1.2 ± 05	1.3 ± 08	0.82
CK (AUC) (mean ± SD)	205 ± 1	358 ± 1	<0.01
Troponin T	3.9 ± 3	5.3 ± 4	<0.05
Ejection fraction			
First 12 h	43 ± 4	41 ± 5	0.87
30 days	51 ± 8	45 ± 6	0.78
9 months	55 ± 9	52 ± 9	0.90

T2DM, Type 2 diabetes mellitus; ACEI, angiotensin-converting-enzyme inhibitor; ARBs, angiotensin receptor blockers; STEMI, ST segment elevation myocardial infarction; LAD, left descending artery; LCX, left circumflex artery; RCA, right coronary artery; PCI, percutaneous coronary intervention; NS, non-significant; UAP, unstable angina.

Figure 3). Accumulation of CPK in the blood up to 5-days post-STEMI was also 56% lower in the LLLT group compared to the NLT group (Figure 3), however, this difference did not reach statistical significance ($P = 0.08$).

There was no statistically significant difference in LVEF in LLLT and the NLT groups 12-hour, 1-month, or 9-month post STEMI. The LVEF gradually increased with time after STEMI in both groups reaching a value of 55 (SD = 9%) and 52 (SD = 9%) in the LLLT and NLT group respectively at 9-month post STEMI ($P = 0.90$).

4 | DISCUSSION

The results of the present study demonstrate that LLLT applied to the BM in patients with STEMI undergoing PPCI was feasible, safe and

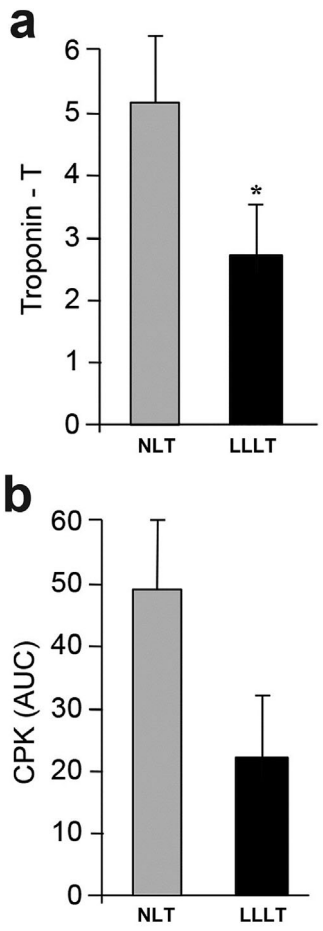

FIGURE 3 Levels of Troponin-T (A) and CPK (area under curve); (B) in the serum of LLLT and NLT groups

easily implemented. Importantly, LLLT therapy applied before reperfusion and as an adjunct to PPCI did not increase D2B times. Furthermore, LLLT did not alter circulating leukocyte and platelet counts after STEMI. LLLT reduced the rise in Troponin-T and did not adversely affect LVEF up to 9-month following STEMI.

LLLT has been safely studied in various animal models. In a previous study, we had demonstrated the safety of LLLT application to the BM in healthy mice over almost their entire lifespan.[21] Moreover, using the infarcted porcine model, multiple laser treatment to the BM had no adverse effects when compared to control non-treated porcine.[19] The results of this trial corroborate these findings and extend its safety into human subjects.

The results of the current study also indicate a possible cardioprotective effect of LLLT exerted on the BM prior to reperfusion. There was a significant 52% reduction in the peak Troponin-T levels after LLLT. A similar non-significant 56% reduction was also noted in the CPK accumulation with LLLT. These results are in accordance with our previous results in the infarcted porcine heart wherein laser application to the BM significantly reduced circulating Troponin-T and CPK in the blood after induction of AMI.[19] These findings must however, be interpreted in the context of a heterogeneous and small sample of patients with varying infarct locations.

In laser treated porcine models, significant improvements in LVEF were observed as early as 2 h after myocardial infarction. This rapid improvement in LVEF after AMI suggests an initial cardioprotective effect mitigated by laser application to the BM. Similar findings have been reported after direct application of LLLT to the myocardium of rats and dogs and to cardiomyocytes in vitro.[11–14] Although we did not observe a significant difference in LVEF with LLLT, LVEF improved to a similar degree in the LLLT and NLT groups. It may therefore be postulated that we were underpowered to determine significant differences in clinical parameters after STEMI, yet LLLT in humans may reduce scarring and infarct size and lead to improvements in LVEF after STEMI.

The mechanism by which LLLT applied to the BM exerts its cardioprotective effects in humans after AMI is not clearly understood. We postulate that LLLT induces various cells types in the BM to proliferate, and under appropriate conditions, migrate to ischemic zones in the heart. Thereafter, these cells may directly exert beneficial effects or indirectly stimulate endogenous cardiac stem cells. Multiple studies in animal models are supportive of this hypothesis. Firstly, after cardiac ischemia in animals[22] and humans,[23] endogenous mononuclear cells are mobilized from the bone marrow suggesting the bone marrow plays an active role in response to cardiac injury. Secondly, transcardial injection of BM-derived MSCs increases endogenous c-kit+ cardiac stem-cells by 20-fold in the porcine infarcted heart when compared to controls. This suggests bone-marrow derived cells have the capacity to engraft, differentiate and stimulate cardiac progenitor cells.[24] Lastly, in porcine models, laser therapy applied to the bone marrow after induction of AMI results in a significantly higher number of c-kit+ stem cells circulating within the blood and within the infarcted zone when compared to controls.[19] This correlates with a lower burden of scar tissue formation and higher density of new blood vessels within the infarcted zone. Therefore, in theory, laser therapy may have the capacity to augment stem-cell mobilization during AMI and promote localization of these cells to ischemic myocardium wherein they may exert their beneficial effects directly or indirectly.

The results of our study are important and clinically relevant. Laser therapy can be applied safely and non- invasively to the pelvic girdle, tibia or other parts of the skeleton containing BM without any delay to the provision of PPCI. This novel and non-invasive approach of utilizing stem-cells circumvents the need to isolate, grow in vitro and to re-inject stem cells into patients. It also avoids the massive loss of cells involved in cell implantation/injection due to insufficient seeding of cells or cell death shortly after implantation. Laser therapy also overcomes the need to grow autologous stem-cell cultures, determine the optimal amount of implanted cells and the optimal timing for their delivery post-STEMI.

In conclusion, to the best of our knowledge we have demonstrated for the first time, the feasibility and safety of adjunctive LLLT treatment to the BM in patients with STEMI managed with PPCI. Application of LLLT does not delay D2B times, and results in lower peak Troponin-T accumulation. This is a novel approach to the induction of autologous stem-cells from the BM of patients with STEMI. Further large-scale studies are needed to determine the clinical

efficacy of this easily implemented treatment adjunct in STEMI patients.

ACKNOWLEDGMENT

Clinical trial name: "low level laser application to the bone marrow in patients with acute myocardial infarction"- No. of trial: 14/111.

CONFLICT OF INTEREST

All authors declare have no conflict of interest to declare.

ORCID

Uri Oron http://orcid.org/0000-0001-5857-8823

REFERENCES

1. Go AS, Mozaffarian D, Roger VL, et al. Heart disease and stroke statistics–2013 update: a report from the American Heart Association. *Circulation*. 2013;127:e6–e245.
2. Mummery CL, Davis RP, Krieger JE Challenges in using stem cells for cardiac repair. *Sci Transl Med*. 2010;2:27ps17.
3. Laflamme MA, Murry CE. Heart regeneration. *Nature*. 2011;473:326–335.
4. Ong SG, Wu JC. Cortical bone-derived stem cells: a novel class of cells for myocardial protection. *Circ Res*. 2013;113:480–483.
5. Traverse JH, Henry TD, Ellis SG, et al. Effect of intracoronary delivery of autologous bone marrow mononuclear cells 2 to 3 weeks following acute myocardial infarction on left ventricular function: the LateTIME randomized trial. *JAMA*. 2011;306:2110–2119.
6. Surder D, Manka R, Lo Cicero V, et al. Intracoronary injection of bone marrow-derived mononuclear cells early or late after acute myocardial infarction: effects on global left ventricular function. *Circulation*. 2013;127:1968–1979.
7. Bolli R, Chugh AR, D'Amario D, et al. Cardiac stem cells in patients with ischaemic cardiomyopathy (SCIPIO): initial results of a randomised phase 1 trial. *Lancet*. 2011;378:1847–1857.
8. Bolli R. Repeated cell therapy: a paradigm shift whose time has come. *Circ Res*. 2017;120:1072–1074.
9. Zhang R, Mio Y, Pratt PF, et al. Near infrared light protects cardiomyocytes from hypoxia and reoxygenation injury by a nitric oxide dependent mechanism. *J Mol Cell Cardiol*. 2009;46:4–14.
10. Quirk BJ, Sonowal P, Jazayeri MA, Baker JE, Whelan HT. Cardioprotection from ischemia-reperfusion injury by near-infrared light in rats. *Photomed Laser Surg*. 2014;32:505–511.
11. Yaakobi T, Shoshany Y, Levkovitz S, Rubin O, Ben Haim SA, Oron U. Long-term effect of low energy laser irradiation on infarction and reperfusion injury in the rat heart. *J Appl Physiol*. 1985;2001:2411–2419.
12. Oron U, Yaakobi T, Oron A, et al. Low-energy laser irradiation reduces formation of scar tissue after myocardial infarction in rats and dogs. *Circulation*. 2001;103:296–301.
13. Oron U, Yaakobi T, Oron A, et al. Attenuation of infarct size in rats and dogs after myocardial infarction by low-energy laser irradiation. *Lasers Surg Med*. 2001;28:204–211.
14. Tuby H, Maltz L, Oron U. Modulations of VEGF and iNOS in the rat heart by low level laser therapy are associated with cardioprotection and enhanced angiogenesis. *Lasers Surg Med*. 2006;38:682–688.
15. Tuby H, Maltz L, Oron U. Low-level laser irradiation (LLLI) promotes proliferation of mesenchymal and cardiac stem cells in culture. *Lasers Surg Med*. 2007;39:373–378.
16. Tuby H, Maltz L, Oron U. Induction of autologous mesenchymal stem cells in the bone marrow by low-level laser therapy has profound beneficial effects on the infarcted rat heart. *Lasers Surg Med*. 2011;43:401–409.
17. Oron U. Light therapy and stem cells: a therapeutic intervention of the future. *J Interv Cardiol*. 2011;3:627–629.
18. Tuby HYT, Malytz L, Delarea Y, Sagi-Assif O, Oron U. Effect of autologous mesenchymal stem cells induced by low level laser therapy on cardiogenesis in the infarcted area following myocardial infarction in rats. *J Biomed Sci Eng*. 2013;6:24–31.
19. Blatt A, Elbaz-Greener GA, Tuby H, et al. Low-level laser therapy to the bone marrow reduces scarring and improves heart function post-acute myocardial infarction in the pig. *Photomed Laser Surg*. 2016;34:516–524.
20. Bourdillon PD, Broderick TM, Sawada SG, et al. Regional wall motion index for infarct and noninfarct regions after reperfusion in acute myocardial infarction: comparison with global wall motion index. *J Am Soc Echocardiogr*. 1989;2:398–407.
21. Tuby H, Hertzberg E, Maltz L, Oron U. Long-term safety of low-level laser therapy at different power densities and single or multiple applications to the bone marrow in mice. *Photomed Laser Surg*. 2013;31:269–273.
22. Asahara T, Masuda H, Takahashi T, et al. Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization. *Circ Res*. 1999;85:221–228.
23. Society of Thoracic Surgeons; American College of Cardiology. STS/ACC TVT registry. https://www.ncdr.com/webncdr/tvt/publicpage/datacollection. Accessed April 17, 2018.
24. Hatzistergos KE, Quevedo H, Oskouei BN, et al. Bone marrow mesenchymal stem cells stimulate cardiac stem cell proliferation and differentiation. *Circ Res*. 2010;107:913–922.

How to cite this article: Elbaz-Greener G, Sud M, Tzuman O, et al. Adjunctive laser-stimulated stem-cells therapy to primary reperfusion in acute myocardial infarction in humans: Safety and feasibility study. *J Interv Cardiol*. 2018;31:711–716. https://doi.org/10.1111/joic.12539