Effectiveness and Safety of Transcranial Laser Therapy for Acute Ischemic Stroke

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Background and Purpose—We hypothesized that transcranial laser therapy (TLT) can use near-infrared laser technology to treat acute ischemic stroke. The NeuroThera Effectiveness and Safety Trial–2 (NEST-2) tested the safety and efficacy of TLT in acute ischemic stroke.

Methods—This double-blind, randomized study compared TLT treatment to sham control. Patients receiving tissue plasminogen activator and patients with evidence of hemorrhagic infarct were excluded. The primary efficacy end point was a favorable 90-day score of 0 to 2 assessed by the modified Rankin Scale. Other 90-day end points included the overall shift in modified Rankin Scale and assessments of change in the National Institutes of Health Stroke Scale score.

Results—We randomized 660 patients: 331 received TLT and 327 received sham; 120 (36.3%) in the TLT group achieved favorable outcome versus 101 (30.9%), in the sham group ($P=0.094$), odds ratio 1.38 (95% CI, 0.95 to 2.00). Comparable results were seen for the other outcome measures. Although no prespecified test achieved significance, a post hoc analysis of patients with a baseline National Institutes of Health Stroke Scale score of <16 showed a favorable outcome at 90 days on the primary end point ($P<0.044$). Mortality rates and serious adverse events did not differ between groups with 17.5% and 17.4% mortality, 37.8% and 41.8% serious adverse events for TLT and sham, respectively.

Conclusions—TLT within 24 hours from stroke onset demonstrated safety but did not meet formal statistical significance for efficacy. However, all predefined analyses showed a favorable trend, consistent with the previous clinical trial (NEST-1). Both studies indicate that mortality and adverse event rates were not adversely affected by TLT. A definitive trial with refined baseline National Institutes of Health Stroke Scale exclusion criteria is planned. (***Stroke***. 2009;40:1359-1364.)

Key Words: acute stroke ■ infrared laser therapy ■ clinical trial

There is an urgent unmet need for safe and effective treatments for stroke that could be administered for extended periods after stroke onset. In most countries, the only treatment for acute ischemic stroke is tissue plasminogen activator (tPA), which must be administered within 3 hours of stroke onset.[1,2] Despite evidence for a 4.5-hour time window for tPA and the availability of thrombectomy de-

vices, fewer than 4% of patients receive any therapy other than rehabilitation and prophylaxis.[3]

Transcranial laser therapy (TLT) is a noninvasive technology that uses near-infrared laser energy delivered transcranially to modulate biochemical changes within neural cells. The initial demonstration of biochemical activity caused by electromagnetic radiation was the discovery of photosynthesis by

Priestley in the 1770s.[3a] In the 1920s, Warburg showed the effects of light on cellular biochemical reactions.[4] In the 1980s, Karu showed that the action spectra of in vitro responses to infrared irradiation matched the wavelength absorption spectra of copper anions of cytochrome c oxidase, a terminal enzyme complex of the electron transport chain located in the inner mitochondrial membrane.[5]

The concept of enhancing mitochondrial function in stroke is based on several lines of evidence suggesting that mitochondrial viability may be critical to protect cells in the ischemic penumbra.[6] Preclinical experiments showed that the net effect of the absorption of infrared energy can include increased adenosine triphosphate (ATP) formation, enhanced recovery, and inhibition of apoptosis.[7,8] TLT has shown significant beneficial and sustained effects in animal stroke models. In rat models, TLT improved neurological outcomes up to 24 hours postocclusion.[9] Further preclinical studies in the rabbit thromboembolic model have shown efficacy at least 6 hours postinfarct.[10] It is known that cells in the brain may continue to die for several hours after stroke onset. Therefore, a technology which rescues impaired mitochondria or prevents apoptosis may similarly benefit patients with ischemic stroke at extended treatment time windows.[11]

Given the evidence regarding the antiapoptotic effect of near infrared irradiation and the apparent extended time window of efficacy, during which apoptotic cell death could still be occurring in the penumbra, it is possible that TLT acts in a neuroprotective fashion. It is also likely that TLT enhances recovery of function because there is evidence that lesion volume is not altered, but neurological function is restored.[9] The absorption of infrared energy by cytochrome c oxidase and increased ATP formation has been well established, but what is not known are possible effects that could be related to absorption by other chromophores which might modulate additional biochemical processes. Further experiments which examine TLT effects on excitotoxicity, gene expression, membrane potentials and inflammation should be helpful to further our understanding of the mechanisms of action of TLT.

The NeuroThera Effectiveness and Safety Trial–1 (NEST-1) evaluated safety and efficacy of TLT for treatment of humans 40 to 85 years of age with ischemic stroke within 24 hours of stroke onset in a small randomized, controlled trial.[12] Results of NEST-1 suggested the efficacy and safety of TLT. The NEST-2 study design was nearly identical to the NEST-1 trial but was larger and included patients 40 to 90 years of age.

Methods

Study Design
NEST-2 was a double blind, placebo (sham) controlled trial in which 660 patients were enrolled at 57 centers in 4 countries. Patients were eligible for inclusion in the study if they were 40 to 90 years of age, had a baseline NIHSS score between 7 to 22, had a clinical diagnosis of ischemic stroke, no evidence of hemorrhagic infarct by CT scan or MRI, and had not received tPA. Initiation of treatment had to occur within 24 hours after stroke onset. The inclusion and exclusion criteria are summarized in Table 1.

Table 1. Major Inclusion and Exclusion Criteria

Inclusion
- 40–90 years of age
- Diagnosis of acute ischemic stroke within 24 hours of onset
- NIHSS ≥7—≤22
- Informed consent

Exclusion
- Evidence of intracranial, subdural, or subarachnoid hemorrhage
- Prestroke ≥3 mRS
- Clinical diagnosis of a brain stem or cerebellar stroke
- Seizure at onset
- Blood glucose >400 or <60
- Sustained systolic BP >220 mm Hg, <80 mm Hg or diastolic >140 mm Hg, <50 mm Hg
- Suspected septic embolus
- CNS tumor (except asymptomatic meningioma)
- Dermatologic conditions (eg, psoriasis) of the scalp
- Thrombolytic stroke therapy
- Head implant (eg, clipped aneurysm, Hakim valve)
- Photodynamic therapy within 14 days (eg, Visudyne)
- Pregnancy
- Severe comorbidities

CNS indicates central nervous system.

Randomization and Treatment
Patients were randomly assigned in a 1:1 ratio to receive either TLT or sham. All patients underwent the identical TLT procedure that involves removal of the patient's scalp hair followed by application of a laser probe to the patient's head. The total procedure time was approximately 2 hours. After consent, an interactive voice randomization system was used, with dynamic randomization at centers to ensure balanced distribution of treatment assignments. All patients received standard of care medical management throughout the course of the trial.

Study Management
This study was conducted in accordance with the FDA/ICH Good Clinical Practice (GCP) guidelines and applicable local regulatory requirements. The trial was also conducted in full conformity with the 1983 revision of the Declaration of Helsinki or with the laws and current regulations in biomedical research involving human patients of the country in which the study was conducted, whichever afforded greater protection to the patients. The study was designed and overseen by the steering committee. Each center's ethics committee or Institutional Review Board and an independent data monitoring committee (DMC) conducted safety reviews. The DMC periodically reviewed serious adverse event data between the groups. The patient or legal representative gave written informed consent before enrollment into the study. Data management and statistical analysis was conducted by an independent contract research organization (Parexel, Waltham, Mass), as well as by the study sponsor (PhotoThera, Carlsbad, Calif). After database lock and independent review by the DMC, the steering committee had complete access to the trial data and assumed responsibility for the analysis and interpretation of the results.

Treatment

The NeuroThera Laser System
The NeuroThera Laser System (NTS) uses energy at a wavelength of 808 nm which is near-infrared, nonionizing, and is invisible to the naked eye. Experiments using cadavers indicated that optimal
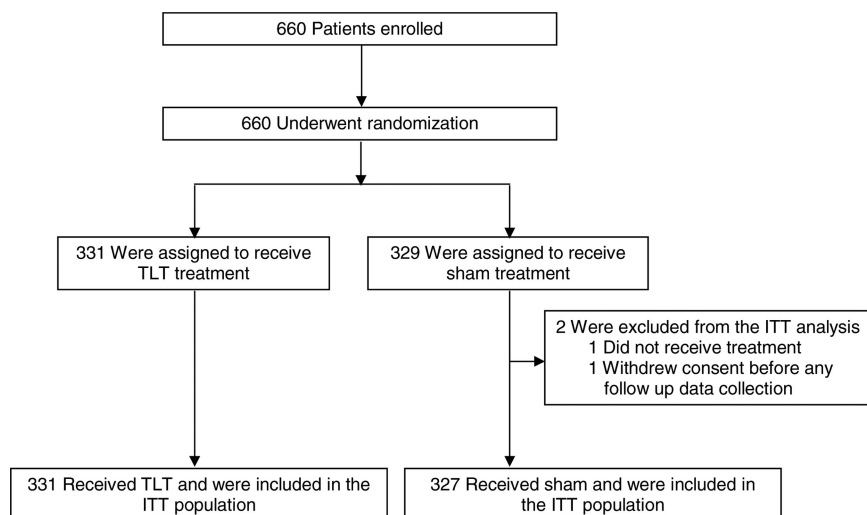
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Figure 1. Enrollment flow chart.

amounts of laser energy are able to penetrate the brain to a depth of approximately 2 cm. The dosimetry of wavelength and irradiance levels was based on transmission experiments with fresh human cadavers without fixation, Monte Carlo simulations, in vitro experiments, review of the literature using infrared therapy for other indications and the results of preclinical studies in validated stroke animal models. A complete treatment regimen consists of NTS application by a hand-held probe on 20 predetermined locations on the shaved scalp for 2 minutes at each site. The predetermined sites, which are irrespective of stroke location, are identified by a cap which is placed on the patients head. The sham procedure is identical to the TLT procedure with the exception that no laser energy is delivered to the patient from the device.

Based on current knowledge of the technology and risk assessment analysis, the most significant known hazard with TLT is potential retinal damage if the beam enters the eye and is focused onto the retina which could result in permanent eye injury. Because of this potential ocular hazard, the procedure must be conducted in a laser safe environment by a trained user.

Clinical Assessments

Patients were assessed by examiners who were unaware of the treatment group. All examiners were trained on the NIHSS and certified on the mRS. The NIHSS is a neurological function scale that ranges from 0 to 42: scores between 7 and 22 are considered to represent moderate to severe neurological impairment. The mRS is a disability index ranging from 0 (no symptoms) to 6 (death). Outcome measures (mRS and NIHSS) were assessed, in addition to baseline, at 5, 30, 60, and 90 days. Baseline data were collected including age, sex, patient demographics, time from stroke onset to arrival at hospital, time to treatment, prestroke mRS, vital signs, and a complete medical history.

Safety Assessments

Vital signs, neurological scores, concomitant medications, adverse events, and serious adverse events were recorded from study entry to day 90. Unresolved serious adverse events were followed for an additional 30 days.

The Data Monitoring Committee examined rates of death, adverse events, and serious adverse events, as well as anticipated and unanticipated device effects during the study. After evaluating the safety data for the first 100 and 400 patients, they did not stop further recruitment into the trial. Because of rapid enrollment, there was no efficacy interim analysis. Neuroimaging assessments were completed at baseline and at 5-day follow-up ±2 days.

Statistical Analysis

All analyses are intention-to-treat. Outcomes were obtained from two 90-day scores, the mRS and the NIHSS score. Patients without

a day-90 visit had their last observation carried forward. The mRS outcomes were: (1) the entire ordinal scale 0 to 6; and (2) a dichotomous outcome with a favorable outcome (success) defined as a 0 to 2 score and an unfavorable outcome (failure) defined as a 3 to 6 score. The NIHSS outcomes were: (1) the change in score from baseline to 90 days using the entire ordinal scale 0 to 42 (with death scored as 42); and (2) a dichotomous outcome for which success could be achieved in 2 ways, either as a 90-day score of 0 to 1 or as a beneficial change from baseline to 90 days of 9 or more points.

The primary efficacy outcome measure was the dichotomous mRS 90-day end point with success (mRS 0 to 2) and failure (mRS 3 to 6). The null hypothesis, that the proportion of successes did not differ by treatment, was tested using multiple logistic regression with 2 prespecified covariates: (1) stroke severity at baseline; and (2) time from stroke onset to time of randomization (TFSO). Baseline stroke severity had 3 levels: NIHSS score 7 to 10 (moderate), 11 to 15 (moderately severe), and 16 to 22 (severe). TFSO had 2 levels: 0 to 12 hours and 12 to 24 hours. The randomization procedure was balanced on (but not stratified by) these factors.

Secondary analyses determined the sensitivity of results to the choice of covariates, of test procedure, and of analytic outcome. The logistic model was run on the primary outcome measure with additional covariates: age, sex, history of CAD, history of diabetes, and history of stroke. To vary the test procedure of logistic regression with a dichotomous outcome, we used the nonparametric 'shift' test with the same dichotomous outcome. Explicitly, the shift test is the Cochran-Mantel-Haenszel (CMH) test with ridit scores to rank outcomes (the van Elteren test).[13] In the stroke literature, the shift test has often only been applied to the full range of the mRS scale, but we applied it to all dichotomous and ordinal outcomes. Logistic regression was run using only dichotomous outcomes. All tests were adjusted for baseline severity and TFSO. Odds ratios were calculated only for tests with dichotomous outcomes.

The safety end points included mortality and all adverse events including procedure and device related adverse events. Safety end points were summarized with descriptive statistics and tables. The analyses were carried out in SAS version 9.1.

Results

Baseline Characteristics

The patients were enrolled between January 2007 and April 2008. A total of 660 patients were randomized (331 TLT and 327 sham), as shown in Figure 1. There were 7 (1.1%) patients lost to follow-up. The groups were balanced with respect to baseline characteristics (see Table 2). Mean time from stroke onset was 14.6±5.9 hours (range 2.7 to 23.9) for the TLT group and 14.7±6.1 hours (range 2.5 to 23.9) for the

Table 2. Demographic and Baseline Characteristics

	TLT (n=331)	Sham (n=327)
Age (y)	70.4±12.6	70.0±11.9
Male (%)	55.3	57.8
Ethnicity (No., %)		
White	256 (77%)	254 (77%)
Black	37 (11%)	38 (12%)
Hispanic	7 (2%)	8 (2%)
Other*	31 (10%)	29 (9%)
Time		
Median time to treatment (h)	15	16
Mean time to treatment (h:min)	14:38±5:55	14:43±6:12
Min (h:min)	2:42	2:30
Max (h:min)	23:54	23:54
Range (h)	3–24	3–24
Time to treatment strata (%)		
<12 h	35.6	35.6
12–24 h	64.4	64.4
NIHSS score		
Mean	13.1±4.7	13.2±4.6
NIHSS score strata (%)		
Median	12	13
Mean 7–10	39.0	38.9
11–15	26.6	27.1
16–22	34.4	34.0
History (%)		
Diabetes	31.4	33.4
Hypertension	82.2	83.9
Atrial fibrillation	38.7	35.6
Current smoker	20.2	19.1

*Largely Mestizo and Native American Indians.

sham group; median times were 15 hours and 16 hours for the TLT group and sham group, respectively. Baseline NIHSS mean scores were 13.1 (range 7 to 22) for TLT and 13.2 (range 7 to 23) for sham. Median NIHSS scores were 12 for TLT and 13 for sham.

Clinical Outcomes

Of the 660 patients: 331 received TLT and 327 received sham; 120 (36.3%) in the TLT group achieved successful outcome versus 101 (30.9%), in the sham group (P=0.094), OR 1.38 (95% CI, 0.95 to 2.00) as shown in Figure 2.

The results of secondary analyses on the prespecified outcomes were consistent with those of the primary analysis. The logistic regression analysis of the primary outcome measure with additional covariates yielded a treatment OR=1.34 (95% CI, 0.94 to 2.03). All covariates except sex and history of stroke were significant. With success defined as a mRS score of 0 to 2, the shift test, stratified for the baseline severity and TFSO, showed a nonsignificant trend toward better outcomes with TLT having a probability value of 0.091 and had an OR 1.38 (95% CI, 0.95 to 2.01). For the ordinal mRS, scores 0 to 6, the shift test trended in favor of
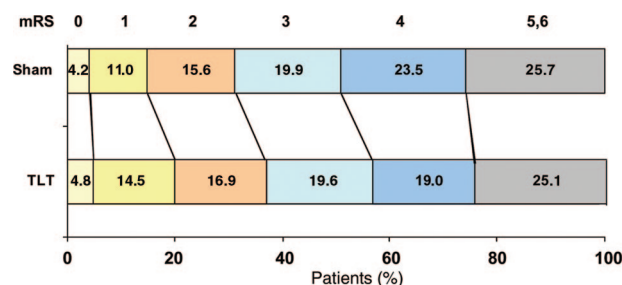

Figure 2. Distribution of scores on the mRS. The scores on the mRS indicate the following: 0, no symptoms at all; 1, no significant disability despite symptoms; 2, slight; 3, moderate; 4, moderately severe; 5, severe disability; 6, death.

TLT with a probability value of 0.113. The odds ratios for all subsets on the primary outcome measure showed a nonsignificant trend favoring TLT treatment except for those patients with severe strokes at baseline (Figure 3).

For the dichotomous NIHSS score outcome for which success had 2 components, the shift test trended in favor of TLT with an OR of 1.23 (95% CI, 0.88 to 1.73) and a probability value of 0.23. Logistic regression analysis results for the dichotomous NIHSS outcome were very similar. An extension of the logistic regression analysis separated the 2 components of success, a 90-day NIHSS score of 0 to 1 and change from baseline ≥9 points, and treated them as correlated outcomes: the OR was 1.30 (95% CI, 0.96 to 1.75) in favor of TLT with a probability value of 0.086. For the ordinal outcome of change in NIHSS score from baseline to 90 days, controlling for baseline severity and TFSO, the shift test indicated the TLT group and had a probability value of 0.65. In patients with deep infarcts, there was no difference in response between the active and sham groups.

Safety Analysis

Mortality rates, serious adverse event (SAE) rates, and adverse event (AE) rates were virtually the same. The TLT and sham groups, respectively, had 58 (17.5%) and 57 (17.4%) deaths, 125 (37.8%) and 137 (41.8%) SAEs; and 92.7% and 93.6% of subjects, respectively, had at least 1 adverse event. No SAEs were directly attributable to TLT. The proportion of patients showing hemorrhagic transformation at day 5 was 49 (14.8%) in the TLT group and 56 (17.1%) in the sham group. There was no difference in the safety outcomes between the 2 groups.

Discussion

The NEST-2 trial results presented here provide evidence of the safety of TLT. The effect size of TLT for the treatment of ischemic stroke in humans within 24 hours of stroke onset was inadequate to meet conventional levels of statistical significance for efficacy, even when the corrections were made for the baseline imbalances in stroke severity and time to treatment, but showed a consistent signal toward better outcomes associated with TLT.

Baseline severity was prespecified into 3 categories: NIHSS=7 to 10, 11 to 15, and 16 to 22. Patients with baseline NIHSS 16 to 22 had a combined dichotomous mRS success rate of 8% (n=224; TLT 7.0%, sham 9.1%). When restricted
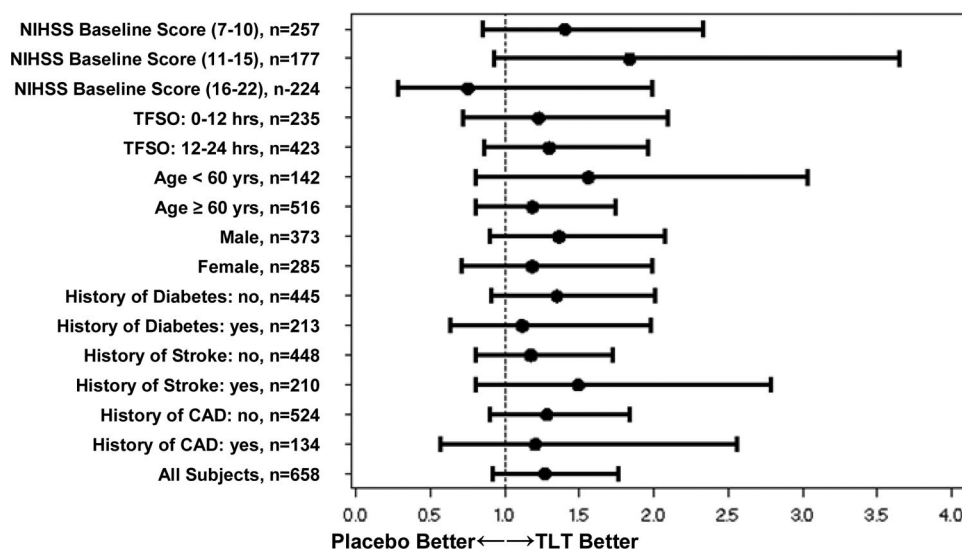
Figure 3. Effects of TLT on the primary end point, on subsets of the data defined by categories of selected baseline characteristics. The simple unadjusted odds ratios for TLT as compared to sham for the 90-day outcome of success defined as mRS score 0 to 2. The horizontal lines indicate 95% CIs. Also shown are the probability values for the test of an interaction between the primary end point and the categories within each subgroup. CAD indicates coronary artery disease; TFSO, time from stroke onset to randomization.

to the 434 patients with moderate and moderately severe strokes (baseline NIHSS score 7 to 15), a post hoc analysis found a significant beneficial effect ($P=0.044$). For these 434 patients, the dichotomous mRS success rate of TLT showed an absolute improvement rate of 9.7% (TLT 51.6%, sham 41.9%). A similar beneficial effect was also found in the NEST-1 trial on the dichotomous mRS.

The failure to initially find the optimal treatment population is reminiscent of the tPA development program. When tPA therapy was administered between 0 and 6 hours, the treated groups improved compared to the placebo ones on all measures, but the final results did not achieve statistical significance. Again, there was a strong signal of efficacy, but it was not until several trials were completed and the proper inclusion and exclusion criteria were established that the clinical benefit of tPA was unequivocally established.[2,3,14] We anticipate that TLT will take additional trial(s) to find the treatment groups that are indisputably helped by the therapy. In contrast to tPA, however, TLT has no untoward side effects, so the barrier to treatment should be much lower.

There are potential weaknesses in the study that should be noted. We had insufficient human experience with TLT to be able to correctly power the study. The trial could have excluded patients with severe strokes at baseline and a prestroke mRS ≥2. Also, it is possible that TLT does not have any effect on stroke recovery. However, substantial preclinical studies, the NEST-1 study, and the trends in the current trial argue against this unfavorable interpretation.

A new feature of this form of therapy is that electromagnetic energy, in the past, has been used almost exclusively for its destructive actions, such as burning out parts of solid tumors with a gamma knife, various types of radiation therapy, and both skin lesion removal and assorted ophthalmologic uses. Preclinical studies have demonstrated that infrared energy produces potential beneficial action by alter-

ation of biochemical pathways, and that these changes are not due to thermal effects. Based on preclinical studies, it is thought that when administering TLT, the temperature of the brain is insignificantly elevated, and the energy is producing its beneficial actions by alteration of some biochemical reactions. It is known that infrared energy can stimulate mitochondria, increase ATP formation, mitigate apoptosis, and possibly enhance neurorecovery mechanisms. The precise nature and balance of these reactions in the ischemic human brain are not fully understood; therefore, the exact mechanism of action of TLT remains unknown. Nevertheless, this does open up a whole new range of potential photobiology therapies for a variety of disorders. Brain trauma and hemorrhagic stroke are obvious extensions; it is also possible that TLT will be useful for a range of neurodegenerative diseases that may involve mitochondrial dysfunction.

Conclusion
The NEST-2 trial included a very broad range of stroke patients with respect to baseline severity, prestroke disability, and time to treatment. In this overall population, TLT was safe but did not significantly improve patient outcomes as measured by both mRS and NIHSS; however, both outcome measures showed positive trends in that direction. Post hoc analyses suggest a meaningful beneficial effect in patients with moderate to moderately severe ischemic stroke within 24 hours of onset. Further clinical studies in this refined population should be considered.

Appendix
Steering Committee: Justin Zivin (chair), Gregory Albers, Natan Bornstein, Bjorn Dahlof, Marc Fisher, Werner Hacke, Robert Lew, Jackson Streeter.

Data and Safety Monitoring Board: Michael Welch (chair), David Fiorella, George Howard, and Steven R. Levine.

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References

1. Thom T, Haase N, Rosamond W, Howard VJ, Rumsfeld J, Manolio T, Zheng ZJ, Flegal K, O'Donnell C, Kittner S, Lloyd-Jones D, Goff DC Jr, Hong Y, Adams R, Friday G, Furie K, Gorelick P, Kissela B, Marler J, Meigs J, Roger V, Sidney S, Sorlie P, Steinberger J, Wasserthiel-Smoller S, Wilson M, Wolf P. Heart disease and stroke statistics–2006 update: A report from the American Heart Association statistics committee and stroke statistics subcommittee. *Circulation.* 2006;113:e85–151.
2. The National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group.Tissue plasminogen activator for acute ischemic stroke. *N Engl J Med.* 1995;333:1581–1587.
3. Hacke W, Kaste M, Bluhmki E, Brozman M, Dávalos A, Guidetti D, Larrue V, Lees KR, Medeghri Z, Machnig T, Schneider D, von Kummer R, Wahlgren N, Toni D; for the ECASS Investigators. Thrombolysis with alteplase 3 to 45 hours after acute ischemic stroke. *N Engl J Med.* 2008;359:1317–1329.
3a. Priestley J. *Experiments and Observations on Different Kinds of Air.* Bowyer LW, Nichols J, eds. 1774–1777.
4. Warburg O. The chemical constitution of respiration ferment. *Science.* 1928;68:437–443.
5. Karu T. *Ten Lectures on Basic Science of Laser Phototherapy.* Grangesberg, Sweden: Prima Books. ISBN 2007;978-91-976478-0-9.
6. Moskowitz M, Lo E. Neurogenesis and apoptotic cell death. *Stroke.* 2003;34:324–326.
7. Mochizuki-Oda N, Kataoka Y, Yamada H, Awazu K. 2004, "Effects of Near-Infrared Laser on Neural Cell Activity," International symposium on portable synchrotron light sources and advanced applications, Japan. AIP Conference Proceedings; 2004;716:1; p 192.
8. Carnevalli C, Soares C, Zangaro R, Pinheiro A, Silva N. Laser light prevents apoptosis on Cho K1 cell line. *J Clin Laser Med Surg.* 2003; 21:193–196.
9. Oron A, Oron U, Chen J, Eilam A, Zhang C, Sadeh M, Lampl Y, Streeter J, DeTaboada L, Chopp M. Low-level laser therapy applied transcranially to rats after induction of stroke significantly reduces long-term neurological deficits. *Stroke.* 2006;37:2620–2624.
10. Lapchak PA, Wei J, Zivin JA. Transcranial infrared laser therapy improves clinical rating scores after embolic strokes in rabbits. *Stroke.* 2004;35:1985.
11. Chan P. Future targets and cascades for neuroprotective strategies. *Stroke.* 2004;35:2748–2750.
12. Lampl Y, Zivin JA, Fisher M, Lew R, Welin L, Dahlof B, Borenstein P, Andersson B, Perez J, Caparo C, Ilic S, Oron U. Infrared laser therapy for ischemic stroke: a new treatment strategy: Results of the NeuroThera Effectiveness and Safety Trial-1 (NEST-1). *Stroke.* 2007;38:1843–1849.
13. Stokes ME, Davis CS, Koch GC. *Categorical Data Analysis Using the SAS System II.* Cary, NC: SAS Institute, Inc; 2000.
14. Clark WM, Wissman S, Albers GW, Jhamandas JH, Madden KP, Hamilton S. Recombinant tissue-type plasminogen activator (alteplase) for ischemic stroke 3 to 5 hours after symptom onset. The ATLANTIS study: A randomized controlled trial. Alteplase thrombolysis for acute noninterventional therapy in ischemic stroke. *J Am Med Assoc.* 1999; 282:2019.