PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Study Protocol for the G-SPIRIT trial: A Randomized, Placebo-Controlled, Double-blinded Phase 3 Trial of Granulocyte Colony Stimulating Factor-Mediated Neuroprotection for Acute Spinal Cord Injury
AUTHORS	Koda, Masao; Hanaoka, Hideki; Sato, Takatoshi; Fujii, Yasuhisa; Hanawa, Michiko; Takahashi, Sho; Furuya, Takeo; Ijima, Yasushi; Saito, Junya; Kitamura, Mitsuhiro; Ohtori, Seiji; Matsumoto, Yukei; Abe, Tetsuya; Watanabe, Kei; Hirano, Toru; Ohashi, Masayuki; Shoji, Hirokazu; Mizouchi, Tatsuki; Takahashi, Ikuko; Kawahara, Norio; Kawaguchi, Masahito; Orita, Yugo; Sasamoto, Takeshi; Yoshioka, Masahito; Fujii, Masafumi; Yonezawa, Katsutaka; Soma, Daisuke; Taneichi, Hiroshi; Takeuchi, Daisaku; Inami, Satoshi; Moridaira, Hiroshi; Ueda, Haruki; Asano, Futoshi; Shibao, Yosuke; Aita, Ikuo; Takeuchi, Yosuke; Mimura, Masaya; Shimbo, Jun; Someya, Yukio; Ikenoue, Sumio; Sameda, Hiroaki; Takase, Kan; Ikeda, Yoshikazu; Nakajima, Fumitake; Hashimoto, Mitsuhiro; Ozawa, Tomoyuki; Hasue, Fumio; Fujiyoshi, Takayuki; Kamiya, Koshiro; Watanabe, Masahiko; Katoh, Hiroyuki; Matsuyama, Yukihiro; Yamato, Yu; Togawa, Daisuke; Hasegawa, Tomohiko; Kobayashi, Sho; Yoshida, Go; Oe, Shin; Banno, Tomohiro; Arima, Hideyuki; Akeda, Koji; Kawamoto, Eiji; Imai, Hiroshi; Sakakibara, Toshihiko; Sudo, Akihiro; Ito, Yasuo; Kikuchi, Tsuyoshi; Osaki, Shuhei; Tanaka, Nobuhiro; Nakanishi, Kazuyoshi; Kamei, Naosuke; Kotaka, Shinji; Baba, Hideo; Okudaira, Tsuyoshi; Hiroaki, Konishi; Yamaguchi, Takayuki; Ito, Keigo; Katayama, Yoshito; Matsumoto, Taro; Matsumoto, Tomohiro; Idota, Masaru; Kanno, Haruo; Aizawa, Toshimi; Hashimoto, Ko; Eto, Toshimitsu; Sugaya, Takehiro; Matsuda, Michiharu; Fushimi, Kazunari; Nozawa, Satoshi; Iwai, Chizuo; Taguchi, Toshihiko; Kanchiku, Tsukasa; Suzuki, Hidenori; Nishida, Norihiro; Funaba, Masahiro; Yamazaki, Masashi

VERSION 1 – REVIEW

REVIEWER	Michael Fehlings Toronto Western Hospital, Canada
REVIEW RETURNED	14-Sep-2017

GENERAL COMMENTS	The authors report a protocol for a prospective, multi-center, phase 3 randomized controlled trial of G-CSF for spinal cord injury. Strengths: - The described study addresses an important topic and the study design (i.e., placebo- controlled RCT) is robust, and indeed, the gold standard in clinical medicine. - The authors use an appropriate array of outcome measures,

	including neurological outcome (ASIA motor and sensory scores, AIS grade), functional status (SCIM), and quality of life (EQ-5D). - A calculation of sample size is provided A few points should be considered: Major: - The authors should provide a more detailed explanation for how they selected the dosage of drug and therapeutic time window (48 hrs) from preclinical / non-randomized clinical studies - It is unclear from the protocol at what time point the drug will first be administered. The authors indicate patients will be re-evaluated at 48 hrs and those improving to ASIA D will be removed from the study. Is the first dose of the test drug given after this re-evaluation, or do patients receive a dose at enrollment? It would be useful to include a schematic diagram outlining the timeline of events from assessment, consent, and enrollment to treatment and follow-up. - The authors should provide at least a brief description of each outcome measure being used in either text, table, or figure form - Several details of the study methodology are missing: □ Are patients who are incapable either because of intubation and/or concomitant head injury eligible to participate with consent from a substitute decision maker? (inclusion criterion 5 would seem to suggest not, but this should be clarified). □ What sort of randomization scheme will be used (e.g., permuted blocks, adaptive randomization, etc.)? □ The dosing is over 5 days, what is the dosing schedule during this time? □ How and where will the drug be stored and in what packaging (to ensure blinding)? □ Is the use of other neuroprotective agents permitted (e.g., methylprednisolone)? If so, it may be appropriate to stratify randomization by use of MP, in addition to age and injury severity. □ Please further detail the clinically-meaningful 'adverse events' that will be assessed related to the use of G-CSF. □ Will outcome assessors receive any formal training with regard to the ASIA/ISNCSCI standards? □ How will losses to follow-up be handled? □ How will missing data be handled in the analyses? □ Will the analysis be by intention to treat or as-treated? Minor: - In the Abstract, the authors state, "The objective of this study is to prove the efficacy of G-CSF for acute SCI". The word "prove" should be replaced with "evaluate", as the former expresses an inherent bias from the authors. The authors should similarly remove terminology in the text making reference to this as a "confirmative" trial as the use of G-CSF in SCI is not established.

REVIEWER	Dzung H. Dinh, MD, MBA Illinois Neurological Institute Peoria, IL

	USA
REVIEW RETURNED	29-Sep-2017

GENERAL COMMENTS	The phase 1/2a result was promising. Looking forward to see the result of this phase 3 trial. There are a few clarifications needed. 1. In phase 1/2a, , the dosage of G-GSF was 5 to 10 µg/kg/day for 5 days. In phase 3, the dose was adjusted to 400 µg/m2/d × 5d. Please clarify. 2. High C1-4 vs. low C5-T1 SCI demonstrate differences in functional recovery in AIS B, C and D. Would the authors consider excluding high cord injury ? If not, the authors should consider stratifying their outcome measurements/assessment and analysis to high and low cord injury for both primary and secondary endpoints. 3. Even in the same AIS class (B or C), there is significant heterogeneity and variability in the structural injury/integrity and potential (or lack there of) for improvement. Please address this point. 4. The AIS scale is not sensitive enough to measure subtle motor and sensory differences within class. The International Standards of Neurological Classification of Spinal Cord Injury (ISNCSCI) or The Graded Redefined Assessment of Strength, Sensibility, and Prehension (GRASSP) could be utilized to enhance the sensitivity and accuracy of motor, sensory and functional measurement. 5. To strengthen the analysis, for every midpoint motor/sensory functional measurement, the therapist/assessor for functional score/classification post-treatment should be blinded to the pre-treatment and every prior score/measurement, and/or not the same person. 6. Even though neuro-imaging was not part of the previous or current trial, did the authors consider including MRIs as part of the the initial (and follow up) evaluation on admission ?

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

1) The authors should provide a more detailed explanation for how they selected the dosage of drug and therapeutic time window (48 hrs) from preclinical / non-randomized clinical studies.

>>> We decided dosage of drug based on our previous non-randomized early phase clinical studies. On phase 1/2a safety and feasibility study, which included SCI patients within 48 hours after the injury, we administered G-CSF in dose-escalation manner. For initial 6 SCI patients, we administered 5 µg/kg/d for 5 days, and we increased the dosage to 10 µg/kg/d for 5 days for next 11 patients (Takahashi et al. *Eur Spine J*, 2012). At the beginning, we planned further escalation of the dosage. However, we decided the dosage of G-CSF 10 µg/kg/d for 5 days because several patients in the second dosage series showed marked elevation of white blood cell number (reached nearly to 50000of which WBC number might cause side effects of G-CSF) during G-CSF administration. In fact, next phase of clinical study, of which design was non-randomized, non-blinded comparative study, showed suggestive efficacy of G-CSF (10 µg/kg/d for 5 days) for acute SCI (Inada et al, *Spine*, 2014).

Based on those results, we finally decided the dosage of G-CSF as 10 μg/kg/d for 5 days (10 μg/kg/d = 400μg/m^2/d).

As for therapeutic time window, we decided to 48hours because our previous non-randomized, non-blinded comparative study, which recruited SCI patients within 48 hours after injury, showed that there was no significant difference in neurological outcome between the patient administered G-CSF very early after the injury and 48 hours after injury.

According to the reviewer's comment, we added those statements in Methods and analysis section, study procedures subsection page 7 line 11-27.

2) It is unclear from the protocol at what time point the drug will first be administered. The authors indicate patients will be re-evaluated at 48 hrs and those improving to ASIA D will be removed from the study. Is the first dose of the test drug given after this re-evaluation, or do patients receive a dose at enrollment? It would be useful to include a schematic diagram outlining the timeline of events from assessment, consent, and enrollment to treatment and follow-up.

>>> The first dose of investigative drugs will be administered to the patients after re-evaluation of neurological status 48 hours after SCI. According to the reviewer's opinion, we added those statements in Methods and analysis section, study procedures subsection, page 7 line 2-10 and the schematic diagram as Figure outlining the timeline of the trial.

3) The authors should provide at least a brief description of each outcome measure being used in either text, table, or figure form.

>>> We agree with the reviewer's opinion. Therefore we added the brief explanation of outcome measures in Methods and analysis section, study procedures subsection, page 8 line 2-3 and page 8 line 27 to page 10 line 12, and added supplementary Figures 1-3 for description of outcome measures.

4) Are patients who are incapable either because of intubation and/or concomitant head injury eligible to participate with consent from a substitute decision maker? (inclusion criterion 5 would seem to suggest not, but this should be clarified).

>>> We will exclude patients with consciousness disorder to maintain accuracy of functional assessment and criteria to obtain patients' own informed consent upon participation to the trial. According to the reviewer, we added exclusion criteria [7] "consciousness disorder" and those statements in Methods and analysis section, exclusion subsection, page 11 line 2-3 and page 11 line 6-9.

5) What sort of randomization scheme will be used (e.g., permuted blocks, adaptive randomization, etc.)?

>>> The method of randomization is dynamic randomization based on age at registration (16-64 years of age, or 65-84 years of age) and severity of paralysis (AIS B or C) at 48 hours after injury in the present trial. According to the reviewer, we added the method of randomization in Methods and analysis section, study procedures subsection, page 6, line 15.

6) The dosing is over 5 days, what is the dosing schedule during this time?

>> > The dosing schedule will be once a day in every morning (9-10 AM) for consecutive 5 days even in case that the first dosing was performed at night because of restriction on practices. According to the reviewer, we added those statements in Methods and analysis section, study procedures subsection, page 7, line 3-5.

7) How and where will the drug be stored and in what packaging (to ensure blinding)?

>>> Investigational drugs including both G-CSF and placebo will be stored in refrigerator which will be kept between 1~6℃ and has temperature-logger in pharmacies in each participating institutes. The investigational drugs is packaged in ample with label only printed as serial numbers, ten ample is packed in one box with label only printed serial numbers. Web-based allocation system will show the serial number of investigational drug which must be used to respective patients to ensure blinding. According to the reviewer, we added those statements in Methods and analysis section, study procedures subsection, page 7, line 5-10.

8) Is the use of other neuroprotective agents permitted (e.g., methylprednisolone)? If so, it may be appropriate to stratify randomization by use of MP, in addition to age and injury severity.

>>> We will exclude the patients who administered MP because we cannot completely contradict the possible interference of MP on neurological outcome assessment. According to the reviewer, we added those statements in Methods and analysis section, exclusion subsection, page 11 lines 4-5 and 8-9.

9) Please further detail the clinically-meaningful 'adverse events' that will be assessed related to the use of G-CSF.

>>> "Clinically meaningful G-CSF's AE" includes anaphylaxis and adult respiratory distress syndrome. According to the reviewer, we added those statements in Methods and analysis section, adverse events subsection, page 13, line 13-15.

10) Will outcome assessors receive any formal training with regard to the ASIA/ISNCSCI standards?

>>> Attending lecture and e-learning (in website of ISCoS) of ASIA/ISNSCI scoring system is mandatory for every investigators participating to the present trial. According to the reviewer's comment, we added those statements in Methods and Analysis section, study procedures subsection, page 8 line 5-8.

11) How will losses to follow-up be handled? How will missing data be handled in the analyses?

>>> Missing data including loss to follow-up and missing measurement will be supplemented with MMRM method. We added those statements in Methods and Analysis section, Statistical analysis subsection, page 12, line 11-12.

12) Will the analysis be by intention to treat or as-treated?
>>> The analyses of data will be intention-to-treat manner as described above. We added this statement in Methods and Analysis section, Statistical analysis subsection, page 12, line 5.

13) In the Abstract, the authors state, "The objective of this study is to prove the efficacy of G-CSF for acute SCI". The word "prove" should be replaced with "evaluate", as the former expresses an inherent bias from the authors. The authors should similarly remove terminology in the text making reference to this as a "confirmative" trial as the use of G-CSF in SCI is not established.

>>> According to the reviewer, we revised the term “prove” to “evaluate” and deleted the word “confirmative”.

Reviewer: 2

1) In phase 1/2a, the dosage of G-GSF was 5 to 10 μg/kg/day for 5 days. In phase 3, the dose was adjusted to 400 μg/m2/d × 5d. Please clarify.

>>> The dosage is written as “400 μg/m2/d” revised from “10 μg/kg/day” according to the instruction by PMDA. In fact, real dosage determined by 10 μg/kg/day and 400 μg/m^2/d is same. We added those statements in Methods and Analysis section, Study procedures subsection, page 7, line 19-22.

2) High C1-4 vs. low C5-T1 SCI demonstrate differences in functional recovery in AIS B, C and D. Would the authors consider excluding high cord injury? If not, the authors should consider stratifying their outcome measurements/assessment and analysis to high and low cord injury for both primary and secondary endpoints.

>>> We agree with the reviewer’ s opinion. Therefore we omitted C1-3 high cervical SCI and below C8 SCI. Therefore we will include cervical SCI C4-7. The reason why we will include C4, one of major patterns of SCI in Japan is high-aged, low-energy fall at floor without bony injury shows C3/4 vertebral level which means C4 SCI. Therefore the exclusion of this population might be unrealistic. More stringent stratification of patients will be discussed in below.

3) Even in the same AIS class (B or C), there is significant heterogeneity and variability in the structural injury/integrity and potential (or lack there of) for improvement. Please address this point.

>>> We completely agree with the reviewer. AIS B/C will include heterogeneous population of SCI patients showing different recovery course. Ideally, more precise stratification of patients is needed to standardize the patients’ background. Unfortunately, we have no precise data about more stringent stratification of SCI patients by now. Moreover, the data of our previous non-randomized, unblended comparative trial suggested that G-CSF is effective for incomplete SCI such as AIS B/C, supporting us to determine inclusion criteria. As for the effort to standardize the participants’ background, we will postpone the enrollment of the patients to 48 hours after SCI. We will initially assess the patients’ neurological status and tentatively enroll the patients with the severity of SCI AIS B/C, will re-assess the patients’ neurological status 48 hours after SCI and will enroll the patients who show severity AIS B/C at the re-assessment, excluding the patients who show recovery to AIS D/E at the re-assessment. Those statements are written in Methods and analysis section, study procedures subsection, page 6, line 17-27.

4) The AIS scale is not sensitive enough to measure subtle motor and sensory differences within class. The International Standards of Neurological Classification of Spinal Cord Injury (ISNCSCI) or The Graded Redefined Assessment of Strength, Sensibility, and Prehension (GRASSP) could be utilized to enhance the sensitivity and accuracy of motor, sensory and functional measurement.

>>> We strongly agree with the reviewer’s comment. AIS is less sensitive and inappropriate for precise neurological evaluation. Therefore we will use ASIA motor score (ISNSCI) instead of AIS as

primary endpoint. At the planning period, we tried to omit AIS from secondary endpoint, however, PMDA instructed us to add AIS as secondary endpoint. As for stratification method, AIS is better than AISA motor score (ISNSCI) even if AIS is not sensitive, because there is no consensus about the precise prediction of neurological outcome using initial ASIA motor score (ISNSCI). Thus we reluctantly use AIS as patients' stratification method in fact.

5) To strengthen the analysis, for every midpoint motor/sensory functional measurement, the therapist/assessor for functional score/classification, post-treatment should be blinded to the pre-treatment and every prior score/measurement, and/or not the same person.
>>> We strongly agree with the reviewer's opinion. However, PMDA instructed us to assess one patients' functional evaluation by one evaluator for consistent assessment in respective patients. PMDA might consider that the consistency of assessment in respective patients is more important than strict blindness as the reviewer pointed out. According to the reviewer, we added those statements in Methods and analysis section, Study procedures subsection, page 8, line 21-26.

6. Even though neuro-imaging was not part of the previous or current trial, did the authors consider including MRIs as part of the initial (and follow up) evaluation on admission?

>>> We will evaluate MRI on screening although we will not evaluate MRI findings as endpoints because the quantitative diagnostic value of MRI for SCI is not established. Those data will contribute to the future investigation of MRI for SCI. We added MRI as a screening in Methods and analysis section, Study procedures subsection, page 6, line 18-20.

VERSION 2 – REVIEW

REVIEWER	Dzung H. Dinh, MD, MBA Illinois Neurological Institute Peoria, IL USA
REVIEW RETURNED	13-Oct-2017

GENERAL COMMENTS	The authors have adequately addressed all the major concerns. One point that was not mentioned was surgical status. For those patients that need emergent or urgent decompression and/or stabilization, how are they randomized or stratified.

REVIEWER	Michael G. Fehlings University of Toronto; Toronto Western Hospital, University Health Network. Canada
REVIEW RETURNED	30-Oct-2017

GENERAL COMMENTS	The authors have written a protocol paper for a phase 3 multi-center, placebo-controlled randomized controlled trial of G-CSF for acute traumatic SCI. This work is predicated upon sound science, with strong pre-clinical work and a prior phase 1/2a trial. We think conduct of this phase 3 trial is a next important step. Strengths of the proposed study include: − Clear inclusion and exclusion criteria − Randomization − Stratification of randomization by age and baseline AIS − Allocation concealment, with separate assessors for

	laboratory data − Blinding of patients and assessors − Inclusion of a robust sample size calculation − The statistical analyses proposed are sound. Analysis will be intention-to-treat − Use of a variety of validated outcome instruments, including standard ISNSCI/ASIA examination, SCIM, and EQ-5D This study provides a rare opportunity to study patients longitudinally. Therefore, the authors should consider inclusion of additional outcome measures, such as the FIM and WISCI.

VERSION 2 – AUTHOR RESPONSE

Reviewer: 1

This study provides a rare opportunity to study patients longitudinally. Therefore, the authors should consider inclusion of additional outcome measures, such as the FIM and WISCI.

>>> We completely agree with the reviewer's opinion. However, alteration of the endpoints is quite difficult even though it is secondary one, because the present study is now ongoing. Therefore we will ask PMDA whether we can add secondary endpoints or not. Nevertheless, we cannot change secondary endpoints in the present manuscript at this time.

Reviewer: 2.

One point that was not mentioned was surgical status. For those patients that need emergent or urgent decompression and/or stabilization, how are they randomized or stratified.

>>> In the present trial, with or without surgery was not included in stratification because there is no apparent consensus about surgical decompression on neurological outcome of SCI patients. Especially, a large part of SCI patients in Japan are SCI without radiological abnormalities (SCIWORA), who has narrow spinal canal and SCI was caused by relatively low energy trauma. Efficacy of decompression surgery for this category of patients is controversial. Therefore we excluded the surgical factor from stratification. We added the statement about surgery in stratification in Methods and Analysis section, page 8 line 18-19.