A randomised, double-masked comparison study of diquafosol versus sodium hyaluronate ophthalmic solutions in dry eye patients

Etsuko Takamura,1 Kazuo Tsubota,2 Hitoshi Watanabe,3 Yuichi Ohashi,4 for The Diquafosol Ophthalmic Solution Phase 3 Study Group

ABSTRACT
Aims To compare the efficacy and safety of 3% diquafosol ophthalmic solution with those of 0.1% sodium hyaluronate ophthalmic solution in dry eye patients, using mean changes in fluorescein and rose bengal staining scores as endpoints.

Trial design and methods In this multicenter, randomised, double-masked, parallel study of 286 dry eye patients with fluorescein and rose bengal staining scores of ≥3 were randomised to the treatment groups in a 1:1 ratio. Efficacy and safety were evaluated after drop-wise instillation of the study drug, six times daily for 4 weeks.

Results After 4 weeks, the intergroup difference in the mean change from baseline in fluorescein staining score was −0.03; this verified the non-inferiority of diquafosol. The mean change from baseline in rose bengal staining score was significantly lower in the diquafosol group (p=0.010), thus verifying its superiority. The incidence of adverse events was 26.4% and 18.9% in the diquafosol and sodium hyaluronate groups, respectively, with no significant difference.

Conclusions Diquafosol (3%) and sodium hyaluronate (0.1%) exhibit similar efficacy in improving fluorescein staining scores of dry eye patients, whereas, diquafosol exhibits superior efficacy in improving rose bengal staining scores. Diquafosol has high clinical efficacy and is well tolerated with a good safety profile.

INTRODUCTION
Dry eye, which is a multifactorial disease of the tear and ocular surface, results in discomfort, visual disturbance and tear film instability in addition to potential damage to the ocular surface.1 Aqueous, mucin and lipid layers constitute the tear film and are essential for maintaining homeostasis of the ocular surface.2 In particular, mucin contributes to aqueous coverage and stability of the ocular surface by making the corneal and conjunctival epithelial surface hydrophilic.3 Dry eye is presumably caused by abnormalities in tear film composition or tear volume. Quantitative and qualitative abnormalities in tear fluid cause tear film destabilisation and keratoconjunctival epithelial disorders, including damage to the mucin layer in the keratoconjunctival epithelium.4 5 This in turn leads to subjective symptoms, such as eye discomfort, foreign body sensation or visual disturbance, eventually lowering patients’ quality of life (QOL).6 7 When treating dry eye, it is recommended to initially supplement the water component by artificial tear. If that is ineffective, treatment with anti-inflammatory agents or secretagogues becomes necessary because simple supplementation of the aqueous component is inadequate; it is essential to improve the tear fluid quality as a whole. Conventional treatment agents for dry eye include artificial tear, sodium hyaluronate solution, corticosteroids and cyclosporine.8 Artificial tear is limited to temporary water and electrolyte supplementation. Although sodium hyaluronate has been used in the treatment of keratoconjunctival disorders by corneal epithelial extension in addition to water supplementation,9 10 it is considered ineffective against conjunctival disorders caused by mucin layer damage.11 While corticosteroids improve ocular surface inflammation in dry eye patients, adverse drug reactions with long-term use, including increased intraocular pressure and cataract progression, are anticipated. Topical cyclosporine A treats tear secretion disorders through anti-inflammatory activity, but does not have a direct tear-enhancing effect.12 13 At present, no adequate therapeutic options are available to treat the individual factors of this multifactorial disease. In clinic practice, there is a great demand for therapeutic agents with a novel mechanism of action, which can improve the quality and quantity of tear fluid in dry eye patients.

Diquafosol is a P2Y2 purinergic receptor agonist. P2Y2 receptor is present at various sites within the ocular surface, including the palpebral and bulbar conjunctival epithelium, goblet cells and adipocytes and ductal epithelial cells in the meibomian gland.14 ATP or uridine triphosphate (UTP) reportedly promotes water and mucin secretion from the conjunctiva by activating this P2Y2 receptor.15 16 Diquafosol has P2Y2 receptor agonist activity, which is equivalent to that of UTP,17 and is more stable in solution form compared with ATP or UTP. Topical instillation of diquafosol reportedly improves corneal epithelial disorders in rat and rabbit dry eye models; this may be due to the promotion of mucin secretion from the conjunctival tissue. In addition, diquafosol induces tear secretion in normal rabbits.18–20 This tear secretion-promoting effect is also confirmed in dry eye patients,21 in whom diquafosol exerts its effects by activating the P2Y2 receptor in the conjunctiva and promoting the secretion of tear fluid with aqueous and mucin components, thereby improving the quality and quantity of tear fluid.

This study aimed to evaluate the efficacy and safety of 5% diquafosol ophthalmic solution in dry eye patients, and to compare its efficacy with that of 0.1% sodium hyaluronate ophthalmic solution as an active control.
MATERIALS AND METHODS

Study design

The efficacy and safety of 3% diquafosol ophthalmic solution in dry eye patients were compared with those of 0.1% sodium hyaluronate ophthalmic solution in a multicenter, randomised, double-masked, parallel-group comparison study. The study drugs were instilled one drop at a time, six times daily for 4 weeks.

The study was conducted in compliance with the Declaration of Helsinki and Good Clinical Practice, and was approved by the Institutional Review Boards at 49 Japanese clinical sites (ClinicalTrials.gov Identifier: NCT01240382). Written informed consent was obtained from all patients prior to study initiation.

Study population

Male and female patients (≥20 years) with unanaesthetised Schirmer test results of ≤5 mm/5 min, fluorescein staining scores of ≥3 points (out of 9 points) and rose bengal staining scores of ≥3 points (out of 15 points) at the end of the run-in period were enrolled. Patients with a history of haematopoietic stem cell transplantation or keratorefractive surgery, Stevens-Johnson syndrome, ocular pemphigoid or ocular disease other than dry eye requiring treatment were excluded.

Study materials

The 3% diquafosol ophthalmic solution was an aqueous solution containing 30 mg diquafosol per 1 ml with benzalkonium chloride as a preservative. The 0.1% sodium hyaluronate ophthalmic solution was an aqueous solution containing 1 mg sodium hyaluronate per 1 ml with benzalkonium chloride as a preservative. Each ophthalmic solution was rendered indistinguishable by outward appearance. The vehicle for the diquafosol ophthalmic solution was used during the run-in period.

Randomisation

The subjects were randomised corresponding to allocation codes generated for the diquafosol and the sodium hyaluronate using the permuted block method by the randomisation manager.

Study treatment

The study comprised a 2-week run-in period and a 4-week treatment period (figure 1). During the run-in period, vehicle ophthalmic solution was instilled one drop at a time, six times daily for 2 weeks. During the treatment period, eligible subjects were randomised, and diquafosol or sodium hyaluronate was instilled, one drop at a time, six times daily for 4 weeks. Concomitant use of therapeutic agents (for any ophthalmic diseases), corticosteroids (excluding local administration on the skin other than the eyelids), other investigational products, punctal plugs, surgical punctal occlusion or moisture chamber spectacles were prohibited throughout the study period.

The subjects visited the clinic every 2 weeks and underwent tests for efficacy parameters, including fluorescein staining of the cornea, rose bengal staining of the cornea and conjunctiva and tear break-up time (BUT); they also underwent interviews to assess subjective symptoms. Other safety evaluations, such as ophthalmologic examinations, were performed at the start and end of the treatment period.

Efficacy and safety evaluation

The primary endpoint for efficacy was the change in fluorescein and rose bengal staining scores from baseline at the 4-week endpoint (last observation carried forward). Fluorescein staining was evaluated according to the Shimamura method, which is the popular method for evaluating Fluorescein staining in Japan, and which was used in the clinical trial for sodium hyaluronate ophthalmic solution in keratoconjunctival epithelium disorders. Three sections, the superior, inferior and mid-cornea were scored on a 0–3-point scale, 0 (without any damage) to 3 (damage in the entire area). Rose bengal staining was evaluated for five sections: the superior, inferior and mid-cornea, and the temporal and nasal bulbar conjunctiva (figure 2).

The secondary endpoints for efficacy included BUT and subjective dry eye symptom score (11 parameters: foreign body sensation, photophobia, itching, eye pain, dryness, heaviness, blurred vision, eye fatigue, eye discomfort, eye discharge and lacrimation). The mean value of three measurements was calculated for BUT, and each of the subjective dry eye symptoms was scored on a 4-point scale from 0 to 3. Change from baseline was evaluated for both endpoints. In addition, the clearing rate of fluorescein and rose bengal stains (percentage of subjects whose scores became zero) was evaluated.

Safety evaluation included adverse events, clinical laboratory tests and ophthalmologic examinations using slit-lamp biomicroscopy.

Statistics

The closed testing procedure was applied for multiplicity considerations of multiple primary endpoints. First, non-inferiority was assessed on the basis of the intergroup difference (diquafosol group mean–sodium hyaluronate group mean) in change in fluorescein staining score, with an inferiority margin of 0.34

Figure 1  Study design. During the run-in period, a vehicle ophthalmic solution was instilled one drop at a time, six times daily for 2 weeks. During the treatment period, eligible subjects were randomised, and diquafosol or sodium hyaluronate ophthalmic solution was instilled one drop at a time, six times daily for 4 weeks under double-masked conditions.
RESULTS

Informed consent was obtained from 332 patients, of whom 287 were randomised and prescribed the study drug (table 1). There were six dropouts in total; two in the diquafosol group because of adverse events and 1, 1 and 2 in the sodium hyaluronate group because of adverse events, fear of adverse events, and lack of efficacy, respectively.

Background characteristics of the efficacy study population are presented in table 2. A significant intergroup difference was found in the baseline fluorescein staining score and BUT of all patients who received the study drug. 286 were analysed for efficacy, excluding one subject who was not evaluated for efficacy properly after receiving the final dose.

Efficacy evaluation

Primary endpoints

Fluorescein staining scores showed a significant improvement from baseline in both groups at all time points. Mean change from baseline (mean±SE; figure 3) at the 4-week endpoint was −2.12±0.14 in the diquafosol group and −2.08±0.13 in the sodium hyaluronate group. The CI for the mean difference was −0.040 to 0.538, and the upper value did not exceed the predetermined non-inferiority limit of 0.34. Therefore, the non-inferiority of diquafosol over sodium hyaluronate was verified. Although an intergroup difference in baseline fluorescein staining scores was observed, baseline-adjusted analysis did not change the above conclusion.

Rose bengal staining scores showed a significant improvement from baseline in both groups at all time points. Mean change from baseline (mean±SE; figure 4) at the 4-week endpoint was −3.06±0.19 in the diquafosol group and −2.58±0.18 in the sodium hyaluronate group. The difference (mean±SE) was −0.67±0.26, showing a statistically significant improvement in the diquafosol group compared with the sodium hyaluronate group (p=0.010). Therefore, the superiority of diquafosol over sodium hyaluronate was verified.

Secondary endpoints

BUT showed a significant improvement from baseline in both groups at all time points. Although the change from baseline was higher in the diquafosol group than in the sodium hyaluronate group, no significant difference was observed between the two groups. Although an intergroup difference in baseline fluorescein staining scores was evaluated every 2 weeks; at the start of the run-in period, at baseline and after 2 and 4 weeks of treatment. A significant improvement from baseline was observed in both groups at all time points, and the non-inferiority of diquafosol over sodium hyaluronate was verified at the 4-week endpoint.

Table 1  Subject disposition

<table>
<thead>
<tr>
<th></th>
<th>Diquafosol</th>
<th>Sodium hyaluronate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enrolled</td>
<td>144</td>
<td>143</td>
</tr>
<tr>
<td>Completed</td>
<td>142</td>
<td>139</td>
</tr>
<tr>
<td>Discontinued</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adverse events</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Fear of adverse events</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Lack of efficacy</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 2  Patient demographics and other baseline characteristics

<table>
<thead>
<tr>
<th></th>
<th>Diquafosol</th>
<th>Sodium hyaluronate</th>
<th>Statistical test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>144</td>
<td>142</td>
<td></td>
</tr>
<tr>
<td>Age (Mean±SD)</td>
<td>55.3±17.1</td>
<td>56.9±16.8</td>
<td>p=0.423*</td>
</tr>
<tr>
<td>Gender, female, n (%)</td>
<td>120 (83.3)</td>
<td>125 (88.0)</td>
<td>p=0.312†</td>
</tr>
<tr>
<td>Sjögren’s syndrome, n (%)</td>
<td>36 (25.0)</td>
<td>32 (22.5)</td>
<td>p=0.678†</td>
</tr>
<tr>
<td>fluorescein staining score baseline (Mean±SD)</td>
<td>4.4±1.5</td>
<td>4.8±1.5</td>
<td>p=0.067*</td>
</tr>
<tr>
<td>Rose bengal staining score baseline (Mean±SD)</td>
<td>6.1±2.4</td>
<td>6.4±2.4</td>
<td>p=0.198*</td>
</tr>
<tr>
<td>Tear break-up time baseline (Mean±SD)</td>
<td>2.7±1.3</td>
<td>2.5±1.1</td>
<td>p=0.045*</td>
</tr>
<tr>
<td>Schirmer’s test (Mean±SD)</td>
<td>2.5±1.7</td>
<td>2.5±1.7</td>
<td>p=0.807*</td>
</tr>
</tbody>
</table>

* Fisher’s exact test.
BUT was observed, baseline-adjusted analysis did not change
the above conclusion. Among the subjective symptoms evaluated, the heaviness
score was significantly decreased in the diquafosol group com-
pared with the sodium hyaluronate group at week 4 (p=0.033; figure 5). On the other hand, eye discharge in the diquafosol
group showed no improvement compared with baseline, whereas, that in the sodium hyaluronate group significantly
decreased compared with baseline.

With regard to the clearing rate of the fluorescein stain from
the mid-cornea, and the rose bengal stain from the whole
cornea, mid-cornea and whole conjunctiva, the diquafosol
group showed significant superiority over the sodium hyaluron-
ate group at the 4-week end point (fluorescein staining: diqua-
ofosol group, 41.7%; sodium hyaluronate group, 30.5%; p=0.049; rose bengal staining: whole cornea; diquafosol group, 27.8%; sodium hyaluronate group, 17.0%; p=0.034; mid-
cornea; diquafosol group, 67.4%; sodium hyaluronate group, 51.1%; p=0.006; whole conjunctiva; diquafosol group, 18.8%; sodium hyaluronate group, 8.5%; p=0.015; figure 6).

Safety evaluation
Adverse event incidence rates during the treatment period were
26.4% for the diquafosol group and 18.9% for the sodium hya-
luronate group, with no significant intergroup difference. Adverse drug reaction incidence rates were 15.3% in the diqua-
ofosol group and 4.9% in the sodium hyaluronate group, and a
significant intergroup difference was observed (p=0.005).

There were no serious adverse events, and the majority of
adverse drug reactions were mild. Eye irritation occurred with
the highest frequency (6.5%) in the diquafosol group, whereas,
blepharitis and eye pruritus (1.4%) occurred with the highest
frequency in the sodium hyaluronate group (table 3). These
were either resolved or recovered to an insignificant level.

With regard to clinical laboratory test values, ophthalmologic
examinations, intraocular pressure, funduscopy, and visual
acuity, no clinically significant changes from baseline were
observed in either group before or after the treatment period.

DISCUSSION
Effective methods of treating dry eye involve improving the
quantity and quality of tear fluid. Diquafosol ophthalmic solu-
tion is a dry eye therapeutic agent which is suitable for the
treatment of dry eye with respect to its pathogenesis.

Diquafosol is clinically important for dry eye treatment in
two ways. First, it improves the rose bengal staining score more
effectively than does sodium hyaluronate. Because rose bengal
stains the ocular surface areas not fully covered by mucin, mucin
disorders of the keratoconjunctival epithelium can be assessed
using this method. Mucin retains tear fluid and lubricates the
ocular surface by converting the corneal epithelial cell surface to
a hydrophilic state. If parts of the corneal surface are not fully
covered by mucin, corneal epithelial disorders may worsen as a
result of low tear retention or reduced ocular surface lubrication.

In a multicenter, double-blind comparative study of sodium hya-
luronate ophthalmic solution in dry eye patients, no significant
improvement in the keratoconjunctival rose bengal staining score
was observed when compared with placebo. In the present
study, diquafosol was shown to be superior to sodium hyaluron-
ate in improving rose bengal staining scores.

Table 3  Adverse reactions observed during the treatment period
(≥1%)

<table>
<thead>
<tr>
<th>Name of event</th>
<th>Diquafosol (n=144)*</th>
<th>Sodium hyaluronate (n=143)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blepharitis</td>
<td>–</td>
<td>2 (1.4)</td>
</tr>
<tr>
<td>Eye discharge</td>
<td>4 (2.8)</td>
<td>–</td>
</tr>
<tr>
<td>Eye irritation</td>
<td>9 (6.3)</td>
<td>1 (0.7)</td>
</tr>
<tr>
<td>Eye pain</td>
<td>2 (1.4)</td>
<td>1 (0.7)</td>
</tr>
<tr>
<td>Foreign body sensation</td>
<td>4 (2.8)</td>
<td>1 (0.7)</td>
</tr>
<tr>
<td>Conjunctival hyperaemia</td>
<td>2 (1.4)</td>
<td>1 (0.7)</td>
</tr>
<tr>
<td>Eye pruritus</td>
<td>2 (1.4)</td>
<td>2 (1.4)</td>
</tr>
<tr>
<td>Ocular discomfort</td>
<td>2 (1.4)</td>
<td>–</td>
</tr>
</tbody>
</table>

* No. of patients (%)
Furthermore, diquafosol was shown to improve the corneal fluorescein staining score more effectively than sodium hyaluronate; in addition, the fluorescein clearing rate from the central cornea was significantly greater with diquafosol than with sodium hyaluronate. Fluorescein allows examination of corneal epithelial disorders, which occur as a result of cell shedding and damaged intercellular spaces in the corneal epithelium. In dry eye, the corneal epithelial disorder is caused by drying of the ocular surface because of reduced lacrimal secretion and volume. Increasing the aqueous component of tear fluid secreted from the lacrimal gland and conjunctival epithelial cells is essential for treating corneal epithelial disorders. Because diquafosol has tear secretion-promoting actions, its effect in improving corneal epithelial disorders, as seen in this study, is presumably caused by the promotion of tear secretion. Because epithelial disorders affecting the central cornea have a significant impact on visual function and lead to a reduced QOL, diquafosol is more effective than sodium hyaluronate in improving the QOL of dry eye patients. Moreover, in this study, the subjective symptom of heaviness was significantly improved following treatment with diquafosol compared with that following treatment with sodium hyaluronate. On the other hand, the subjective symptom of eye discharge was not improved following treatment with diquafosol compared with that following treatment with sodium hyaluronate. This may possibly be related to the mucin secretion of diquafosol. No significant intergroup differences were seen for other subjective symptoms. A long-term study is required to provide further follow-up on QOL improvements, and to elucidate the optimal cure for this condition.

In the BUT assessment, the diquafosol group showed significant improvement from baseline, and the change was greater than that in the sodium hyaluronate group; however, the difference was not significant. The sample size, which was selected in order to detect any significant inferiority in fluorescein staining score, may have been too small to detect any statistical difference in BUT. Further studies are needed to evaluate the effect of diquafosol ophthalmic solution on BUT.

Safety evaluations showed that the incidence rates of mild adverse drug reactions, eye discharge and eye irritation were higher in the diquafosol group than in the sodium hyaluronate group; however, owing to their mild nature, these were considered clinically non-problematic and allowed study continuation. Moreover, the symptoms disappeared with continuing administration of the study drug, or with completion/discontinuation of treatment with the study drug. With regard to clinical laboratory test values, no clinically significant changes from baseline were observed in either group before or after the treatment period.

The results of our study demonstrate that, in the treatment of epithelial disorders in dry eye patients, diquafosol ophthalmic solution causes an equivalent improvement in fluorescein staining score and a superior improvement in rose bengal staining score when compared with sodium hyaluronate. Diquafosol is believed to exert its therapeutic effect in dry eye patients by activating the P2Y2 receptor in the conjunctiva, thereby improving the quality and quantity, promoting the secretion and increasing the aqueous and mucin content of tear fluid.

Acknowledgements The Diquafosol Ophthalmic Solution Phase 3 Study Group: Akihito Kuroda (Hormouchi Eye Clinic, Tokyo), Fukuchi Oura (Miyazaki Chubu Eye Hospital, Miyazaki), Hidehito Kawabata (Kawabata Eye Clinic, Chiba), Hidenao Ideta (Ideta Eye Hospital, Kumamoto), Hidetoshi Yamashita (Yamagata University, Yamagata), Hiroko Shimizu (Ryukyu University, Okinawa), Hiroko Shimizu (Shimizu Eye Clinic, Tokyo), Hironori Aoki (Kumamoto Medical Center, Kumamoto), Hiroshi Mishima (Nara Hospital Kinki University Faculty of Medicine, Nara), Hiroshi Takahashi (Nippon Medical School, Tokyo), Hitoshi Watanabe (Kansai Rosai Hospital, Hyogo), Ikue Takagi (Kyushu Medical Center, Fukuoka), Jun Kozaki (Kozaki Eye Clinic, Osaka), Jun Shimazaki (Tokyo Dental College Ichikawa General Hospital, Chiba), Katsura Kimura (Iwate Medical University, Iwate), Kazunori Miyata (Miyata Eye Hospital, Miyazaki), Kazuo Ichikawa (Social Insurance Chukyo Hospital, Aichi), Kazuo Tsutoba (Keio University, Tokyo), Kazutaka Kamiya (Kitasato University Hospital, Kanagawa), Ken Hayashi (Hayashi Eye Hospital, Fukuoka), Kenji Inoue (Inoue Eye Hospital, Tokyo), Kenji Sakagami (Hoshigaoka Koseinenkin Hospital, Osaka), Koji Hirano (Baburtanbe Houtoukai Hospital, Aichi), Makoto Obara (Yamagata University Hospital, SASEIKEN, Yamagata), Masahiko Yamaguchi (Ehime University, Ehime), Masakazu Yamada (Tokyo Medical Center, Tokyo), Miho Enoki (Ogaki Daichi General Hospital, Yamaguchi), Miki Sakata (Goken-Chou Kozawa Eye Clinic, Ibaraki), Miki Uchino (Ryogoku Eye Clinic, Tokyo), Misaki Ishioka (Ryogoku Eye Clinic, Tokyo), Naoki Hamada (Omiyahamada Eye Clinic, Saitama), Naoko Kato (Shimawahara East Medical Clinic, Tokyo), Naoyuki Maeda (Osaka University, Osaka), Norihiko Yokoi (Kyoto Prefectural University of Medicine, Kyoto), Naoji Horii (Tokyo Women’s Medical University, Tokyo), Shigeki Okamoto (Kobamoto Eye Clinic, Ehime), Shiro Amamo (Yuya University, Tokyo), Taro Kimura (Ueno Eye Clinic, Tokyo), Teruo Nishida (Yamaguchi University, Yamaguchi), Tetsuro Oshika (University of Tsukuba, Ibaraki), Tomoko Goto (Takanoko Hospital, Ehime), Torao Sugira (Sugiura Eye Clinic, Osaka), Toshio Kodama (Matsutsuya Red Cross Hospital, Ehime), Yasuko Nakamura (Kagoshima Miyata Eye Hospital, Kagoshima), Yutaka Uchihori (Osaka General Medical Center, Osaka), Yoshikazu Shimanuma (Kinki University, Osaka), Yoshinori Takahashi (Tachikawadori Clinic, Tokyo), Yoshitsugu Inoue (Tottori University, Tottori), Yoshitsugu Tagawa (Hokkaido University, Hokkaido), Yu Monden (Kurume University, Fukuoka), Sadao Komemushi (Kinki University Faculty of Agriculture, Nara).


Contributors ET: acquisition of data, drafting the article, critical for important intellectual content, final approval of the version published. JT: acquisition of data, revising the article critically for important intellectual content, final approval of the version published. KH: acquisition of data, revising the article critically for important intellectual content, final approval of the version published. The Diquafosol Ophthalmic Solution Phase 3 Study Group: acquisition of data, revising the article critically for important intellectual content, final approval of the version published.

Funding This study was sponsored and funded by Santen Pharmaceutical Co, Osaka, Japan.

Competing interests KT, HW and YO were consultants to Santen Pharmaceutical Co.

Ethics approval This manuscript does not contain personal medical information about an identifiable living individual.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES


