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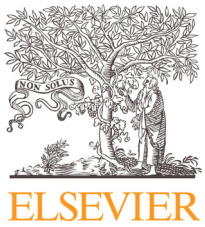

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Phase 1 Lymfactin® Study: 24-month Efficacy and Safety Results of Combined Adenoviral VEGF-C and Lymph Node Transfer Treatment for Upper Extremity Lymphedema

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KEYWORDS
Breast cancer-related lymphedema;
BCRL;
Breast reconstruction;
Reconstructive surgery;
Microvascular lymph node transfer;
VEGF-C

ABSTRACT *BACKGROUND:* Lymphedema is a common problem after breast cancer treatment. Lymfactin® is a prolymphangiogenic growth factor vector inducing the expression of human vascular endothelial growth factor C (VEGF-C). It promotes growth and repair of lymphatic vessels.
METHODS: Lymfactin® was combined with microvascular lymph node transfer surgery (VLNT) to study the safety and efficacy of the treatment in breast cancer-related upper limb lymphedema (BCRL) patients. This is a continuation study with a 3 year efficacy and 5 year safety follow-up.
RESULTS: Fifteen patients were recruited in the study between June 2016 and February 2018. Three patients received a lower dose (1×10^{10} viral particles (vp)), and 12 patients received a higher dose (1×10^{11} vp) of Lymfactin®, respectively. In the higher dose group, the reduction of excess arm volume was on average 46% after the 12 month follow-up, and the transport index was improved in 7/12 patients. At baseline, removal of the compression garment for 7 days resulted in significant arm swelling (105.7±161.0 ml, p=0.0253). However, at 12 months, there was less and not significant swelling after removal of the garment (84.4±143.0 ml, p=0.0682). Lymphedema Quality of Life Inventory (LQOLI or LyQLI) questionnaire showed significant and sustained improvement of quality of life.
CONCLUSIONS: During 24 months' of follow-up, the results indicate that Lymfactin® is well tolerated. The most promising findings were a 46% reduction in excess arm volume and a nonsignificant volume increase after garment removal at 12 months, suggesting that there is potential for the reduction of lymphedema.

INTRODUCTION

Surgery including an axillary lymph node dissection followed by radiation therapy is a common treatment of primary breast cancer with lymph node metastases. The incidence of lymphedema is more than 20% in patients who have undergone an axillary lymph node dissection,[1] and this risk significantly increases in patients receiving additional postoperative radiotherapy.[1,2] There is no cure for lymphedema, and the current treatment focuses on conservative decongestive therapy using manual lymphatic drainage and compression garments.

Vascularized lymph node transfer surgery (VLNT) has been gaining popularity during the recent years providing at least some benefit to the patients.[3-7] The regrowth of lymphatic vessels is expected to occur spontaneously after the operation, and therefore the incorporation of the transferred lymph nodes into the existing lymphatic vasculature may fail.[8,9] Lymphoscintigraphic studies have shown that autologous lymph nodes incorporate only at a low frequency into the existing lymphatic vasculature, and most of the operated patients must continue to use compression garments.[4,10]

Vascular endothelial growth factor C (VEGF-C) is the most selective growth factor for lymphatic vessels, and it plays an important role in lymphangiogenesis. In experimental models, VEGF-C seems to have potential to induce growth of new lymphatic vessels and reduce edema volume.[11] The transfer of VEGF-C leads to the local production of therapeutic proteins during the first two weeks after administration,[12] thereby stimulating a robust growth of lymphatic capillaries. Thereafter, the adenoviral vector is eliminated by the host immune system, and VEGF-C downregulation leads to the regression of some of the generated lymphatic vessels.[13] However, the newly formed vessels with lymphatic flow stabilize and mature into collecting lymphatic vessels spontaneously over the course of six months.[8,9,13,14] Novel lymphangiogenic growth factor therapies have shown promising results in previous preclinical models of lymphedema. The transplantation of VEGF-C transfected lymph nodes results in the restoration of a functional lymphatic network in the damaged area.[8,14,15,16]

In breast cancer-related lymphedema (BCRL), the affected lymphatic network is usually restricted to the axillary area.[17] Results from the experimental models described above suggest that in this setting, the expression of the VEGF-C vector for 1–2 weeks is sufficient to rebuild damaged lymphatic vessels. Human lymph nodes express VEGF-C, which is also found in the axillary wound exudate after microvascular lymph node transfer.[4,5]

Lymfactin® is an adenovirus type 5 based gene therapy vector that induces local expression of human VEGF-C. It aims to correct the deficient lymphatic flow by promoting the growth and repair of lymphatic vessels. In combination with VLNT, it aims to incorporate the transferred lymph nodes into the pre-existing lymphatic vessel network. In this article, we present the 24-month efficacy and safety results of the Lymfactin® Phase I trial, where both VLNT surgery and adenoviral VEGF-C treatment were combined.

METHODS

The study protocol was approved by the Finnish Medical Agency (FIMEA) and the Ethics Committee (EC) of Helsinki Hospital District. The study identifier number at ClinicalTrials.gov is NCT02994771.

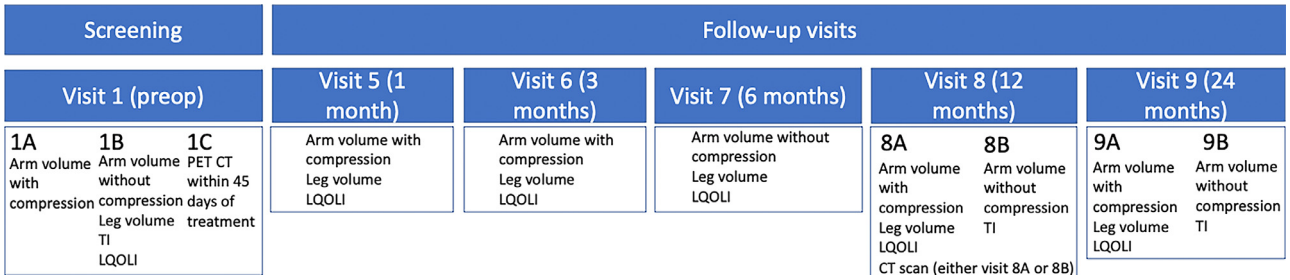

Figure 1 Phase I study design. TI=transport index LQOLI=quality of life questionaire.

Study design

This study was performed in Helsinki, Turku, and Tampere University Hospitals. The study was a first-in-human Phase I multi-center, open-label, uncontrolled dose escalation study to evaluate the safety, tolerability, and biodistribution of the vector as a single dose of Lymfactin® in female patients with secondary lymphedema associated with breast cancer treatment. This novel gene therapy treatment was combined with VLNT surgery. The study-related procedures to assess the effects of Lymfactin® were standardized across all participating sites. Two dose cohorts were included as previously reported.[18] Please see reference[18] and supplemental digital content 1 for patients selection.

Study visits and data collection

At baseline, a written informed consent was obtained. Demographic data and medical history, including a history of breast cancer and lymphedema, were recorded at the baseline visit. A complete physical examination was carried out at the baseline visit, at days 0 and 7, and at months 1, 3, 6, and 12. A ^{18}F-fluorodeoxyglucose (FDG) positron emission tomography-computed tomography (PET-CT) scan of body was performed 45-15 days before treatment as the final baseline procedure. Arm volumes and LQOLI (The Lymphedema Quality of Life Inventory) were done at the baseline visit and at months 1, 3, 6, 12, and 24. Donor limb volumes were measured at the baseline visit and at months 1, 3, 6, and 12. Quantitative lymphoscintigraphy measuring the transport index was carried out at the baseline visit and at 12 and 24 months postoperatively (Figure 1).

Adverse events (AEs) and serious adverse events (SAEs) were carefully documented up to 12 months and have already been published in short-term safety article of Lymfactin®.[18] Thereafter, only adverse events that were considered to be study related by the investigator were documented.

Operative technique and administration of Lymfactin®

Reverse sentinel node mapping were done as described earlier[18] to minimize possible donor site morbidity.[19,20] The sentinel nodes were detected with a gamma detector, and dissection near these nodes was avoided during the operation.

The VLNT flap based on the superficial circumflex iliac vessels was raised as previously described.[4,5,19] For some patients, this was combined with breast reconstruction using a lower abdominal flap (DIEP or ms-TRAM flap). Preparation, perinodal injection of Lymfactin®, and anastomoses of vessels were done as described earlier.[18] Please see supplemental digital content 1 for post-treatment therapy.

Study objectives

The aim of this study was to evaluate the safety and efficacy of a single dose of Lymfactin® administered as a perinodal injection in association with VLNT surgery in patients with BCRL. The efficacy was monitored up to 24 months post-treatment using volumetry measurements, lymphoscintigraphy, and Lymphedema Quality of Life Inventory questionnaires (LQOLI or LyQLI).

Volume measurements

The volume of each limb was quantified using the Brorson and Höijer measurement method.[21] An edema volume of the affected and unaffected arm were recorded, and an excess volume (ml and %) was calculated by substraction.

The excess volume was measured immediately after compression removal and recorded at baseline, after 1, 3, 6, 12, and 24 months. A 7-day swelling volume was calculated by the substraction of edema volume 7 days after removal of the compression garment and edema volume with compression at baseline, 12 and 24 months after treatment. This depicts how much the affected arms swells when the garment is off for 7 days. In this article, we use systematically the terms excess volume and 7 days swelling volume as described above. After the measurements were performed, the patients resumed garment use until the next scheduled measurement. An excess volume measurement of both legs was done to identify the development of any potential edema development in the limb of the donor site.

Qualitative lymphoscintigraphy

The lymphoscintigraphy of the upper limbs was performed at baseline, at 12 and 24 months postoperatively, as previously described.[5] For a qualitative evaluation of lymphatic drainage, a numerical transport index (Ti) was used as described previously.[22,23]

Quality of Life

The patients completed the Lymphedema Quality of Life Inventory (LQOLI or LyQLI) questionnaire[24] at the baseline and 1, 3, 6, 12, and 24 months post-treatment visits. The LQOLI measures how much lymphedema may impact on the patient's quality of life in three domains: physical, psychosocial, and practical. The three domains have been validated separately, but the total score has not been validated. The LQOLI contains a total of 45 questions that are filled out by the patients themselves. The maximum amount of points is 123, and a low score represents a better quality of life. The LQOLI has originally been developed in Australia and later translated, adapted, and validated in Swedish.[24] In this study, the validated Swedish questionnaire was used together with a translation into Finnish (unvalidated).

Statistical analysis

Statistical analysis was performed using GraphPad Prism 8.0 (GraphPad Software, Inc., CA, USA) and SAS software, Version 9.4 of the SAS System for Windows (SAS Institute Inc., Cary, NC, USA). Data normality was assessed using D'agostino-Pearson normality test. Data that was not normally distributed were reported using median and IQR. Volume data were compared between 0 and 12 months using Wilcoxon matched-pairs signed rank test. Repeated measures ANOVA followed by Dunnett's multiple comparison test were used to compare follow-up timepoints to baseline measurements to analyze quality of life and transport indexes. Statistical significance was set at $p \leq 0.05$. For without compression minus compression analysis, the mean changes over time were analysed using linear mixed models for repeated measurements (Kenward Rogers), where time was handled as a within factor, and time points 0 and 12 months were included in the analysis.

RESULTS

Participants

A total of 15 female BCRL patients were included in the study between June 2016 and February 2018. In the results, we report only Cohort 2 patients (n=12) who received the aimed therapeutic dose of Lymfactin®. The presence of first symptoms of lymphedema ranged from 1 to 4 years after breast cancer diagnosis. All patients had compression garments as a conservative treatment for lymphedema. The average excess volume at baseline was 527±450 ml with compression, and the average TI of the affected arm in lymphoscintigraphy was 28.8±14.6. Only 1 patient had cellulitis in the affected arm in the preceding 12 months. See table, supplemental digital content 2, of patients' breast cancer and lymphedema history.

Safety profile

Results of safety at 12-month follow-up have been published earlier.[18] The study was completed with the predetermined maximum dose of Lymfactin, and no dose-limiting toxicities were observed.[18] During 12-24 months, there were no SAEs reported. None of the AEs reported after 12 months follow-up were considered study related.

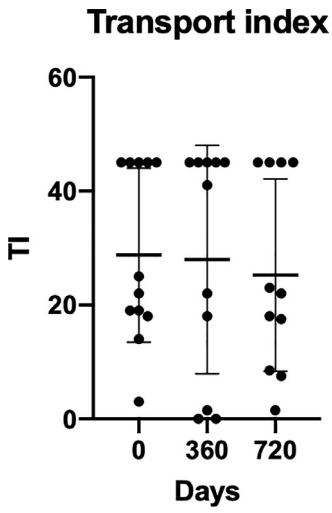

Figure 2 Transport index measured at baseline, 12 months post-treatment and 24 months post-treatment. Data are shown as mean ± SD. TI=transport index.

Lymphoscintigraphy

The transport index was improved in 7/12 patients during 12 months follow-up and 4/11 patients during 24 months follow-up. In all of the patients with improvement during 24 months the TI decreased by over 10 points, and this was considered clinically significant. At 24 months timepoint, one patient was excluded because of lacking measurements. The mean Ti values at baseline were 28.8±15.3, 12 months post-treatment were 28.0±20.1, and 24 months post-treatment were 25.3±16.9 (Figure 2). There were no statistical significances with Dunnett's multiple comparisons test. The deviation of the results was large.

Volume measurements - with garment and 7 days after garment removal

We obtained a full set of measurements (with compression, 7 days without compression, 7 days swelling volume) at the 1-year follow-up, and after that the patients were allowed to reduce or discontinue the use of their compression garments if the symptoms had improved. After 1 year, the data show that either a reduced or discontinued compression garment usage in 5/12 patients, and due to a limited amount of data, the 24 months was not included in all the subanalyses.

The excess of volume **with** compression was decreased in 11/12 patients after 12 months follow-up, and in 7/11 of them, the decrease was >25% compared to the baseline measurement, which was considered clinically significant. After 24 months follow-up, the excess volume was decreased in 9/12 patients. The deviation of results was

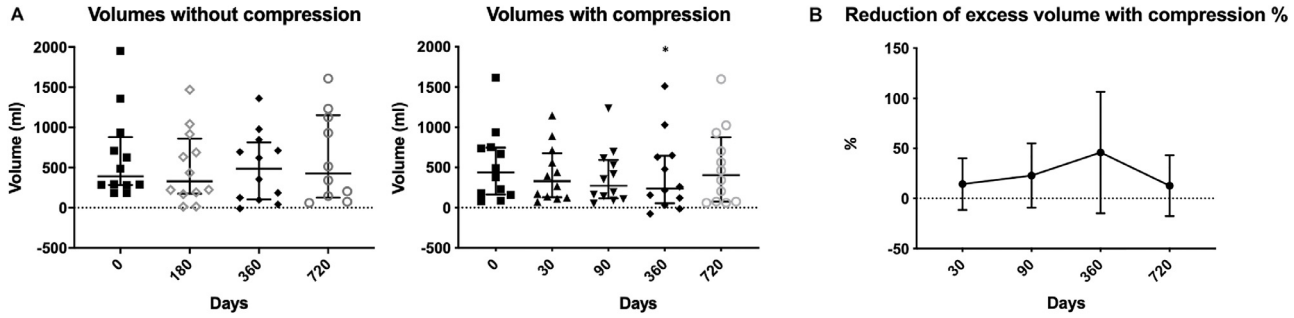

Figure 3 A-B. A. The median excess volumes without compression measured at baseline, 6 months post-treatment, 12 months post-treatment and 24 months post-treatment and with compression measured at baseline, 1 month post-treatment, 3 months post-treatment, 12 months post-treatment (*=p=0.0269) and 24 months post-treatment. Data are shown as median with interquartile range. **3A-B. B.** The mean reduction of excess volume with compression (%) was 46.0% ± 60.7 12 months post-treatment. Data are shown as mean ± SD.

large. The median volume excess percentage **with** compression was 20.6% (IQR 9.4-36.1), 435.5 ml (IQR 165.3-746.8) at baseline, 3 months post-treatment was 16.7% (IQR 6.1-26.2), 273.5 ml (116.5-529.3), and 12 months post-treatment was 9.4% (IQR 3.3-34.0), 239.5 ml (54.5-643.8). Twenty-four months post-treatment, the median volume excess percentage with compression was 20.3% (IQR 4.3-33.7), 403.5 ml (77-875), when even the patients who had reduced/discontinued their use of compression garments were included (Figure 3A). The excess volume reduction at 12 months was statistically significant compared to the baseline situation (p=0.0269). Twelve months post-treatment, the mean reduction of excess volume with compression was 46±61% (Figure 3B). The median volume excess percentage **without** compression was 22.4% (IQR 11,8-39,0), 390.0 ml (IQR 285,3-880,3) at baseline, 19.1% (IQR 8,7-40,3), 330.5 ml (175,5-860,5) 6 months post-treatment, and 18.5% (IQR 6,2-41,3), 487.0 ml (104.8-813,8) 12 months post-treatment. Twenty-four months post-treatment, the median volume excess percentage without compression was 20.5% (IQR 7,7-42,6), 428.0 ml (IQR 128,5-1152). At this timepoint, two patients were excluded because of lacking measurements without compression. Without compression, the mean reduction of excess volume at 12 months was 23.3±46.1%, and at 24 months 10.3±43.6%.

At baseline, the 7 days swelling volume was 105.7±161.0 ml, and 12 months post-treatment was 84.4±143.0 ml. There was statistically significant swelling at baseline (p=0.0253) but interestingly not at 12 months post-treatment (p=0.0682) (Figure 4).

Volume measurements and clinical symptoms of the donor limb

To detect any possible complications to the donor area, lower limb excess volumes were measured pre- and post-treatment. The median volume excess percentage at baseline was -0.85% (IQR -3.1-1.6), -1.85% (IQR -3.0-(-0.8)) 12 months post-treatment (ns) and 0.75% (IQR -2.5-2.1) 24 months post-treatment (ns), indicating no measurable edema of the donor limb (Figure 5). No clinical symptoms of the donor site were observed.

Quality of Life

Significant lymphedema-associated morbidity was observed at baseline. A significant and sustained reduction (41.0 (IQR 28.5-75.5) at baseline, 25.5 (IQR 14.3-41.8) 12 months post-treatment (p=0.0055), and 18.0 (IQR 11.5-43.0) 24 months post-treatment (p=0.0056)) was observed in the total score (Figure 6A). Significant and sustained reductions were also observed in the subdomains (physical, psychosocial, and practical) of cohort 2, during the follow-up period (Figure 6B-D).

DISCUSSION

We present the 24-month follow-up efficacy and safety results of a Phase I multi-center study of the prolymphangiogenic growth factor vector Lymfactin® in female patients with BCRL. The study will continue with a 3-year efficacy and 5-year safety follow-up and the 12 months safety results have been published earlier.[18] Lymfactin® was combined with VLNT surgery after complete scar removal from the axilla. In previous reports, no dose-limiting toxicities were observed.[18] The results of this article show that Lymfactin® is well tolerated during 24 months follow-up. Four of 11 patients showed marked improvements in their lymphoscintgraphy results, the excess volume was significantly reduced at 12 months, and the excess volume reduction was sustained in most patients at the 24 months. Twelve months post-treatment, the removal of the garment did not result in statistically significant increase in arm excess volume as opposed to the baseline situation. Quality of life was significantly improved, and there was no edema of the donor limb in these patients. These results suggest that Lymfactin® has the potential for the resolution of lymphedema in BCRL patients.

After 12 months follow-up, the median excess volume with compression decreased significantly. At 24 months, there was no further reduction in the median excess volume when compared to the 12 month results. However, the patients with improved symptoms and reduced edema had been encouraged to discontinue the use of compression garments after 12 months. The reduced usage of compression

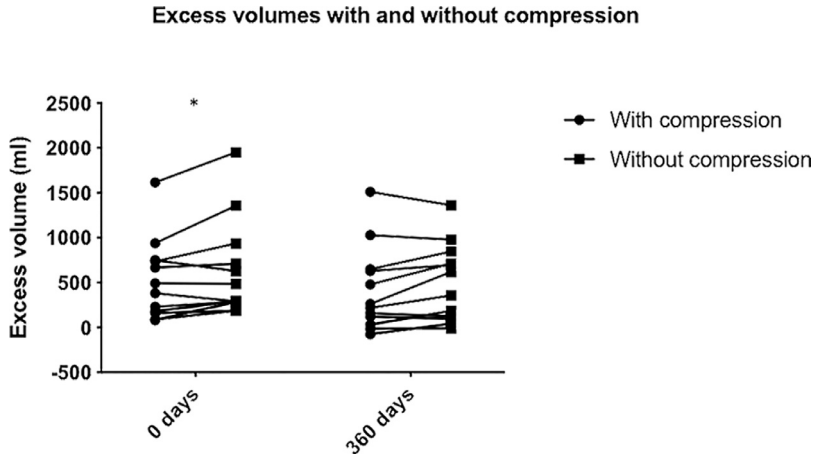

Figure 4 Excess edema volume with compression and without compression. At baseline, the swelling was statistically significant (*=p=0.0253), as opposed to 12 months follow-up (p=0,0682).

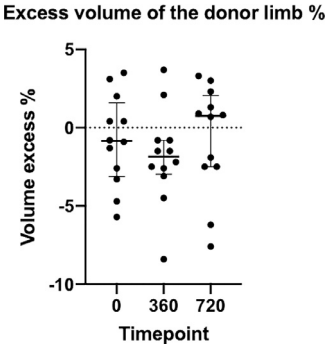

Figure 5 Excess volume of the donor limb (%). There was no measurable edema of donor limb. Data are shown as median with interquartile range.

garments due to improved symptoms indicates better lymphatic flow after treatment even though the median excess volume slightly increased after 24 months follow-up. For the first year, the compression treatment was managed by the University Hospitals, but thereafter the compression treatment was managed by local health care centers. Some patients have reported that in some centers there was lower availability of the compression garments. Thus, in the following phase II study, we have standardized the use of compression garments across centers.

In a recent paper, Beederman et al.[25] have analyzed the results of lymphatic surgery in one of the largest patient series published. The mean reduction in excess volume after VLNT, lymphovenous bypass, or a combined treatment modality was 25.7%, at 12 months. In our results, the mean reduction of excess volume at 12 months was as good as 46.0%. There was still improvement in the results of Beederman et al. after 24 months, but in our results this improvement was not detected. The results cannot be directly compared because there are differences in surgical treatment modalities and compression treatment protocols, but the volume reduction at 12 months in our study still seems very promising.

The swelling of the affected arm was statistically significant when the compression garment was removed for 7 days at baseline. After 12 and 24 months, there was less and not statistically significant swelling after garment removal. If the gained volume reduction is maintained when removing the compression garment, the result can be regarded as a resolution of lymphedema.[10,26] This indicates that after one year of Lymfactin® and combined VLNT surgery treatment, the tendency of edema decreases.

The patients included in this study had different stages of lymphedema (range of preop volume excess 81 ml to 1617

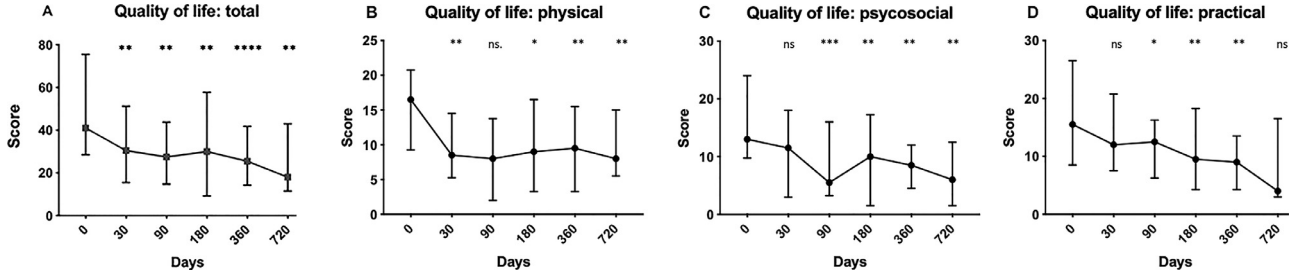

Figure 6 **A-D.** Quality of life was assessed with the LQOLI questionnaire. **A**=total score, **B**=physical, **C**=psycosocial, **D**=practical. There was significant reduction on LQOLI points in every time point. A lower score represents better quality of life. There was also significantly reduction on LQOLI points of each subdomain. Data are shown as median with interquartile range. *=p=<0.05 **=p=<0.01 ***=p= <0.001 ****=p=<0,0001.

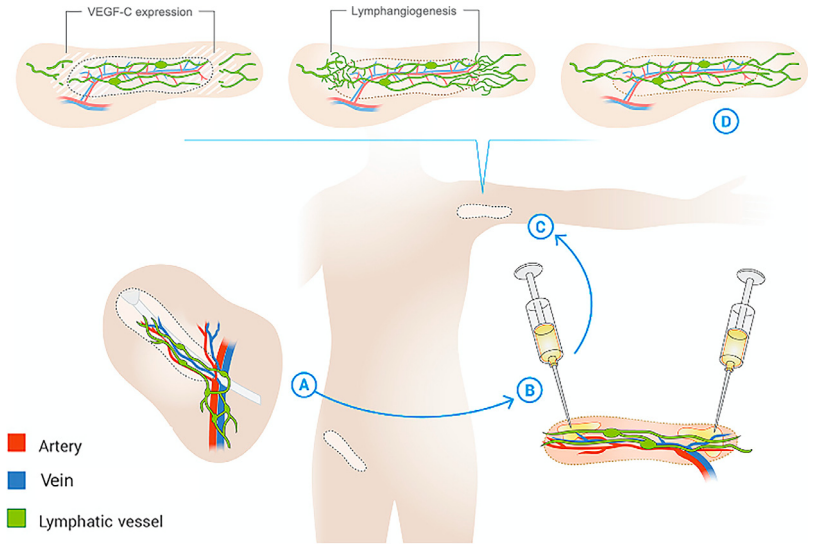

Figure 7 Operation and treatment protocol. A) A soft tissue flap containing several lymph nodes was harvested from the patient's lower abdominal wall or groin area using the superficial circumflex iliac artery perforator (SCIP) vessels. B) Lymfactin® was injected into the flap ex vivo as a single dose to enhance lymphangiogenesis and subsequent lymphatic network maturation. C) The tissue flap was transferred into the axillary region of the patient's affected upper limb. D) Inducing the growth of a functional lymphatic network in order to treat the underlying cause of secondary lymphedema.

ml), which clearly affect the results. The edema cannot be completely resolved in patients who in addition to pitting edema also have excess of fibrofatty tissue at baseline. Lymfactin® treatment is not considered to remove the fibrofatty tissue of the lymphedema arm, and because of this the arm volume cannot be completely normalized after the treatment in stage II patients. In patients with ISL Stage II lymphedema[27] liposuction could be combined with this experimental treatment in the future.[10,26,28] However, as excess volume reduction was an endpoint in the study, liposuction was not included as a method of treatment in the Phase I protocol.

At this stage, it can also be deduced that Lymfactin® in combination with VLNT surgery is safe immediately after the operation and during 24 months follow-up. However, the ultimate safety results and especially the oncological safety of Lymfactin® will require a long 5-year follow-up and a larger patient population.

The limitation of the study in light of the efficacy data is that it was performed as a safety study without a control group. In the Phase I trial, one cannot separate the effects of VLNT surgery and Lymfactin® treatment as all patients have received Lymfactin®. As the patients were allowed to discontinue their garment use if their symptoms had improved, a complete excess volume data set was not obtained at 24 months. Many studies have reported good outcomes in excess volume reduction after VLNT surgery alone, but as discussed in many systematic reviews, the comparison of results between studies is difficult because of different measurement methods and data analysis tools.[6,7,29-31] The Phase II trial, an ongoing randomized placebo controlled trial, aims to answer the question about efficacy as half of the patients are randomized to receive only placebo in combination with VLNT surgery. Measurements and compression treatment protocols in the Phase II trial are standardized across centers.

No curative treatment modalities for lymphedema exist at present. Thus, Lymfactin® treatment combined with lymph node transfer surgery could offer new hope for lymphedema patients (Figure 7). The most promising findings in this study were a 46% reduction in excess arm volume and a nonsignificant swelling volume after garment removal at 12 months, suggesting that there is a potential for reduction of lymphedema. A randomized placebo controlled Phase II study, in which the patients will be evaluated for the efficacy and safety of Lymfactin® for several years, is now ongoing.

CONFLICTS OF INTEREST

PH, SS, ES, IK, JK, TV, and AS have received honoraria for participating in advisory boards of Herantis Pharma Plc. (Espoo, Finland). KA has been a consultant for Herantis Pharma Plc. AV and OL are employees of Herantis Pharma Plc. (Espoo, Finland). IL, MM, and MS report no conflict of interest.

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FUNDING

This trial was supported by Herantis Pharma Plc. (Espoo, Finland). The sponsor has been responsible for trial management and has also reviewed this manuscript.

ETHICAL APPROVAL

The research protocol was approved by the Ethical Committee of the Helsinki University Hospital.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.bjps.2022.08.011.

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