Drug therapy for lymphedema:
Drug-induced lymphangiogenesis

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Abstract for the 3rd Swiss Lymphsymposium, Zurich, September 4, 2021.

The lymphatic vasculature regulates the fluid balance between blood and interstitium by absorbing excess tissue fluid and transporting it back into the blood. The growth and function of both the blood vasculature and the lymphatic vasculature are regulated by signaling molecules. The arguably most important signaling molecules for vessels are the vascular endothelial cell growth factors (VEGFs), and - when lymphatic vessels are concerned - VEGF-C is the primary growth factor.

Lymphedema is the swelling of body parts due to excess fluid in the interstitium which is not drained by the lymphatic network. A distinction can be made between primary and secondary lymphedema: Primary lymphedema is caused by genetic defects and is relatively rare. Secondary lymphedema, on the other hand, is the result of environmental factors, such as accidents, surgery, or parasites.

In first-world countries, the perhaps most common cause of secondary lymphedema is breast cancer surgery, affecting mostly the upper extremities. However, by no means do all breast cancer surgeries result in lymphedema of the arm. Similarly, not everyone who walks barefoot on laterite soil for years develops from the mineral dust podoconiosis, which is a secondary lymphedema condition, which is almost exclusive to certain third world countries.

Although both podoconiosis and breast cancer surgery-associated lymphedema are considered examples of secondary lymphedema, genes play a predisposing role in both. Conversely, there are also subjects with classic "lymphedema mutations" in whom lymphedema is not (yet?) clinically diagnosable. It is thought, that the interactions with certain alleles of protective genes might prevent the phenotypic expression of lymphedema. Therefore, 100% primary or 100% secondary lymphedema are the extremes on a spectrum, and many - if not all - lymphedema cases likely result from an interaction between genetic and environmental factors.

It is often emphasized that there are no pharmacologic substances for the causal treatment of lymphedema. This is not entirely correct, depending on the lymphedema cause. For example, if increased permeability of the capillary walls leads to edema, substances similar to those used for chronic venous insufficiency may be used to reduce the permeability of the blood vessels and thus the formation of tissue fluid. And without a doubt, antiparasitic drugs such as avermectin and its derivatives causally control lymphedema caused by parasitic nematodes.

However, the main focus of this presentation is on those drugs that are themselves capable of stimulating the growth of lymphatic vessels (lymphangiogenesis). There are only a few substances that are directly capable of doing this. The best known of these is the growth factor VEGF-C.

VEGF-C is produced by the human body as an inactive precursor. Before VEGF-C can stimulate the growth of new lymphatic vessels, it must be activated by the enzymatic removal of parts of its polypeptide chain (the propeptides). During embryonic development, the two proteins responsible for the final activation step are the protein-cleaving ADAMTS3 enzyme and the helper protein CCBE1. The mature VEGF-C released during this process can activate VEGFR-3, thereby stimulating the formation of new lymphatic vessels.

Two of the drugs that were tested in clinical trials in recent years are Ketoprofen/Ubenimex and Lymfactin. That Ketoprofen causes volume reduction in acute surgically induced lymphedema was shown in mice in 2009. After it became clear that this effect was due to inhibition of the enzyme 5-lipoxygenase, subsequent studies were conducted with the similarly effective but more specific drug Bestatin.

Although two clinical pilot trials with Ketoprofen showed little evidence of efficacy, a phase II trial with Bestatin was initiated and, as expected, showed that it was not suitable for the treatment of chronic lymphedema. The reasons for the failure probably lie in the mechanism of action of Bestatin, which supports physiological regeneration of lymphatic vessels after their destruction (i.e., in acute lymphedema), but is incapable of stimulating new growth of lymphatic vessels in chronic lymphedema.

It is precisely this stimulation of growth that is the mechanism of action of Lymfactin. Lymfactin is a gene therapy in which the gene for VEGF-C is introduced into the body using a modified first-generation adenovirus (AdVEGF-C). Cells infected by the virus begin to produce VEGF-C until they are eliminated by the immune system after a few weeks. Therefore, this therapy is suitable for short-term stimulation of lymphangiogenesis. AdVEGF-C was developed in a collaboration between the Universities of Helsinki and Kuopio that dates back to the year 1999.

The first clinical trials with Lymfactin were initiated in 2016. The aim was to improve the success rate of lymph node transplants. Lymph node transplants are the current treatment of choice for lymphedema, not least because lymph nodes

produce VEGF-C and thus stimulate lymphatic vessel growth. Mouse experiments showed that the integration rate of transplanted lymph nodes increased using treatment with AdVEGF-C.

Because the therapy was safe and well-tolerated, phase 2 trials began in Finland in 2018 and expanded to Sweden in 2019. In spring 2021, the Lymfactin clinical trials were stopped because the sponsoring company wanted to focus on its CNS drug development pipeline. At the same time, it became apparent that the Phase 2 results regarding efficacy could not be statistically evaluated because the distribution of patients into the Lymfactin and placebo groups had not been at random. Since then, Herantis Pharma has been looking for a partner willing to restart the interrupted studies.

What would make a lymphedema drug successful? Ketoprofen/Bestatin was a pill whose effectiveness was limited to a short interval after surgery. Lymfactin administration, on the other hand, is logistically and practically more challenging compared to a pill. In addition, we do not know whether a short VEGF-C boost is sufficient for chronic lymphedema patients. What we need is a combination: an orally administered medication that has a lymphangiogenic effect similar to Lymfactin. Does such a medicine exist? The fact remains that the lion's share of all potential lymphedema drugs has never been tested for efficacy. The key molecular events of lymphangiogenesis can be recapitulated in cell culture, and cell culture can be automated. Thus, automated screening could be used to identify novel lymphedema drugs, but nobody has done this yet at scale (high throughput screening, HTS).

Lymphangiogenesis assays exist and "just" need to be adapted for HTS. An HTS robot and quite a few chemical compound libraries are available to us. We are currently looking for funding to finance this project.

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