Drug-induced Lymphangiogenesis

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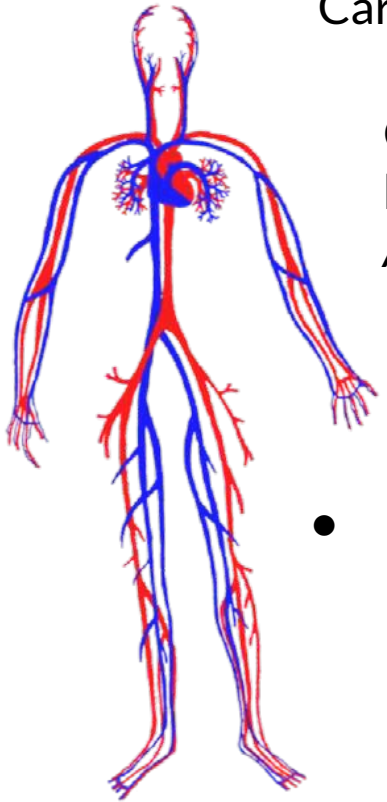
Websites: mjlab.fi (laboratory), jeltsch.org (private)

https://mjlab.fi/dil

- **(Markku) Michael Jeltsch**
- Moved from Germany to Finland in 1995
- PhD (University of Helsinki 2003 with Kari Alitalo)

  Identification of growth factors for lymphatic vessels VEGF-C and VEGF-D; Jeltsch et al. 1997, *Science*; Achen et al. 1998, *PNAS*
- Experience in three biotech startups with several patents for biologics (VGX-100, VGX-300/OPT-302, Lymfactin)
- Associate professor for pharmaceutical protein drug research at the University of Helsinki since 2020

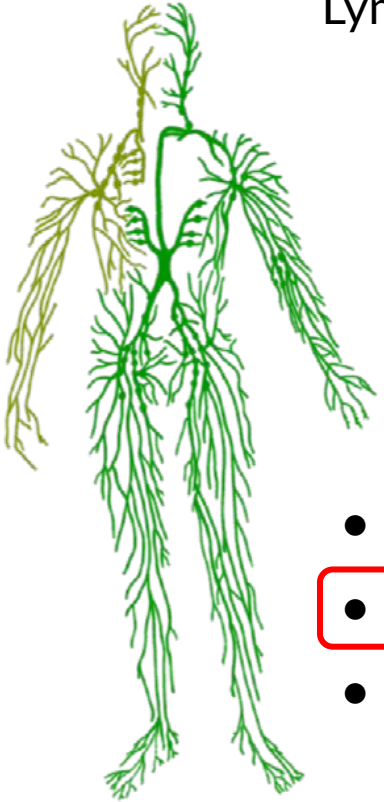
Cardiovascular system



Growth of new blood vessels: *Angiogenesis*

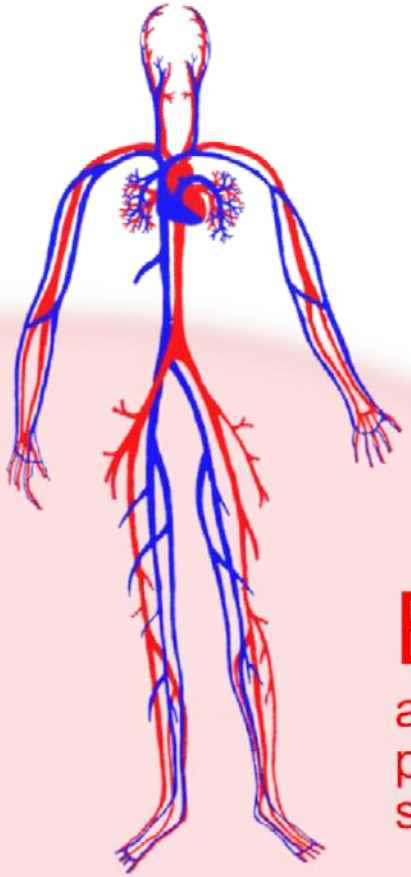
- Distribution of oxygen and nutrients

Lymphatic system



Growth of new lymphatic vessels: *Lymphangiogenesis*

- Immune defense
- Fluid balance
- In the gut: absorption and transport of dietary fat



Interstitial fluid

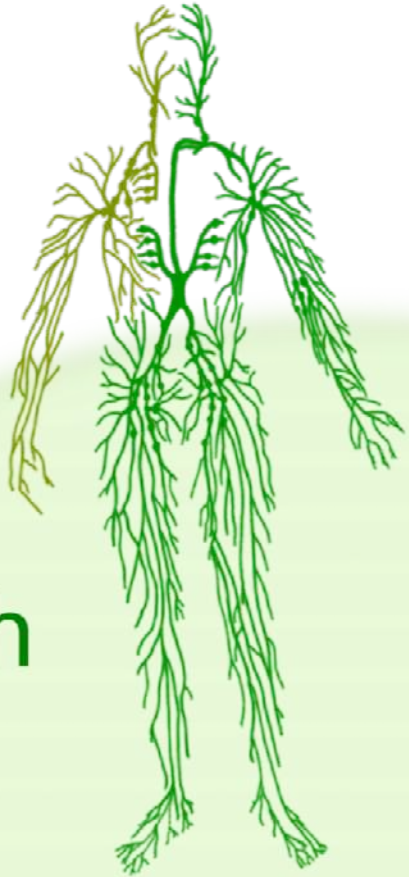
leakage

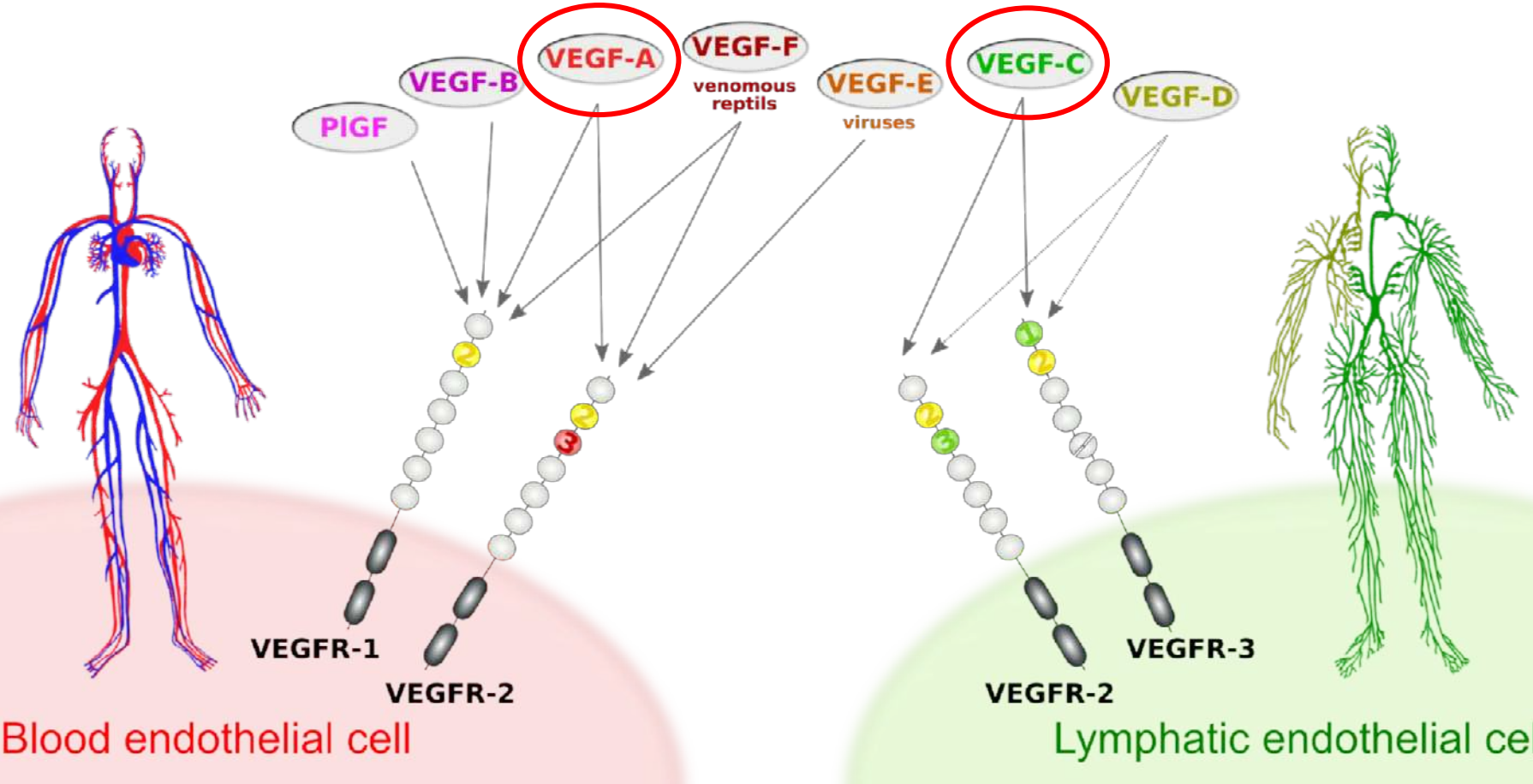
absorption

Blood

arterial high pressure system

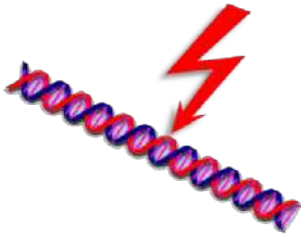
Lymph




PlGF
VEGF-B
VEGF-A
VEGF-F
venomous reptils
VEGF-E
viruses
VEGF-C
VEGF-D
VEGFR-1
VEGFR-2
VEGFR-2
VEGFR-3
Blood endothelial cell
Lymphatic endothelial cell

Primary (hereditary) Lymphedema

Relatively rare:
1-5 in 10,000*



Secondary Lymphedema

surgery/trauma

Most common cause in Europe/US:
Breast cancer surgery (~20%)*

Filariasis

Infection with Nematodes

Podoconiosis

Barefoot walking on laterite soil
30-40 Mio. / ~4 Mio. cases

* Vastly different estimates by different studies.

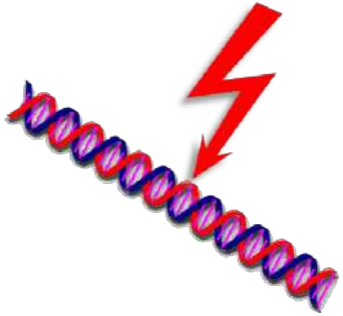
Primary and secondary lymphedema are extremes on a spectrum

Not everybody walking for years on laterite soils develops podoconiosis.

Predisposition from certain alleles of the HLA/MHC genes (DRB1, DQA1, DQB1, DPB1)[1].

Not every breast cancer surgery results in lymphedema.

Some alleles of certain genes (“lymphedema-genes”) seem to predispose[2].



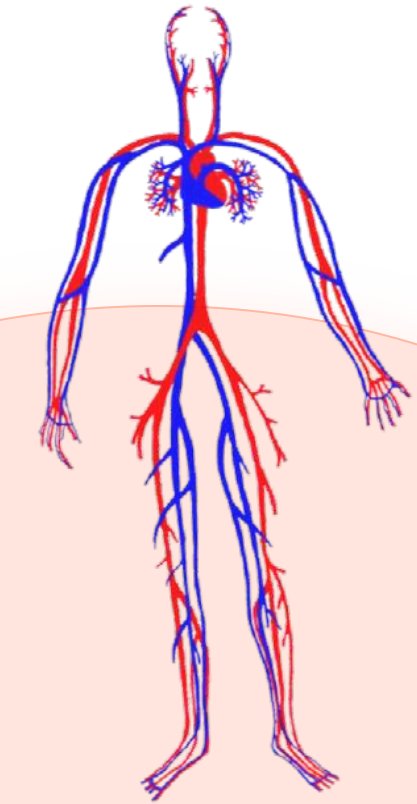
Not everybody carrying a mutation in a “lymphedema gene” is clinically diagnosed with lymphedema

Some alleles of certain genes can protect from or exacerbate the effect of a mutation (modifier genes)[3].

Environment ↔ Genome

1 Vanquishing "Mossy Foot" with Genetic Epidemiology and Shoes - Scientific American Blog Network
2 Prox-1 alleles can cause congenital disease, but also remain subclinical and only manifest only upon environmental insult (https://doi.org/10.1002/mgg3.1424)
3 Review: https://doi.org/10.1089/lrb.2017.0083. Further causes for the variable penetrance and expressivity of mutations are epigenetic factors and chance.

No drug therapy for lymphedema?!



Interstitial fluid

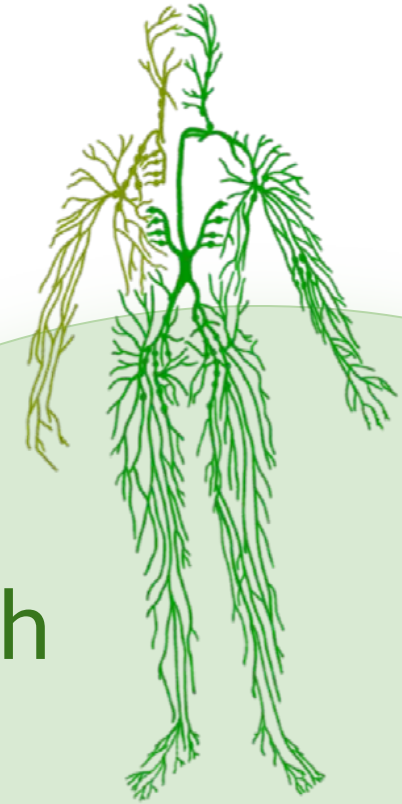
Increased vessel wall permeabilty

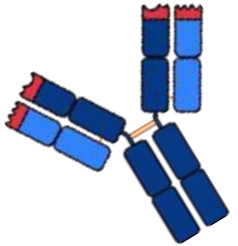
Reduced uptake

Blood

Lymph

Reduced transport



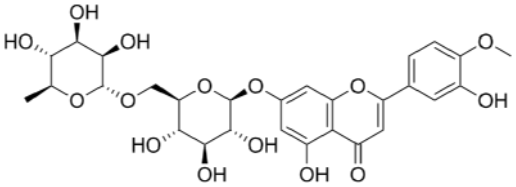


- **Bevacizumab (**Antibody taregting VEGF-A)
  VEGF-A increases the permeability of blood vessels increasing tissue fluid generation
  2006: NCT00318513, 35 participants, open study
  2006: NCT00393497, 11 participants, open study

- **Pazopanib** (Tyrosine kinase inhibitor that blocks i.a. VEGF-A receptor VEGFR-2)
  2009: NCT00827372, 10 participants, open study

- **“Stem cells”: Adipose tissue-derived regenerative cells (ADRC)**
  2015: NCT02592213, 10 participants, open study
  doi:10.1002%2Fsctm.20-0394, no objective measurable improvement, subjective improvement
  Assumed mechanism: ADRCs produce VEGF-C und VEGF-D

- **Tissue Engineering**
  2016: NCT02734979, BioBridge (collagen fibre scaffold), 1 participant
  2020: NCT04606030, BioBridge (collagen fibre scaffold), 60 participants, partially blinded
  FaciliFlow (“artificial lymph node”), EP3223750A1, no clinical studies yet



- **Flavonoids (diosmin, hesperidin, troxerutin, etc.)**
  Exact mechanism unknown, likely a combination of reduced Vascular permeability, increased lymphatic pumping, inhibition of inflammation and eNOS inhibition.
  Venous blood vessels were targeted in most studies, as flavonoids are commonly used to treat chronic venous insufficiency (CVI). An effect on lymphatics is plausible due to the molecular similarity of lymphatic and venous vessels and based on a few studies[1].

- **Selenium**
  Exact mechanism unknown, likely inhibition of inflammation (antioxidative effect). It remains unclear, whether the positive clinical studies result from the quite common dietary selenium deficiency (worlwide estimate: 1 billion cases). A specific effect beyond deficiency supplementation is not known[2].

- **Interleukin-7 (IL-7)**
  No clinical studies (doi:10.1182/blood-2013-01-478073)
  Mechanism: Increases the uptake of interstitial fluid by the lymphatics.
  IL-7 mediates inflammatory reactions and increases via TNF-α vascular permeability.

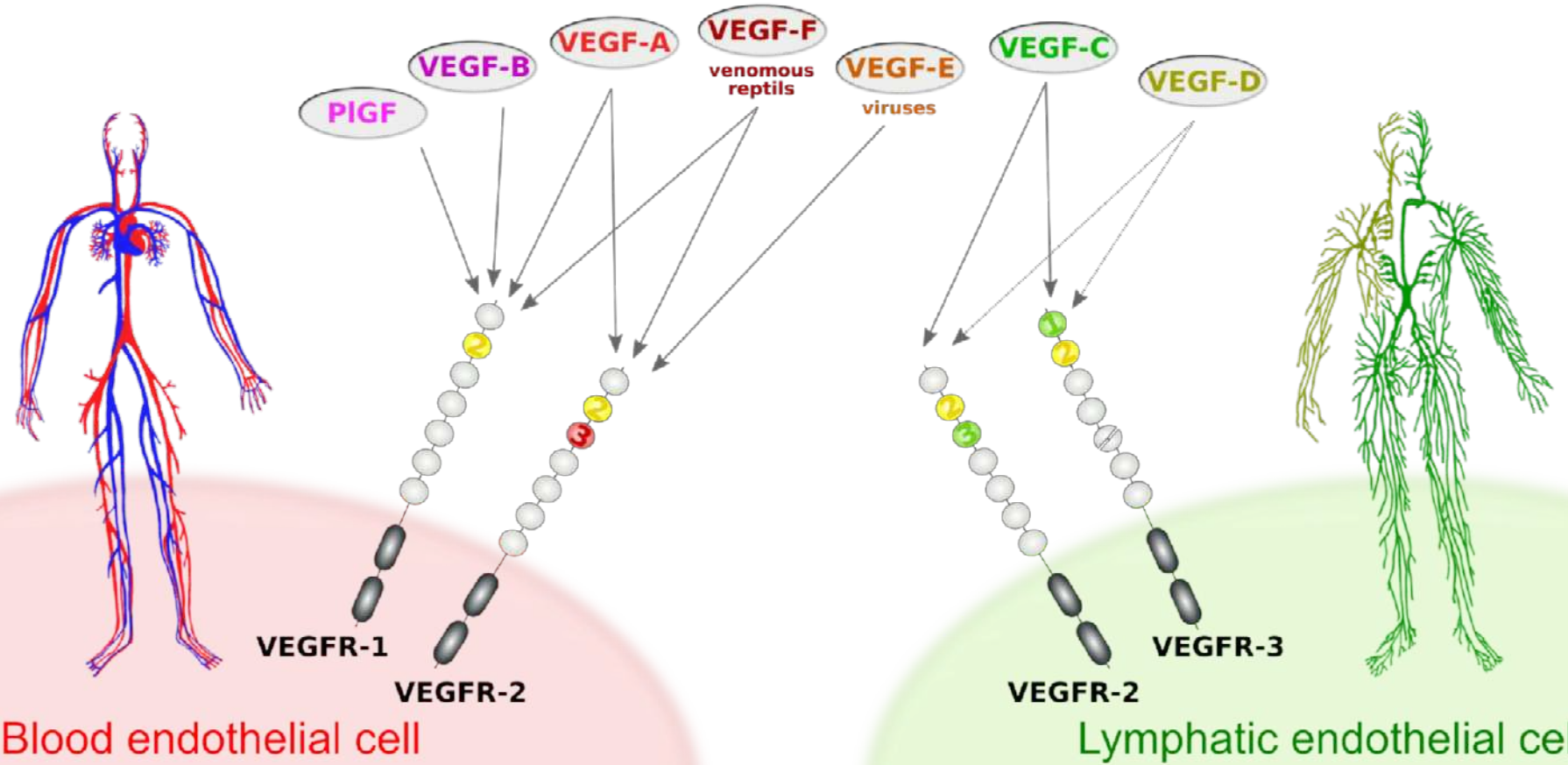
1 The fact that these drugs/nutritional supplements are available without prescription does not mean that they do not have side effects, especially when taken over a longer period of time! Before stocking up on selenium supplements, it makes sense to measure whether you have a deficiency at all. You can also poison yourself (fatally) by overdosing of selenium preparations and flavonoids.

2 The enzyme glutathion peroxidase contains selenocystein, the “21. amino acid”; glutathion peroxidase: $2GSH + H_2O_2 \rightarrow GSSG + 2H_2O$; glutathion reductase: $GSSG + NADPH + H^+ \rightarrow 2\ GSH + NADP^+$

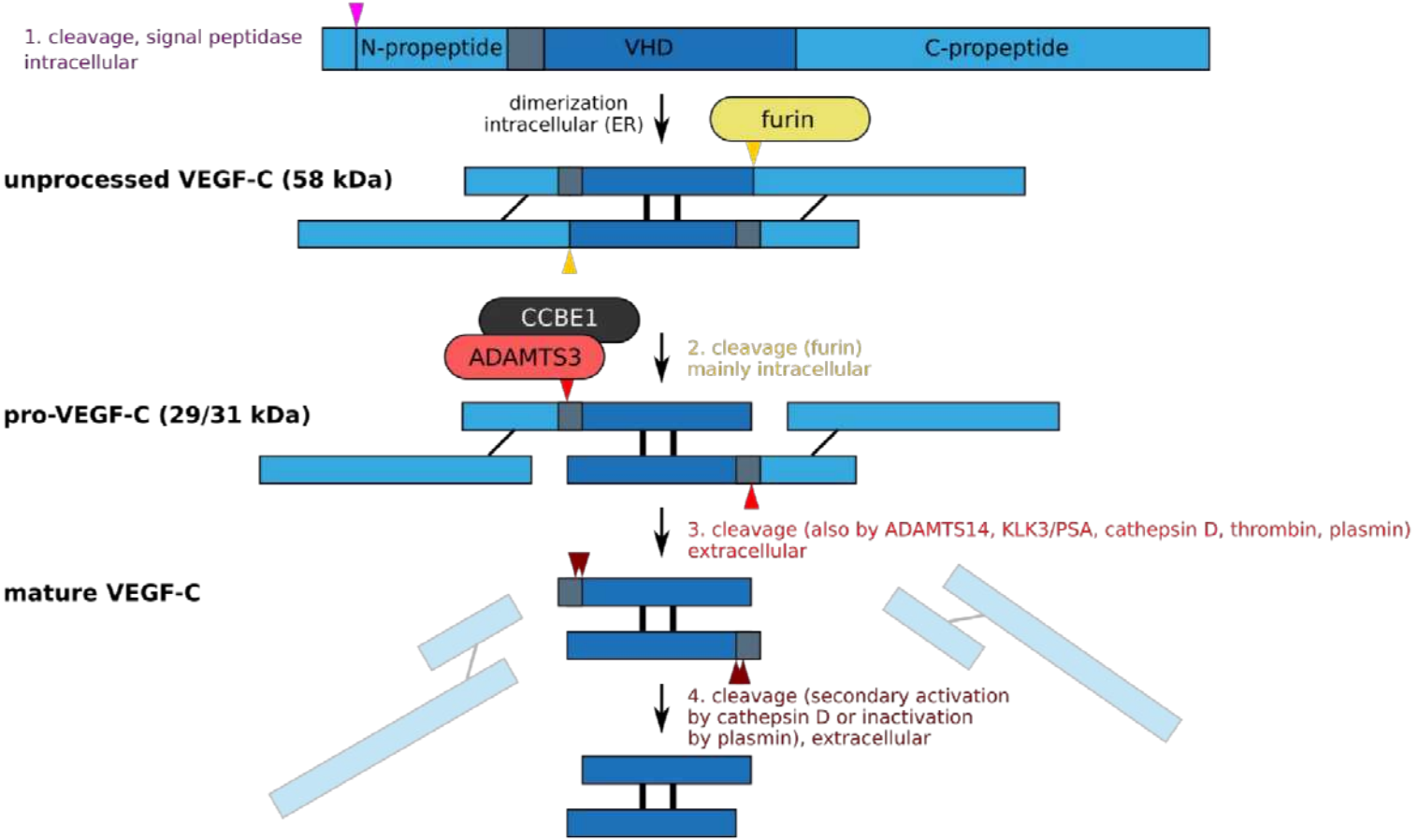
- **Platelet-rich plasma (PRP)**
  2017: NCT03080207, 45 participants, open study
  Assumed effect mediated by the high concentration* of VEGF-C in thrombocytes
  doi:10.1089/lrb.2019.0064, no significant differences between CDT and CDT+PRP
- **Lymphocyte injektions (Tregs)**
  1984-1999: Several case studies in Japan, doi:10.7759%2Fcureus.5638
  2016: A newer, technically superior** study in mice, doi:10.1172/jci.insight.89081
- **Avermectin and other antiparasitika**
  2015: Nobel prize for physiology/medicine
- **Coumarin*****
  Assumed mechanism: Reduction of vascular permeability
  Studies are of low quality (P. Mortimer: “Meta-analysis impossible”: doi:10.1002/14651858.cd003140.pub2. The perhaps best study (140 participants, “RCT”****) finds no effect (doi:10.1056/nejm199902043400503). Liver toxicity!

* Although the concentration of VEGF-C is high, it is unlikely that the amount is sufficient to have a significant effect on lymphangiogenesis. In the protocol that was used, the pro-VEGF-C is activated by thrombin before injection, i.e., mature VEGF-C is released, which diffuses much more readily, further reducing the local concentration.
** In the more recent study, a subpopulation of T cells (Tregs) was used. Other T cells (TH1 and TH2) have a negative effect on lymphedema because TH1 cells inhibit the expression of pro-lympangiogenic growth factors and TH2 cells are pro-fibrotic by secreting TGF-β1, IL-14 and IL-13.
*** Not to be confused with the chemically and namesimilar bis-4-hydroxycoumarins, which act as anticoagulants.
**** Randomized controlled trial

VEGF growth factors and VEGF receptors



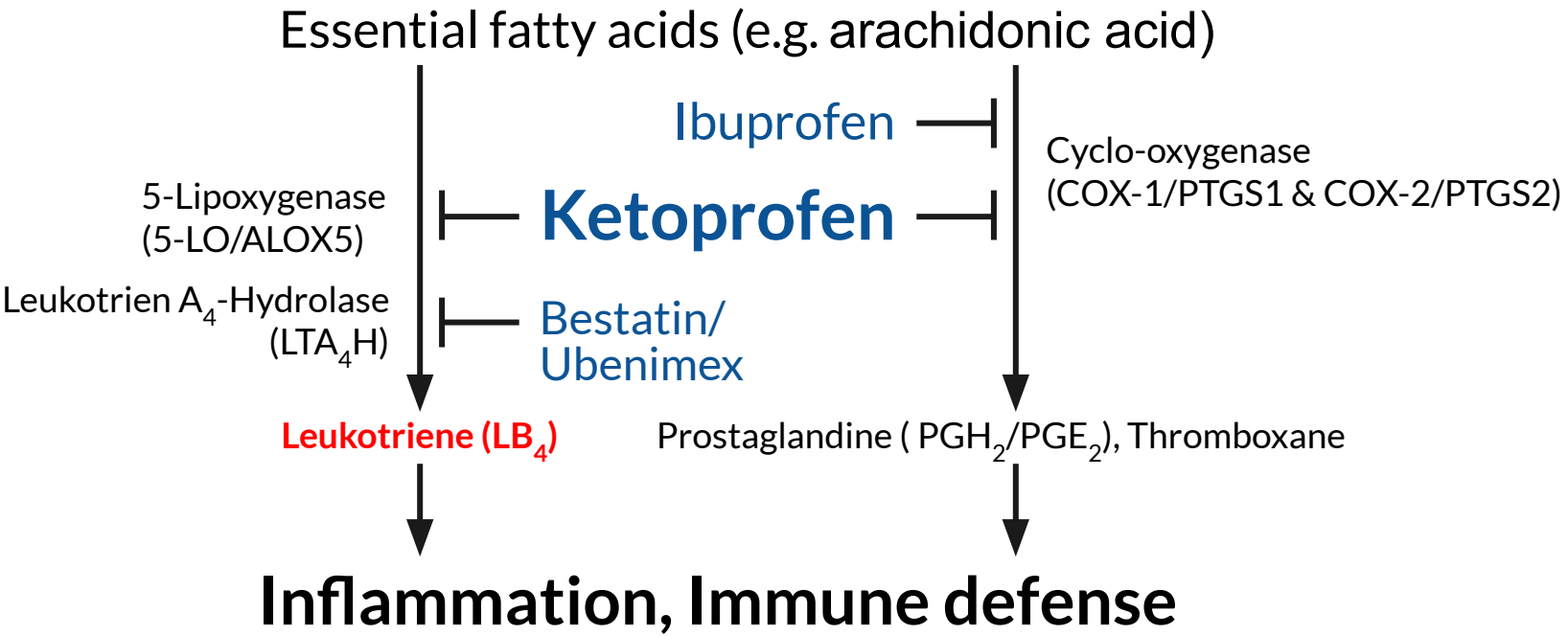
The activation of VEGF-C



Ketoprofen/ Ubenimex

Lymfactin

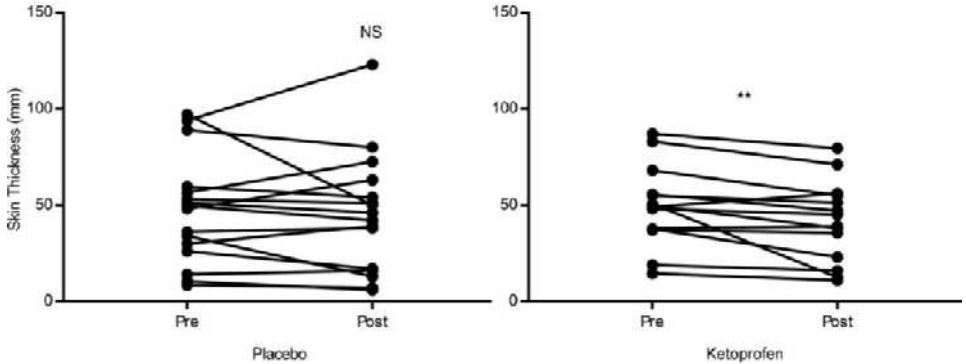
Drug X



doi:10.1126/scitranslmed.aal3920

“Pilot studies demonstrate the potential benefits of antiinflammatory therapy in human lymphedema” (Ketoprofen)

- 2010-2011: Open study with 21 participants, 2011-2015: Blinded study with 34 participants
- Primary endpoint: skin thickness
- Secondary endpoints: histopathology, volumen, bioimpedance, systemic inflammation markers
- Most results were not significant
- Some results were inconsistent (e.g. the lack of a volume reduction)
- Statistic analysis problematic: No adjustment of significance levels due to multiple comparisions

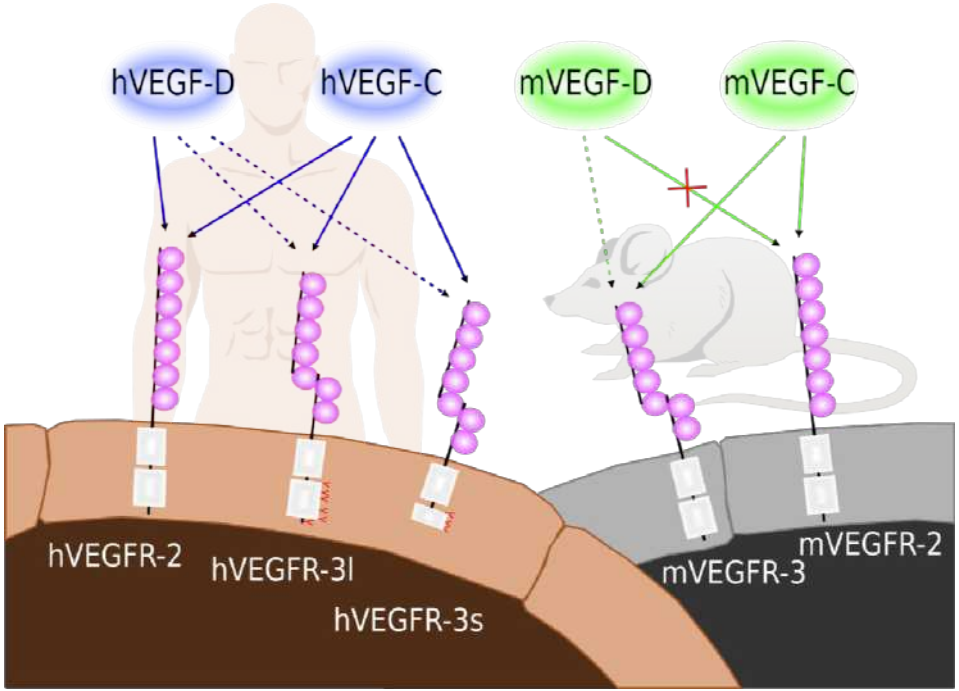


doi:10.1172/jci.insight.123775

- 2016-2018: Blinded study with 46 Teilnehmern
- Primary endpoint: skin thickness
- Secondary endpoints: patient reported outcome measures (questionnaire), leg volume, extracellular leg fluid volume (bioimpedance), skin thickness (biopsy)
- 10/2018: “Study Did Not Meet Primary or Secondary Endpoint”
- “Underpowered”, “The ULTRA (Ubenimex) clinical trial for lymphedema failed as expected - but there is still hope”
- The acute, surgically induced lymphedema in the mouse tail is not comparable with the most common typical chronic lymphedema in humans.

https://clinicaltrials.gov/ct2/show/NCT02700529

- Acute, post-traumatic edema: 3 days after surgery the LTB_4 rises to anti-lymphangiogenic levels: Bestatin therapy is likely effective only within this period! The mouse tries to heal the surgically induced lymphedema, and Bestatin supports these attempts by inhibiting the inflammatory mediator LTB_4.
- Whether edema, that has persisted already for weeks, will respond to Bestatin, was not testet. The differences are large: e.g. fibrosis, fat tissue, etc. torontophysiotherapy.ca/ubenimex-bestatin-for-treating-lymphedema
- A prevention study would be better! Tacrolimus (externally applied!): [NCT04390685](NCT04390685)
- Differences between mice and humans at the molecular level.



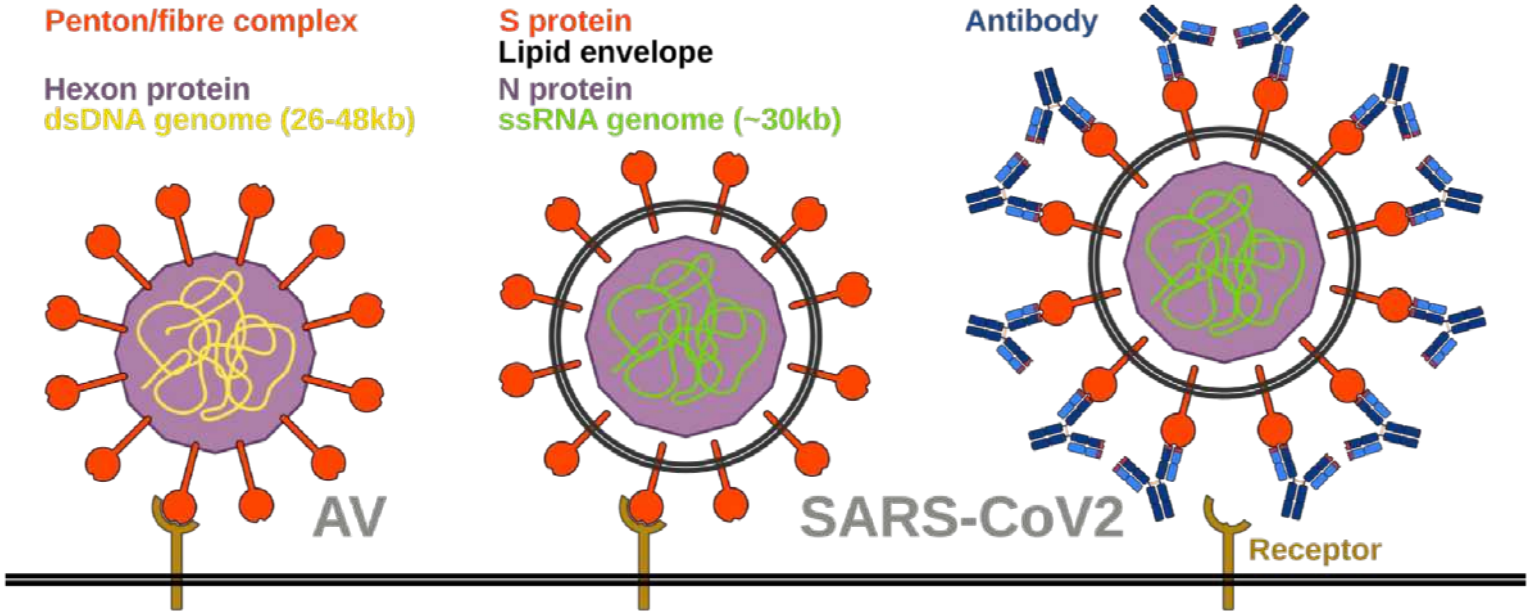
Ketoprofen/ Ubenimex

Lymfactin

Drug X

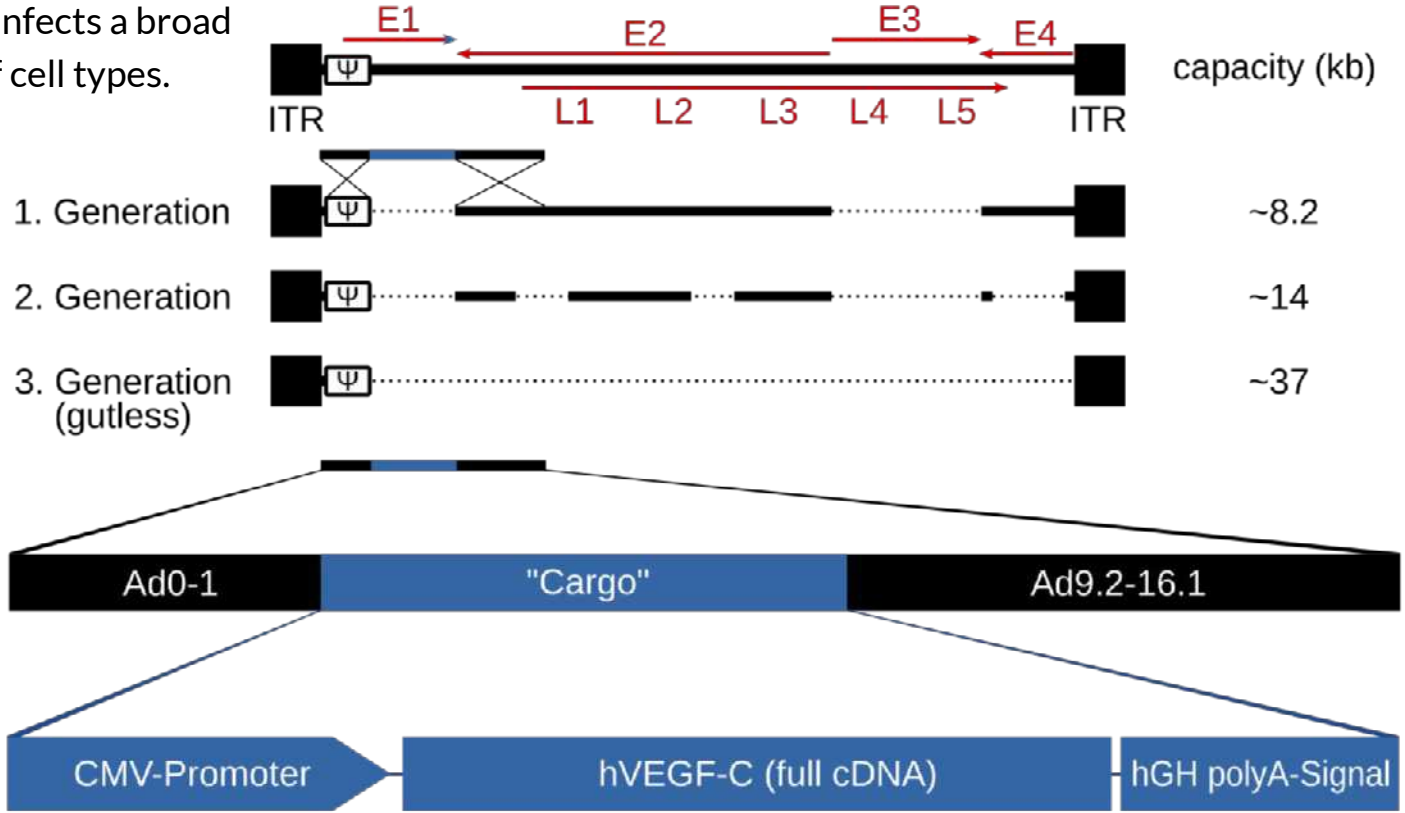
- 2000: The first VEGF-C-expressing virus (AdVEGF-C) was made in 1998 (University of Helsinki & University of Kuopio; doi:10.1161/01.CIR.102.18.2262); first application in rabbits to prevent restenosis upon balloon dilatation.
- 2001: The same adenovirus was used to grow new lymph vessels in the skin of mice (doi:10.1161/01.RES.88.6.623).
- 2001: First gene therapy of primary lymphedema in mice using AAV-VEGF-C (doi:10.1073/pnas.221449198)
- 2002: Preclinical gene therapy trials in mice using Ad- and AAV-VEGF-C_{C156S} (doi:10.1084%2Fjem.20020587)
- 2007: VEGF-C used as adjuvant during lymph node transplantation in mice (doi:10.1038/nm1689)
- 2015: Ad-VEGF-C & -VEGF-C_{C156S} in a pig model (doi:10.1007/s10456-015-9469-2)
- 2016- Phase I (NCT02994771, doi:10.1016/j.bjps.2020.05.009)
- 2018- Phase II (NCT03658967)

- To produce designer viruses is nowadays neither very difficult nor expensive or lengthy*.
  Lymfactin: “Serotype #5 1st generation adenoviral vector with VEGF-C cargo”
- No lipid envelope, only protein capsid
- Genome: a single linear, double-standed DNA molecule
- Serotype (= which cells can the virus infect?) is defined by the capsid proteins.



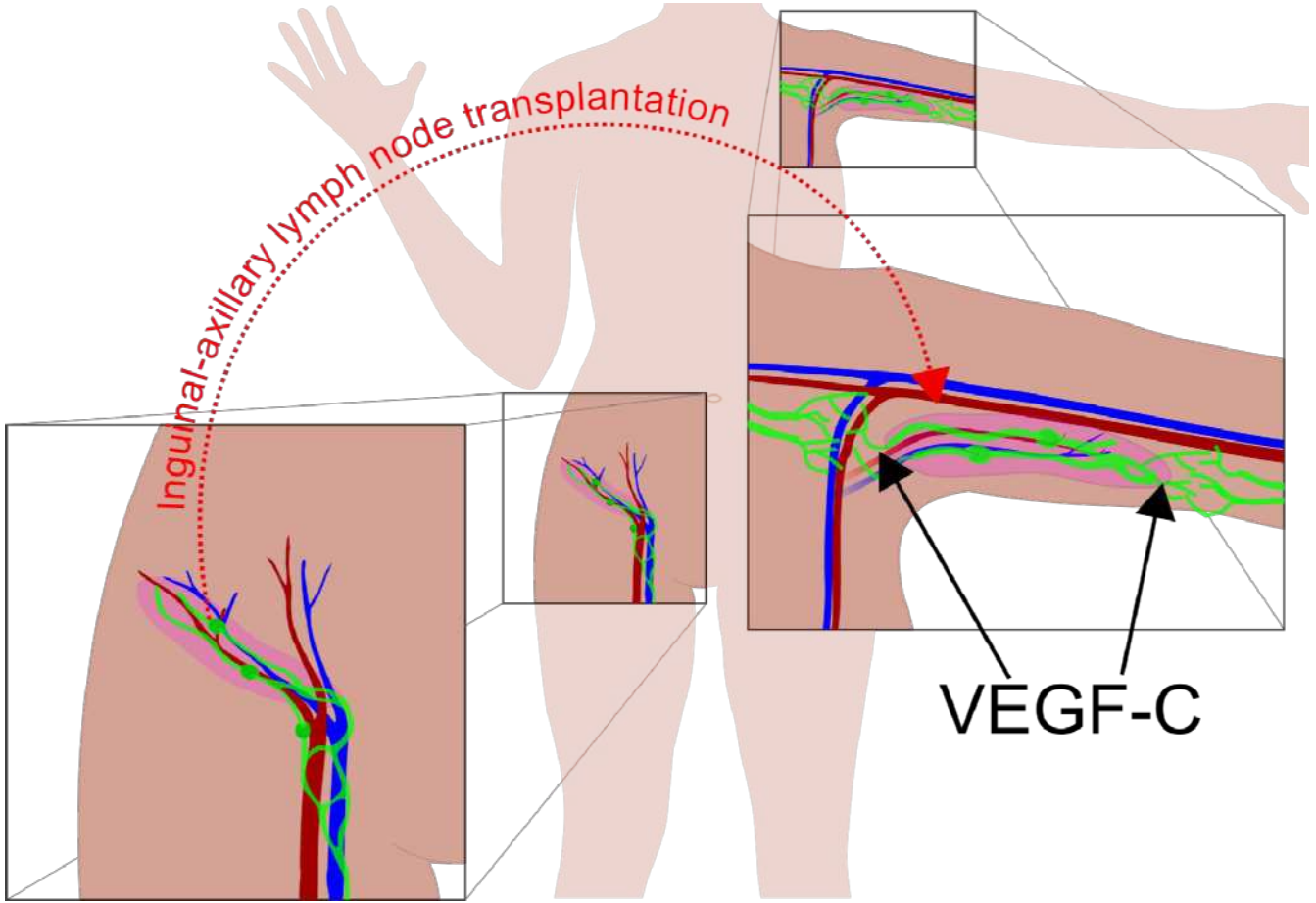
Lymfactin is a Serotype 5 1. Generation adenovirus vector*

Serotype 5 infects a broad spectrum of cell types.



* It is very similar to the adenoviruses, that are used as vaccines against SARS-CoV2, like the vaccines from Oxford/AstraZeneca (ChAdOx1 nCoV-19), Johnson&Johnson/Janssen (Ad26.COV2.S), and Sputnik V (Ad26/Ad5).

Lymfactin, Phase 1 study: Combination with lymph node transplantation



- Why are lymph node transplantations helpful in the treatment of lymphedema? Lymph nodes produce VEGF-C (the removal of lymph nodes results in a strong reduction of the local VEGF-C concentration, doi:10.1097/GOX.0b013e318293a532).
- Lymph node transplantations are not always successful. Only ~30% of the transplanted nodes are integrated into the local lymphatic network!

  VEGF-C increases a) the chance of integration
  b) affects directly the growth of lymphatic vessels
- Injection of the virus ex-vivo into the (fat-)tissue flap, that contains the lymph node
- Phase 1: Safety, tolerance und biodistribution
  Any observed efficacies are mostly not significant due to the low numbers of participants.
- The virus is inactivated by the immune system (VEGF-C is only produced for 1-2 weeks), which should be sufficient according to the mouse studies.

- Safe and well tolerated
- Virus remains local (not detectable in the blood by PCR)
- No or only minimal activation of the immune system (unchanged Ad5 antibody concentrations)
- Phase 2 studies started in Finland in 2018
- 2019: Expansion of phase 2 studies to Sweden
  https://herantis.com/press-releases/herantis-pharma-announces-expansion-of-its-phase-2-study-adele-in-breast-cancer-associated-lymphedema-with-two-centers-in-sweden/
- Spring 2021: Phase II studies discontinued

  https://herantis.com/press-releases/herantis-announces-inconclusive-results-from-phase-ii-study-with-lymfactin-in-breast-cancer-related-lymphedema/

  https://herantis.com/press-releases/herantis-pharma-to-focus-on-cdnf-and-xcdnf-programs/
- **Reasons for the ambiguous results**

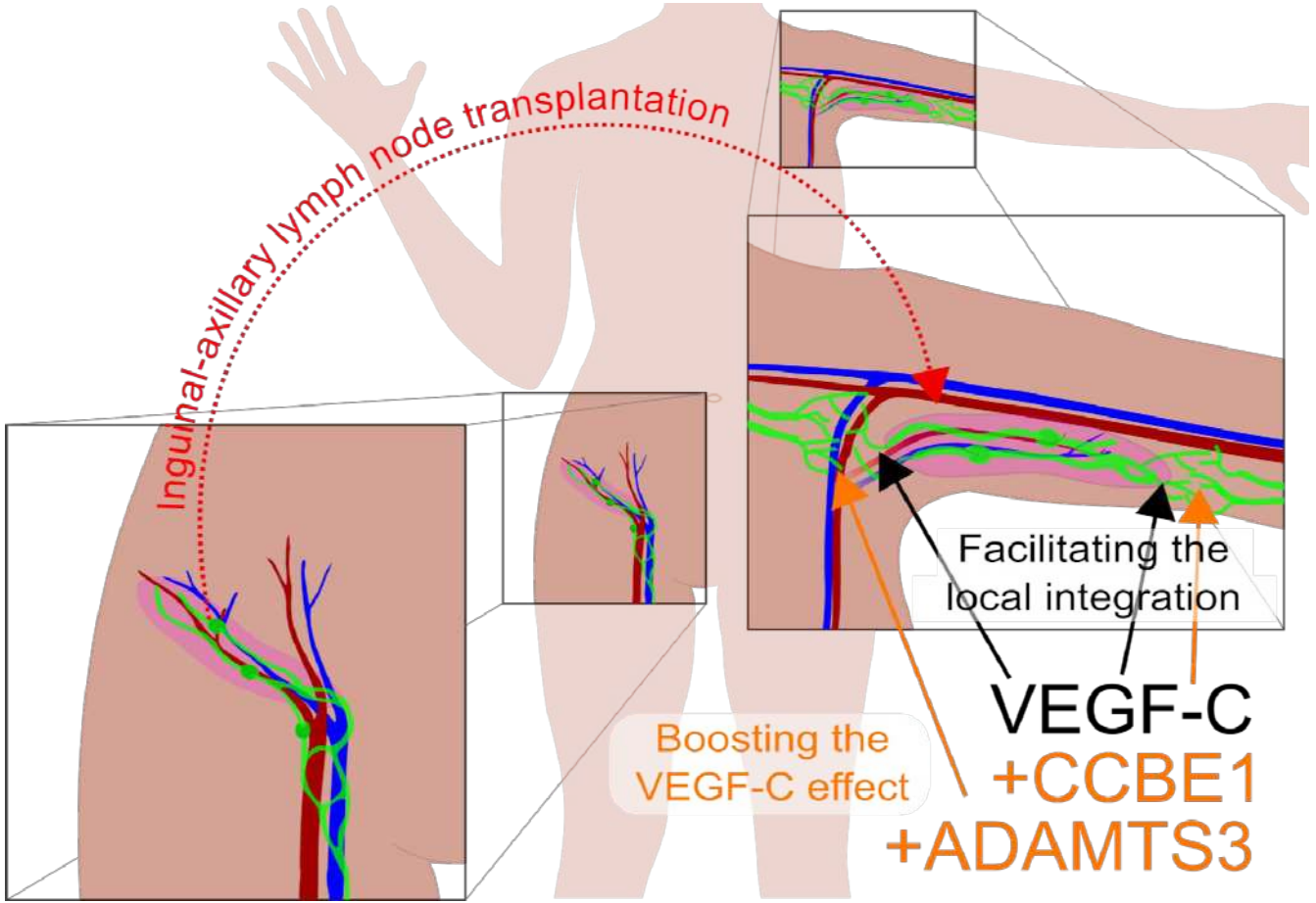
  The assignment of the participants into the Lymfactin or the placebo arm did not happen at random

  Some inconsistent data (e.g. a lower arm volume, while the lymphoscintigraphy indicated a worse lymphatic function)

Needed:
A partner who will pick up the Phase II studies!

Significance and effect size: The larger the effect,
the easier it is to show that the effect really exists.

Contact at Herantis Pharma: Antti.Vuolanto@herantis.com

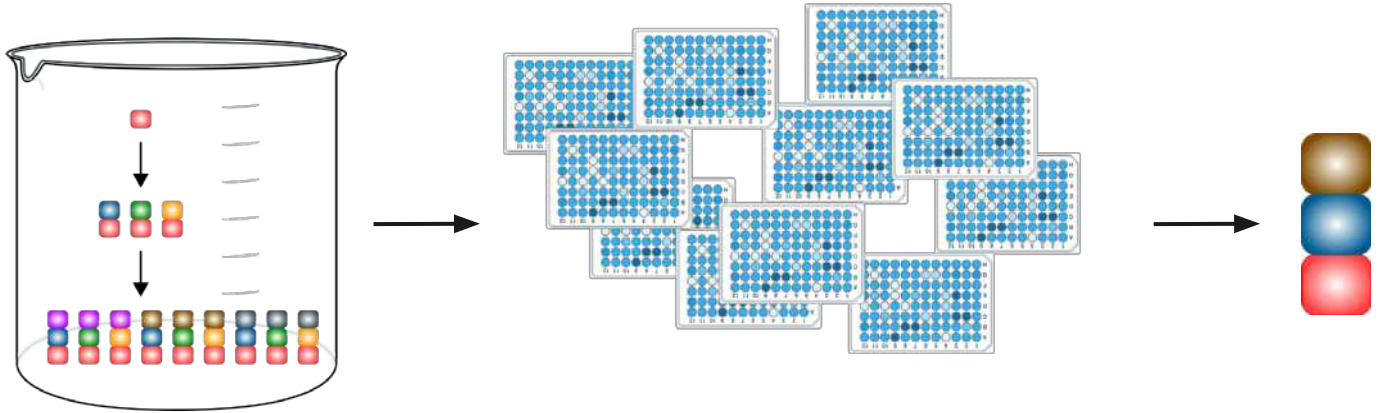

Inguinal-axillary lymph node transplantation
Facilitating the local integration
Boosting the VEGF-C effect
VEGF-C
+CCBE1
+ADAMTS3

Ketoprofen/ Ubenimex

Lymfactin

Drug X

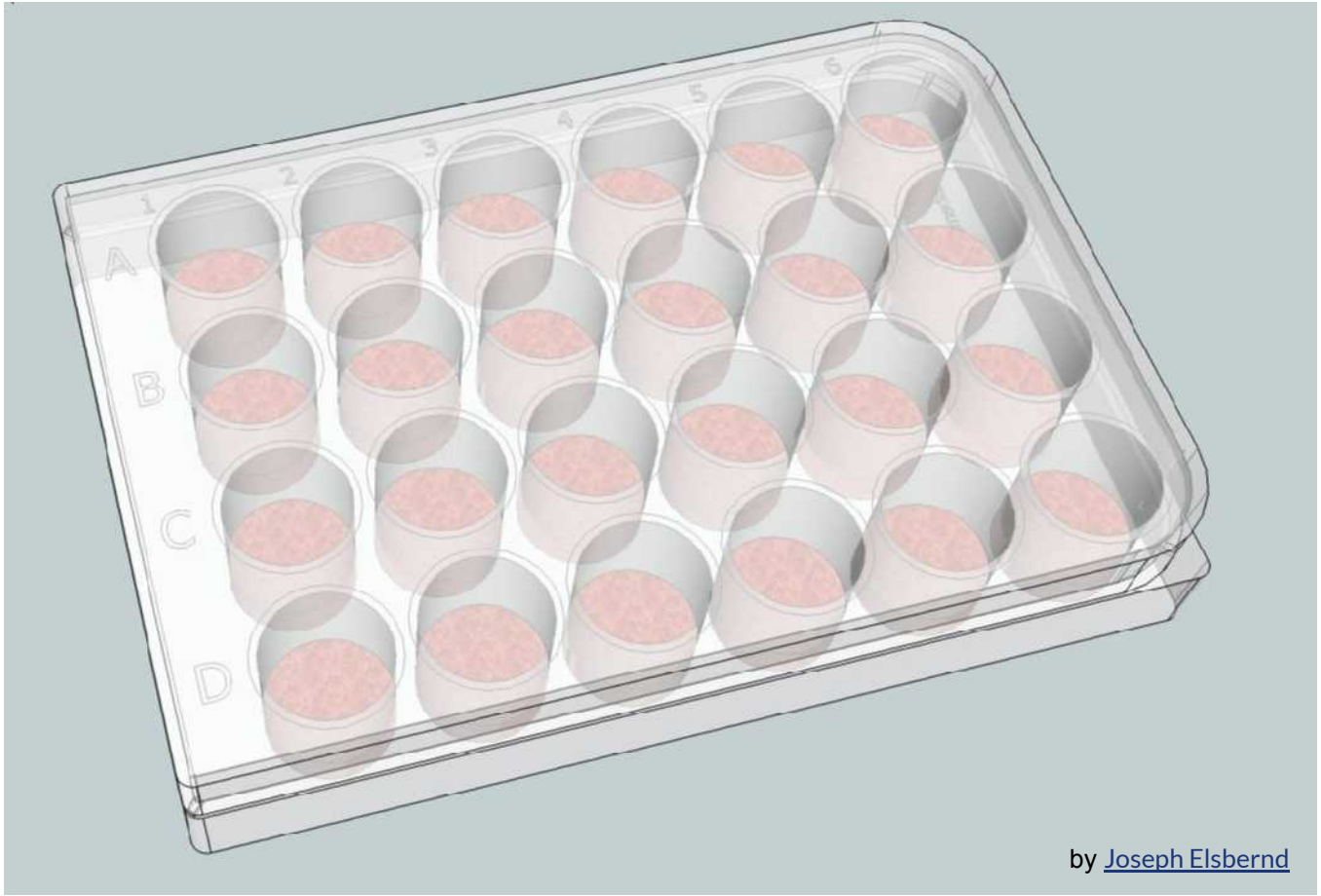
More than 99.9999% of all potential lymphedema drugs have never been tested in-vitro or in-vivo



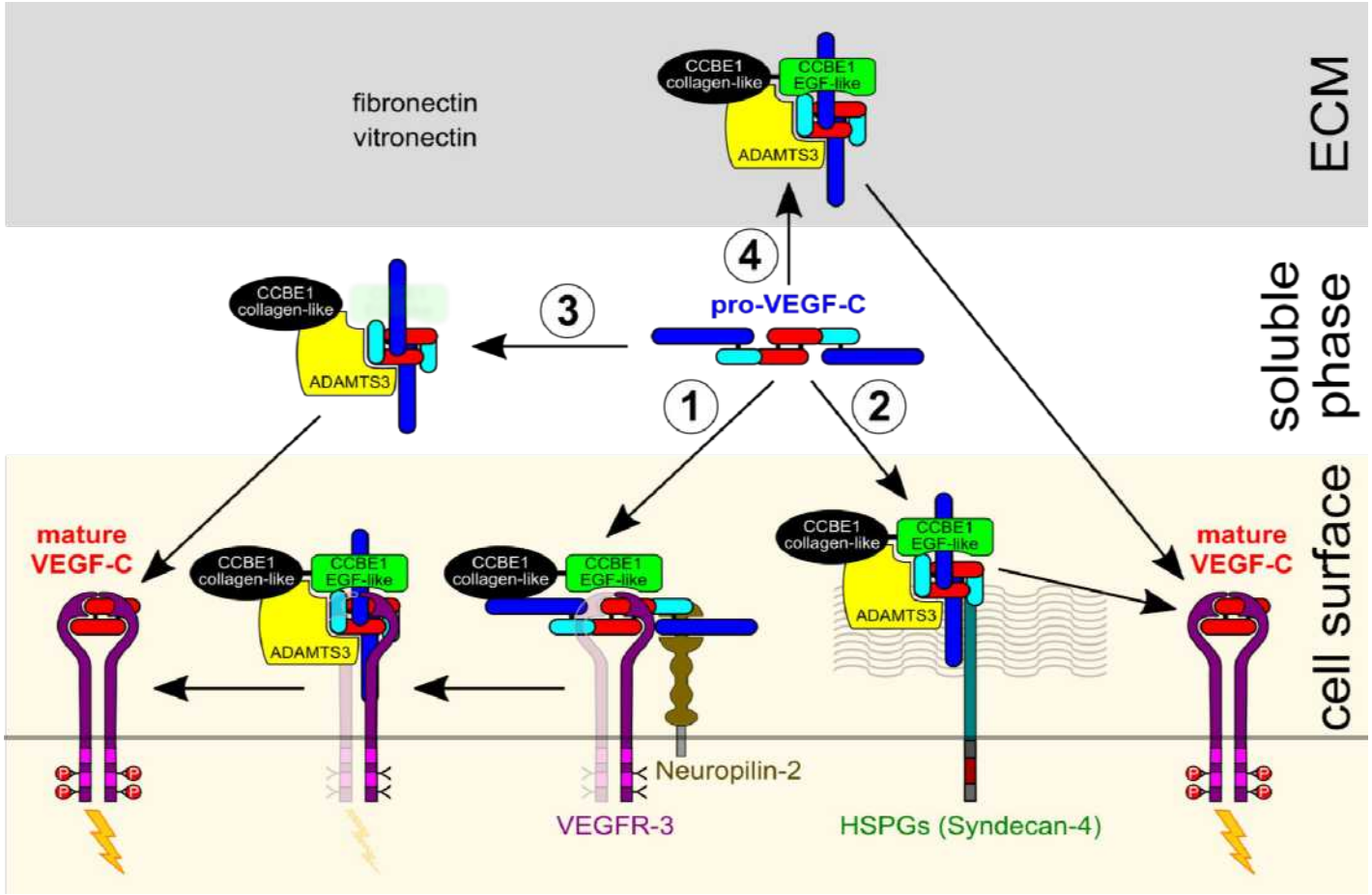
>20 Mio. characterised chemical compounds

High-throughput-screening

candidate molecules


1
2
3
4
5
6
A
B
C
D
by Joseph Elsbernd

Activation of the lymphangiogenic VEGF receptor-3 by VEGF-C



- Which library? https://images.jeltsch.org/drug_origin_deutsch.html
- Adapting our existing methods (10 cm cell culture dish, Western blot) to the requirements of high-throughput screening (microtiterplate, chromogenic readout)
- Financing...

Päivikki ja Sakari Sohlbergin Säätiö

Magnus Ehrnrooth Foundation

novo nordisk fonden

German Society of Lymphology

Sydäntutkimussäätiö
Stiftelsen för hjärtforskning
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Drug Research Program, Individualized Drug Therapy Research Program, & **Wihuri Research Institute**

That’s all folks!

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