

# Clinical translation of a novel treatment to enhance recovery and improve long-term outcome after stroke

## Kort sammanfattning

About 50% of stroke survivors suffer from long-lasting or permanent disability. No efficient therapy is currently available, except for the removal of the occluding blood clot during the first hours after ischemic stroke. Plasticity responses in spared brain regions are a major contributor to functional recovery, while secondary neurodegeneration in remote brain regions impedes the long-term outcome after stroke.

We showed that treatment with C3a started a full week after stroke accelerates functional recovery and inhibits stroke-induced neurodegeneration. The positive effects of treatment are sustained weeks after treatment cessation. These findings provide strong evidence for multiple beneficial effects of C3a in the post-stroke brain and point to the utility of neuroimaging and blood levels of biomarkers of neurodegeneration such as NfL for objective evaluation of treatment efficacy in clinical studies. The broad time window means that such a treatment could help to reduce neurological impairment and improve the quality of life for the majority of, if not all, stroke survivors.

To translate this experimental treatment strategy into clinical practice, we aim to scale up production of fully active recombinant C3a, perform the regulatory in vivo toxicity studies, and conduct Phase 1b clinical study with recombinant C3a. The project has the potential to provide a desperately needed pharmacological treatment to enhance recovery and improve long-term outcome after stroke.

## Populärvetenskaplig sammanfattning

Vi har utvecklat en farmakologisk strategi att öka återhämtningen och förbättra det långsiktiga utfallet efter stroke. Ur ett kliniskt perspektiv är det särskilt viktigt att behandlingen visat sig vara effektiv även när den påbörjas en hel vecka efter stroke. I en tänkt framtid skulle därmed de flesta strokepatienter kunna behandlas med C3a, även de som inte är hjälpta av trombolys eller trombektomi.

Det övergripande målet med detta projekt är att överföra denna nya behandlingsstrategi till klinisk praxis.

## Bakgrund och forskningsläge

**C3a** is a peptide generated by the proteolytic activation of the third complement component (C3) **and functions through a G-protein coupled receptor called C3aR**. In a multicenter pre-clinical study, we showed that intranasal treatment with C3a accelerates functional recovery after ischemic stroke and the positive effects of treatment are sustained weeks after treatment cessation [1, 2]. For the clinical translation, it is particularly important that the treatment was **effective even when it was started a full week after stroke**. Our unpublished data show that ischemic stroke in the cortex triggers long-lasting inflammatory response and neurodegeneration in the ipsilesional thalamus. These stroke-induced processes are enhanced in mice lacking C3aR and reduced in mice that received daily intranasal treatment with C3a starting 7 days after stroke (Fig. 1). The beneficial effect of C3a treatment in terms of reduced secondary neurodegenerative changes was independently confirmed by our neuroimaging study (Fig. 2).

## Syfte och frågeställningar

We developed and patented (US 11,266,715, EP 3541402) a clinically feasible pharmacological strategy that (1) accelerates functional recovery and (2) inhibits post-stroke secondary neurodegeneration in mice. The therapeutic window of 1 week, as opposed to the time limit in hours for the blood clot removing interventions, means that the majority, if not all, stroke survivors would potentially benefit from intranasal treatment with C3a.

**The overall goal of this project is to translate this novel treatment into clinical practice.**

The C3a used in our experimental studies was prepared by purification from human plasma. For clinical testing, we have now established the feasibility of large-scale production of fully active recombinant human C3a.

**As a next step in translating our experimental stroke therapy to the clinic and in preparation for phase 1 clinical study, we aim to** perform the necessary pharmacokinetics and toxicology studies and regulatory safety assessment in animals with recombinant C3a

## Material och metod

**Pharmacokinetics studies** to determine the absorption and distribution of intranasally administered recombinant C3a are performed in mice.

## Toxicology and safety studies in animals

Maximum tolerated dose (MTD) and the effects of 3 week-long treatment with MTD of recombinant C3a will be determined in mice. As we did not observe any adverse effects of intranasal administration of human plasma-derived C3a in mice, we do not anticipate

any serious side effects of recombinant C3a. These studies will be conducted under *good laboratory practice (GLP)* protocol by an accredited contract research organization - discussions with RISE and Charles River are ongoing. Satisfactory results of studies in mice will be followed by studies in larger animals such as dogs or pigs.

### **First-in-human Phase 1b clinical trial with intranasally administered C3a**

The primary goal of the Phase 1 study is (A) **to identify possible side effects** and (B) **to study the absorption and distribution of intranasally administered recombinant human C3a in humans to ensure its safety**. As the secondary goal and proof-of-concept, we aim to assess functional recovery and secondary neurodegeneration.

The integrated protocol (single and multiple doses) for first-in-human Phase 1b study will be prepared together with Gothia Forum and Clinical Trial Center, Sahlgrenska University Hospital (SU) and in a dialogue with experts at Läkemedelsverket. Between 10 and 20 individuals discharged with or without home rehabilitation and supported by the Stroke support team and the Neurology clinic, SU will be recruited (responsible physician K. Jood). The first dose will be given one week after stroke with the treatment given once daily for up to 3 weeks and follow-up for 1 month at the Clinical Trial Center, SU and up to 6 months (Neurology clinic, SU).

Permit for the study will be obtained from Läkemedelsverket and the Ethical Review Authority (Etikprövningsmyndigheten). Further we will apply for approval to by the Regional Biobank Centre. The trial will be conducted at Clinical Trial Center and Neurology clinic, SU and monitored by Gothia Forum.

**Outcome measures** (most relevant for Phase 2/3 studies):

#### **Primary outcome measures:**

Changes from baseline score in NIH Stroke Scale

Modified Rankin scale (ordinal shift) at 90±7 days after incident stroke

#### **Secondary outcome measures:**

Changes from baseline score in Stroke Impact Scale

#### **Surrogate markers of efficacy:**

Serum NfL level change between day 7 and 3/6 months after stroke (biomarker of secondary neurodegeneration, Clinical Neurochemistry, SU, collab. with Prof. H. Zetterberg).

Diffusion-weighted MRI of the brain (lesion size and microstructural damage within major white matter tracts and thalamus, Dept. of Radiology, SU prior to treatment and 3 and 6 months after stroke, collab. with Prof. I. Björkman-Burtscher).

## Tidsplan för det kommande året

Pharmacokinetics studies in mice, analytical method development (Jan-March 2026)

Pilot in vivo toxicity studies (non-GLP) to determine MTD (April-June 2026)

Study planning, protocol finalization for regulatory in vivo toxicology studies in mice (in a dialogue with Läkemedelsverket and e.g RISE) (May-Sept. 2026)

Regulatory in vivo toxicology in mice (GLP; e.g. RISE) (Oct. 2026 – May 2027)

Study planning, protocol finalization for regulatory in vivo toxicology and pharmacokinetic studies in larger animals (in dialogue with Läkemedelsverket and e.g. RISE) (Dec. 2026 – June 2027)

## Betydelse för strokepatienter

The project has the potential to provide a desperately needed pharmacological treatment to **enhance recovery and improve long-term outcome after stroke. In addition, our findings point to the use of C3a as an attractive strategy for the prevention of stroke-induced cognitive impairment and depression.** The much broader time window, as opposed to the time limit in hours for the blood clot removing interventions, means that such a treatment could help to reduce neurological impairments and improve the quality of life for the majority of, if not all, stroke survivors.

## Etiska överväganden

The project has the potential to enhance recovery and improve long-term outcome after stroke and thus to brake new grounds in the post-acute care of stroke survivors. The animal studies described here have been approved by the Animal ethics committee in Gothenburg (it was not possible to select that option under the Ethical permit section of this application. Permit for the clinical study will be obtained from Läkemedelsverket and the Ethical Review Authority (Etikprövningsmyndigheten) after the completion of the regulatory animal studies. Further we will apply for approval to by the Regional Biobank Centre. The trial will be conducted at Clinical Trial Center and Neurology clinic, SU and monitored by Gothia Forum.

## Referenser

1. Stokowska A, Atkins AL, Moran J, Pekny T, Bulmer L, Pascoe MC, et al. Complement peptide C3a stimulates neural plasticity after experimental brain ischemia. *Brain*. 2017;140:353-69.
2. Stokowska A, Aswendt M, Zucha D, Lohmann S, Wieters F, Morán Suarez J, et al. Complement C3a treatment accelerates recovery after stroke via modulation of astrocyte reactivity and cortical connectivity. *J Clin Invest*. 2023;133(10):e162253.

## Relevanta publikationer

### **[C3a Receptor Signaling Inhibits Neurodegeneration Induced by Neonatal Hypoxic-Ischemic Brain Injury.](#)**

Pozo-Rodrigálvarez A, Li Y, Stokowska A, Wu J, Dehm V, Sourkova H, Steinbusch H, Mallard C, Hagberg H, [Pekny M](#) et al [ [Pekna M](#) Nr. 11 ].

Front Immunol 2021:12:768198.

### **[Complement C3a treatment accelerates recovery after stroke via modulation of astrocyte reactivity and cortical connectivity.](#)**

Stokowska A, Aswendt M, Zucha D, Lohmann S, Wieters F, Morán Suarez J, Atkins AL, Li Y, Miteva M, Lewin J et al [ [Torinsson Naluai Å](#) Nr. 13 ] [ [Pekny M](#) Nr. 18 ] [ [Pekna M](#) Nr. 19 ].

The Journal of clinical investigation 2023:133(10):e162253.

### **[Plasma neurofilament light chain levels predict improvement in late phase after stroke.](#)**

Stokowska A, [Bunketorp Käll L](#), Blomstrand C, [Simrén J](#), Nilsson M, [Zetterberg H](#), [Blennow K](#), [Pekny M](#), [Pekna M](#).

Eur J Neurol 2021:28(7):2218-2228.

### **[Inflammation, Anti-inflammatory Interventions, and Post-stroke Cognitive Impairment: a Systematic Review and Meta-analysis of Human and Animal Studies.](#)**

Tack RWP, Amboni C, van Nuijs D, [Pekna M](#), Vergouwen MDI, Rinkel GJE, Hol EM.

Transl Stroke Res 2023