Neurotrophic factor mimetics as drug candidates against neurological diseases

The burden of neurological diseases

Current treatments of Parkinson's disease (PD) are symptomatic and none of the available drugs can attenuate or stop neurodegeneration. Neurotrophic factor glial cell line-derived neurotrophic factor (GDNF) not only protects, but also repairs the brain dopaminergic system in animal models of PD, and has validated therapeutic potential also for the treatment of amyotrophic lateral sclerosis (ALS) and addiction. Artemin (ARTN) is an homologous protein to GDNF and has great potential for the treatment of neuropathic pain. Neurodegenerative diseases, like Alzheimer's, PD and ALS, are caused by progressive loss of neurons in the brain or in the spinal cord. The estimation is that 23.4 million people live with Alzheimer's disease and more than 6 million live with PD today. Every 7 seconds there is a new case of Alzheimer's disease and every 36 seconds a new case of PD (Van Den Eeden et al., 2003). The incidence of ALS is much lower and does not exceed 130,000 new cases annually. The number of people affected by Alzheimer's disease will double every 20 years to 81.1 million by 2040 (Ferri et al., 2005). The prevalence of PD is also likely to rise sharply because of the rapidly aging population. The prevalence of peripheral neuropathy is estimated as 2.4%. However, the statistical data on the occurrence of this disorder are not always reliable. Because peripheral neuropathy can accompany a great number of other disorders, many cases go undiagnosed. It is thought that centralized and peripheral chronic pain disorders together were estimated to affect one-sixth of the population (Campbell and Meyer, 2006). The major problem for the management of these diseases is that only symptomatic treatments are currently available for patients. The drugs (molecules) used by clinicians can neither stop nor reverse the progression of these devastating disorders. In other words, none of the existing drugs is able to stop or even slow down the death of neurons.

Glial cell line-Derived Neurotrophic Factor (GDNF) family ligands can protect and repair brain neurons

Several proteins that were in clinical or pre-clinical trials were reported to slow down the rate at which neurons die and, in some cases, reversed the disease progression by promoting the survival and sprouting of the remaining neurons (Love et al., 2005). The molecule that does this for PD is termed GDNF for short. GDNF is a protein that supports the survival of dopamine producing neurons in the brain and motor neurons in the spinal cord. The GDNF and ARTN-based therapies hold a great promise, because in addition to the promotion of neuronal survival they also induce axonal regeneration, support the formation of synapses and stimulate neuronal functioning. For these reasons GDNF neurotrophic factors are probably the best candidates for the treatment of PD. In addition, GDNF is one of the few growth factors that not only protects, but also repairs dopamine neurons and enhances their functional activity in animal models of PD (Bespalov and Saarma, 2007; Lindholm et al., 2007). GDNF has also many other functions in the organism but it is mainly due to its ability to support dopamine and motor neurons that GDNF is attracting much attention. These neuronal populations are affected in Parkinson's disease and ALS, respectively. The related to GDNF molecules neurturin (NRTN), ARTN and persephin (PSPN) also support various neuronal populations i.e. they are all neurotrophic factors (Fig. 1). NRTN, similarly to GDNF, can support the survival of dopamine and motor neurons. ARTN is a potent neurotrophic factor for the peripheral nervous system (Airaksinen and Saarma, 2002; Andressoo and Saarma, 2008). In animal experiments ARTN has potential for the treatment of chronic pain (Figure 1).

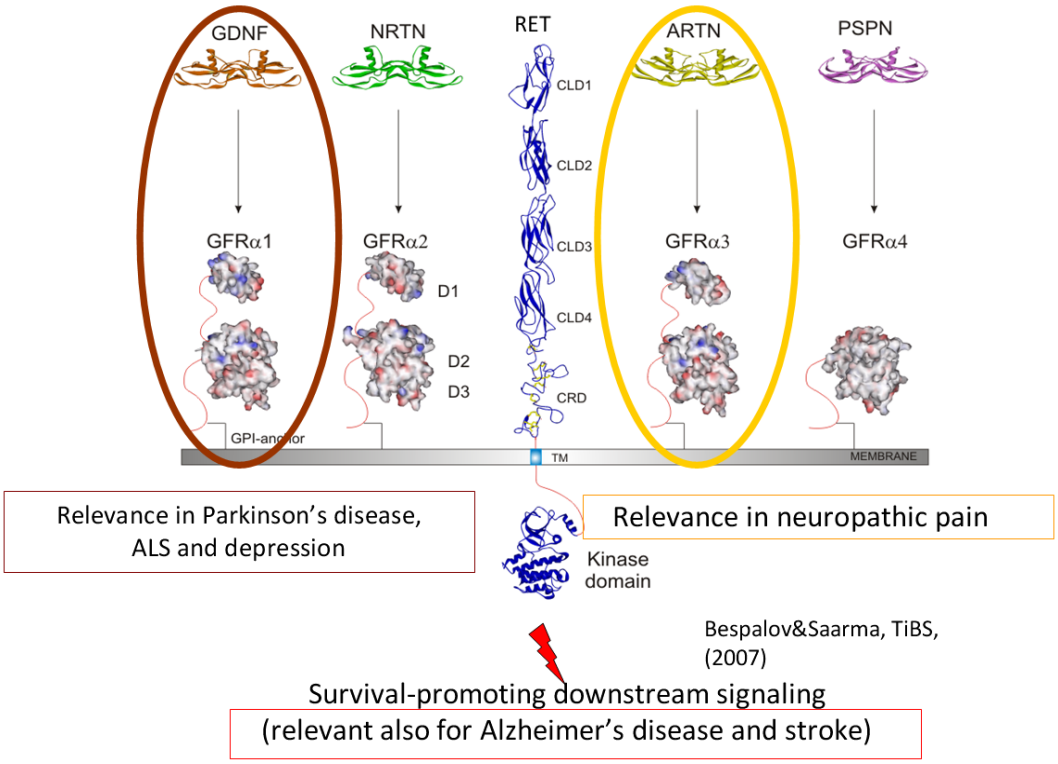


Figure 1. Clinical indications related to the GDNF family neurotrophic factors.

Discovery of the GDNF mimetics as agents against Parkinson's disease

The development of small molecule drugs mimicking GDNF action is crucially important for the development of conceptually new drugs that can slow down or even reverse neurodegeneration. GDNF and related proteins as therapeutic proteins are not ideal pharmacological agents. They are sticky proteins of more than 100 amino acids in length, which are unable to penetrate the blood-brain barrier. Therefore, brain surgery is required to deliver GDNF or NRTN to treat Parkinson's disease. Furthermore, exogenous GDNF may induce inflammation and formation of anti-GDNF antibodies (Lang et al., 2006) and the price of recombinant GDNF is high. Finally, GDNF is promiscuous; it activates RET not only through its own co-receptor GFRα1, but weakly also through GFRα2 and GFRα3, the co-receptors for NRTN and ARTN. Furthermore, GDNF can also activate completely different receptors: neural cell adhesion molecule NCAM and syndecan glycoproteins that carry GDNF-binding heparan sulphate side chains.These pleiotropic GDNF actions might lead to multiple side-effects. Small molecule GDNF-mimetics that specifically bind to the GFRα1 co-receptor and activate RET would be devoid of many of these problems.

Using the knowledge of the crystal structure of GDNF-GFRα1 complex (Leppänen et al., 2004; Parkash et al., 2008) and by the combination of structure-based drug design and chemoinformatics it was possible to find the first small molecules mimicking GDNF (Bespalov and Saarma, 2007). We first developed a range of novel cell-based methods that allow monitoring RET activity. From low-throughput RET phosphorylation assay based on Western blotting to a medium-throughput immunological method, which also monitors RET activity directly (RET-ELISA) to the high-throughput reporter-gene assay to monitor activity of RET downstream kinases. The assays have average Z' value of 0.6 and 0.7 for RET-ELISA and reporter-gene, respectively. We used the latter assay for hit identification and the former assay for hit validation. Using the novel RET-ELISA assay and the high-throughput reporter-gene assay in combination with molecular docking and with the original structure-activity relationship (SAR) analysis developed at MolCode Ltd. we first discovered 21 diverse

chemical compounds that mimic GDNF and ARTN action. The optimal concentration for the most potent compounds is 5-10 μM, but with optimization we have developed few compounds having activity below 500nM. The discovered molecules can, similarly to GDNF, activate the GFRα/RET receptor complex (Fig. 2).

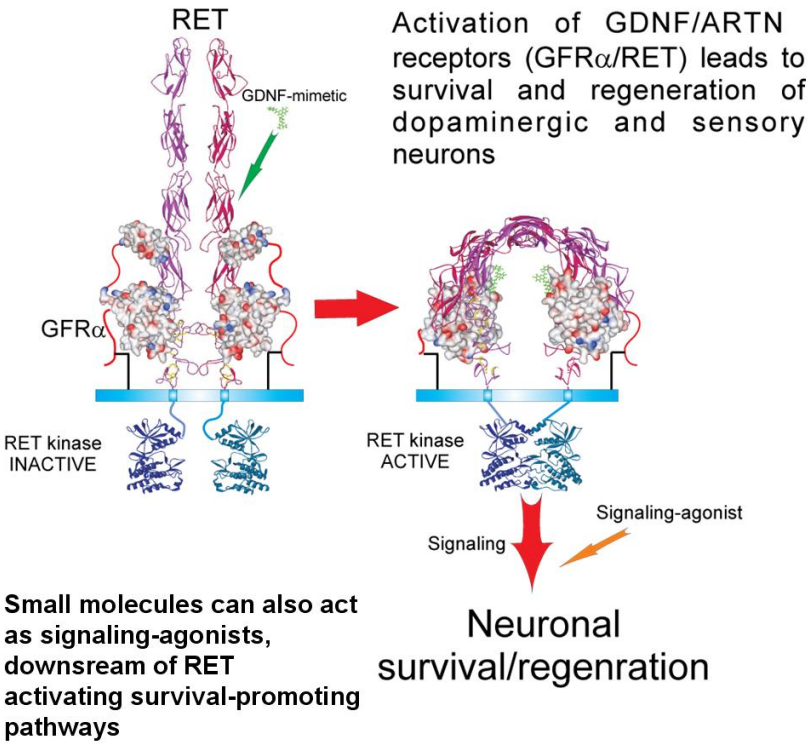


Figure 2. Putative mechanism of action of GDNF/ARTN-mimetics.

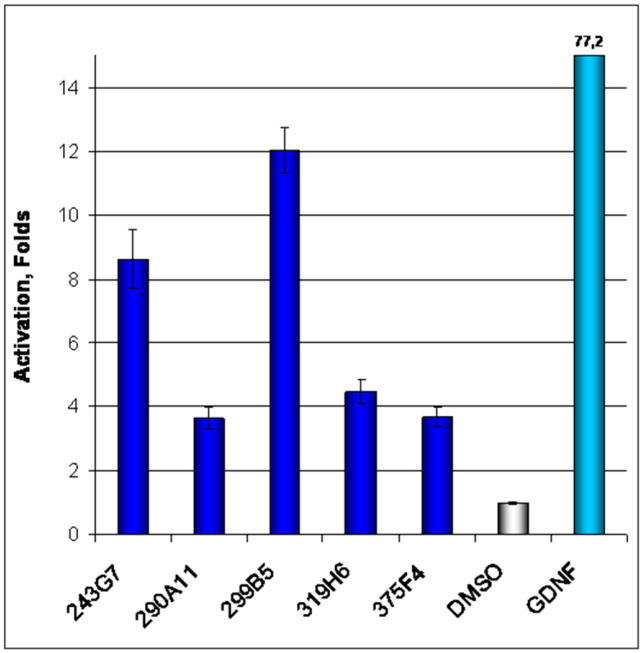


Figure 3. Small molecules potently activate RET downstream signaling at 5 μM concentration.

To further diversify the set of active molecules we performed a high-throughput screening of a large “diversity” chemical library. As a result we identified 5 molecules that potently induce RET downstream signaling (independently of GFRα1) at concentrations below 5 μM (our preliminary data demonstrate that some compounds are active at 100 nM) (Fig. 3). These compounds are non-toxic and do not induce proliferation in fibroblasts.

To further characterize the neurotrophic and neuroprotective efficacy of developed compounds their survival promoting effects on rat embryonic mesencephalic dopaminergic neurons in culture were assessed after neurotoxin MPP^+ treatment. In this classical dopaminotrophic assay we tested six GFRα1-RET agonists and two RET downstream activators and compared their effects with those of GDNF and brain-derived neurotrophic factor (BDNF). Five out of six GFRα1-RET agonists and both RET downstream activators efficiently promoted the survival of dopamine neurons already at 50nM concentrations. The best compounds were almost as effective as BDNF.

The second generation of GDNF mimetics was recently developed by using Molcode, Ltd. unique fragment-based in silico technology (QSARSOFT© Wizard) and some of the selected compounds have much higher activity than the compounds of the first generation (Figure 4).

The neuroprotective and neurorestorative efficacy of five most potent compounds are currently tested in the rat 6-OHDA model of PD.

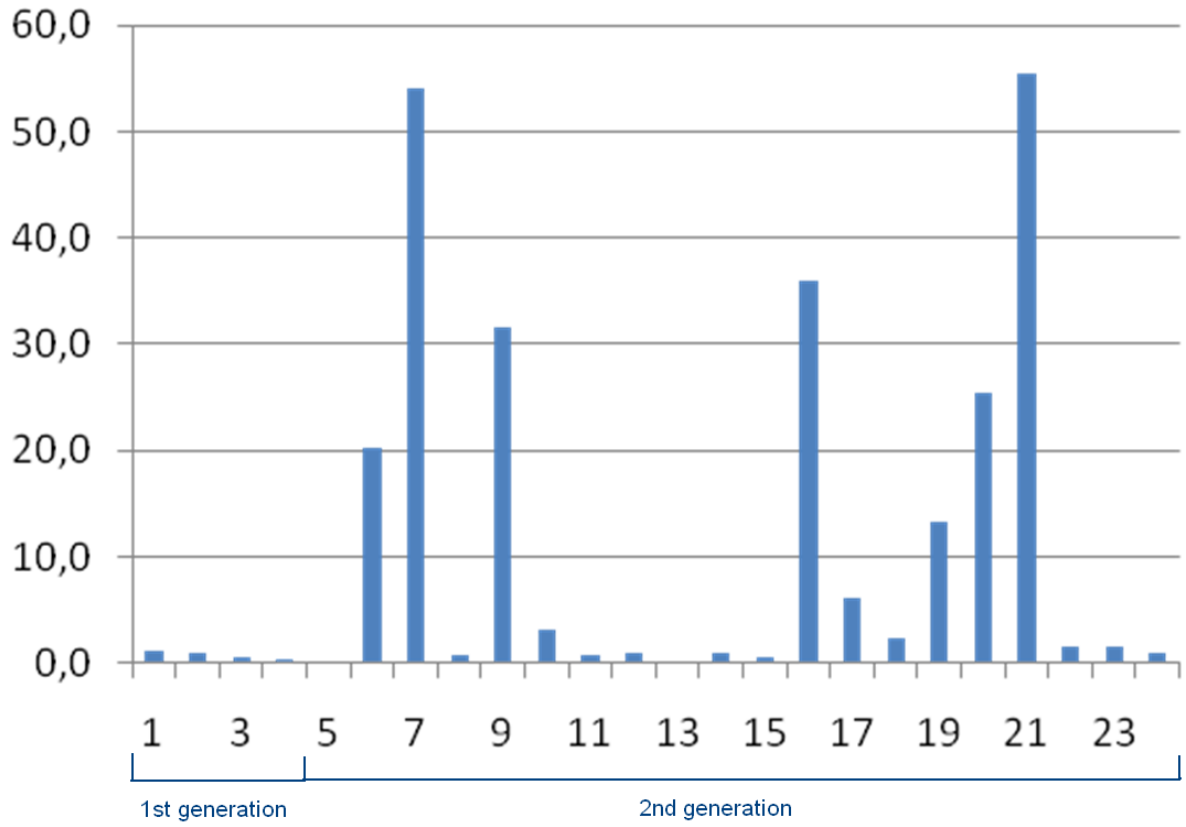


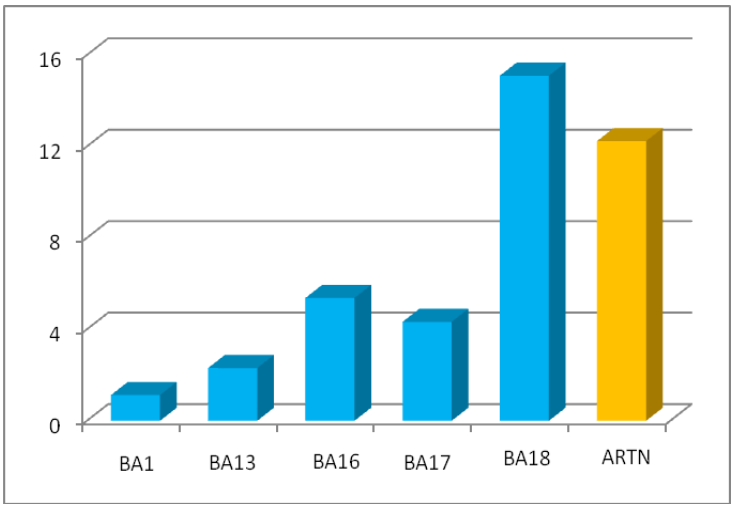
Figure 4. Activation in GFRα1/RET-expressing cells. Agonists at 10 μM concentration.

Discovery of artemin (ARTN) mimetics as agents against neuropathic pain

The chronic pain or neuropathy was estimated to affect up to one sixth of the Western population. The current therapies poorly control the chronic pain and a major breakthrough is required to improve the quality of life for millions of affected people. The neuropathy can be caused by the lesion of the peripheral sensory neurons or of the central neurons. Neuropathy can also develop as a result of diseases e.g. diabetes. Several proteins supporting neuronal survival were effective against this devastating disorder in animal models and in clinical trials (e.g. artemin (ARTN) or anti-NGF antibodies). As currently available therapies for the treatment of neuropathy are largely symptomatic, the neurotrophic factor-based therapies hold a great promise, because in addition to the promotion of neuronal survival they also induce axonal regeneration, support the formation of synapses and stimulate neuronal functioning. However, the proteins are large, often labile molecules with poor pharmacokinetic properties. Therefore, our goal was to find small molecule ARTN-mimetics.

By using QSARSOFT© Wizard, 15 active compounds were predicted, the best of them exhibiting the comparable activity with ARTN or even exceeding it! In Figure 5, the representative data are given for several small-molecule mimetics as compared to ARTN. The measurements were carried out by BTD's proprietary assays described above.

Figure 5. Activation in GFRα3/RET-expressing cells by ARTN and ARTN mimetics.



We believe that the developed compounds will be much more efficient and safer alternatives to current symptomatic treatments of Parkinson's disease, ALS, addiction and pain, but possibly also for several other neurological diseases.

Contact

Dr. Mart Saarma
Professor
Institute of Biotechnology
Viikinkaari 9 (P.O.Box 56)
00014 University of Helsinki
FINLAND
Tel: +358-9-1915 9359
Fax: +358-9-1915 9366
Cell: +358-5-0500-2726
E-mail: mart.saarma@helsinki.fi

Dr. Mati Karelson
Professor
Chairman
Baltic Technology Development Ltd.
Ahtri Street 8, 10151 Tallinn
ESTONIA
Phone: +372 5 371 536
Fax: +372 7 371 535
Cell: +372 5 021 519
E-mail: mati.karelson@molcode.com