Aligning cures across neurodegenerative diseases (ACAND)

The proposed project aims at treating neurodegenerative diseases (NDs) like Alzheimer's Disease (AD), Parkinson's Disease (PD) and in polyglutamine diseases, including Huntington's disease (HD), spinobulbar muscular atrophy (SBMA), and dominant forms of spinocerebellar ataxia (SCA).
We performed *in vitro* and preclinical studies in rodent models of NDs and developed three therapeutic approaches ("Verticals"). Crucially, these approaches are highly complementary and can be used for multiple diseases.
We have also developed tools ("Horizontals") that are instrumental to consolidate our findings and take them to a clinical setting.

Vertical 1 - Gene therapy for polyGlutamine diseases
Graziano Martello - UniPD (4 mesi/uomo)
Maria Pennuto - UniPD (1 mesi/uomo)

Long polyGlutamine (polyQ) tracts cause incurable neurodegenerative diseases, such as HD, SBMA and SCAs.
We identified gene modifiers suppressing polyQ toxicity. Their delivery, via mRNAs and AAV vectors, ameliorated the phenotype of two animal models of HD, acting on the homeostasis of metals (Zn, Cd, Fe, Cu) and Ca^{2+}.
Our aim is to deliver by AAVs these suppressor genes to human neurons and brain organoids, and test their efficacy in human cells, optimize their delivery and enter TRL 6.

For SBMA, we identified epigenetic regulators, whose silencing by artificial miRNA (amiR, patent pending) ameliorates the symptoms of SBMA mice. Our aim is to optimise the delivery of amiRs by AAVs in human organoids and animal models and translate our amiRs to the clinics (TRL6).

Synergies
Testing our candidates in human neurons and brain organoids will be based on Horizontal 1, while the optimisation of the delivery will be based on Horizontal 2.
Moreover, we will test the efficacy of our gene modifiers in multiple diseases (i.e.,SBMA, SCAs, PD, AD) to extend the range of applications, given that altered homeostasis of metals and Ca^{2+} are associated with such pathologies.

Vertical 2 - Targeting neuroinflammation in NDs
Paola Pizzo - UniPD (4 mesi/uomo)
Stefano Salmaso - UniPD (1 mesi/uomo)
Dorianna Sandonà - UniPD (1 mese/uomo)

Neuroinflammation is a main pathogenic factor in AD and other NDs. Extracellular ATP (eATP) is a major proinflammatory agent, reaching high concentrations (even hundreds of micromoles/L) at inflammatory sites and stimulating P2 purinergic receptors, with P2X7R being the subtype most frequently involved. ATP can leak passively following cell injury or death, but more frequently is released via non-lytic pathways, such as pannexins (Panx)-mediated channels.
The P2X7R, mostly silent at low, physiological [eATP] but activated at [eATP] found at inflammatory sites, is expressed by microglia, astroglia and oligodendroglia and is one of the most potent activators of the NLRP3 inflammasome, driving the release of IL-1β and other cytokines (TNFα, IL-6, CCL3). P2X7R is upregulated in human AD brains and in AD mouse reactive microglia surrounding Aβ plaques. Of note, *P2rx7* deletion or P2X7R pharmacological blockade in AD mice alleviated memory loss, synaptic impairment and neuropathological alterations, including Aβ deposition and inflammation. On the same line, downmodulation of eATP-release pathways by pharmacological inhibition of Panx-1 mitigated neuronal damage in AD mice, suggesting that the eATP-P2X7R axis is a crucial pathogenic pathway and an appealing therapeutic target in AD.
Our aim is to delivery specific siRNA-nanocarriers, targeting either P2X7R or Panx-1, in primary microglia/astrocytes isolated from WT and AD (B6.152H) mice (activated by Aβ peptides), and *in vivo* in AD mice by intrathecal administration.

Synergies
Delivery in mice will be optimised using the splitGFP system described in Horizontal 2.
Since neuroinflammation is common across NDs, we will test whether the eATP-P2X7R axis could be a feasible target also in PD, HD and SBMA cellular/animal models.
Furthermore, the efficacy of the therapeutic approaches described in Verticals 1 and 3 could be verified by investigating neuroinflammation via i*n vivo* measurements of [eATP] in the brains of different ND animal models. We will use the luminescent ratiometric probe pmeLUC/nilla specifically targeted to the brain (by AAV2/9 stereotactic injection).
Moreover, since P2X7R is shed and accumulates into circulation during several disease conditions in parallel with C-reactive protein, thus behaving in principle as a marker of inflammation, shed P2X7R (sP2X7R; an indirect indication of [eATP]) levels could be measured in both CSF and blood from ND animal models upon different treatments.

Vertical 3 - Targeting protein aggregation by RNA vaccines
Angelo Antonini - UniPD (2 mesi/uomo)
Luigi Bubacco - UniPD (2 mesi/uomo)
Elisa Greggio - UniPD (2 mesi/uomo)
Miryam Carecchio – UniPD (1 mese uomo)
Roberta Biundo – UniPD (1 mese uomo)

Disease modification is an unmet need in NDs, like PD, HD and AD, which all share common mechanisms of protein aggregation and neuronal degeneration.

The possibility to induce active immunization against α-synuclein has been demonstrated many years ago in animal models, with reduction of accumulated α-synuclein in neurons with mild microglia activation.
Neither neuroinflammation nor neurotoxicity was noted. The levels of physiologic α-synuclein were not affected and the animal models showed improved motor and cognitive function

We aim at developing an RNA-based vaccine stimulating active immunization against pathological alpha-synuclein and, subsequently, use the same approach to find immunizing peptides to be used also for AD.
First, we will identify the most suitable antigenic polypeptides specifically inducing a sustained immune response against pathological alpha-synuclein. Second, we will develop an RNA transcript that carries the sequence of the antigenic polypeptides and test it in PD animal models. Finally, we will optimise their delivery in human neurons and brain organoids derived from iPS cells.

Synergies
Preclinical testing of immunizing peptides in PD mouse models (PFFs mouse,1-120 syn mouse and LRRK2 G2019S mouse).
Possible immunizing peptides against Ab plaques will be also tested in AD (B6.152H) mice.

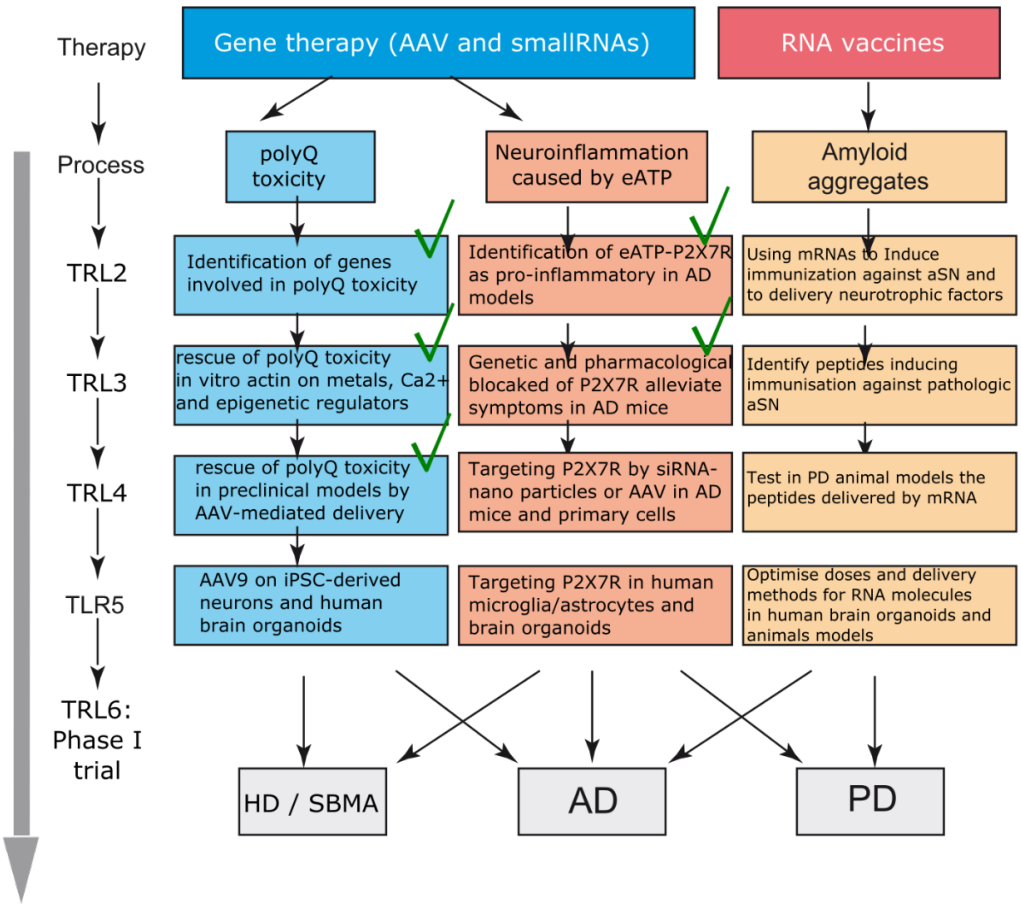
Horizontal 1
Graziano Martello - UniPD (4 mesi/uomo)
Nicola Elvassore - UniPD (2 mesi/uomo)

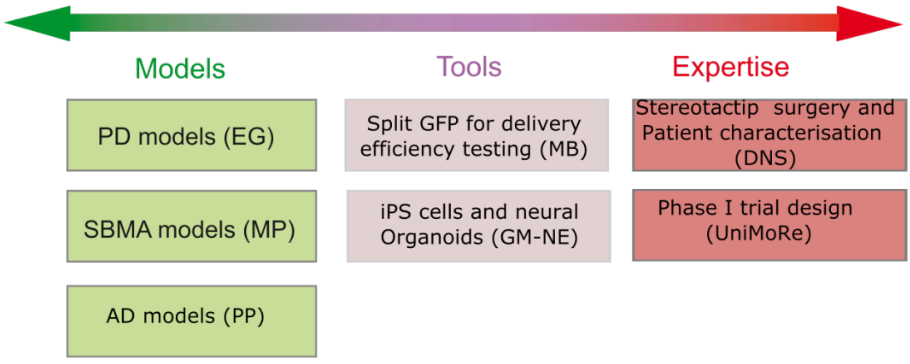
The development of therapeutic approaches requires the testing of efficacy in human neurons, specifically carrying mutations causing the diseases under study.
Thus, we will exploit the capacity to generate transgene-free high quality iPS cells from primary cells of the Martello and Elvassore lab.
After obtaining patient specific iPS cells for HD, SBMA and SCA, AD, we will conduct direct differentiation via delivery of mRNAs to obtain human neurons. Furthermore, we will generate brain organoids from patient specific iPS cells using 3D ECM-based systems optimised in the Elvassore laboratory.

Horizontal 2
Marisa Brini - UniPD (1 mesi/uomo)
Tito Calì - UniPD (1 mesi/uomo)

The evaluation of the efficiency of gene modifiers, siRNA-nanocarriers and RNA-based vaccine delivery is a key step in selecting promising approaches with potential clinical relevance. The cutting-edge splitGFP based technology developed at the University of Padua will be exploited to explore either in vitro or in vivo the efficiency and intracellular selectivity of the proposed tools.

Aligning Cures Across Neurodegenerative Diseases (ACAND)





CENES Centro Studi per la Neurodegenerazione