Editorial
Need of translational research on hearing loss recovery

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In its report on deafness and hearing loss, updated to August 2021, the World Health Organization estimates that 5.5% of the world population is affected by these diseases and this percentage is expected to increase up to 25% in 2050 (WHO, 2021a). Untreated hearing impairment leads to poor life quality, affecting health-care systems and educational support, leading to a significant decrease in productivity and in familiar and social life. The economic global impact of untreated hearing loss, including direct and indirect costs and intangible/societal ones was estimated in 2019 at 981 billion dollars per year (McDaid et al., 2021). Considering the costs of hearing devices (around $ 2,000 per device) (hearingtracker.org, 2021), of cochlear implants and consequently of hearing rehabilitation, the resulting economic impact has been estimated as 0.003% of GDP per capita in Australia and 0.022% in Netherland (data referring to 2015) (WHO, 2017). In this economic framework, the most important impact is related to the benefit of hearing rehabilitation, which could lead to a 5% reduction in the prevalence of people with hearing loss and therefore a reduction of social costs of at least $ 50 billion a year (McDaid et al., 2021).

The incidence of hearing loss and deafness could be reduced thanks to the timeliness and accuracy of diagnosis and interventions. At present, hearing can be recovered by applying hearing aids or cochlear implants since, unfortunately, a definitive pharmacological intervention has not yet been developed (Haile et al., 2021; McDaid et al., 2021). This is the main reason why researchers are increasing studies on molecular biology and physiology of hearing pathologies, such as sudden hearing loss, noise-induced hearing loss, ototoxicity and age-related hearing loss, applying new technologies, surgical approaches and compounds aimed to develop innovative pharmaceutical treatments or improve existing ones (Le et al., 2017; Ding et al., 2019; Gentilin et al., 2019; Freyer et al., 2020; Carlyon and Goehring, 2021; Lei et al., 2021; Peixoto et al., 2021; Sharma et al., 2021).

The development of an effective pharmaceutical therapy to treat the inner ear is a scientific challenge as well as a collective necessity with invaluable economic and social implications (Haile et al., 2021; McDaid et al., 2021). Gene therapy, regenerative medicine and nanotechnologies are emerging research fields that address this need. However, in all cases investigators must consider how to administer the compounds to the inner ear in a non-traumatic way (Simoni et al., 2017; Valente et al., 2017; Plontke and Salt, 2018; Patel et al., 2019; Delmaghani and El-Amraoui, 2020). In addition to the systemic and oral route, the local administration of substances has become a preferential route based on intratympanic injection of substances subsequently released near or through the round membrane (Patel et al., 2019). Inside the cochlea, compounds could be released dispersed in solutions, conjugated to nanoparticles or embedded into controlled-release matrices, or else delivered through infusion pumps (Forouzandeh et al, 2019; Lee et al.,

2020), cochlear electrodes (Astolfi et al., 2014,2016; Plontke et al., 2017; Simoni et al., 2020), and microneedle injections (Watanabe et al., 2016; Chiang et al., 2020; Aksit et al., 2021).

Concerning translational research, there are few clinical trials aimed at recovering hearing loss through local drug delivery. Two of them are based on gene therapy (NCT02132130, NCT03996824) three on stem cells delivery (one completed NCT02038972 and two "suspended"), and only one is dealing with a round window injection of a new compound, FX-322 (NCT03300687; McLean et al., 2021).

The last study is divided in five trials and is presently in Phase 2. The FX-322 is a combination of a glycogen synthase kinase-3 (GSK3) inhibitor (CHIR99021) and valproic acid (VPA). Previous investigations in *in vitro* and mice models (McLean et al., 2017) showed the potential synergy of the two compounds in directing hair cell regeneration. The same authors published in 2021 the results of pharmacokinetics in Guinea pig and human perilymph and the phase 1b study (NCT03616223), showing the safety of the compounds locally delivered in selected patients affected by unilateral sensorineural hearing loss (SNHL) (McLean et al., 2021). The study involved 15 patients treated with FX-322 vs 8 with placebo. Among patients treated with a single dose of FX-322, five significantly improved their scores in word recognition in quiet (WR) and signal-to-noise ratio (SNR) after 90 days from treatment. The study had nevertheless several limits, among which multiple levels of baseline hearing loss and variability and imbalance of the basic scores of vocal perception between the experimental groups and the limited sample size (McLean et al., 2021). The improved WR and SNR scores encouraged the authors to test the positive effects over a longer time frame and in a larger sample, and to verify the effectiveness of treatment even in patients with different aetiologies and severities of SNHL, with the aim of understanding which conditions could benefit most from treatment with FX-322 (Frequency therapeutics, May 2021).

Intermediate results obtained from Phase 2a study (NCT04120116, FX-322 in Adults With Stable Sensorineural Hearing Loss, FX-322-202) in which 95 participants with mild to moderately severe SNHL were treated with one to four repeated doses, were unfavourable. Although the safety of the treatment was confirmed and a favourable increase in hearing ability was detected, unexpected improvements were also detected in the placebo group. Frequency Therapeutics (the company that is founding the study) declared that the results were probably due to a bias in the trial design (Frequency therapeutics, May 2021). At the same time, the follow-up of five patients whose hearing ability had improved in a previous study showed positive results in four of them. These results encouraged the authors to return to single-dose treatments, since repetitive injections may have led to vascular alterations in the cochlear microcirculation (Frequency therapeutics, May 2021). The inclusion criteria for patient enrolment were noise-induced or sudden SNHL. The aetiology of sudden SNHL is unknown in 70% of cases, but a common factor of known cases is the alteration of cochlear microcirculation leading to hypoxia (Cho et al., 2012; Li et al., 2015). The treatment of sudden SNHL is therefore based on associations of different classes of drugs, such as vasoactive substances (aspirin, heparin), corticosteroids (prednisolone, dexamethasone), diuretics, vitamins and antioxidant factors, with the aim of increasing deformability of erythrocytes, reduce blood and plasma viscosity, and inhibit the production of oxygen radicals (Schreiber et al., 2010; Greco et al., 2011). Previous clinical trials about alternative to drugs reported the application of selective apheresis for LDL and fibrinogen (Heparin-induced Extracorporeal Low-density lipoprotein Precipitation, HELP-apheresis), or rheopheresis (Bianchini et al., 2011; Greco et al., 2011; Canis et al., 2012). The importance of the vascular component in causing hearing loss and in its recovery was also confirmed by a study emphasizing the equal efficacy of treatment with pharmacological therapy associated with HELP-apheresis or with rheopheresis (Klingel et al., 2009).

About studies on effectiveness of FX-322, two new trials were planned with a single injection: the trial FX-322-112 (NCT04601909), focused on presbycusis patients, and the trial FX-322-113 (NCT04629664), focused on patients with severe SNHL. Currently, no results are yet available on the second trial,

and from those on the first trial no significant effects were unfortunately detected when comparing patients treated with FX-322 with those with placebo. The importance of finding a cure for presbycusis lies in the fact that about one third of people over the age of 65 have disabling hearing loss, and the total number of elderly people is estimated to increase from 1 billion in 2019 to 1. 4 billion by 2030 and 2.1 billion by 2050 (WHO 2021b). In addition, there is increasing evidence of the neurodegenerative implication in age-related hearing loss, thus age-related cognitive decline could be slowed by treatments for hearing loss (Castiglione et al, 2019, Erb et al., 2020). Probably, the authors did not obtain positive results in their study because presbycusis may be due not only to sensorineural damage, but also to metabolic (vascular stria) and mechanical (spiral ligament) factors (Schuknecht and Gacek, 1993). It would probably be informative for researchers to have more details on the clinical and scientific features of the study.

In conclusion, pending the publication of the final results of FX-322, this study divided into several clinical trials emphasizes the need for further research on the effectiveness of new therapies, carefully considering the different etiologies and severities that characterize deafness.

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