Published in final edited form as:

Curr Otorhinolaryngol Rep. 2018 March ; 6(1): 15–23. doi:10.1007/s40136-018-0186-4.

The Role of Tumor Necrosis Factor Alpha (TNF α) in Hearing Loss and Vestibular Schwannomas

Yin Ren, MD, PhD^{1,2}, Konstantina M. Stankovic, MD, PhD^{1,2,3,4}

¹Department of Otolaryngology, Massachusetts Eye and Ear, 243 Charles Street, Boston, MA 02114, USA

²Department of Otolaryngology, Harvard Medical School, 25 Shattuck St, Boston, MA 02115, USA

³Eaton Peabody Laboratories, Massachusetts Eye and Ear, 243 Charles Street, Boston, MA 02114, USA

⁴Harvard Program in Speech and Hearing Bioscience and Technology, 25 Shattuck Street, Boston, MA 02115, USA

Abstract

Purpose of review: The aim of this review is to highlight relevant literature on the role of tumor necrosis factor alpha (TNF α) in sensorineural hearing loss (SNHL) and vestibular schwannomas (VS).

Recent Findings: A comprehensive review of publically available databases including PubMed was performed. The mechanism by which hearing loss occurs in VS is still unknown and likely multifactorial. Genetic differences between VSs and tumor secreted proteins may be responsible, at least in part, for VS-associated SNHL. TNF α has pleiotropic roles in promoting inflammation, maintaining cellular homeostasis, inducing apoptosis, and mediating ototoxicity in patients with sporadic VS. TNF α -targeted therapies have shown efficacy in both animal models of sensorineural hearing loss and clinical trials in patients with immune-mediated hearing loss. Efforts are underway to develop novel nanotechnology-based methods to target TNF α and other pathogenic molecules in VS.

Summary: Development of molecularly targeted therapies against TNF α represents an important area of research in ameliorating VS-associated hearing loss.

Keywords

TNF alpha; TNF α ; vestibular schwannoma; nanotechnology; inflammation; hearing loss

CORRESPONDING AUTHOR: Konstantina M. Stankovic, MD, PhD, Eaton Peabody Laboratories, Department of Otolaryngology, Massachusetts Eye and Ear, 243 Charles Street, Boston, MA 02114, USA, konstantina_stankovic@meei.harvard.edu **Phone:** 617-573-3972.

CONFLICT OF INTEREST: Dr. Y. Ren and Dr. K. Stankovic declare that they have no conflicts of interest.

HUMAN AND ANIMAL RIGHTS AND INFORMED CONSENT: This article does not contain any studies with human or animal subjects performed by any of the authors.

Introduction

Vestibular schwannomas (VSs) are the most common tumors of the cerebellopontine angle (CPA) and the fourth most common intracranial tumors overall [1]. They arise from Schwann cells lining cranial nerve VIII (vestibular nerve). Although benign in nature, they are associated with substantial morbidity due to their location within the CPA and proximity to the brainstem. These include asymmetric sensorineural hearing loss (SNHL) which affects up to 95% of patients, tinnitus, dizziness, cranial neuropathies such as facial palsy, brainstem compression, and hydrocephalus [2]. Today, surgical resection and stereotactic radiation remain the main therapeutic modalities for growing VSs; however, neither can address VS-associated SNHL. While several recent clinical trials on systemic therapies for neurofibromatosis type 2 (NF2)-associated VSs have shown promising results, there are currently no FDA-approved drugs to halt VS growth or ameliorate VS-associated hearing loss [3, 4].

Tumor necrosis factor alpha (TNF α) is a pro-inflammatory cytokine with important key functions in diverse cellular processes, such as regulation of pro-inflammatory responses and maintenance of cellular homeostasis. Aberrant signaling in TNF α or in its cognate receptor (TNFR) has been implicated in diseases including rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, and inflammatory bowel disease. Dysregulation of TNF function has also been shown to be associated with autoimmunity and cancer. While much is known about the role of TNF in these disorders and several therapeutics targeting TNF α have been approved for clinical use, little is known about the association of TNF signaling with hearing loss.

The functions of the TNF α cytokine in cancer are complex and have been subjects of intensive laboratory investigation. Since its initial discovery as a serum factor that induced necrosis of tumor cells, TNF α was thought to exert its downstream signaling effects by acting on the tumor vasculature to compromise its blood supply. Recombinant TNF α exhibited cytostatic effects on certain cancer cell lines *in vitro*, an effect that was augmented by interferon-gamma (IFN- γ) [5]. More recent work suggested that effects of TNF on tumor cells may also be secondary to induction of senescence, a state of permanent growth arrest in cancer cells observed in animal models [6]. In the context of established tumors, TNF α has been shown to promote tumorigenesis by activating survival signaling *via* upregulation of NF κ B, contribute to immunosuppression by enabling escape from immunosurveillance, and facilitate metastasis by inducing the synthesis of matrix metalloproteinases [7, 8].

In this report, the pathophysiology of VS tumorigenesis and VS-associated SNHL are reviewed, in the context of TNF signaling. The roles of TNF in mediating inflammation and hearing loss are examined. Finally, studies aiming to develop targeted TNF-based therapies for treatment of VS-associated hearing loss are summarized.

Vestibular Schwannomas and Hearing Loss

The overall incidence of VS is estimated at approximately 1.09 per 100,000 population in the US. While the vestibular nerve, not the cochlear nerve, typically serves as a site of

predilection for tumor growth, up to 95% of patients with VSs are affected by SNHL [9, 10]. VS was first observed at an autopsy in 1777 and the first case of VS and associated SNHL was described by Sir Charles Bell in 1833 [11]. Nevertheless, the precise mechanism by which SNHL occurs is still unknown and likely multifactorial. One hypothesis suggests that SNHL is caused, at least in part, by mechanical compression of the adjacent auditory nerve and compromise of the cochlear vascular supply. However, while some correlations exist between tumor size and the degree of SNHL, especially for NF2-associated VSs, neither the overall radiographic dimension nor the intracannalicular extent of VSs fully correlates with the degree of SNHL. Furthermore, a subset of patients with non-growing VSs develop progressive hearing loss [12, 13]. Therefore, nerve compression does not appear to fully account for the degree of SNHL in sporadic VS.

A second hypothesis postulates that cochlear damage occurs because of ototoxic and neurotoxic tumor metabolites reaching the cochlea. This is supported by the findings that decreased distortion product otoacoustic emissions (DPOAEs), generated by outer hair cells (OHCs), are observed in VS patients with mild SNHL [14], suggesting that OHC dysfunction could be a primary event rather than secondary to blockade of auditory neurons. Furthermore, damage to hair cells and cochlear neurons is a common post-mortem finding, affecting ~90% of human temporal bones with ipsilateral untreated VS [9].

More recently, studies have suggested that differences in the inherent genetic landscape of VSs and secretions from VS may be responsible in the hearing loss observed in patients. When our laboratory compared gene expression profiles between VS with good hearing versus poor hearing, we found several differences, including in the expression of genes associated with peroxisomal biogenesis [15]. The expression of platelet-derived growth factor (PDGF-A) was found to be inversely correlated with the degree of SNHL in VS patients [16]. Focusing on VS tumor secretions, the overall protein levels in the perilymphatic fluid was 5–15 times higher in patients with VS than healthy controls, the basis for the diagnosis of VS prior to the advent of MRI scans [17, 18]. Our laboratory and others have used liquid chromatography tandem mass-spectrometry based proteomic analysis to reveal a specific pattern of expression of proteins in the perilymph and differential expression between patients with VSs and those without, suggesting that proteins in VS secretions may modulate cochlear degeneration [19, 20].

Tumor angiogenesis governed by secreted pro-angiogenic factors such as vascular endothelial growth factor (VEGF) and inflammation mediated by TNF are two distinct but closely interlinked processes in the context of tumor survival and growth. TNF α signaling is dependent on the VEGF pathway to recruit inflammatory macrophages and activate lymphatic endothelial cells, thereby promoting lymphangiogenesis and metastasis [21]. Therapeutics targeting VEGF may therefore reduce the level of TNF α secreted by VS, and mitigate cochlear hair cell and spiral ganglion neuron damage caused by ototoxic cytokines [13, 22]. The most promising results reported in the literature involved NF2 patients treated with Bevacizumab, a monoclonal antibody against VEGF. Hearing improvement was observed in approximately 57% of NF2 patients which was independent from a concomitant decrease in tumor size [23, 24].

Molecular Biology of TNF α

In a landmark paper from Carswell *et al.* in 1975, a factor made by host cells in response to endotoxin destroyed tumors in animals with experimental cancers. This factor was named “tumor necrosis factor” due to its functional activity [25]. The gene was cloned and its full spectrum of activity was characterized in the mid-1980s [26–28]. TNF α is a member of the TNF superfamily (TNFSF) that consists of 19 ligands, which interacts with 29 receptors as a part of the TNF receptor superfamily (TNFRSF) including TNF receptor 1 (TNFR1) and TNFR2. Newly synthesized TNF is initially expressed as a trimeric transmembrane protein, which is cleaved by the metalloproteinase TNF-converting enzyme to release soluble extracellular TNF. The generation of soluble TNF is a tightly regulated process that occurs as a response to various types of stimuli, and is controlled in part by rhomboid protein 2.

TNFR1 is expressed ubiquitously in almost every cell type, bears conserved death-domain motifs, and is activated by both soluble and membrane-bound TNF. Binding of TNF to TNFR1 recruits the adapter molecule TNFR1-associated death domain protein (TRADD) and leads to activation of transcription factors including NF κ B and mitogen-activated protein kinases which induce inflammation, tissue degeneration, and apoptosis. TNFR2, which lacks a death-domain motif, is only expressed in specific cell types such as endothelial cells, immune cells, and neurons. Activation of TNFR2 is primarily by transmembrane TNF *via* cell-cell interactions. TNFR2 recruits TNFR-associated factor 2 (TRAF2) and triggers activation of NF κ B, MAPKs, and AKT, which is mainly responsible for promoting cellular homeostasis including survival and proliferation [29].

TNF plays a critical role in diseases characterized by chronic inflammation and thereby serves as an attractive drug target. Currently, five molecular therapeutics against TNF α have been approved for the treatment of rheumatoid arthritis (RA), inflammatory bowel disease (IBD) such as Crohn disease and ulcerative colitis, psoriasis, psoriatic arthritis, juvenile idiopathic arthritis, ankylosing spondylitis, and hidradenitis suppurativa (Table 1). Overproduction or dysregulated hyper-function of TNF activates vascular endothelium, recruits immune cells and ultimately results in tissue destruction. In addition, TNF signaling has also been implicated in cellular homeostatic functions, including defense against pathogens and induction of tissue regeneration such as neuronal remyelination and cartilage repair [30, 31]. By contrast, the complex role TNF plays in the organs of hearing and balance, both in maintaining normal functions and in conditions of hearing loss, is less well-understood.

TNF α in Animal Models of Hearing Loss

The expression of TNF α and the efficacy of TNF α blockade in animal models of hearing loss have been investigated extensively (Table 2). TNF α is typically undetectable in the normal cochlea [32], but is up-regulated with aging, inflammation, or acoustic trauma. In mice, increased expression of HIF-1 targets, including TNF α , was found in the modiolus, spiral ganglion, and stria vascularis at 4 weeks of age, possibly related to hypoxic stress-induced inflammation [33]. Intracholear perfusion of keyhole limpet hemocyanin (KLH) in a mouse model of immune-mediated labyrinthitis led to a rapid accumulation of TNF α -

producing inflammatory cells, which was reduced with systemic injection of Etanercept, a TNF α receptor blocker [34]. In a mouse model of acoustic trauma, TNF α and IL-6 were elevated in the cochlea within hours after noise exposure, and followed a time course similar to that in other CNS organs after ischemia or trauma [35]. Similarly, transcanal vibration in guinea pigs led to increased cochlear shear stress and increased expression of TNF α , TNFR1 and TNFR2 [32].

TNF α is also up-regulated in acute or chronic otitis media (COM) and thereby contributes to inflammation-related middle ear remodeling. Specifically, both TNF α and IL-1 were significantly elevated by over four-fold in the middle ear and over two-fold in the inner ear of mice with COM [36]. Similarly, middle ear TNF α levels doubled following trans-tympanic injections with *Haemophilus influenza* to mimic acute otitis [37]. Direct inoculation of cerebral spinal fluid (CSF) with *S. pneumoniae* in rats led to bacterial meningitis and subsequent rise in numerous pro-inflammatory cytokines, with TNF α increased by over 10-fold. Auditory brainstem response (ABR) hearing thresholds were also elevated and correlated with TNF α levels in the CSF. Histopathological analyses showed that both spiral ganglion neurons and OHCs are affected, especially in the cochlear basal turn [38].

Activation of TNF α was also seen with treatment with cisplatin, a platinum-based chemotherapeutic and gentamicin, an aminoglycoside antibiotic. The ototoxic effects of cisplatin were thought to be due to, at least in part, by the generation and release of pro-inflammatory cytokines. In immortalized cochlear cell culture, cisplatin decreased cell viability and increased synthesis of TNF α , IL-1, and IL-6 [39]. Immunohistochemical staining demonstrated that TNF α expression was localized to the spiral ligament, spiral limbus and organ of Corti [39, 40]. These cytokines were also elevated in the serum of cisplatin-injected rodents, an effect that was rescued by administration of Etanercept. Interestingly, pre-treatment with Etanercept *via* intra-tympanic instillation protected OHC damage and reduced ABR threshold shifts [41]. In rat organ of Corti explants, treatment with dexamethasone led to reduction of both TNF α and TNFR1, decreased generation of reactive oxygen species and expression of pro-apoptosis genes [42]. *In vivo* infusion of Rat cochleae with dexamethasone protected against gentamicin induced OHC loss and hearing damage [43].

While substantial research has attempted to elucidate the role of TNF α in mediating inflammatory responses in auditory hair cells *in vitro*, little is known about the *in vivo* auditory phenotype in transgenic animals where TNF signaling is altered. Two transgenic mouse models have been established [44–47]. Mice deficient in TNF α developed more variable and incomplete frequency representations in the cortex, weaker cortical response to tones, and less refinement after exposure to multiple frequencies compared to wild type [48]. TNF α -deficient homozygous mutant mice exhibit higher ABR thresholds and reduced DPOAE amplitudes, which was attributed to defects in OHC function as evidenced by sporadic absence of stereocilia in the cochlear basal turn and distortion in the middle turn on electron microscopy [49]. Nevertheless, hearing in mice deficient in TNFR1 or TNFR2 has not been well characterized.

TNF α may also affect hearing *via* a vascular-based mechanism through modulation of the cochlear microcirculation, and inhibition of TNF α may improve cochlear blood flow. Blood flow in the cochlear lateral wall vessels in guinea pigs was reduced in a dose-dependent fashion after perfusion with TNF α as measured by intravital microscopy, which was abrogated when the animals received Etanercept either as pretreatment prophylaxis or post-treatment rescue [50, 51]. In guinea pigs exposed to acoustic trauma, administration of Etanercept led to increased cochlear blood flow and preservation of ABR thresholds [50, 52]. In a separate study, guinea pigs undergoing cochlear implantation received instillation of Etanercept into the perilymph *via* an osmotic mini-pump. This resulted in preservation and recovery of hearing thresholds as early as 3 days post-operatively, with maximal benefits at 28 days [53].

TNF α in Hearing Disorders

Much of the existing literature has focused on the efficacy of TNF antagonist therapies in disorders of the inner ear where autoimmunity may play an important role, including autoimmune inner ear disease (AIED), idiopathic sudden sensorineural hearing loss (ISSNHL), and Meniere's disease (MD). AIED is a syndrome of progressive, often fluctuating, rapid bilateral SNHL. In AIED, immunosuppressive therapies such as high-dose corticosteroids [54], methotrexate [55], and cyclophosphamide [56] have been shown to have some benefit albeit with significant toxicity. In ISSNHL, Demirhan *et al.* reported that patients who did not respond to glucocorticoids had higher serum levels of TNF α 6-weeks after treatment, suggesting that TNF α may play a role in the steroid response [57]. In a small group of subjects with non-glucocorticoid responsive sudden hearing loss, treatment with Etanercept led to some recovery of hearing within two weeks [58]. Nonetheless, controversies remain regarding the levels of pro-inflammatory cytokines, including TNF α , IL-6 and IL-8, in patients with SSNHL [59].

Meniere's disease (MD) is an inner ear disorder characterized by intermittent episodes of vertigo, fluctuating SNHL, tinnitus, and aural fullness. While the precise etiology of MD is still unclear, autoimmunity appeared to be closely associated with its pathogenesis. To support this hypothesis, elevated levels of circulating immune complexes were found in a group of 30 patients with MD [60]. Elsewhere, elevated thyroid autoantibodies were found in a higher proportion of MD patients than healthy subjects [61]. In a large series of patients with both MD and SNHL, there was a higher prevalence of autoimmune disorders including RA, systemic lupus erythematosus and ankylosing spondylitis than expected for the general population. However, no significant difference in the serum levels of pro-inflammatory cytokines including TNF α was observed in patients with MD versus those without [62].

In patients with VS, TNF α has been identified as a putative ototoxic molecule in tumor secretions. This was demonstrated in several ways by Dilwali *et al.* [63]. There was a positive correlation between serum TNF α level and the degree of SNHL in patients with sporadic unilateral VS. When tumor secretions were applied to murine cochlear explants, the extent of cochlear cellular damage correlated with the severity of hearing loss. Secretions from tumors with the most substantial SNHL led to the most significant damage to hair cells and spiral ganglion neuronal fibers, whereas secretions from matched tumors without

hearing loss led to minimal damage. Finally, to investigate whether tumor-secreted TNF α has a direct role in mediating ototoxicity, TNF α was neutralized in tumor secretions with a blocking antibody, which significantly reduced secretion-induced damage in cochlear explants. Applying recombinant TNF α directly to cochlear explants resulted in neurite loss and disorganization in the cochlear basal turn. Together, these results suggested that TNF α plays a key role in mediating SNHL in VS by directly damaging hair cells and neurites.

Anti-TNF α Therapy in Hearing Restoration and Vestibular Schwannomas

Given the pleotropic role of TNF α in orchestrating the inflammatory response in animal models of hearing loss, there have been numerous investigations on the efficacy of pharmacological targeting of TNF, albeit with mixed results (Table 3). In a series of 9 patients with AIED dependent on steroid treatment, intratympanic administration of Infliximab, a monoclonal anti-TNF α antibody, allowed tapering of steroids and improvement in pure tone average (PTA) thresholds [64]. Elsewhere in a series of 10 patients with steroid-dependent AIED, intratympanic injection of Golimumab, an antibody that binds both soluble and membrane-bound TNF α , resulted in hearing stabilization [65]. There is a case report of a patient with both RA and steroid-refractory SNHL where simultaneous resolution of arthritis and improvement of hearing were seen after the administration of Adalimumab, an antibody that binds TNF α and blocks receptor interactions [66]. In a retrospective small series of 12 patients with bilateral, long-standing immune-mediated SNHL treated with Etanercept, 92% of patients had stabilization or improvement of hearing and 90% had improvement of vertigo over short-term follow-up [67]. Similar results were reported in an open-label study of Etanercept in immune-mediated hearing loss and Meniere's disease, where hearing was stabilized in 87% of patients [68]. However, conflicting results were reported in a randomized, placebo-controlled study of 20 patients with AIED, where 8-week treatment with Etanercept did not show any significant benefit in hearing restoration [69]. Therefore, larger series are still needed to validate the efficacy of TNF biologics in immune-mediated hearing loss.

Latest work from our laboratory and others have begun to uncover the ototoxic properties of TNF α in VS tumor secretions as well as therapeutic strategies targeting TNF α for hearing loss, both in cochlear explants *in vitro* and in animal models *in vivo*. In one study, over 70% of VS tissues expressed TNF α , whereas only 5% of healthy vestibular nerve controls stained positive by immunohistochemistry [70]. To ascertain whether neutralization of TNF α in VS tumor secretions can rescue hair cell damage and neurite loss, an anti-human TNF α antibody was added to secretions from patients with VS and significant SNHL that also contained high levels of TNF α . Reduction of TNF α by nearly 80% prevented the loss of IHCs in the apical and basal turns of murine cochlear explants but did not affect the fiber organization of neurites [63].

Recently, we have also begun to develop a nanotechnology-based platform for preclinical testing of therapeutics in VS [71]. Fresh tumor cells derived from patients with sporadic VS were found to express two unique receptors, α v β 3/ β 5 integrins and neuropilin-1, which distinguish them from normal tissue counterparts. Nanoparticles were generated from a mixture of tandem peptides consisting of a targeting domain that selectively binds α v β 3/ β 5

integrins, and a membrane-penetrating domain that complexes with short interfering RNA (siRNA) against TNF α . The nanoparticles were taken up by primary VS cultures, delivered TNF α -specific siRNA, and resulted in potent suppression of TNF α secretion by VSs when exposed to a pro-inflammatory stimulus. This is the first study that demonstrated tumor-targeting nanotechnology and RNA interference can be leveraged synergistically to mitigate the secretion of ototoxic molecules including TNF α . As more promising therapeutic targets for VS and VS-associated hearing loss emerge from large-scale genome-wide screens [72], the nanoparticle system can be adapted to validate other genes of interest, and ultimately identify those suitable for *in vivo* testing and clinical translation.

Furthermore, targeting TNF α in VS may also have beneficial effects on tumor growth through interactions with other molecular pathways, which in turn regulate TNF α levels. The transcription factor NF κ B mediates the induction of COX-2 expression and production of reactive oxygen species *via* proinflammatory cytokines including TNF α [73, 74]. Specifically, anti-inflammatory medications that signal through NF κ B [75] or COX-2 [76] were found to reduce VS cellular proliferation *in vitro*, in line with our findings that aspirin intake correlated with reduced growth of VS *in vivo* [77]. Taken together, our findings have not only motivated the ongoing prospective, randomized, placebo-controlled phase II clinical trial of aspirin for VSs, but also further investigation in the role of TNF inhibition as an anti-tumor therapy to suppress VS growth [78].

Given the immunosuppressive nature of anti-TNF therapy, there is concern of increased incidence of adverse events such as serious and opportunistic infections including tuberculosis, and malignancies such as skin cancers and lymphomas in patients undergoing therapy. Importantly, studies suggest that chronic inflammation in these conditions is itself associated with an increased risk for lymphoma [79–81]. Indeed, long-term safety studies from clinical trials showed the overall incidence of lymphoma was approximately 105.9 per 100,000 person-years, similar to the population of RA patients not on anti-TNF biologics, but greater than age- and gender-matched general populations without RA [82–84].

Conclusions

TNF α is a pleiotropic cytokine with diverse functions, including maintaining cellular homeostasis and mediating the inflammatory response in various disease states. Insights into the underlying biology of TNF signaling have led to the development of molecularly-targeted therapeutics in inflammatory conditions where aberrant TNF activation plays an important role. Selective inhibition of TNF has yielded moderate benefits in disorders of hearing loss although results are limited to animal models and early clinical trials.

Recent insights into VS biology have further enriched our understanding of TNF-mediated cellular injury, especially in the context of VS-associated cochlear damage and hearing loss. Further, large-scale genome-wide screens have led to the discovery of additional pathways aberrantly activated to promote tumor survival and hearing loss. Deployment of new delivery strategies such as tumor-targeted nanotechnology to address these targets, including TNF α , represents a new therapeutic paradigm for the treatment of VS and VS-associated hearing loss.

Many important challenges remain in our understanding of the complex roles TNF α plays in hearing loss, and how to best translate this knowledge into better treatments. Looking forward, several important research directions include development of a deeper understanding of the pathways that underlie and regulate TNF α responses, discovery of TNF-induced molecules that may be responsible for sustaining chronic inflammation and cochlear damage, and identification of additional markers in patients that may predict clinical response to anti-TNF therapy. Future challenges ahead lie in creating biologics that selectively blunt the deleterious effects of TNF α and can be delivered specifically to tumor cells in patients with VS and debilitating hearing loss.

Acknowledgments

FUNDING: No external sources of funding.

References

Papers of particular interest, published recently, have been highlighted as: * Of importance

1. Mahaley MS, Mettlin C, Natarajan N, Laws ER, Peace BB (1990) Analysis of patterns of care of brain tumor patients in the United States: a study of the Brain Tumor Section of the AANS and the CNS and the Commission on Cancer of the ACS. *Clin Neurosurg* 36:347–352 [PubMed: 2295209]
2. Thakur JD, Banerjee AD, Khan IS, Sonig A, Shorter CD, Gardner GL, Nanda A, Guthikonda B (2012) An update on unilateral sporadic small vestibular schwannoma. *Neurosurg Focus* 33:E1
3. Jacob A, Oblinger J, Bush ML, et al. (2012) Preclinical validation of AR42, a novel histone deacetylase inhibitor, as treatment for vestibular schwannomas. *Laryngoscope* 122:174–189 [PubMed: 22109824]
4. Goutagny S, Raymond E, Esposito-Farese M, Trunet S, Mawrin C, Bernardeschi D, Larroque B, Sterkers O, Giovannini M, Kalamarides M (2015) Phase II study of mTORC1 inhibition by everolimus in neurofibromatosis type 2 patients with growing vestibular schwannomas. *J Neurooncol* 122:313–320 [PubMed: 25567352]
5. Sugarman BJ, Aggarwal BB, Hass PE, Figari IS, Palladino MA, Shepard HM (1985) Recombinant human tumor necrosis factor- α : effects on proliferation of normal and transformed cells in vitro. *Science* 230:943–945 [PubMed: 3933111]
6. Braumüller H, Wieder T, Brenner E, et al. (2013) T-helper-1-cell cytokines drive cancer into senescence. *Nature* 494:361–365 [PubMed: 23376950]
7. Zhao X, Rong L, Zhao X, et al. (2012) TNF signaling drives myeloid-derived suppressor cell accumulation. *J Clin Invest* 122:4094–4104 [PubMed: 23064360]
8. Elinav E, Nowarski R, Thaïs CA, Hu B, Jin C, Flavell RA (2013) Inflammation-induced cancer: crosstalk between tumours, immune cells and microorganisms. *Nat Rev Cancer* 13:759–771 [PubMed: 24154716]
9. Roosli C, Linthicum FH, Cureoglu S, Merchant SN (2012) Dysfunction of the cochlea contributing to hearing loss in acoustic neuromas: an underappreciated entity. *Otol Neurotol* 33:473–480 [PubMed: 22377650]
10. Matthies C, Samii M (1997) Management of 1000 Vestibular Schwannomas (Acoustic Neuromas): Clinical Presentation. *Neurosurgery* 40:1–10 [PubMed: 8971818]
11. (1996) The Early History of the Neurofibromatoses: Evolution of the Concept of Neurofibromatosis Type 2. *Arch Otolaryngol Head Neck Surg* 122:1240–1249 [PubMed: 8906061]
12. Nadol JB, Diamond PF, Thornton AR (1996) Correlation of hearing loss and radiologic dimensions of vestibular schwannomas (acoustic Neuromas). *Am J Otol* 17:312–316 [PubMed: 8723968]
13. Caye-Thomasen P, Dethloff T, Hansen S, Stangerup S-E, Thomsen J (2007) Hearing in patients with intracanalicular vestibular schwannomas. *Audiol Neurotol* 12:1–12

14. Gouveris HT, Victor A, Mann WJ (2007) Cochlear origin of early hearing loss in vestibular schwannoma. *Laryngoscope* 117:680–683 [PubMed: 17429875]
15. Stankovic KM, Mrugala MM, Martuza RL, Silver M, Betensky RA, Nadol JB, Stemmer-Rachamimov AO (2009) Genetic determinants of hearing loss associated with vestibular schwannomas. *Otol Neurotol* 30:661–667 [PubMed: 19546833]
16. Lassaletta L, Martínez-Glez V, Torres-Martín M, Rey JA, Gavilán J (2009) cDNA microarray expression profile in vestibular schwannoma: correlation with clinical and radiological features. *Cancer Genet Cytogenet* 194:125–127 [PubMed: 19781445]
17. Silverstein H (1972) A rapid protein test for acoustic neurinoma. *Arch Otolaryngol* 95:202–204 [PubMed: 5013262]
18. Silverstein H (1973) Labyrinthine tap as a diagnostic test for acoustic neurinoma. *Otolaryngol Clin North Am* 6:229–244 [PubMed: 4220279]
19. Schmitt HA, Pich A, Schröder A, Scheper V, Lilli G, Reuter G, Lenarz T (2017) Proteome Analysis of Human Perilymph Using an Intraoperative Sampling Method. *J Proteome Res* 16:1911–1923 [PubMed: 28282143]
20. Lysaght AC, Kao S-Y, Paulo JA, Merchant SN, Steen H, Stankovic KM (2011) Proteome of human perilymph. *J Proteome Res* 10:3845–3851 [PubMed: 21740021]
21. Ji H, Cao R, Yang Y, et al. (2014) TNFR1 mediates TNF- α -induced tumour lymphangiogenesis and metastasis by modulating VEGF-C-VEGFR3 signalling. *Nat Commun* 5:4944 [PubMed: 25229256]
22. Brieger J, Bedavanija A, Lehr H-A, Maurer J, Mann WJ (2016) Expression of Angiogenic Growth Factors in Acoustic Neurinoma. *Acta oto-laryngologica* 123:1040–1045
- 23*. Plotkin SR, Stemmer-Rachamimov AO, Barker FG, Halpin C, Padera TP, Tyrrell A, Sorensen AG, Jain RK, di Tomaso E (2009) Hearing improvement after bevacizumab in patients with neurofibromatosis type 2. *N Engl J Med* 361:358–367 [PubMed: 19587327] First demonstration that VEGF blockade can improve hearing in patients with NF2 vestibular schwannomas.
24. Plotkin SR, Merker VL, Halpin C, Jennings D, McKenna MJ, Harris GJ, Barker FG (2012) Bevacizumab for progressive vestibular schwannoma in neurofibromatosis type 2: a retrospective review of 31 patients. *Otol Neurotol* 33:1046–1052 [PubMed: 22805104]
25. Carswell EA, Old LJ, Kassel RL, Green S, Fiore N, Williamson B (1975) An endotoxin-induced serum factor that causes necrosis of tumors. *Proc Natl Acad Sci USA* 72:3666–3670 [PubMed: 1103152]
26. Pennica D, Nedwin GE, Hayflick JS, Seeburg PH, Derynck R, Palladino MA, Kohr WJ, Aggarwal BB, Goeddel DV (1984) Human tumour necrosis factor: precursor structure, expression and homology to lymphotoxin. *Nature* 312:724–729 [PubMed: 6392892]
27. Gray PW, Aggarwal BB, Benton CV, et al. (1984) Cloning and expression of cDNA for human lymphotoxin, a lymphokine with tumour necrosis activity. *Nature* 312:721–724 [PubMed: 6334807]
28. Aggarwal BB, Kohr WJ, Hass PE, Moffat B, Spencer SA, Henzel WJ, Bringman TS, Nedwin GE, Goeddel DV, Harkins RN (1985) Human tumor necrosis factor. Production, purification, and characterization. *The Journal of biological chemistry* 260:2345–2354 [PubMed: 3871770]
29. Probert L (2015) TNF and its receptors in the CNS: The essential, the desirable and the deleterious effects. *Neuroscience* 302:2–22 [PubMed: 26117714]
30. Papathanasiou S, Rickelt S, Soriano ME, Schips TG, Maier HJ, Davos CH, Varela A, Kaklamanis L, Mann DL, Capetanaki Y (2015) Tumor necrosis factor- α confers cardioprotection through ectopic expression of keratins K8 and K18. *Nat Med* 21:1076–1084 [PubMed: 26280121]
31. Zhao S, Yin KW, Goodson NJ (2014) FRI0134 Association between Vitamin D Deficiency and Markers of Disease Activity in Axial Spondyloarthritis: Table 1. *Annals of the Rheumatic Diseases* 73:430.2–430
32. Zou J, Pyykko I, Sutinen P, Toppila E (2005) Vibration induced hearing loss in guinea pig cochlea: expression of TNF-alpha and VEGF. *Hear Res* 202:13–20 [PubMed: 15811694]
33. Riva C, Donadieu E, Magnan J, Lavieille J-P (2007) Age-related hearing loss in CD/1 mice is associated to ROS formation and HIF target proteins up-regulation in the cochlea. *Experimental Gerontology* 42:327–336 [PubMed: 17141999]

34. Satoh H, Firestein GS, Billings PB, Harris JP, Keithley EM (2002) Tumor necrosis factor- α , an initiator, and etanercept, an inhibitor of cochlear inflammation. *Laryngoscope* 112:1627–1634 [PubMed: 12352677]
35. Fujioka M, Kanzaki S, Okano HJ, Masuda M, Ogawa K, Okano H (2006) Proinflammatory cytokines expression in noise-induced damaged cochlea. *Journal of neuroscience research* 83:575–583 [PubMed: 16429448]
36. MacArthur CJ, Pillers DA, Pang J, Kempton JB, Trune DR (2011) Altered expression of middle and inner ear cytokines in mouse otitis media. *Laryngoscope* 121:365–371 [PubMed: 21271590]
37. Trune DR, Larrain BE, Hausman FA, Kempton JB, MacArthur CJ (2011) Simultaneous measurement of multiple ear proteins with multiplex ELISA assays. *Hear Res* 275:1–7 [PubMed: 21144888]
38. Perny M, Roccio M, Grandgirard D, Solyga M, Senn P, Leib SL (2016) The Severity of Infection Determines the Localization of Damage and Extent of Sensorineural Hearing Loss in Experimental Pneumococcal Meningitis. *Journal of Neuroscience* 36:7740–7749 [PubMed: 27445150]
39. So H, Kim H, Lee JH, et al. (2007) Cisplatin cytotoxicity of auditory cells requires secretions of proinflammatory cytokines via activation of ERK and NF- κ B. *Journal of the Association for Research in Otolaryngology* 8:338–355 [PubMed: 17516123]
40. Kim HJ, Oh GS, Lee JH, et al. (2011) Cisplatin ototoxicity involves cytokines and STAT6 signaling network. *Cell research* 21:944–956 [PubMed: 21321603]
41. Kaur T, Mukherjee D, Sheehan K, Jajoo S, Rybak LP, Ramkumar V (2011) Short interfering RNA against STAT1 attenuates cisplatin-induced ototoxicity in the rat by suppressing inflammation. *Cell Death Dis* 2:e180 [PubMed: 21776018]
42. Hoang KN, Dinh CT, Bas E, Chen S, Eshraghi AA, Van De Water TR (2009) Dexamethasone treatment of naive organ of Corti explants alters the expression pattern of apoptosis-related genes. *Brain research* 1301:1–8 [PubMed: 19747459]
43. Bas E, Van De Water TR, Gupta C, Dinh J, Vu L, Martinez-Soriano F, Lainez JM, Marco J (2012) Efficacy of three drugs for protecting against gentamicin-induced hair cell and hearing losses. *British journal of pharmacology* 166:1888–1904 [PubMed: 22320124]
44. Pasparakis M (1996) Immune and inflammatory responses in TNF α -deficient mice: a critical requirement for TNF α in the formation of primary B cell follicles, follicular dendritic cell networks and germinal centers, and in the maturation of the humoral immune response. *Journal of Experimental Medicine* 184:1397–1411 [PubMed: 8879212]
45. Erickson SL, de Sauvage FJ, Kikly K, Carver-Moore K, Pitts-Meek S, Gillett N, Sheehan KCF, Schreiber RD, Goeddel DV, Moore MW (1994) Decreased sensitivity to tumour-necrosis factor but normal T-cell development in TNF receptor-2-deficient mice. *Nature* 372:560–563 [PubMed: 7990930]
46. Pfeffer K, Matsuyama T, Kündig TM, Wakeham A, Kishihara K, Shahinian A, Wiegmann K, Ohashi PS, Krönke M, Mak TW (1993) Mice deficient for the 55 kd tumor necrosis factor receptor are resistant to endotoxic shock, yet succumb to *L. monocytogenes* infection. *Cell* 73:457–467 [PubMed: 8387893]
47. Rothe J, Lesslauer W, Lötscher H, Lang Y, Koebel P, Köntgen F, Althage A, Zinkernagel R, Steinmetz M, Bluethmann H (1993) Mice lacking the tumour necrosis factor receptor 1 are resistant to IMF-mediated toxicity but highly susceptible to infection by *Listeria monocytogenes*. *Nature* 364:798–802 [PubMed: 8395024]
48. Yang S, Zhang LS, Gibboni R, Weiner B, Bao S (2014) Impaired Development and Competitive Refinement of the Cortical Frequency Map in Tumor Necrosis Factor- α -Deficient Mice. *Cerebral Cortex* 24:1956–1965 [PubMed: 23448874]
49. Oishi N, Chen J, Zheng H-W, Hill K, Schacht J, Sha S-H (2013) Tumor necrosis factor- α -mutant mice exhibit high frequency hearing loss. *J Assoc Res Otolaryngol* 14:801–811 [PubMed: 23996384]
50. Sharaf K, Ihler F, Bertlich M, Reichel CA, Berghaus A, Canis M (2016) Tumor Necrosis Factor-induced Decrease of Cochlear Blood Flow Can Be Reversed by Etanercept or JTE-013. *Otology & Neurotology* 37:e203–e208 [PubMed: 27295443]

51. Ihler F, Sharaf K, Bertlich M, Strieth S, Reichel CA, Berghaus A, Canis M (2013) Etanercept prevents decrease of cochlear blood flow dose-dependently caused by tumor necrosis factor alpha. *Annals of Otology, Rhinology & Laryngology* 122:468–473
52. Arpornchayanon W, Canis M, Ihler F, Settevendemie C, Strieth S (2013) TNF-alpha inhibition using etanercept prevents noise-induced hearing loss by improvement of cochlear blood flow in vivo. *International Journal of Audiology* 52:545–552 [PubMed: 23786392]
53. Ihler F, Pelz S, Coors M, Matthias C, Canis M (2014) Application of a TNF-alpha-inhibitor into the scala tympany after cochlear electrode insertion trauma in guinea pigs: Preliminary audiologic results. *International Journal of Audiology* 53:810–816 [PubMed: 25311100]
54. Lobo D, García-Berrocal JR, Trinidad A, Verdaguer JM, Ramírez-Camacho R (2013) Review of the Biological Agents Used for Immune-Mediated Inner Ear Disease. *Acta Otorrinolaringologica (English Edition)* 64:223–229
55. Matteson EL, Tirzaman O, Kasperbauer J, Facer GW, Beatty CW, Fabry DA, McDonald TJ (2016) Use of Methotrexate for Autoimmune Hearing Loss. *Annals of Otology, Rhinology & Laryngology* 109:710–714
56. McCabe BF (1979) Autoimmune sensorineural hearing loss. *Annals of Otology, Rhinology & Laryngology* 88:585–589
57. Demirhan E, Eskut NP, Zorlu Y, Cukurova I, Tuna G, Kirkali FG (2013) Blood levels of TNF- α , IL-10, and IL-12 in idiopathic sudden sensorineural hearing loss. *Laryngoscope* 123:1778–1781 [PubMed: 23382065]
58. Scherer EQ, Yang J, Canis M, et al. (2010) Tumor necrosis factor- α enhances microvascular tone and reduces blood flow in the cochlea via enhanced sphingosine-1-phosphate signaling. *Stroke* 41:2618–2624 [PubMed: 20930159]
59. Haubner F, Martin L, Steffens T, Strutz J, Kleinjung T (2011) The role of soluble adhesion molecules and cytokines in sudden sensorineural hearing loss. *YMHN* 144:575–580
60. Derebery MJ, Rao VS, Siglock TJ, Linthicum FH, Nelson RA (1991) Ménière's disease: an immune complex-mediated illness? *Laryngoscope* 101:225–229 [PubMed: 1825685]
61. Nacci A, Dallan I, Monzani F, Dardano A, Migliorini P, Riente L, Ursino F, Fattori B (2010) Elevated antithyroid peroxidase and antinuclear autoantibody titers in Ménière's disease patients: more than a chance association? *Audiol Neurotol* 15:1–6 [PubMed: 19451704]
62. Gazquez I, Soto-Varela A, Aran I, Santos S, Batuecas A, Trinidad G, Perez-Garrigues H, Gonzalez-Oller C, Acosta L, Lopez-Escamez JA (2011) High prevalence of systemic autoimmune diseases in patients with Ménière's disease. *PLoS ONE* 6:e26759 [PubMed: 22053211]
- 63*. Dilwali S, Landegger LD, Soares VY, Deschler DG, Stankovic KM (2015) Secreted Factors from Human Vestibular Schwannomas Can Cause Cochlear Damage. *Sci Rep* 5:18599 [PubMed: 26690506] First demonstration that VS secretions rich in TNF α can cause direct cochlear damage.
64. van Wijk F, Staecker H, Keithley E, Lefebvre PP (2006) Local Perfusion of the Tumor Necrosis Factor α Blocker Infliximab to the Inner Ear Improves Autoimmune Neurosensory Hearing Loss. *Audiol Neurotol* 11:357–365
65. Derebery MJ, Fisher LM, Voelker CCJ, Calzada A (2014) An Open Label Study to Evaluate the Safety and Efficacy of Intratympanic Golimumab Therapy in Patients With Autoimmune Inner Ear Disease. *Otology & Neurotology* 35:1515–1521 [PubMed: 25203561]
66. Gazeau P, Saraux A, Devauchelle-Pensec V, Cornec D (2014) Long-term efficacy of infliximab in autoimmune sensorineural hearing loss associated with rheumatoid arthritis. *Rheumatology* 53:1715–1716 [PubMed: 24625506]
67. Rahman MU, Poe DS, Choi HK (2001) Etanercept Therapy for Immune-mediated Cochleovestibular Disorders: Preliminary Results in a Pilot Study. *Otology & Neurotology* 22:619–624 [PubMed: 11568668]
68. Matteson EL, Choi HK, Poe DS, Wise C, Lowe VJ, McDonald TJ, Rahman MU (2005) Etanercept therapy for immune-mediated cochleovestibular disorders: A multi-center, open-label, pilot study. *Arthritis Rheum* 53:337–342 [PubMed: 15934127]

- 69*. Cohen S, Shoup A, Weisman MH, Harris J (2005) Etanercept Treatment for Autoimmune Inner Ear Disease: Results of a Pilot Placebo-Controlled Study. *Otology & Neurotology* 26:903–907 [PubMed: 16151336] Randomized controlled trial of etanercept treatment in patients with AIED.
70. Taurone S, Bianchi E, Attanasio G, et al. (2015) Immunohistochemical profile of cytokines and growth factors expressed in vestibular schwannoma and in normal vestibular nerve tissue. *Mol Med Rep* 12:737–745 [PubMed: 25738867]
- 71*. Ren Y, Sagers JE, Landegger LD, Bhatia SN, Stankovic KM (2017) Tumor-Penetrating Delivery of siRNA against TNF α to Human Vestibular Schwannomas. *Sci Rep* 7:12922 [PubMed: 29018206] First demonstration that tumor-targeted nanotechnology and RNA interference can be leveraged synergistically to mitigate the secretion of ototoxic molecules including TNF α .
72. Agnihotri S, Jalali S, Wilson MR, et al. (2016) The genomic landscape of schwannoma. *Nat Genet* 48:1339–1348 [PubMed: 27723760]
73. Jobin C, Morteau O, Han DS, Balfour Sartor R (1998) Specific NF-kappaB blockade selectively inhibits tumour necrosis factor-alpha-induced COX-2 but not constitutive COX-1 gene expression in HT-29 cells. *Immunology* 95:537–543 [PubMed: 9893042]
74. Feng L, Xia Y, Garcia GE, Hwang D, Wilson CB (1995) Involvement of reactive oxygen intermediates in cyclooxygenase-2 expression induced by interleukin-1, tumor necrosis factor-alpha, and lipopolysaccharide. *Journal of Clinical Investigation* 95:1669–1675 [PubMed: 7706475]
75. Dilwali S, Briët MC, Kao S-Y, Fujita T, Landegger LD, Platt MP, Stankovic KM (2015) Preclinical validation of anti-nuclear factor-kappa B therapy to inhibit human vestibular schwannoma growth. *Mol Oncol* 9:1359–1370 [PubMed: 25891780]
76. Dilwali S, Kao S-Y, Fujita T, Landegger LD, Stankovic KM (2015) Nonsteroidal anti-inflammatory medications are cytostatic against human vestibular schwannomas. *Transl Res* 166:1–11 [PubMed: 25616959]
77. Kandathil CK, Dilwali S, Wu C-C, Ibrahimov M, McKenna MJ, Lee H, Stankovic KM (2014) Aspirin intake correlates with halted growth of sporadic vestibular schwannoma in vivo. *Otol Neurotol* 35:353–357 [PubMed: 24448296]
78. Stankovic KM (2017) Study of aspirin in patients with vestibular schwannoma In: <https://clinicaltrials.gov/ct2/show/NCT03079999?term=stankovic&rank=1>. *ClinicalTrials.gov* Identifier: . Accessed 30 Nov 2017
79. Hellgren K, Smedby KE, Feltelius N, Baecklund E, Askling J (2010) Do rheumatoid arthritis and lymphoma share risk factors?: a comparison of lymphoma and cancer risks before and after diagnosis of rheumatoid arthritis. *Arthritis Rheum* 62:1252–1258 [PubMed: 20155827]
80. Hellgren K, Smedby KE, Backlin C, Sundstrom C, Feltelius N, Eriksson JK, Baecklund E, Askling J (2014) Ankylosing spondylitis, psoriatic arthritis, and risk of malignant lymphoma: a cohort study based on nationwide prospectively recorded data from Sweden. *Arthritis Rheumatol* 66:1282–1290 [PubMed: 24782185]
81. Baecklund E, Iliadou A, Askling J, et al. (2006) Association of chronic inflammation, not its treatment, with increased lymphoma risk in rheumatoid arthritis. *Arthritis Rheum* 54:692–701 [PubMed: 16508929]
82. Burmester GR, Panaccione R, Gordon KB, McIlraith MJ, Lacerda APM (2013) Adalimumab: long-term safety in 23 458 patients from global clinical trials in rheumatoid arthritis, juvenile idiopathic arthritis, ankylosing spondylitis, psoriatic arthritis, psoriasis and Crohn's disease. *Annals of the Rheumatic Diseases* 72:517–524 [PubMed: 22562972]
83. Wolfe F, Michaud K (2007) The effect of methotrexate and anti-tumor necrosis factor therapy on the risk of lymphoma in rheumatoid arthritis in 19,562 patients during 89,710 person-years of observation. *Arthritis Rheum* 56:1433–1439 [PubMed: 17469100]
84. Ramiro S, Sepriano A, Chatzidionysiou K, et al. (2017) Safety of synthetic and biological DMARDs: a systematic literature review informing the 2016 update of the EULAR recommendations for management of rheumatoid arthritis. *Annals of the Rheumatic Diseases* 76:1101–1136 [PubMed: 28298374]

Table 1.

Currently FDA-approved TNF-based therapeutics

Drug (Trade name)	Description	Indications
Etanercept (Enbrel)	TNFR2 fused to IgG1 Fc	AS, JIA, plaque psoriasis, PsA, RA
Adalimumab (Humira)	Human whole monoclonal antibody against TNF	AS, Crohn disease, hidradenitis suppurativa, JIA, plaque psoriasis, PsA, RA
Certolizumab pegol (Cimzia)	PEGylated Fab' fragment of a humanized monoclonal antibody against TNF	AS, Crohn disease, PsA, RA
Golimumab (Simponi)	Human whole monoclonal antibody against TNF	AS, PsA, RA, ulcerative colitis
Infliximab (Remicade)	Chimeric whole monoclonal antibody against TNF	AS, Crohn disease, plaque psoriasis, PsA, RA, ulcerative colitis

Abbreviations: AS – ankylosing spondylitis, JIA – juvenile idiopathic arthritis, PsA – psoriatic arthritis, RA – rheumatoid arthritis

Table 2.TNF α blockade in animal models of hearing loss

Model [Ref]	Indication	Drug	Route	Treatment	Outcome
Mouse [34]	KLH induced labyrinthitis	Etanercept	Intraperitoneal	Pre (10 min before)	Decreased number of inflammatory cells
Rat [41]	Cisplatin induced ototoxicity	Etanercept	Trans tympanic	Pre (30 min before)	20–37 dB improvement in ABR thresholds at day 3
Rat [43]	Gentamicin induced ototoxicity	Dexamethasone	Intrachochlear perfusion via RW	Simultaneous	20–25 dB improvement in ABR thresholds at day 20
Guinea pig [51]	TNF α cochlear perfusion	Etanercept	Intracochlear perfusion	Pre (5 min before)	Cochlear blood flow maintained
Guinea pig [53]	Cochlear implantation	Etanercept	Perilymph infusion	Post (immediately after)	28–48 dB improvement in ABR threshold at day 28
Guinea pig [52]	Noise-induced trauma	Etanercept	Intraperitoneal	Post (15 min after)	18 dB improvement in ABR thresholds; cochlear blood flow maintained

Table 3.Anti-TNF α therapy for hearing loss

Study (ref)	Indication	N	Agent	Route	Outcomes
Prospective, non-randomized, open-label [64]	AIED	9	Infliximab	Intratympanic (4 weeks)	80% tapered off steroids; 75% PTA improved to 22.6 dB; reduced recurrence of hearing loss episodes
Prospective, non-randomized, open-label [65]	AIED	10	Golimumab	Intratympanic (5 weeks)	71% stable PTA, 57% stable WR; 28% improved WR
Randomized, placebo-controlled [69]	AIED	20	Etanercept	Subcutaneous (8 weeks)	Treatment no better than placebo (1 of 8 treatment vs. 2 of 9 placebo improved PTA >10 dB and/or improved WR>12%)
Case report [66]	AIED; RA	1	Infliximab	Systemic (indefinite)	Long-term stabilization of hearing loss
Retrospective [67]	IMCVD	12	Etanercept	Subcutaneous (28 weeks)	92% improved hearing and tinnitus, 88% improved vertigo, 89% improved aural fullness
Prospective, non-randomized, open-label [68]	IMCVD; MD	23	Etanercept	Subcutaneous (24 weeks)	30% improved PTA > 10dB at least one ear; 26% improved WR > 12% at least one ear
Prospective, non-randomized, open-label [58]	ISSNHL	12	Etanercept	Subcutaneous (12 weeks)	85% with acute hearing loss improved PTA by 30.3 dB
N/A [63]	VS	2	Anti-TNF α antibody	Applied to cochlear explants	Prevented IHC and OHC loss