In Germany alone, 800,000 people currently suffer from Alzheimer’s disease. LMU biochemist Christian Haass has been studying the causes of the dementia for years. Great progress has been made, but the search for a therapy that can halt nerve cell loss remains an arduous task.

Three of the small aquaria have bits of yellow tape stuck to them, marked “VIP”. Others are labeled with red tape bearing the same acronym. The labels express something of the relaxed and familiar relationship laboratory researchers develop with their experimental subjects, which reflects the fact that they work with them every day. – And the animals in these tanks certainly are “Very Important Piscines”. Whole batteries of aquaria are arrayed on shelves in the tiled climate chamber – over 1000 in all. In each tank, dozens of small fish are restlessly darting about. A little over 3 cm long, they shimmer like mica in the light. They owe their common name, zebrafish, to the dark longitudinal stripes on their flanks. They all look much the same but, to adopt a phrase referring to a different “Animal Farm” (George Orwell’s), some are more equal than others. The genetic make-up of the fish varies from tank to tank, but those in the VIP tanks are the stars of the whole swarm, although the tag might seem cynical, because these fish carry an artificial gene that is associated with a devastating disease. Some of their offspring rapidly develop the primary hallmark of Alzheimer’s disease. As in humans with this form of dementia, characteristic protein precipitates form in their nerve cells.

The construction of this fish strain was not the result of some weird whim. The experiment is part of a carefully thought-out scientific strategy. The VIP zebrafish and the other genetically engineered strains kept here at LMU’s Adolf Butenandt Institute provide an ideal system in which to study many aspects of Alzheimer’s disease – on a greatly compressed time scale. The larvae of Danio rerio (the scientific name for the zebrafish) are transparent, so the researchers can actually see inside them. Indeed, they have been able to follow the neurodegeneration
that is the basic feature of Alzheimer’s, in real time, in the living animal. Under the microscope, the nerve cells fluoresce in red, and some of them are stained with a yellow dye that is taken up only by damaged cells. The tiny yellow spots can be seen to increase in size and coalesce, as the dye accumulates in the dying cells. With the aid of their fish model system, the LMU team can search for agents that inhibit this nerve cell death.

Where and when does nerve cell death begin? Why do so many people develop Alzheimer’s as they get older? Can the characteristic progressive loss of memory be prevented? There are, as yet, no definitive answers to these questions – partly because, from a molecular biological standpoint, Alzheimer’s has turned out to be a very complicated puzzle. Biochemist Christian Haass has been assembling the pieces for almost 20 years, but their number continues to grow. Haass, who is now 50, holds the Chair of Metabolic Biochemistry at LMU Munich, and is one of the most highly regarded scientists in the field of dementia research. He has also been the Coordinator of the Munich Branch of the German Center for Neurodegenerative Diseases (DZNE), a Federal Health Research Center, since 2009. “We actually have a pretty good understanding of the disease mechanism,” he says, “and more and more of the details are being filled in.”

Yet, with every conference that Haass attends to discuss the current state of knowledge, the more sobering the picture becomes. Despite the years of research work, the drugs that are
now available have, at best, only a palliative effect on the condition. Each new forecast of the incidence of Alzheimer’s, the most prevalent form of dementia, is higher than the last. At present, some 800,000 people suffer from the disease in Germany alone, and medical experts expect this number to double by the year 2030 – a demographic time-bomb. “No society can cope with such a huge number of patients,” says Haass.

Furthermore, it has become clear that every conceivable approach to therapy will face certain fundamental obstacles. The disease begins to develop perhaps as much as 20 years before the first overt symptoms appear. This at any rate is one alarming implication of recent publications. Moreover, it is seldom possible to proactively identify those at risk. This makes the outcome of the race between research and demography even more open. Haass himself believes that it will be extremely difficult to translate research findings into effective treatments for patients. “In the beginning I too thought that the problem could be solved in a decade. Now, I wouldn’t hazard any prognosis at all.”

**Following in the Footsteps of Alois Alzheimer**

What then is the present state of play? What do researchers know about the illness and its origin? Postmortem examinations of the brains of Alzheimer’s patients reveal the presence of two types of deposits. In fact, Alois Alzheimer, who first described the disease over 100 years ago, had already noticed them. From his sixth-floor office in the Adolf Butenandt Institute, Christian Haass has a good view of the grounds of the old medical campus and can see the gable of the building in which Alzheimer examined his histological preparations.

So-called tangles are one class of accretion that was noted by Alzheimer. These are protein aggregates that accumulate in nerve cells, and are left behind as insoluble clumps when the cells die. We now know that they consist of so-called tau proteins. In healthy cells, tau proteins form part of an intracellular transport system that delivers essential components to sites where they are needed. Since nerve cells form long processes called axons, and must keep them supplied, they are especially vulnerable to any breakdown in this system.

Microtubules, the tracks that form the system, are made up of long arrays of linked sub-units, which are held together by tau proteins. In Alzheimer patients, the tau proteins detach from the microtubules, so that the “railroads” disintegrate, and the whole supply network collapses. The axons shrink and the cell ultimately dies. The unattached tau proteins form tangles, but it is not clear whether these are toxic to the cell. Haass and his team have shown in zebrafish that nerve cell death begins long before large tangles have formed.

What accounts for the loss of tau function? It is now clear that so-called amyloids, short peptides produced by fragmentation of a larger precursor protein and secreted by nerve cells, play a crucial role. These extracellular peptides give rise to the second kind of pathological
deposit in the Alzheimer brain, the plaques. However, other cell types also produce amyloids. Haass has found the same peptides secreted from kidney cells, and even in the blood. Furthermore, similar levels of amyloid are present in the blood of healthy and ill subjects. As Haass explains, he made this disturbing discovery early in his career, and in the end it was this finding that put him on the right track. “The observations led to three papers in Nature in one year,” he recalls – no easy feat for a junior researcher to pull off. In these papers, Haass concluded that the mechanism underlying Alzheimer’s disease operates in all of us, and that slight alterations in metabolism can cause it to have catastrophic consequences. This model would explain why the risk of developing Alzheimer’s disease as we get older is so high. About one-third of those over 80 suffer from dementia. “Alzheimer’s is a part of the normal ageing process,” notes Haass.

Only a few years ago there were two opposing schools of thought among Alzheimer experts. One regarded tangles as the primary culprit in pathogenesis, the other held amyloids to be the key to nerve cell death. Haass says the feud is now over. The two types of aggregate are part of one cascade. The amyloid first forms so-called oligomers, consisting of two or three copies of the peptide. The oligomers are soluble, can diffuse through the extracellular space, and may even come into contact with brain cells far from their site of origin. They perturb the function of the synapses, the specialized structures that transmit the nerve impulse from cell to cell. This functional disturbance in turn induces a stress reaction in the nerve cell, which leads to the addition of excess amounts of phosphate to tau proteins, causing them to

„Our understanding of the disease is improving all the time," says Haass. The zebrafish is one of the model systems researchers use to determine what exactly goes wrong in nerve cells and triggers formation of the characteristic protein aggregates.

Source: LMU Munich
lose their normal function and facilitating their aggregation. The amyloid oligomers are the real toxin, says Haass. In contrast to what was once thought, the plaques may not be toxic.

What then is it that turns the amyloid into a toxin? The peptide unit that forms the oligomers is called beta-amyloid, and is cleaved from a much longer protein named APP (Amyloid Precursor Protein) found in the nerve cell membrane. Enzymes called secretases, acting as molecular scissors, release it by cutting the precursor at specific positions. But they sometimes make mistakes, and snip off amyloid variants that differ in length from the normal form and are particularly prone to aggregation. Errors can occur because the scissors or the APP is deformed due to a genetic mutation, as in some hereditary forms of dementia. But they also reflect the intrinsic complexity of the enzymes. For instance, one of the scissors, gamma-secretase, is put together in a such a complicated way that it took Haass and his team years to dissect. Haass points out that, even in young organisms, the assembly of the gamma secretase frequently goes wrong. Gamma-secretase is a membrane protein consisting of four subunits. Each of these must be threaded back and forth through the cell membrane several times and then linked to the others. “Biochemically, much can go wrong in older individuals.”

**SYNAPSIS, SCISSORS AND STRESS**

Haass and his team have studied the secretases and their potential as targets for therapeutic drugs for many years. Dozens of candidate inhibitors are now being tested by scientists all over the world; many are in clinical trials. These agents block the active center of the enzyme; essentially they jam the scissors by sticking between the blades. Some look promising but, in almost all cases, there have been problems with side-effects. This is because secretases have important functions in healthy organisms, and inhibitors threaten to knock these out too. For instance, gamma-secretase plays an essential role in cell differentiation. In mid-2010 a clinical trial being conducted by the American drug company Eli Lilly, involving 2000 Alzheimer’s patients, had to be terminated prematurely. The gamma-secretase inhibitor being tested, code-named LY450139, actually enhanced the rate of memory loss, and increased the risk of skin cancer. “Millions of dollars went out the window,” says Haass. Setbacks like this always raise doubts as to whether the toxic amyloid variants are responsible for the induction and progression of the disease. “But the hypothesis is 100% correct,” Haass insists. Beta-secretase, the other relevant scissors, plays a crucial role in the process that wraps the so-called myelin sheath around axons. The sheath acts as an electrical insulator, and is indispensable for proper transmission of nerve impulses, as the LMU investigators have shown. In an extensive series of experiments on mice in which the gene for beta-secretase had been specifically mutated, they were able to observe the effect of enzyme loss under the microscope. Transverse sections of the sciatic nerve of healthy mice showed a clearly defined myelin sheath. In the mutant mice, in contrast, the sheath was extremely thin, Haass reports. However, since myelination is already complete in mature organisms,
In 1999, Haass was appointed to the Chair of Metabolic Biochemistry in the Medical Faculty at LMU. Born in 1960, he studied biology and obtained his PhD in Heidelberg. In 1990, he went to Harvard Medical School as a Postdoctoral Fellow, and became an Assistant Professor there in 1993. After his return to Germany, he served as Professor of Molecular Biology in Mannheim, before moving to his present position in Munich. In 2002 Haass was awarded the Leibniz Prize by the German Research Foundation (DFG), the highest honor for scientific achievement awarded in Germany, and in 2010 he received an honorary doctorate from the University of Zürich. Since 2009 Haass has been Scientific Coordinator of the Munich Branch of the German Center for Neurodegenerative Diseases (DZNE).

Haass assumes that this is one side-effect that should not complicate efforts to block beta-secretase in elderly Alzheimer patients.

In the case of another neurodegenerative disease, frontotemporal dementia (FTD), the LMU researchers have evidence for a promising therapeutic approach. Here, Haass believes the risk of serious side-effects is lower. In FTD nerve cells in the frontal and temporal lobes of the brain degenerate, and patients develop speech defects and personality disturbances. Some forms of FTD are hereditary, and are caused by inadequate levels of a hormone-like growth factor named progranulin, which acts as a neuroprotective factor. In patients with congenital FTD one of the two progranulin gene copies is missing, and levels of the factor

Source: LMU Munich
are only half those found in normal subjects. Haass and his team discovered that progranulin production can be boosted by reducing the acidity of the medium in the vesicles (tiny membrane sacs) from which it is secreted. They then tested a series of established pharmacological agents that are known to have such an effect. And indeed, in experiments done in mice and in cultured human cells, they found that chloroquine, which is used to treat malaria, markedly enhances the production of progranulin. “Chloroquine is tried and tested, and has been prescribed millions of times. I actually had to take it over a considerable period myself.” Doctors at a specialist clinic at London University are now testing whether the drug has a beneficial effect in FTD patients.

A TEST IS URGENTLY NEEDED
The nagging problem of side-effects is especially acute in the case of Alzheimer’s because therapy must be started long before the first symptoms appear. Finding a drug that can be tolerated perhaps for decades is a very tall order. Let’s assume for a moment that one were to be found. How would one decide who should be treated with it? As yet there is no test that can reliably detect the disease early enough to have a chance of preventing it from progressing to full-blown Alzheimer’s. According to Haass, a test for a biomarker that measures the risk of dementia is “urgently needed”. “Otherwise, therapy will always come too late.” Immunization against plaques has been much discussed, and passive immunization of elderly patients with antibodies raised against highly purified amyloid “gets rid of plaques,” reports Haass, but unfortunately it doesn’t slow down the dramatic pace of memory loss.

In the case of familial Alzheimer’s, which is relatively rare, the risk of developing the condition can be determined using a simple genetic test. In people who carry a copy of the defective gene, the disease takes a particularly aggressive course, and massive memory loss begins between the ages of 30 and 40. An international network has been set up to compile a registry of cases, and to recruit patients for various studies. Haass himself has long argued the case for such a strategy. At first, ethical objections were raised against the idea of using a gene test to identify the population at risk and against the stated intention to treat healthy participants also. But Haass contends that, for those at risk, drug trials are “the only chance they have,” and their readiness to take part in such trials is correspondingly high. Haass has observed this at first hand In the case of FTD. “When we published our paper early this year and the first media reports appeared,” he says, “the telephones here never stopped ringing.”

This raises the basic issue of how the results of basic research can best be translated into real advances for patients. How does one set about developing new therapeutic approaches on the basis of laboratory findings, and then designing effective treatments? These questions of translation from bench to bedside have taken up much of Haass’ time since he became the Coordinator of the Munich Branch of the German Center for Neurodegenerative Diseases (DZNE) in 2009. The Center has its headquarters in Bonn and branches in seven other cities,
and is one of several National Health Research Centers in which the Federal Government has invested a great deal of money over the past few years. Their common goal is to promote clinical research into disorders that place major burdens on our society – and accelerate the translation of basic research into clinical practice. In addition to LMU and its University Hospitals, the Technische Universität München and the Helmholtz Center are involved in the Munich Center. Some of the planned research groups are already hard at work, while others still await the appointment of laboratory heads. On the campus in Großhadern, a new building is under construction and is scheduled to open in 2014. In the meantime, the researchers are using rented laboratory space in the area. All DZNE groups in Munich will ultimately be housed in the new building, and Haass’ own institute will also move in. “The work done there will address all aspects of neurodegenerative disease – from molecular analysis to the treatment of patients,” he says. An outpatient department and a day clinic are also planned.

The Center will provide a home for the new Clinical Institute for Stroke and Dementia Research, which went into operation this year. The concentration of so many specialist disciplines in a single location promises to change conventional views of neurological disease. Haass has high hopes for such an integrated interdisciplinary approach, which he calls systems neurology, as the strategy addresses the interaction of diverse pathological mechanisms. It has, for instance, become clear that inflammatory processes and restriction of microcirculation play an important role in Alzheimer’s. Stroke and other cerebrovascular syndromes result in the death of nerve cells, and neurodegenerative mechanisms facilitate the progression of multiple sclerosis, a disease which is generally regarded as a neuroinflammatory syndrome. In the DFG Priority Program which he now coordinates, Haass has been applying a concerted approach to the analysis of several neurodegenerative diseases, and finds many parallels between the molecular mechanisms of their pathogenesis. He is confident that seeing them in the light of systems neurology will lead to new insights into all of these conditions.

Translated by Paul Hardy