Leading Edge
Essay
Cell

Lessons from a Failed γ-Secretase Alzheimer Trial

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γ-Secretase proteases have been associated with pathology in Alzheimer disease (AD), but we are just beginning to understand their basic mechanisms and physiological roles. A negative drug trial with a broad spectrum γ-secretase inhibitor in AD patients has severely dampened enthusiasm for the potential of pursuing γ-secretase research therapeutically. This pessimism is unwarranted: analysis of available information presented here demonstrates significant confounds for interpreting the outcome of the trial and argues that the major lessons pertain to broad knowledge gaps that are imperative to fill.

Rising too fast to fame is dangerous for artists, scientists, politicians, and is now the unhappy fate of the γ-secretases. These proteases have been eagerly investigated by pharmaceutical companies, as inhibitors would block the production of amyloid β-peptide (Aβ), widely thought to play a key pathological role in Alzheimer disease (AD) (Haass and Selkoe, 1998). Recently, however, γ-secretase research has fallen into disgrace because a phase III clinical trial testing the inhibitor semagacestat failed dramatically (Doody et al., 2013) leading to abrupt closure of promising lines of γ-secretase work. Unfortunately, this effect has not been limited to industry, but has also cast a shadow over research agendas in academia. In this essay I present arguments for why the takehome message of the semagacestat trial should be the exact opposite, particularly in light of the prodigious evidence for the relevance of γ-secretase in physiology and disease (Jurisch-Yaksi et al., 2013). The field (and its granting bodies) should instead learn from the trial what is needed to fill our huge knowledge gaps, and the pharmaceutical industry must be persuaded to sustain their interest.

A Hasty Start

The semagacestat trial by Eli Lilly is significant as an example of how we are moving forward to treat AD in a rational way. All the trial data have been made available to the community. However, it may be argued that the study was premature for several reasons. γ-Secretase inhibitors had become the subject of intense focus in pharmaceutical research many years ago because they were readily identified in screens for compounds that lower Aβ cleavage in various cell lines (Haass et al., 1992). Only several years later were the proteases responsible for the release of Aβ identified (De Strooper et al., 1998; Vassar et al., 1999). With very little knowledge of the biology of γ-secretases, compounds were pushed forward as drug candidates. Lack of structure-function information on the different γ-secretases, no understanding of how they interact with their many substrates, and simplistic assumptions on their physiology have without any doubt hampered progress of the field.

Semagacestat was one out of the many candidate drugs that advanced as a γ-secretase inhibitor blocking Aβ generation in cell cultures, animals, and finally human. Only relatively late in the studies did it become clear that the potential side effects caused by blocking Notch-signaling were severely limiting the clinical use of semagacestat (Henley et al., 2014). In fact, from a scientific point of view, it remains puzzling why the company decided to move forward toward phase III tests (Doody et al., 2013) with a dose (maximal 140 mg/day) that in none of the phase II tests had shown any significant impact on Aβ levels in the cerebrospinal fluid (CSF) of humans (Karran and Hardy, 2014). Looking back, it seems clear that such a phase III trial was unlikely to test the amyloid hypothesis, as elaborated below.

Dosing, Kinetics, Toxicity, and Cognitive Decline

The really unexpected finding of the trial was that the patients actually experienced cognitive decline as assessed by two clinical scoring systems (Doody et al., 2013). This toxic effect remains unexplained but is cited repeatedly as a major counter argument against the further development of γ-secretase inhibitors. The interpretation of the cognitive alterations is complicated, however, and deserves in depth analysis and further research before jumping to such a drastic conclusion.

Based on the fact that chronic but partial lowering of γ-secretase activity causes no major phenotypes in heterozygote mice mutated in one of the γ-secretase genes (see for instance Ma et al., 2005; Serneels et al., 2005), one would expect that a chronic, moderate lowering of this enzymatic activity in human could be tolerated. Unfortunately, the trial did

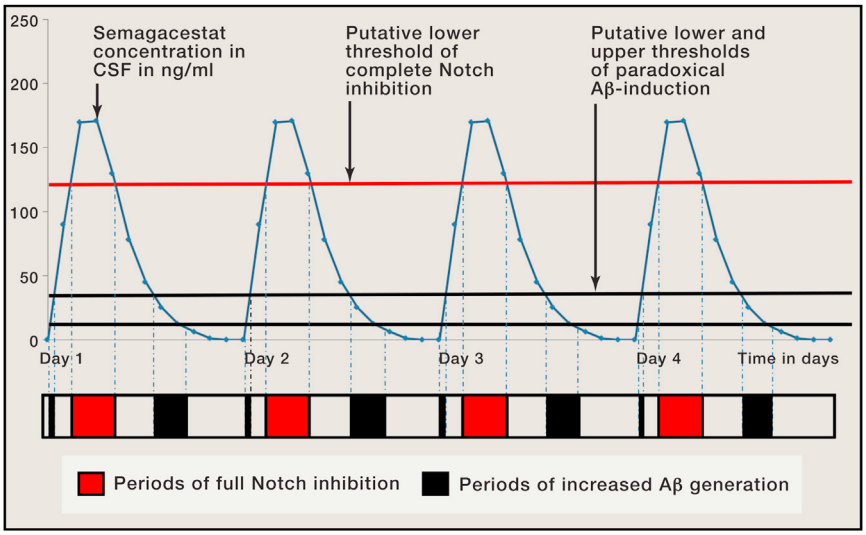

Figure 1. Theoretical Flucutations of Semagacestat in CSF of Patients

The pharmacokinetic curve for semagacestat is modeled based on measurements and figure 2 in (Bateman et al., 2009), using the data for the 140 mg dose. The thresholds for Aβ induction (black lines) and Notch inhibition (red line) are only illustrative as the critical concentrations for these effects in human in vivo are not known. As discussed in the text, the IC_{50} for γ-secretase in cell culture is 5.9 ng/ml while the maximum concentration reached in the CSF was 194 ng/ml. The bar below the figure shows the fluctuations in γ-secretase activity under this drug treatment: black blocks indicate stimulation (i.e. increased Aβ generation) while red blocks indicate complete inhibition of γ-secretases. The precise scale of these effects is not known and the figure is only meant to illustrate the strong oscillations in γ-secretase activity that were induced by the drug treatment in the trial.

not test this hypothesis, but tested, as explained below, the effects of short peaks of complete γ-secretase inhibition in the brain alternated with periods of normal activity (Figure 1). This problem was accentuated by the fact that the fear of side effects led the investigators to dose the drug just once a day, rather than the twice a day regimen originally planned. These bursts of full inactivation are likely problematic. Indeed, severe Notch phenotypes are seen after complete genetic inactivation of γ-secretases in mice (Bammens et al., 2011; Li et al., 2003; Saura et al., 2004; Shen et al., 1997). Notch signaling is part of ultradian oscillations, best studied in somitogenesis (Kageyama et al., 2010) but also involved in neuronal stem cell differentiation (Kageyama et al., 2009). Short pulses of full inhibition could have disastrous effects in such oscillating systems. Notch signaling oscillations have indeed been implicated in memory formation (Ables et al., 2011; Alberi et al., 2011; Zhang et al., 2013), potentially explaining at least part of the cognition problems in the treated patients. It must be said that misprocessing of other γ-secretase substrates like N-cadherin, syndecan, neuregulin etc, could also contribute to the problem, but little is known about whether and how their misprocessing is related to cognition and memory processes. In any event, the tantalizing question arises whether a more moderate but continuous inhibition of γ-secretase would have preserved such ultradian signaling mechanisms to an extent sufficient to avoid the adverse effects on cognition observed now in the trial.

A second critical and important question is to what extent confounding factors contributed to the cognitive alterations in these patients. γ-secretase activity was very strongly inhibited in peripheral tissues during the trial and many of the patients showed skin problems, weight loss, vomiting, etc. (Doody et al., 2013). These already frail Alzheimer patients might have underperformed in those tests simply because they felt sick from the treatment. Again, lower dosing of the drug in a more chronic regimen would have avoided part of this peripheral toxicity and patients probably would have felt better. The issue that side effects of test medication might compromise the interpretation of cognitive assays certainly needs further scrutiny as it could lead to the premature arrest of other promising drug development programs in the Alzheimer field for the wrong reasons.

These peripheral side effects may reflect the strong inhibition of Notch signaling and maybe other γ-secretase substrates in all tissues. It has been observed that the oral dosing used in the trial yields very high (2,160 ng/ml) concentrations for drug in plasma (Yi et al., 2010). The half maximal inhibitory concentration (IC_{50} of semagacestat) for γ-secretase inhibition in cell culture is 5.9 ng/ml (Bateman et al., 2009). It is therefore likely that complete Notch inhibition was achieved in a number of peripheral organs for relatively long periods. Obviously the one-dose/day regimen also resulted in drug free periods, and it can be argued that these are beneficial in the context of γ-secretase treatment. Indeed, continuous inhibition of γ-secretases might result in toxic accumulation of its direct substrates like the APP carboxyterminal fragments (Mitani et al., 2012). However, it seems very likely that the complete inhibition of γ-secretase during the peak periods caused by the one-a-day dosing contributed to extensive side-effects.

The effects on the brain deserve separate discussion. In the trial no effects on Aβ levels in the CSF were recorded (Doody et al., 2013). Isotope labeling kinetics experiments indicated, however, that with one dose of 140 mg semagacestat, the rate of Aβ generation in the brain would be inhibited by 52% over a 12 hr time period (Bateman et al., 2009). In these experiments it was also found that peak drug concentrations of 194 ng/ml were reached in the CSF (Bateman et al., 2009). Although the IC_{50} in vivo is not known, it can be safely assumed based on the cellular IC_{50} that during the trial and with this dose almost complete inhibition of all γ-secretase activity in the brain was reached albeit for short periods (Figure 1). Additional measurements of alternative $Aβ_{1-15/16}$ fragments in the CSF further indicated that γ-secretase is indeed inhibited in the brain under the conditions of the trial (Portelius et al., 2012). On the other hand, given the rather short half-life of

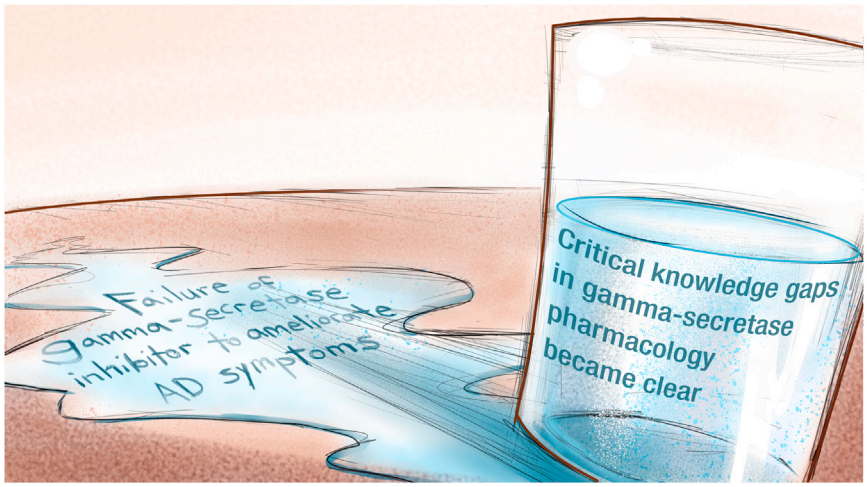

Figure 2. The Semegacestat Clinical Trial: Taking the Glass Half-Full Perspective

semagacestat ($t_{1/2}$ ~2–3 hours), a single dose of the drug is completely removed from CSF after 12 hr (Bateman et al., 2009).

From this brief overview it can be deduced (1) that most of the day, patients had no drug in the brain and produced normal amounts of Aβ; (2) each day patients went through a drug concentration cycle, twice hitting a critical low concentration of drug that is known, as explained below, to paradoxically stimulate Aβ secretion; and (3) this treatment regimen generated sharp oscillations in γ-secretase activity (Figure 1) that probably also affected the kinetics of Aβ generation. It should be mentioned that inhibition of Notch signaling might have exacerbated the situation. Very different estimates have been published for the selectivity of semagacestat toward amyloid precursor protein (APP) versus Notch (Karran and Hardy, 2014), but from a quantitative cell free assay with which IC_{50} could be directly compared, it appears that semagacestat has an intrinsic bias toward inhibiting Notch cleavage (IC_{50}APP/IC_{50}Notch: 0.1) (Chávez-Gutiérrez et al., 2012). Other γ-secretase inhibitors including avagacestat also turn out to be far less selective for APP versus Notch processing (Chávez-Gutiérrez et al., 2012; Crump et al., 2012) than sometimes claimed (Probst et al., 2013). Therefore novel drugs in the future should be profiled more carefully against Notch but also versus the other different substrates of γ-secretase.

Steady-State Levels of Aβ Were Not Lowered in the Brain

As mentioned, the observation that Aβ levels were not affected in the CSF is very puzzling. It could be argued that most of the time γ-secretase activities were not inhibited in the patients, and that therefore no extreme effects on Aβ levels in the CSF were anticipated. However, one would still predict a lowering of Aβ if semagacestat would have worked as a classical protease inhibitor. The terminology "inhibitor" is in fact quite misleading. Semagacestat targets an uncharacterized allosteric mechanism that affects Aβ generation in a biphasic way causing a significant increase in Aβ release at low concentrations (Lanz et al., 2006). Thus, in the pulsed drug regimen used in the trial, patients would have experienced transient periods of increased Aβ-generation twice a day (Figure 1), which might have neutralized further the effects on Aβ in the CSF, and might have led to a "zero-sum game".

Transient increases in Aβ peptides might have caused toxic effects although this is speculative. Yet another confounding problem, however, is that semagacestat affects generation of the $Aβ_{40}$ peptide product more than that of $Aβ_{42}$ (Lanz et al., 2004; Lanz et al., 2006). This was confirmed in the plasma samples of the patients in the trial: $Aβ_{40}$ was down by 48% while $Aβ_{42}$ decreased only by 18% (Doody et al., 2013). This increase in $Aβ_{42}/Aβ_{40}$ ratio while lowering the total Aβ load mimics the effects of several genetic mutations in γ-secretase which cause inherited Alzheimer disease (Chávez-Gutiérrez et al., 2012). Relatively small alterations in the ratio strongly influence toxic and aggregation properties of Aβ peptide mixes (Kuperstein et al., 2010; McGowan et al., 2005). While it remains unclear whether this effect was played out in the brain of the patients during the few hours per day that γ-secretase activity was affected, it is very clear that semagacestat was not a simple inhibitor of γ-secretase activity and that the effects on Aβ in the brain were complex.

A New Deal for γ-Secretase Research

The thought pendulum in the field regarding the critical pathological factor in AD has been shifting slowly away from the amyloid hypothesis toward Tau, inflammation, and vascular problems. However, it is premature to move completely into other directions, and different approaches should not be considered mutually exclusive. The arguments that causally link Aβ peptides to the disease are strong (Karran et al., 2011), and it therefore remains mandatory to test the amyloid hypothesis exhaustively in the clinic. The reasons behind the failures of the semagacestat and other amyloidocentric phase III trials are, as discussed here and elsewhere (Karran and Hardy, 2014), quite complicated. We need to thoroughly address the many new questions that are arising if we want to make real scientific progress (Figure 2). More time for our thinking to mature and to evaluate different clinical hypotheses in smaller clinical trials seems a prerequisite to make the best out of the few options for treatment that are currently available. However, even more important at this moment is profound basic research. Although only recognized by few, the current knowledge gap in γ-secretase biology, physiology, and structural biology is appallingly wide. Novel insights and deeper understanding of these enzymes will provide a basis for more rational drug design and for a less speculative analysis of results obtained in clinical trials. It is also likely that such work will broaden the scope of potential applications, i.e., in acute leukemia (Groth and Fortini, 2012), or, unexpectedly, as a topical treatment for regeneration of inner

ear hair cells after acoustic trauma (Mizutari et al., 2013).

Back to the Basics

γ-Secretases are indeed very intriguing molecular machines. We know for instance that they consist of four protein subunits (presenilin, nicastrin, presenilin enhancer 2, and anterior pharynx 1) encoded by four different genes (*PSEN*, *NCT*, *PEN2*, and *APH1*) (De Strooper, 2003), but we still do not understand why the presenilin subunits need the other subunits to become catalytically active. This question is not trivial as their full cousins, the signal peptide peptidases (SPPs) (Grigorenko et al., 2002; Ponting et al., 2002; Weihofen et al., 2002) are fully active as single proteins or as homomultimers (Li et al., 2013; Miyashita et al., 2011; Nyborg et al., 2004 reviewed in Voss et al., 2013). It has been also very misleading to discuss γ-secretases as if they were one protease, which has been stated in most of the available literature. Four major variant complexes are generated with the two different PSEN and two different APH1 subunits (De Strooper, 2003), and additional complexity comes from alternative splicing of the subunits. Semagacestat, as well as other clinically tested inhibitors such as avagacestat, might block all different γ-secretase complexes indiscriminately which might contribute to the overall toxicity of these drugs. Also the question of the specific function of the different enzymes remains hardly explored (Serneels et al., 2009), although we already know that major toxic effects caused by such broad spectrum γ-secretase inhibitors can be circumvented in mice when using more selective compounds (Borgegård et al., 2012).

The fundamental cell biology of the γ-secretase complexes needs a revival: how does the differential composition of the complexes determine subcellular, cellular and tissue distribution of the γ-secretases? Can a specific localization of the complexes in specific membrane compartments be related to specificity toward different substrates? Given the critical roles of γ-secretases in the synapse (Zhang et al., 2009), cell biological questions are particular relevant in the context of the neuron with its long axons and complex dendritic compartments. Evidence links conformational changes in γ-secretase with synaptic electrical activity and Aβ generation (Dolev et al., 2013). Ultimately the link between brain activity, functions of γ-secretases, and alterations in Aβ production is one of the real core questions in the field.

Additionally, it is key to understand what are the physiologically relevant substrates of γ-secretases. A number have been proposed and this promiscuity is often cited as a rationale against the development of γ-secretase inhibitors, but with very little knowledge about which substrates matter in vivo. This lack of basic understanding of the function of such important enzymes renders the interpretation of the results obtained in clinical trials virtually impossible, and better understanding will lead to more effective and safer drug candidates.

Another path to greater understanding and better drug development is structural insights. This has been challenging but progress is underway: Last year Shi and collaborators provided a first ultrastructure of a presenilin homologue (Li et al., 2013). This year the same group, using single particle cryoelectron microscopy, published a structure of an entire γ-secretase complex at 4.5 Angstrom resolution, and a higher resolution map of the nicastrin subunit based on its similarity to a glutamate carboxypeptidase (Lu et al., 2014). These are major steps forward, although we need even higher resolution to provide full atomic detail.

A Bright Future

It is clear that exciting times for γ-secretases are ahead of us. Progress in the areas discussed above will allow us to understand in precise detail how genetic mutations causing AD affect the function of these enzymes. They also will provide real insight in how drugs like semagacestat target allosteric sites in the complexes and how this modulates their activities. This will facilitate optimization of the γ-secretase modulators still under clinical development. Finally, we might be able to understand how different substrates dock into different γ-secretases and to generate very specific compounds that only interfere with APP processing and not with the processing of other substrates. It is clear that the currently prevailing pessimism in the γ-secretase field is not warranted. While we need the help from investors, government, and public opinion to achieve our goals, the first and major drive for progress is the conviction and enthusiasm of the researchers, the pioneers that try to chart the many unknowns and uncertainties in this difficult but very important area of Alzheimer disease research.

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