The trials and tribulations in the drug discovery cycle

A particular case for Alzheimer's disease

As people live longer lives, the spectre of Alzheimer's disease will only grow. Scientists now know that the amyloid beta (Aβ) peptides are important for treating Alzheimer's, particularly Aβ42 which aggregates into the amyloid plaques that trigger the disease. Researchers have been looking to target γ-secretase – the enzyme that creates pathogenic Aβ42 affecting the relative abundance of short Aβ peptides. However, they have struggled to balance the competing and often contradictory requirements, in the drug discovery cycle. Dr Rosa María Rodríguez Sarmiento (Hoffmann La Roche) uses multi-dimensional drug optimization to create a drug without safety flags, that has the physiochemical properties and pharmacokinetic profile to be an effective potential clinical candidate.

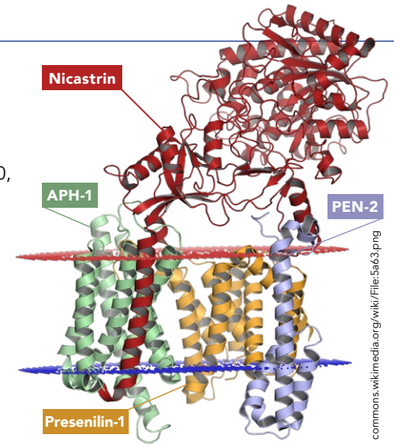
Alzheimer's disease is a neurodegenerative condition that falls under the umbrella term dementia, and comprises 60-70% cases of dementia. The condition targets the elderly, with 30% of people over the age of 80 showing a loss of cognitive capabilities, which slowly affects their memory, decision-making and personality until they can no longer independently support themselves. Alzheimer's can be a devastating disease; a manifestation of the most terrifying nightmares that haunt our collective imagination – the loss of your identity. Our art and literature are dominated by our desire to craft and cement an identity on the path of self-actualisation. Nothing is quite as scary as the inexorable loss of an identity defined by a lifetime of choices, and by an inability to remember the memories of the past or activities of the present which imbue meaning in our lives.

Alzheimer's disease is also becoming more prevalent. The World Health Organization projects there to be 152 million dementia patients by 2050. The cost of care for Alzheimer's patients is estimated to quintuple in the next 30 years from $226 billion to potentially $1.1 trillion. As the populations of many countries grow older, as their citizens live longer, a disease like Alzheimer's that develops in an elderly population will only become a bigger problem.

AMYLOID-β AND γ-SECRETASE ENZYME

In their bid to find drug treatments against Alzheimer's disease and on the basis of compelling genetic evidence researchers have focused on brain amyloid beta (Aβ). Aβ peptide is produced by sequential cleavage of amyloid precursor protein (APP) by beta amyloid cleavage enzyme 1 (BACE1) and gamma secretase (GS). They are peptides – molecules built from short (< 50) chains of amino acids, the building blocks of the longer proteins, The Aβ peptides come in many different shapes and sizes, the most common being Aβ38, Aβ40 and Aβ42 – the numbers denoting the number of amino acids in the peptide. The longer Aβ42 peptides are more likely to aggregate into amyloid plaques within the cerebral spinal fluid, which drive the neurocognitive symptoms that define Alzheimer's disease.

Alzheimer's research has identified a clear relationship between an increase in toxic Aβ42 peptides, relative to Aβ40 peptides. They're created in an intricately complex biochemical process. Multiple enzymes cut, reshape, and add new chemicals to the amyloid precursor protein (APP), pruning it into the Aβ peptide, like a bonsai tree. Targeting this process which produces Aβ peptides has been the goal of drug development. This has yielded a variety of approaches, including targeting the γ-secretase enzyme which is primarily responsible for cutting the protein into its final lengths (Aβ42, Aβ40, or Aβ38).



commons.wikimedia.org/wiki/File:5a63.png

Human gamma-secretase protein complex (cryo-electron microscopy), with its 4-subunits in the endoplasmic reticulum membrane (lumen in red, cytosol in blue). GS is responsible for cutting amyloid precursor protein (APP) to Aβ peptide.

Since its discovery in 2001, researchers have targeted γ-secretase with gamma secretase modulators (GSMs). However, this is a much trickier task than it may seem. γ-secretase has its catalytic finger in many physiological pies – in other words, γ-secretase is involved in multiple chemical reactions and molecular pathways. It cleaves a broad type of protein (type-I transmembrane proteins), including Notch molecules, which are vital for cell development. It was important to observe that gamma secretase allosteric binders ("GSMs") decrease Aβ42 and Aβ40 peptides without affecting the total amount of Aβ produced. As a result, there is an increase of shorter and beneficial Aβ38, Aβ37 peptides, offering additional therapeutic benefit for Alzheimer´s disease.

Targeting γ-secretase becomes a very dextrous affair, as you balance the need to reduce its output for Aβ42 without disrupting other vital cellular processes.

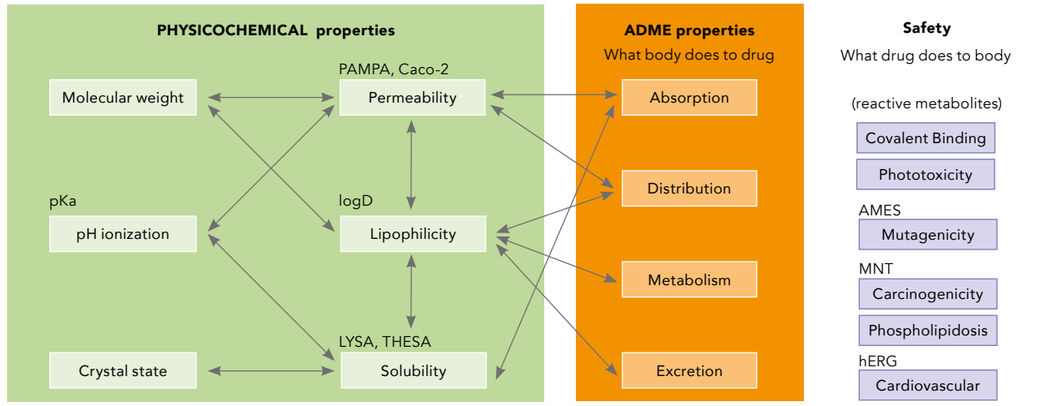
You can't just turn it off – drugs that inhibit γ-secretase outright were abandoned after clinical trials found they were toxic. Targeting γ-secretase becomes a very dextrous affair, as you balance the need to reduce its output for Aβ42 without disrupting other vital cellular processes. This hints at the fundamental complexities involved in any effort to discover and develop new drugs.

THE DRUG DISCOVERY CYCLE, BUT WITHOUT NEW DISCOVERIES

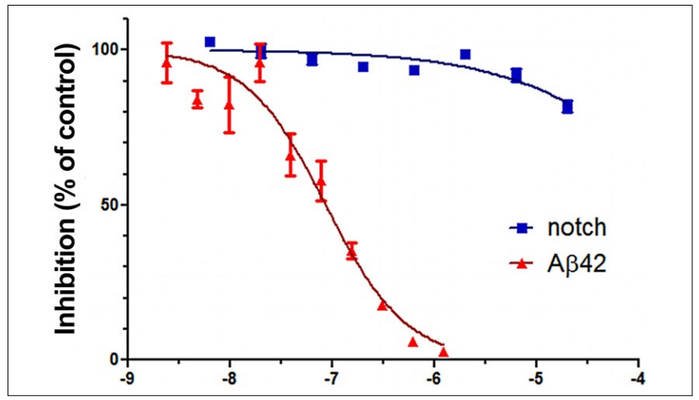
The fundamental problem the drug discovery cycle poses is one of conflict, an almost contradiction in terms. The drug must simultaneously stop the process inadvertently causing the disease, but none of the other processes in the body. Drug discovery becomes a "multi-parameter" or a "multi-dimensional optimization problem", as each candidate compound has to meet many different requirements to fulfil the desired target profile according to the disease, for its approval.

A drug candidate for Alzheimer's disease needs to have acceptable physicochemical properties (such as its solubility and lipophilicity) and tick off the ADME list – absorption, distribution, metabolism and elimination of the drug. Researchers have to juggle this together with safety concerns by reducing the risk of toxicity eliminating observed flags, alongside the additional caveat of being able to pass through the blood-brain barrier to reach the central nervous system where the amyloid plaques form. Medicinal chemists, within a team with diverse backgrounds, need to integrate available *in vitro* and *in vivo* multi-parameters to design the next round of optimized compounds.

It becomes a see-saw of priorities; you can improve the potency of your drug, but at the expense of a long list of properties that could influence its absorption, safety, efficacy or other parameters. Juggling multiple parameters in medicinal chemistry is highly demanding and requires not only expertise but creativity and precision. Optimization should be accomplished before going into advanced stages where safety margins are evaluated in toxicity studies, before entering human clinical trials to evaluate the risk/benefit of the drug. This is a major factor behind the stagnating research and development (R&D) scene, as the screening for ADME and low toxicity drives up costs in research, now estimated to be $1.1 billion to bring a drug to the market. Many smaller companies are locked out of drug research entirely into the Valley of Death,



Interplay of physicochemical and pharmacokinetics molecular properties.



Dose response of RO6800020 on Aβ42 without notch inhibition in Hek cells.

whilst large companies and institutes will balk at the cost of drug research, which isn't even guaranteed to pay off.

THE MULTI-PARAMETER OPTIMIZATION PROBLEM IN DRUG DISCOVERY (CASE OF AN ALZHEIMER´S DRUG)

A good example of the complexity of multi-parameter optimization in drug discovery can be found in the published medicinal chemistry work led by Dr Rosa María Rodríguez Sarmiento at Hoffmann La Roche Ltd. The Roche team, based on the data from biology, has long been interested in a GSM compound that can specifically alter the production of amyloid beta peptides, decreasing the proportion of Aβ42 in favour of shorter Aβ peptides that attenuate Aβ42 toxicity.

One compound which showed promise was RO6800020, as it had a strong affinity for selectively reducing the production of Aβ42, Aβ40 peptides, while increasing Aβ38, Aβ37, without interfering with Notch signalling in γ-secretase. However, it was also plagued with problems. There were multiple structural characteristics which led to toxicity, as well as a limited potency of the drug. A limited potency means, in this example, a moderate and unpredictable concentration of the drug is required to have an impact, which exacerbates existing concerns about its toxicity and makes it harder for researchers to precisely predict its effect on the patient.

Dr Rodríguez Sarmiento saw the promise RO6800020 held and decided to look deeper into its structure, breaking it down to its core chemical constituents running through any molecules which could replace the original constituent. Through a slow, but methodical series of recombinations, drawing up new structures, Dr Rodríguez Sarmiento and her colleagues and collaborators were able to optimize its structure into RO7101556 – a much better drug in terms of properties and safety flags which was ready to be evaluated on *in vivo* toxicity studies as a prerequisite to go forward clinical trials.

Medicinal chemists, within a team with diverse backgrounds, need to integrate available in vitro and in vivo multi-parameters to design the next round of optimized compounds.

THE MULTI-PARAMETER OPTIMIZATION SOLUTION

One important change was the central piperidine ring. This was key to increasing its potency, and its fraction unbound to acceptable levels as it meant a greater proportion of the drug molecules given in a dose weren't attached to proteins in the plasma - therefore could do their pharmacological job of reducing Aβ42 concentrations. This also led to improving its efficacy as a drug.

In addition, the researchers realised the risk of metabolic activation of the terminal rings ("head heterocycle") which could produce toxicity in mice liver, and replaced them with less reactive ones. In this way, they reduced toxicity by preventing covalent binding liability (CVB) to cellular macromolecules due to the ring. They also made the novel adjustment of adding a 5-substituent that prevents the drug from binding to other enzymes, specifically the PIK4CB kinase, which play an important role in triggering the immune response, preventing side effects from unselective binding to those enzymes.

However, the CF_3 alkoxy is arguably the key adjustment that was made due to its ability to perfectly balance the characteristics a drug candidate needs to succeed. The Roche researchers replaced a phenyl ring with the CF_3 alkoxy, which also has fluoro substituents, whilst providing many improvements through its structural differences. It can maintain the compound's potency and selectivity towards blocking γ-secretase's creation of amyloid beta, and Aβ42, and balances this with the traits that make it a viable preclinical drug candidate for further exploration in dose range toxicity before becoming a clinical candidate.

OCF_3 absorbs significantly less ultraviolet A waves, thus reducing toxicity from UV radiation. Alongside this, the less aromatic structure of OCF_3 makes it less lipophilic and more soluble, helping it flow in the bloodstream and reducing the chance of the drug binding to plasma proteins. Finally, the CF_3 alkoxy is slowly metabolised so it maintains a good pharmacokinetic (ADME) profile and a low P-glycoprotein (Pgp) mediated efflux, ensuring it can pass through the blood-brain barrier and reach the amyloid plaques.

It's undeniable that the complex balancing act makes drug discovery and development a time-consuming and resource intensive process. For diseases like Alzheimer's, there is a pressing need for a detailed and thorough approach which can investigate an abundance of potential changes to meet the many requirements for drug treatments. The only solution to a multi-faceted problem is a multi-faceted solution.

Behind the Research

Dr Rosa María Rodríguez Sarmiento

E: rosa_maria.rodriguez_sarmiento@roche.com **T:** (+41) 61-688-3585
W: https://www.linkedin.com/in/rmrodriguezsarmiento

Research Objectives

Rosa María Rodríguez Sarmiento is Senior Principal Scientist, Project Leader in Medicinal Chemistry at Hoffmann La Roche Ltd. She is currently leading several projects in drug discovery.

Detail

Address
Dr Rosa María Rodríguez Sarmiento
Pharmaceutical Research and Early Development
F. Hoffmann-La Roche AG, 4070
Basel, Switzerland

Bio

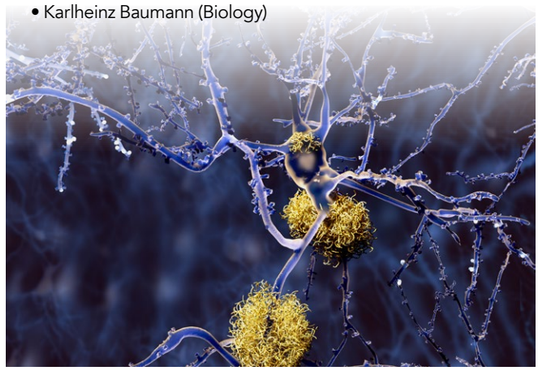
Rosa María Rodríguez Sarmiento, born in the Canary Islands, has worked at Hoffmann La Roche since 2000. She is currently a project-team leader with more than 20 years' experience in medicinal chemistry in a wide array of targets, covering all stages of drug discovery, with direct contribution to the discovery of five compounds in advanced clinical phases or marketed.

Funding
Hoffmann La Roche Ltd.

Collaborators

A multifunctional team was involved in this research:

- Caterine Bissantz and Johan Bylund (DMPK)
- Anja Limberg, Werner Neidhart, Roland Jakob-Roetne (Medicinal Chemistry)
- Lisha Wang (Molecular Modelling)
- Karlheinz Baumann (Biology)



References

Rodríguez Sarmiento, R.M., *et al.* (2020). Stepwise Design of γ-Secretase Modulators with an Advanced Profile by Judicious Coordinated Structural Replacements and an Unconventional Phenyl Ring Bioisostere. *Journal of Medicinal Chemistry,* 63 (12), 8534–8553. doi:10.1021/acs.jmedchem.0c00909

Moore, B., *et al.* (2018). Short Aβ peptides attenuate Aβ42 toxicity *in vivo*. *Journal of Experimental Medicine,* 215 (1), 283-301. doi:10.1084/jem.20170600

Pajouhesh, H., Lenz, G. (2005). Medicinal Chemical Properties of Successful Central Nervous System Drugs. *The Journal of the American Society for Experimental Neurotherapeutics,* 2, 541-553. doi:10.1602/neurorx.2.4.541

Segall, M. (2014). Advances in Multi-parameter Optimisation Methods for de Novo Drug Design. *Expert Opinion on Drug Discovery,* 9 (7), 803-17. doi:10.1517/17460441.2014.913565

Personal Response

Do there need to be any changes to practice within research science or funding to overcome the challenges in drug discovery? If so, what do you think should change?

" Probably to invest more in "basic research" e.g. with collaborations. Also by evaluating additional or alternative scientific "risk taking" approaches and following key programmes even if they are no longer within a new disease strategy. In addition, a proper investment in manpower, considering the attrition rate in generating key data in order to be highly competitive. "

Where do the specific ideas for changing or replacing chemicals/molecules in a compound come from? (for example, changing the phenyl ring to the bioisosteric CF_3 alkoxy ring?)

" From medicinal chemists within a multifunctional team, usually they design the next compound to be prepared based on data available for a compound-series. They are not only chemists that design synthetic routes, as "medicinals" they need to understand and integrate the data from other functions. We use knowhow (e.g. on functional group effects and relationships) and trends based on available data from particular compound-series and predictions. The idea of using the OCF_3 and analogues came from me through an observation. "