New technology for stereoisomeric synthesised drugs

NOXXON Pharma is a biopharmaceutical company developing a new class of proprietary therapeutics called Spiegelmers, a chemically synthesised, non-immunogenic alternative to antibodies. The Spiegelmer platform has generated a number of lead oligonucleotide compounds that are under preclinical investigation as well as a diversified portfolio of clinical-stage therapeutics.

A significant pitfall to the optimisation of RNA aptamers as therapeutics is the instability of RNA. As such, a PhD project at the Free University Berlin in the late 1990s researched how best to solve this issue. The researchers took a novel approach that combined the evolutionary screening technology known as SELEX (systematic evolution of ligands by exponential enrichment) with the use of mirror-image RNA made from non-natural, mirror-image L-nucleotides. The research and subsequent analysis demonstrated that a) functional mirror-image RNA oligonucleotides were generated and that b) such molecules displayed the expected high biostability in a biological environment. On that basis, NOXXON Pharma AG was formed to exploit this drug discovery platform in order to identify better RNA-based therapeutics, which were named Spiegelmers from the German word 'Spiegel', meaning 'mirror'.

Mirror-image oligonucleotides

Spiegelmers are mirror-image oligonucleotides either consisting of L-RNA or L-DNA building blocks, and therefore these molecules have the exact mirror-image behaviour compared to natural nucleic acids. The phenomenon of 'handedness' or chirality is an important property of a large number of organic chemical compounds and the majority of small-molecule drugs are chiral. However, apart from NOXXON's drug candidates, fully functional mirror-image macromolecules based on either a nucleic acids backbone or made from amino acids are not yet in development.

It is important to note that Spiegelmers cannot base-pair with natural RNA or DNA, nor do they interact with the components of the immune system that normally react to extracellular nucleic acids (for example, the toll-like receptors or TLRs).

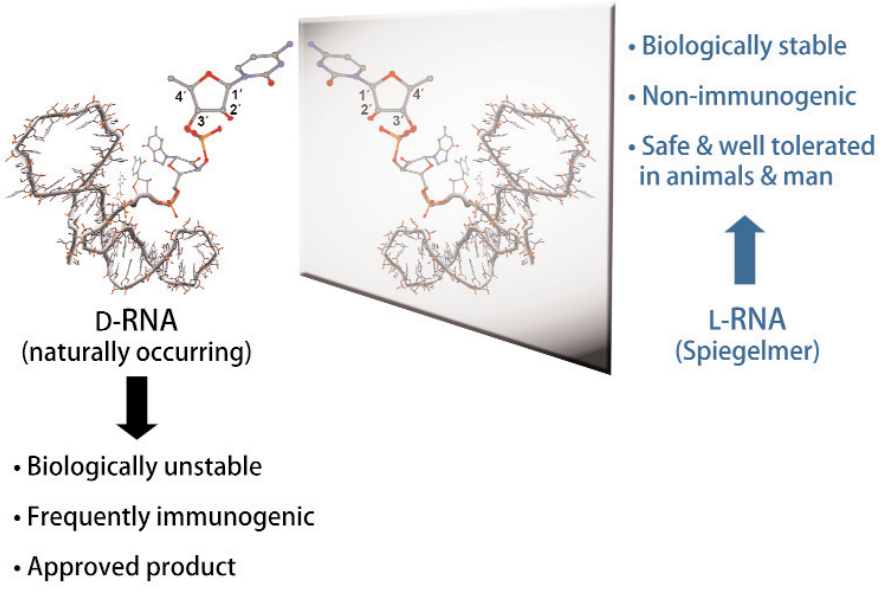
The founders of NOXXON started with an in-licensed patent application that was generated at the Free University Berlin. As with other platform technologies, overlapping and complementing IP has been added to the NOXXON portfolio, giving the company full freedom-to-operate which, in turn, it can pass on to its partners.

Screening for functional structures

"The basis for the identification of new Spiegelmers is the screening of vast oligonucleotide libraries for functional structures. The enormous diversity of these libraries almost guarantees that a few sequences will fold into a suitable structure that can bind and inhibit a target molecule of interest. It is assumed that the larger the size of the library the higher the likelihood of finding a well-suited sequence as a drug candidate lead. The huge number of sequences can be easily handled through means of PCR (polymerase chain reaction) and even if only a single molecule in the starting library may provide the desired properties, powerful PCR may be able to help to select it from the 'noise' of other sequences," explains NOXXON's CSO, Dr Sven Klussmann. "The screening of libraries is performed using natural D-RNA on a mirror-image target, for example a D-amino acid protein. Once the candidate is identified, the corresponding sequence is used but made with L-oligonucleotides and it is this therapeutic which binds to the natural target, for example an L-amino acid protein."

Desirable properties

As described above, the SELEX process is an evolutionary screening technique to identify functional structures (aptamers or their mirror-image counterpart: Spieglemers) based on short nucleic acid sequences. These oligonucleotide structures can be used to block strong pathological protein-protein interactions very efficiently. As such, their properties in terms of affinity and selectivity are comparable to those of monoclonal antibodies and other protein-based scaffolds. A major point of differentiation, however, is the immunogenicity of the products. Whereas oligonucleotides with a natural configuration



Conventional RNA aptamers vs Spiegelmers.

often induce innate immune responses via the toll-like receptor pathway, and non-natural protein sequences almost always elicit strong immune reactions in the body through anti-drug antibodies, Spiegelmers do not seem to cause such reactions.

The molecular weight of a Spiegelmer (or equally an aptamer) sequence is usually in the range of 10-15 kDa. Therefore, systemic administration will lead to rapid renal clearance within a few hours but elimination from the body can be easily prolonged by days through adding polyethylene glycol (PEG) moieties of different sizes. Such a conjugation reaction is particularly easy for oligonucleotides as, compared to protein scaffolds, it is a site-specific reaction that does not require cumbersome optimisation processes for GMP manufacturing. Other modifications are also possible, for example with hydroxyl ethyl starch.

Delivering drug candidates

"The technology platform has now 'matured' into a process that is able to not only deliver drug molecules but also to prepare them for clinical development, as downstream processes in terms of bioanalytics (GLP assays), chemical analytics and GMP manufacturing have been established," says Klussmann.

The technology platform has delivered several drug candidates, three of which are currently in clinical development: NOX-E36 (anti-MCP-1 / CCL2) for diabetic nephropathy (Phase 2a ongoing); NOX-A12 (anti-SDF-1 / CXCL12) for multiple myeloma (Phase 2a ongoing), chronic lymphatic leukemia (Phase 2a ongoing), and glioblastoma; and NOX-H94 (anti-hepcidin) for iron-restriction anemia (Phase 2a to begin in Q3 2012).

"NOXXON is very active in terms of collaborating with academic partners, particularly through our provision of very strong proprietary drug candidates. In return, we benefit from the data our partners provide in elaborated animal models that are often not otherwise available," says Klussmann.

"Partnerships are an essential part of NOXXON's strategy and will be an important means to develop candidate molecules" he says. "With three compounds in clinical trials across a range of potential indications, risk-sharing approaches will be required to mitigate the expense burden of Phase 3 studies. Based on postive data emerging from one or more of the ongoing Phase 2 studies, we expect to sign a partnership agreement on a clinical programme in the first half of 2013. As to what we hope to achieve with such a partnership, NOX-A12 provides a good example. We have compelling preclinical data with NOX-A12 in a generally refractory animal model of brain cancer and a clinical trial design that has been reviewed with clinical experts in the field and regulatory agencies in Europe. We would like very much to start a clinical study of NOX-A12 in this indication, and we have similar examples for NOX-E36.

"Our short-term objective is to achieve proof-of-concept in clinical programmes," Klussmann continues. "Therefore, most efforts and capacities within NOXXON are dedicated to this goal and currently less resources are devoted to preclinical research. Since anything beyond a clinical proof-of-concept requires significant resources, it is planned to do further (co)development steps together with a partner.

"Our primary mission is to develop Spiegelmers as drugs and to bring this new and unique chemical class of therapeutics to the pharmaceutical market place.

The significant resources that are needed will almost certainly need the buy-in of a pharma partner in order to conduct Phase 3 clinical trials. In five years time, it should be possible to have a Spiegelmer product approved and on the market.

"In the past 20 years, fewer small-molecule drugs and increasingly more biological products or biopharmaceuticals were approved as drugs," he adds. "That does not mean that chemical drug discovery has become less important, but rather more elaborated approaches in drug discovery have had to be developed for difficult targets. Here, larger molecules that were based on protein scaffolds, for example monoclonal antibodies, succeeded. The latter is precisely an area where NOXXON will position its drugs as a chemical alternative to monoclonal antibodies."

Klussmann concludes: "NOXXON is at a critical juncture in its development. Three Spiegelmer-based compounds have now advanced through the first stages of clinical development. They appear to be generally safe and well tolerated, and importantly we can already see that they hit their targets because of shifts in biomarkers.

"In June and July 2012 NOXXON started Phase 2a clinical trails for NOX-E36 in patients with diabetic nephropathy and for NOX-A12, treating the first cohort of three chronic lymphocytic leukemia (CLL) patients.

A trial for NOX-H94, which targets hepcidin, the key regulator of iron metabolism, is also planned to start in 2012, so we hope to get answers to the key question in the coming months, if modulation of those targets in patients will result in the expected therapeutic effect."

Meet Sven Klussmann of NOXXON Pharma AG

Dr Sven Klussmann is a world leading expert in the field of therapeutic aptamers and one of the original founders of NOXXON Pharma AG. As a PhD student at the Free University Berlin, Dr Klussmann was the first person to demonstrate the principles of the Spiegelmer technology. He received the Carl Ramsauer Award for this work and, following the completion of his academic studies, went on to transfer the Spiegelmer discovery process from an academic environment into a robust, therapeutic lead-generating industrial process. Since the foundation of NOXXON, Dr Klussmann has held positions as Chief Technology Officer and Chief Scientific Officer. Under his scientific leadership, NOXXON automated the drug discovery process and demonstrated in-vivo proof-of-concept for Spiegelmers' utility as therapeutics in animal models. During this time he has authored more than 40 articles and more than 40 patents on oligonucleotides and their application and has edited a book on aptamers. He is a reviewer for several scientific journals.

From 2006 until January 2008, Dr Klussmann served NOXXON in a twofold function as Chief Scientific Officer and Chief Executive Officer. In this dual role he initiated the transition of NOXXON from a technology-focused drug discovery company into an innovation-driven drug development organisation. To drive this endeavour, Dr Klussmann raised €37 million in venture capital from top-tier biotechnology investors, completing one of the biggest financing rounds in Europe in 2007. Since beginning of 2008, Dr Klussmann has refocused his efforts on scientific development.

Further information

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