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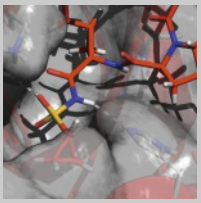

Medicinal Chemistry and Chemical Biology Highlights

Division of Medicinal Chemistry and Chemical Biology
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Drugs Based on *de novo*-developed Peptides are Coming of Age

Kaycie Deyle and Christian Heinis*

*Correspondence: Prof. C. Heinis, École Polytechnique Fédéral de Lausanne (EPFL), Laboratory of Therapeutic Peptides and Proteins, BCH 5305 CH-1015 Lausanne, E-mail: christian.heinis@epfl.ch

Abstract: Naturally evolved peptides, such as the hormone oxytocin or the anti-bacterial vancomycin, have seen decades of success as powerful therapeutics due to many of the favorable properties of peptides. Not every desired target has a naturally occurring bioactive peptide, so rational design and random *in vitro* evolution techniques have been developed and applied to generate peptide leads *de novo*. However, can these artificially created peptides be translated into successful therapeutics? Several drug development programs involving *de novo*-generated peptide ligands have made important progress recently, and we report here on these exciting activities.

Keywords: Cyclic peptide · Drug development · *in vitro* evolution · Peptide · Phage display · Protein epitope mimetics · Ribosome display

Peptides have several qualities that make them an attractive format for the development of drugs, such as good binding properties, low inherent toxicity and ease of synthesis. More than 50 peptide drugs are currently used as therapeutics, such as the well-known human hormones or hormone derivatives oxytocin and octreotide or the antibiotics vancomycin and polymyxin B.[1] All these peptide drugs are natural products or derivatives thereof, and as such, originate from peptides evolved in nature. Given the success of these peptides and the lack of natural peptide ligands for a broad range of desired disease targets, efforts were undertaken for the *de novo* generation of peptide ligands, either based on existing protein ligands or by screening libraries of random peptides. Several of these peptides have entered clinical testing recently and are showing promising results, as illustrated through the following examples.

Peptide drug candidates based on so-called protein-epitope mimetics (PEMs) developed by Polyphor (Allschwil, Switzerland) have recently made important advances at different stages of clinical development. The PEM format, developed by John Robinson at the University of Zurich in collaboration with Polyphor, is based on hairpin loop sequences taken from binding faces of folded proteins that are transplanted onto hairpin-stabilizing templates, such as the dipeptide D-Pro-L-Pro (Fig. 1a).[2] Polyphor's most advanced PEM drug candidate, the antibiotic murepavadin (POL7080), has entered a phase III clinical trial in March of this year. Murepavadin targets specifically *Pseudomonas aeruginosa* by binding to an outer membrane protein.[3] A second clinical-stage PEM developed by Polyphor, the immuno-oncology candidate balixafortide (POL6326), has successfully completed a phase I clinical trial this year. Balixafortide is a potent and selective antagonist of CXCR4, a G-protein coupled receptor that regulates the trafficking and homing of both cancer and immune system cells. In addition to these two programs, Polyphor is developing the peptide POL6014, currently in phase I, which is a potent and selective inhibitor of human neutrophil elastase that was licensed to Santhera Pharmaceuticals (Liestal, Switzerland) this year.

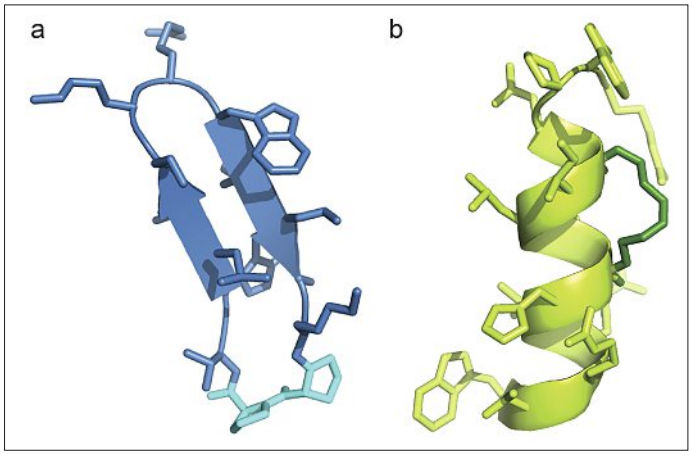

Fig. 1. Structure of a β-strand peptide (blue) stabilized by D-Pro-L-Pro (cyan) (a) and an α-helix (light green) stabilized by a hydrocarbon linker (dark green) (b). The structures are based on PDB files 2M7I and 4DJS.

Another *de novo*-developed peptide in clinical testing is ALRN-6924, an α-helical peptide that disrupts both MDMX- and MDM2-mediated inhibition of the p53 tumor suppressor gene. ALRN-6924 was engineered by Aileron Therapeutics through the use of a 'peptide stapling' technique developed by Greg Verdine (Harvard University) (Fig. 1b)[4] to improve the stability and cell penetrability of the α-helical peptide while maintaining its high affinity for the target proteins.[5] Aileron Therapeutics has tested ALRN-6924 in multiple clinical trials, the most advanced currently a phase IIa. In an IPO one year ago, the company raised money for the further clinical development of the drug candidate.

Biological display techniques, such as phage display or mRNA display, have been employed to genetically encode billions of random peptides, which enables the identification of lead peptides through affinity selections performed against immobilized proteins. These biological display techniques encode the peptide library with DNA or RNA to allow for the rapid deconvolution of lead sequences. In phage display, this encoding is achieved by fusing the peptides to a bacteriophage coat protein, such as pIII.[6] In mRNA display, the mRNA is modified with puromycin, which is incorporated into the peptides during ribosomal translation and physically links the mRNAs and peptides.[7] In terms of therapeutic leads, these techniques are shifting such that cyclic peptides that tend to bind to targets with a higher affinity and selectivity and have a better proteolytic stability are becoming the standard over their linear counterparts.

Several of these peptides created 'from scratch' through the screening of these large random peptide libraries have entered the clinical phase in recent years. A clinical stage peptide that was generated by the mRNA display approach of Jack Szostak (Harvard Medical School)[7] is RA101495, an inhibitor of complement component 5 (C5) that was developed for the treat-

ment of paroxysmal nocturnal hemoglobinuria (PNH) and other diseases. This cyclic peptide generated by Ra Pharmaceuticals (Cambridge, USA) is currently being evaluated in a phase II clinical trial. The peptide was made for subcutaneous self-administration by patients, which would provide an advantage over the approved anti-C5 antibody drug, Soliris, which is applied by infusion.

Another peptide generated by mRNA display that has reached the clinical stage was developed jointly by the Japanese company PeptiDream and Bristol-Myers Squibb (BMS). PeptiDream is using an elegant approach developed by Hiroaki Suga (University of Tokyo) that is based on a ribozyme (termed flexizyme) that enables incorporation of unnatural amino acids into mRNA-encoded peptide libraries (Fig. 2a).[8] The PeptiDream and BMS peptide was developed for an undisclosed immuno-oncology application; in 2016, the two companies announced the dosing of a first patient in a clinical trial.

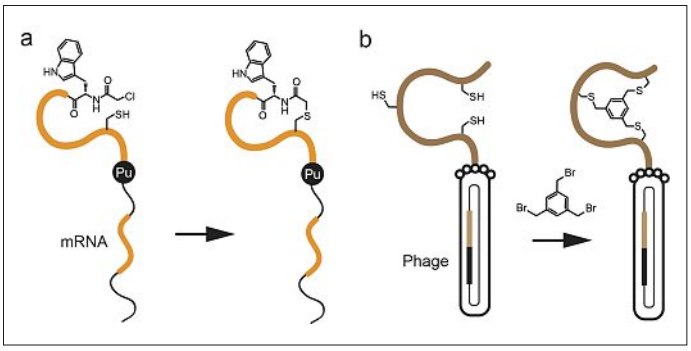

Fig. 2. Display techniques used to evolve peptide ligands to targets of interest. (a) Ribosome display of cyclic peptides. The example shows a cyclization based on a chloroacetamide-functionalized tryptophan with cysteine. (b) Phage display of bicyclic peptides. The example shows the reaction of three cysteines with 1,3,5-tris-(bromomethyl)benzene.

A peptide-based drug candidate engineered by phage display, the bicyclic peptide-drug conjugate BT1718 developed by Bicycle Therapeutics (Cambridge, UK), has entered clinical evaluation this year. Bicyclic peptides binding to targets of interest can be generated by chemically cyclizing random peptides displayed on phage prior to affinity selections (Fig. 2b).[9] BT1718 is a conjugate between a bicyclic peptide that binds to membrane type 1 matrix metalloproteinase (MT1-MMP/MMP-14) and a toxin. This strategy of guiding toxic chemical payloads specifically to malignant tumors by peptide ligands is a promising way of minimizing systemic toxin exposure through renal clearance. BT1718 is being evaluated by Bicycle Therapeutics and Cancer Research UK in a phase I/IIa trial in patients with advanced solid tumors.

In addition to the above clinical programs, a wide range of *de novo*-developed peptide ligands are currently in pre-clinical stages, meaning that many more promising peptides should reach the clinical phase soon. In parallel, techniques for engineering new peptide ligands are continuously improving and new strategies are being invented. This past year alone, several new peptide formats and encoding strategies were presented, such as a plasmid-encoded lanthipeptide library developed by the lab of Wilfred van der Donk,[10] DNA-encoded macrocycles by the group of Dario Neri[11] and phage-encoded double-bridged peptides by our lab.[12] The development of ever better peptide ligands to more and varied targets will deliver new candidates for clinical development and promises a bright future for *de novo*-developed peptide therapeutics.

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Can you show us your Medicinal Chemistry and Chemical Biology Highlight?
Please contact: Dr. Cornelia Zumbrunn, Idorsia Pharmaceuticals Ltd., Hegenheimermattweg 91, CH-4123 Allschwil,
E-mail: cornelia.zumbrunn@idorsia.com