VEGF reflects on itself

By Tim Fulmer, Senior Writer

North American researchers have chemically synthesized a D-protein ligand that blocked the binding of VEGF to its receptor *in vitro*.¹ **Reflexion Pharmaceuticals Inc.** has licensed the compound and thinks it could have better stability and bioavailability than anti-VEGF antibodies that are L-isomer proteins. The company is optimizing its molecule before doing head-to-head comparisons with Lucentis and Avastin in animal models of age-related macular degeneration.

Protein and peptide molecules expressed in all organisms, including recombinant antibodies produced in bacteria, occur only as the left-handed L-isomer and never as the mirror-image, right-handed D-isomer.

Although biologics developers generally have not been concerned with that distinction, protein therapeutics based on the D-isomer should have greater stability and serum half-life than the L-isomer because the D-isomer is not recognized by the body's proteases. The ability to resist proteolytic degradation also raises the possibility of delivering D-isomers orally.

The open question is whether a D-protein therapeutic can be synthesized and optimized to hit its target. To date, researchers have studied only small D-peptides² and

not D-proteins that potentially consist of hundreds of amino acids and could be quite challenging to synthesize and optimize.

In 2009, Stephen Kent, Sachdev Sidhu and Dana Ault-Riché founded Reflexion with the goal of developing D-protein-based therapeutics. As initial proof of principle, they decided to design a D-protein that bound VEGF and prevented it from binding the VEGF receptor (VEGFR).

Kent is a professor of chemistry at **The University of Chicago**. Sidhu is co-investigator at the **Ontario Institute for Cancer Research** and associate professor at the **University of Toronto**. Ault-Riché is CEO of Reflexion.

They chose VEGF because it is a validated therapeutic target and would allow for a direct comparison between a D-protein VEGF antagonist and an antibody-based VEGF antagonist such as Avastin

bevacizumab. Moreover, Kent had experience working with VEGF. In 2011, he published in *Angewandte Chemie International Edition* the total chemical synthesis of the L-isomer of the 204-amino-acid growth factor.³

Avastin is marketed by **Roche**, its **Genentech Inc.** unit and **Chugai Pharmaceutical Co. Ltd.** to treat multiple solid cancers.

For their D-protein antagonist screening assay, Reflexion licensed technology from the **Massachusetts Institute of Technology's Whitehead Institute for Biomedical Research** that allowed them to screen ligand and protein libraries against the D-isomer of the target protein. The method, dubbed mirror image phage display, originally was developed by Peter Kim in the mid-1990s when he was a researcher at Whitehead.^{4,5} Kim now is EVP and president of **Merck & Co. Inc.**'s Merck Research Laboratories.

Mirror image phage display uses the D-isomer of the target protein to screen a library of L-isomer ligands. The top hits are then chemically converted to D-isomers.

The final D-isomer ligand binds the original protein with high affinity and specificity.

Reflexion synthesized D-VEGF using the chemical synthesis method described in the 2011 paper. Next, the researchers used D-VEGF to screen a large library of L-protein ligands. The scaffold for the library was derived from the B1 domain of streptococcal protein G (GB1), a 56-amino-acid protein that is known to interact strongly with VEGF and is sufficiently stable to be used in a phage display assay.

Following multiple screening rounds, the L-protein ligand that bound D-VEGF with the highest affinity was chemically converted to the mirror image D-protein ligand, which was predicted to bind VEGF with high affinity.

Indeed, the D-protein blocked the binding of VEGF to VEGFR *in vitro*. Moreover, protein X-ray crystallography studies showed that the D-protein bound the region of VEGF that interacts with VEGFR, confirming the D-protein was an antagonist of the VEGF-VEGFR interaction.

The findings were published in the *Proceedings of the National Academy of Sciences*.

The key difference between the PNAS findings and prior work in the field "is that the authors constructed a protein library for their screen, as opposed to a peptide library, thus obtaining a D-protein inhibitor," Wuyuan Lu told *SciBX*. He is professor of biochemistry and molecular biology at the **University of Maryland School of Medicine**.

"D-Protein inhibitors enjoy a huge advantage over D-peptide inhibitors when it comes to targeting extracellular proteins such as receptors, as it is generally more difficult to design high-affinity small peptide antagonists of receptors," said Lu. The reason, he said, is that thermodynamic factors generally cause small peptides to interact weakly with receptors.

Earlier this year, Lu and colleagues published in the *Journal of Medicinal Chemistry* that mirror image phage display identified a high affinity D-peptide antagonist of the cancer target mdm2 p53 binding protein homolog (MDM2; HDM2).⁶

D-Protein inhibitors also have a potential cost advantage over antibodies because they are produced as a totally synthetic product, unlike biologics that require cell culture, said Michael Kay, assistant

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—Wuyuan Lu,
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School of Medicine

professor of biochemistry at **The University of Utah School of Medicine**.

Kay and colleagues have developed D-peptide HIV entry inhibitors that are exclusively licensed to **Navigen Pharmaceuticals Inc.**^{7,8} The company's lead D-peptide inhibitor, PIE12-trimer, is in preclinical development to prevent and treat HIV.

Because the *PNAS* paper has no *in vivo* data, "obviously a lot more needs to be done to study the PK, PD and toxicity profiles" of the D-protein antagonist, said Lu.

Also important will be "studies in suitable transgenic animal models," added Dieter Willbold, professor of physical biology at the **Heinrich Heine University of Duesseldorf**. Willbold and colleagues have shown that an orally available D-peptide ligand of β -amyloid (A β) reduced pathology and improved behavior in transgenic mouse models of Alzheimer's disease (AD).⁹

The right-hand path

Reflexion "has already produced affinity-matured, next-generation molecules based on the initial lead in the *PNAS* article," Sidhu told *SciBX*. He added that the best antagonists "will be validated in cell-based assays to assess inhibition of VEGF activity in physiologically relevant systems and also tested in animal models of age-related macular degeneration and cancer, in comparison with Lucentis and Avastin."

The anti-VEGF mAb Lucentis ranibizumab is marketed by Roche, Genentech and **Novartis AG** to treat age-related macular degeneration (AMD) and diabetic macular edema (DME).

Reflexion will also test its D-protein antagonists for immunogenicity. "We believe the D-amino-acid nature of our molecules should significantly reduce immunogenicity, since our molecules are resistant to proteolysis and cannot be recognized by MHC," said Sidhu. The major histocompatibility complex (MHC) plays a central role in triggering the host immune response.

To help carry out the animal testing, Reflexion is collaborating with Calvin Kuo, associate professor of medicine at the **Stanford University School of Medicine**. Kuo has expertise in animal models of angiogenesis, said Ault-Riché.

Sidhu said he is assembling a large panel of diverse scaffolds that will expand the power of the mirror image phage display technology.

Reflexion also is developing a D-protein compound to treat the orphan disease lymphangioleiomyomatosis (LAM), which causes the

lungs of women in their thirties and forties to develop cysts that destroy lung function.

"Studies suggest that LAM patients could benefit from a compound that antagonizes the VEGF-D homolog," said Ault-Riché. "We should be able to do mirror image phage display much like what we did in the *PNAS* paper to identify D-protein antagonists of VEGF-D," he said.

The compounds in the *PNAS* paper are covered by patents and are available for licensing from Reflexion.

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REFERENCES

1. Mandal, K. *et al. Proc. Natl. Acad. Sci. USA*; published online Aug. 27, 2012; doi:10.1073/pnas.1210483109
Contact: Sachdev S. Sidhu, University of Toronto, Toronto, Ontario, Canada
e-mail: sachdev.sidhu@utoronto.ca
Contact: Stephen B.H. Kent, The University of Chicago, Chicago, Ill.
e-mail: skent@uchicago.edu
2. Sun, N. *et al. J. Biotechnol.* **161**, 121–125 (2012)
3. Mandal, K. & Kent, S.B. *Angew. Chem. Int. Ed.* **50**, 8029–8033 (2011)
4. Schumacher, T.N.M. *et al. Science* **271**, 1854–1857 (1996)
5. Funke, S.A. & Willbold, D. *Mol. Biosyst.* **5**, 783–786 (2009)
6. Zhan, C. *et al. J. Med. Chem.* **55**, 6237–6241 (2012)
7. Welch, B.D. *et al. J. Virol.* **84**, 11235–11244 (2010)
8. Welch, B.D. *et al. Proc. Natl. Acad. Sci. USA* **104**, 16828–16833 (2007)
9. Aileen Funke, S. *et al. ACS Chem. Neurosci.* **1**, 639–648 (2010)

COMPANIES AND INSTITUTIONS MENTIONED

Chugai Pharmaceutical Co. Ltd. (Tokyo:4519), Tokyo, Japan
Genentech Inc., South San Francisco, Calif.
Heinrich Heine University of Duesseldorf, Duesseldorf, Germany
Massachusetts Institute of Technology, Cambridge, Mass.
Merck & Co. Inc. (NYSE:MRK), Whitehouse Station, N.J.
Navigen Pharmaceuticals Inc., Salt Lake City, Utah
Novartis AG (NYSE:NVS; SIX:NOVN), Basel, Switzerland
Ontario Institute for Cancer Research, Toronto, Ontario, Canada
Reflexion Pharmaceuticals Inc., San Francisco, Calif.
Roche (SIX:ROG; OTCQX:RHHBY), Basel, Switzerland
Stanford University School of Medicine, Palo Alto, Calif.
The University of Chicago, Chicago, Ill.
University of Maryland School of Medicine, Baltimore, Md.
University of Toronto, Toronto, Ontario, Canada
The University of Utah School of Medicine, Salt Lake City, Utah
Whitehead Institute for Biomedical Research, Cambridge, Mass.