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REPRINT FROM OCTOBER 31, 2013

Building meditope-enabled mAbs

By Kai-Jye Lou, Senior Writer

Linker technologies for generating antibody-drug conjugates typically require a labor-intensive process of reengineering the antibody for every application. Researchers at the **Beckman Research Institute at City of Hope** have published proof-of-concept data on a platform to generate mAb conjugates with other molecules of interest that eliminates the need to reengineer the antibody for each conjugate.[1]

Institute spinout **Meditope Biosciences Inc.** is commercializing the technology, which involves building a site into mAbs that can bind to peptides called meditopes that serve as linkers to various payloads. The startup raised $3.6 million in a series A round in July and is seeking industry partnerships.

Antibody-drug conjugate (ADC) linkers can disrupt a mAb's native properties in an undesirable manner—for example, by decreasing antigen-binding affinity, specificity and stability or increasing immunogenicity.[2]

Thus, researchers need to reassess and likely reoptimize the properties of a mAb each time they reengineer it to carry a different molecule. Doing so is labor intensive and can become cost prohibitive when generating a series of candidates.

A research group led by John Williams has been studying the structure of various antibodies in order to develop a more efficient approach to link compounds to mAbs. Williams is director of the X-ray Crystallography Core Facility, co-director of the Drug Discovery and Structural Biology Core and an associate professor in the Department of Molecular Medicine at the Beckman Research Institute.

In a City of Hope press release in June, Williams' team announced the chance discovery of a peptide-binding site unique to Erbitux cetuximab and then showed that a peptide called a meditope could bind to the site.

Williams' group hypothesized that it might be possible to use the site and meditopes as a convenient means to attach different compounds to a mAb by first conjugating a compound to a meditope.

Eli Lilly and Co., **Bristol-Myers Squibb Co.** and **Merck KGaA** market Erbitux, a chimeric antibody against epidermal growth factor receptor (EGFR), to treat head and neck cancer and colorectal cancer.

Now, the City of Hope researchers have characterized the binding site along with its interaction with the meditope peptide and have shown proof of concept on how to exploit it (*see* **Figure 1, "Meditope-enabled mAbs"**).

Analysis of the crystal structure of the Erbitux Fab domain in complex with meditope peptides showed that the meditope-binding site is located within a cavity formed by the mAb's light and heavy chains. Follow-up biophysical and sequencing studies identified the key amino acid residues that mediate meditope binding. The studies also showed that binding of the meditope to the mAb occurs via a noncovalent interaction that does not disrupt the mAb's ability to bind to its target antigen.

With the structural and sequence data in hand, the researchers were then able to engineer the meditope-binding site into another marketed mAb—the humanized anti-HER2 (EGFR2; ErbB2; neu) mAb Herceptin trastuzumab. Importantly, creating the binding site and binding a meditope to it did not significantly disrupt the ability of the meditope-enabled trastuzumab to bind its target antigen.

However, the researchers found that monovalent meditopes only bound to mAbs with moderate affinity and could be removed with a wash procedure—an issue that could preclude their potential use for attaching imaging agents and therapeutic payloads to the mAb.

Thus, the researchers engineered a bivalent meditope-Fc construct and showed that it could bind to Erbitux and the meditope-enabled Herceptin with high enough affinity to resist an extensive washing procedure without significantly disrupting native antigen binding.

Data were published in the *Proceedings of the National Academy of Sciences*. **Roche**'s **Genentech Inc.** unit markets Herceptin to treat breast and gastric cancers.

"The strategy presented in the report clearly offers a novel mAb linker technology platform, where any mAb could easily be turned into a site-specific, Lego-like system that is able to attach small molecules or toxins to antibodies without the need for chemical conjugation," said Iqbal Grewal, CSO at **ImmunGene Inc.**

"If you're trying to make an antibody-drug conjugate, current methods generally involve chemically locking a payload to the antibody. But doing this can disrupt the antibody's native properties, such as antigen binding," added Williams, the corresponding author and a cofounder of Meditope. "What we have developed is a system to hitch a molecule onto an antibody via a noncovalent interaction that does not interfere with antigen binding."

Williams said that the meditope platform could have utility across a range of settings, including the development of new ADCs or

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SciBX is produced by BioCentury Publications, Inc. and Nature Publishing Group Joint Steering Committee: Karen Bernstein, Ph.D., Chairman & Editor-in-Chief, BioCentury; David Flores, President & CEO, BioCentury; Bennet Weintraub, Finance Director, BioCentury; Steven Inchcoombe, Managing Director, Nature Publishing Group; Peter Collins, Ph.D., Publishing Director, NPG; Christoph Hesselmann, Ph.D., Chief Financial Officer, NPG.

reagents for antibody-pretargeted imaging and immunoprecipitation assays.

Grewal said that bivalent meditopes also could be prepared and that these could be used to cross-link meditope-enabled mAbs to improve the efficacy or signaling properties of the antibodies. ImmunGene is developing mAbs fused to immune effector molecules to treat various types of cancer.

Meditope benefits

Williams said that the meditope platform obviates the need to reengineer a meditope-enabled mAb every time the user wants to attach a different compound. Instead, all the chemistry and conjugation work now occurs on the meditope peptide, which Williams said is easier to work with than a mAb.

The other key benefit of meditope-enabled mAbs is that users can swap out one meditope conjugate for another. This enables the generation and evaluation of a series of candidates more efficiently than methods that involve chemical modifications to the mAb itself.

"What we've basically done is installed a hitch onto an antibody that would allow it to carry a variety of payloads," Williams told *SciBX*. "We could build libraries with hundreds of meditopes conjugated with different drugs and imaging agents, all of which could be attached to a single meditope-enabled mAb without making additional modifications to the mAb itself."

He also said that building a meditope-binding site into a mAb should not increase its immunogenicity. The reason is that the location of the binding site and the specific amino acid sequences that mediate meditope binding are unlikely to be seen or recognized by the host immune system.

In contrast, Williams said that reengineered mAbs often result in the exposure of new antibody surfaces to the host immune system, which can be immunogenic.

Grewal said that the meditope platform also enables the design of ADCs with specific and predictable drug-to-antibody ratios, which could allow for better control over efficacy and toxicity compared with current ADC linker technologies.

He also said that a meditope could noncovalently bind to a mAb under a broad range of settings, such as aqueous, nonaqueous, hydrophobic, acidic or basic conditions.

"This provides for a very easy process in which drug-loaded meditopes and antibodies can simply be mixed to create fully functional antibody-drug conjugates," he said.

Making more mAbs

Williams said that the group at City of Hope plans to report additional examples of meditope-enabled mAbs in an upcoming publication. He said that the group also is evaluating various strategies to improve the affinity of meditope-mAb interactions.

Meditope Biosciences president and CEO Stephanie Hsieh said that the company will focus on developing the meditope platform for clinical applications. She said that the most immediate application will be using the platform as a new way to generate ADCs.

Grewal said that he now wants to see candidates generated with the meditope platform tested *in vivo*.

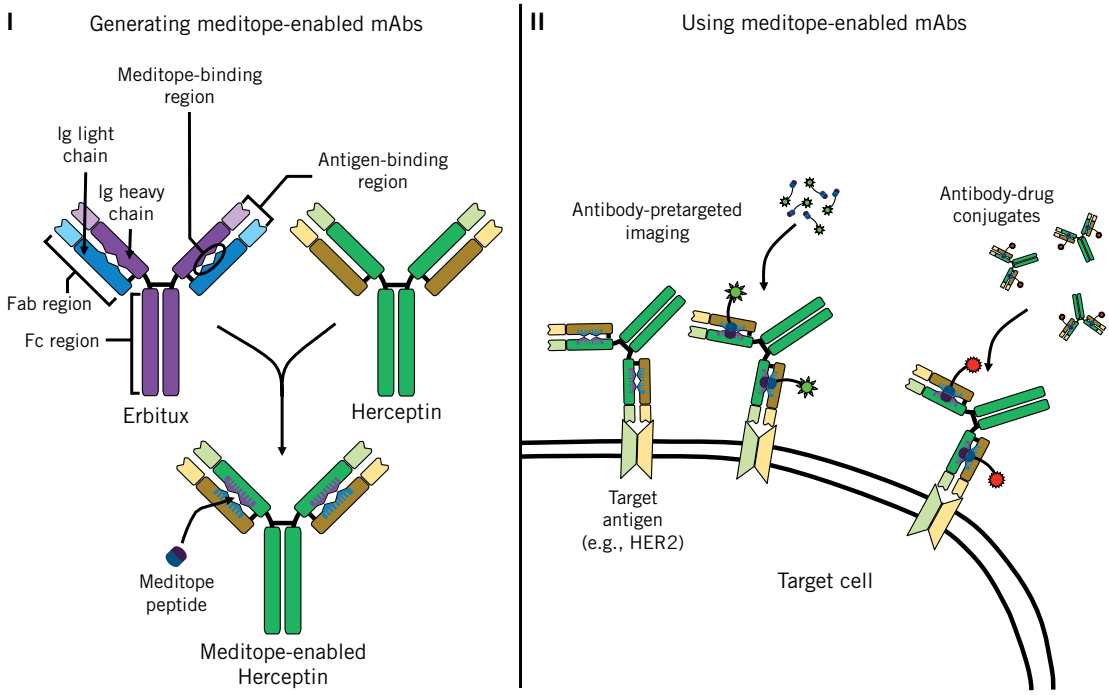

Figure 1. Meditope-enabled mAbs. As reported by Donaldson et al., the Fab framework of the chimeric anti–epidermal growth factor receptor (EGFR) mAb Erbitux cetuximab contains a unique peptide-binding site that is distinct from the antibody's antigen-binding sites. This site is located in the center of a cavity formed by the mAb's light and heavy chains and can bind to engineered peptides called meditopes without interfering with antigen binding.

[I] The meditope-binding site can be engineered into other mAbs, such as the anti-HER2 (EGFR2; ErbB2; neu) mAb Herceptin trastuzumab.

Meditope-enabled mAbs can be conjugated to a broad range of molecules and thus represent a new avenue in antibody-drug conjugates. A meditope-enabled mAb can be used to target many otherwise nonspecific agents without the need to make additional modifications to the mAb itself.

[II] Potential applications include using meditope-enabled mAbs plus meditope-peptide conjugates to deliver imaging agents [green stars] or therapeutic payloads [red stars] to target cells.

"In order to validate the clinical utility of the meditope technology, it must be studied through an *in vivo* evaluation using well-established model systems," he told *SciBX*. "In addition, toxicity studies in animals are necessary to validate its viability for potential therapeutic use."

City of Hope has filed patents covering the meditope platform, which have been exclusively licensed to Meditope Biosciences.

Hsieh said that Meditope is seeking deals to generate meditope-enabled versions of a partner's mAbs.

Lou, K.-J. SciBX 6(42); doi:10.1038/scibx.2013.1178
Published online Oct. 31, 2013

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