Linker Domain Size Does Not Impact Bivalent HER3 Targeting Affibody Efficacy

Jinan M. Oubaid, John S. Schardt, Ph.D., Steven M. Jay, Ph.D.
Fischell Department of Bioengineering, University of Maryland College Park

A. James Clark School of Engineering

PURPOSE

HER3 Receptor:

- Tyrosine kinase receptor that is overexpressed in various cancers including breast, ovarian, prostate, and lung cancer
- Has a weak intracellular kinase domain and relies on forming heterodimers with other members of the HER family to promote downstream signaling
- Responsible for patient resistance to therapeutics that target other members of the HER family

Clinical Need:

- No HER3 targeting therapeutic has received FDA approval
- HER3 overexpression can result in poor prognosis for several cancers, including lung adenocarcinomas
- Targeted therapy allows for site-directed therapy; reduces risk of affecting healthy tissue

Objectives:

- Engineer and optimize bivalent HER3 affibodies for enhanced therapeutic potential
- Modify bivalent HER3 affibody linker domain length and test affibody efficacy

METHODS

Affibodies Used for Cell Assays:

- Affibody with linker domain comprised of 1, 2, 3, or 4 repeating units (Figure 1)
- A minimal linker made with 3 glycine amino acids

Creating the Constructs:

- Original bivalent HER3 affibody was made up of 3 repeats of the following sequence called L20: ASGAGGSEGGGSEGGTSGAT
- Each repeat unit has a length of ~7nm
- The 1 link, 2 link, 3 link, 4 link, and minimal linker bivalent HER3 affibody constructs were created using mutagenesis
- Vectors were transformed into and expressed in BL21 (DE3) competent *E. coli* cells
- Competent cells were induced, lysed, and purified

Cell Signaling Assay:

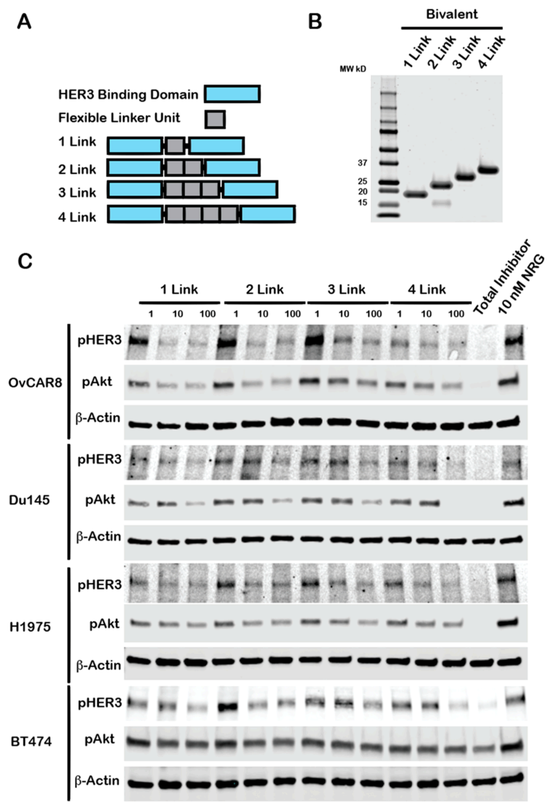
- Cells were serum starved for 3 hours treated with indicated concentrations of affibodies, stimulated with NRG, lysed, and prepped for immunoblotting
- Immunoblots were probed with indicated primary antibodies

Downregulation Assay:

- Cells were treated with indicated concentrations of affibodies for 3 or 24 hours, lysed, and prepped for immunoblotting
- Immunoblots were probed with indicated primary antibodies

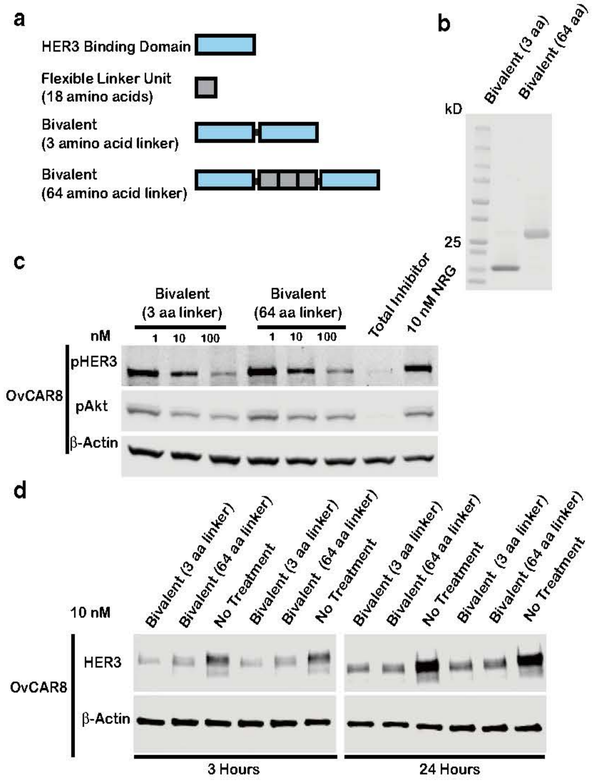
RESULTS

Figure 1: Immunoblots comparing pHER3/pAkt inhibition of the 1, 2, 3, and 4 link bivalent affibodies



- **Figure 1A:** Abstract representation of affibody constructs
- **Figure 1B:** Molecular weights of 1, 2, 3, and 4 linker bivalent HER3 affibodies are 16.0, 17.5, 19.1, 20.6 kDa, respectively
- **Figure 1C:** Immunoblots of cell signaling study comparing efficacy of 1, 2, 3, and 4 link Bivalent HER3 affibodies
- Bivalent HER3 affibodies inhibited pHER3/pAkt for all linker lengths tested in multiple cell lines
- 1 link bivalent HER3 affibody (~7nm) also maintained efficacy even though it theoretically is not long enough to cross the 10nm distance between homodimerized HER3 binding domains

Figure 2: Immunoblots comparing pHER3/pAkt inhibition and HER3 downregulation of the GGG minimal linker and the original 3 link bivalent affibody for the OvCAR8 cell line



- **Figure 2A:** Abstract representation of affibody constructs
- **Figure 2B:** MW of 3 aa linker and 64 aa linker affibodies are 14.4 and 19.1 kDa, respectively
- **Figure 2C:** Immunoblots of cell signaling study comparing efficacy of the bivalent HER3 affibodies with the 3 aa and the 64 aa linker (3 link)
- **Figure 2D:** Immunoblots of HER3 downregulation study comparing efficacy of bivalent HER3 affibodies with the 3 aa and 64 aa linker
- pHER3/pAkt inhibition and HER3 downregulation was similar for both the original 3 link linker bivalent HER3 affibody (64 aa linker) and the minimal linker bivalent HER3 affibody (3 aa linker)
- 64 aa linker is ~20nm in length
- 3 aa linker is ~1nm in length

CONCLUSIONS

- Linker domain design such as altering flexibility and length are important for developing optimized therapeutics; however, bivalent HER3 affibody efficacy is not affected by linker domain length
- Affibody constructs including the 1 link, 2 link, 3 link, 4 link, and minimal link (3 aa linker) were all effective at promoting inhibition of HER3 and Akt phosphorylation
- The 1 link, 2 link, 3 link, and 4 link affibodies effectively inhibited HER3 phosphorylation in OvCAR8, Du145, H1975, and BT474 cell lines
- The bivalent HER3 affibody with the 3 aa linker also demonstrated comparable HER3 downregulation as seen with the original bivalent HER3 affibody (3 link) design
- Smaller proteins, such as the bivalent HER3 affibody with the 3 aa linker, result in better tissue penetration
- Minimal linkers may also result in therapeutics with lower immunogenicity risks
- The reduction in immunogenicity risks allows for enhanced translational potential

Future Work:

- Optimize affibodies using an albumin binding domain to increase affibody half-life for *in vivo* testing

REFERENCES

- A. Martin, T. A. Baker, and R. T. Sauer, "Rebuilt AAA + motors reveal operating principles for ATP-fuelled machines," (in eng), Nature, vol. 437, no. 7062, pp. 1115-20, Oct 2005, doi: 10.1038/nature04031.
- C. J. Liu and J. R. Cochran, "Engineering Multivalent and Multispecific Protein Therapeutics," in Engineering in Translational Medicine, W. Cai Ed. London: Springer London, 2014, pp. 365-396.
- E. S. Yi et al., "High c-erbB-3 protein expression is associated with shorter survival in advanced non-small cell lung carcinomas," (in eng), Mod Pathol, vol. 10, no. 2, pp. 142-8, Feb 1997.
- F. Y. Frejd and K. T. Kim, "Affibody molecules as engineered protein drugs," (in eng), Exp Mol Med, vol. 49, no. 3, p. e306, 03 2017, doi: 10.1038/emm.2017.35.
- G. Sithanandam and L. M. Anderson, "The ERBB3 receptor in cancer and cancer gene therapy," (in eng), Cancer Gene Ther, vol. 15, no. 7, pp. 413-48, Jul 2008, doi: 10.1038/cgt.2008.15.
- H. Lyu, A. Han, E. Polsdofer, S. Liu, and B. Liu, "Understanding the biology of HER3 receptor as a therapeutic target in human cancer," (in eng), Acta Pharm Sin B, vol. 8, no. 4, pp. 503-510, Jul 2018, doi: 10.1016/j.apsb.2018.05.010.
- "How Targeted Therapies Are Used to Treat Cancer." American Cancer Society. https://www.cancer.org/treatment/treatments-and-side-effects/treatment-types/targeted-therapy/what-is.html (accessed March 7, 2020).
- J. S. Schardt *et al.*, "Engineered Multivalency Enhances Affibody-Based HER3 Inhibition and Downregulation in Cancer Cells," (in eng), *Mol Pharm,* vol. 14, no. 4, pp. 1047-1056, 04 2017, doi: 10.1021/acs.molpharmaceut.6b00919.
- J. S. Schardt *et al.*, "HER3-Targeted Affibodies with Optimized Formats Reduce Ovarian Cancer Progression in a Mouse Xenograft Model," (in eng), *AAPS J,* vol. 21, no. 3, p. 48, Apr 2019, doi: 10.1208/s12248-019-0318-x.
- K. Mujoo, B. K. Choi, Z. Huang, N. Zhang, and Z. An, "Regulation of ERBB3/HER3 signaling in cancer," (in eng), Oncotarget, vol. 5, no. 21, pp. 10222-36, Nov 2014, doi: 10.18632/oncotarget.2655.
- S. M. Jay *et al.*, "Engineered bivalent ligands to bias ErbB receptor-mediated signaling and phenotypes," (in eng), *J Biol Chem,* vol. 286, no. 31, pp. 27729-40, Aug 2011, doi: 10.1074/jbc.M111.221093.

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PI: Dr. Steven Jay

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Other contributors: Sonya Williams, Madeleine Noonan-Shueh, Riyadh Ali, Eli Pottash