Engineered Multivalency Enhances Efficacy of Affibody-Based HER3 Inhibition for Cancer Therapy

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Purpose
The receptor tyrosine kinase HER3 has emerged as a therapeutic target in ovarian, prostate, breast, lung and other cancers due to its ability to potently activate the PI3K/Akt pathway and mediate drug resistance. Enhanced efficacy of HER3-targeted therapeutics would therefore benefit a wide range of patients. In this study, we evaluated the potential of multivalent presentation, through protein engineering, as a strategy to enhance the therapeutic effectiveness of HER3-targeted antibody fragments (affibodies).

Methods
The Z05413 affibody, evolved through display technology with a sub-nanomolar affinity for HER3 and the ability to block neuregulin-1β (NRG) -mediated activation, was selected as the ligand binding domain. Coding DNA for affibody constructs was inserted into pET45b vector and expressed in E. coli strain BL21 (DE3). The affibodies were purified from soluble protein lysates by immobilized metal affinity chromatography, and buffer exchanged in PBS using size exclusion HPLC. For signaling assays, cells were treated with affibodies or small molecule total ErbB inhibitor at the indicated concentrations, stimulated with NRG, lysed, and probed for pHER3, pHER2, and pAkt by immunoblot. To examine HER3 receptor levels, cells were treated with affibodies at the indicated concentrations, lysed at the indicated time points, and HER3 protein was probed by immunoblot. For proliferation assays, cells were seeded at 2,500 cells per well in a 96 well plate, treated with affibodies or control, and analyzed by Alamar Blue assay.

Results
We hypothesized that engineered multivalency would improve HER3-targeted therapeutic efficacy via enhanced avidity and HER3 sequestration into cytostatic homotypic interactions, given that HER3 requires hetero-dimerization with other HER members for signal transduction. We thus engineered multivalent affibodies, multiple HER3 binding domains linked by flexible spacer peptides, and evaluated their ability to block NRG-mediated HER3 activation. Our results demonstrate that multivalency enhances pHER3 signal inhibition in a variety of cancer cell lines: OvCAR8 (ovarian), DU145 (prostate), BT474 (breast), and H1975 (lung) (Figure 1A).

Based on the ability of multivalency to improve pHER3 signal inhibition, we next examined the effect of multivalent ligands on in vitro proliferation, and discovered that multivalency reduces proliferation both as a single agent and in combination with vertical and horizontal inhibitor strategies in the OvCAR8 and DU145 cell lines, whereas BT474 and H1975 cell lines were not responsive to affibody treatment. OvCAR8 cell response to multivalent affibody alone or in vertical combination with Akt inhibitor Mk2206 or PI3K inhibitor NVP-BKM120 is shown in Figure 1B. Mechanistically, we found that the ability of multivalent affibodies to reduce in vitro proliferation correlates with downstream pAkt inhibition, but not pHER2 inhibition. We further identified that multivalent affibodies cause HER3 downregulation (Figure 1C), presumably as a result of receptor clustering and differential trafficking. The monovalent affibody did not impact HER3 protein levels.

Conclusion
Overall, these results highlight the promise of engineered multivalency for enhanced efficacy of HER3-targeted affibodies against a variety of cancers and establish the potential of this strategy for broad utility in myriad anticancer applications and against additional therapeutic targets.