EMB-01: An innovative bispecific antibody targeting EGFR and cMet on tumor cells mediates a novel mechanism to improve anti-tumor efficacy

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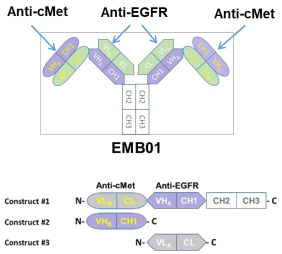
Introduction

Interruption of EGFR signaling, either by blocking EGFR binding sites on the extracellular domain of the receptor or by inhibiting intracellular tyrosine kinase activity, can prevent the growth of EGFR-expressing tumors. EGFR tyrosine kinase inhibitors (TKIs) provide a favorable treatment outcome in EGFR mutation positive NSCLC patients. However, many patients eventually develop progressive disease after treatment. Such acquired resistance limits the long-term efficacy of these EGFR TKIs in the clinic. The mechanisms of acquired resistance include a variety of mutations of the EGFR and crosstalk with the adjacent cMet receptors that allow the tumor to partially compensate the EGFR activity.

EMB-01 is an innovative bispecific antibody developed based on EpimAb's proprietary FIT-Ig® platform to target EGFR and cMet on tumor cells simultaneously. The anti-EGFR and anti-cMet Fab-domains in each EMB-01 arm are fused directly in-tandem in a unique crisscross orientation without any mutations or use of peptide linkers to form a final tetravalent binding complex with the corresponding receptors on cell surface. We demonstrated the potential benefit of EMB-01 in treating EGFR and/or cMet driven cancers, particularly in NSCLC PDX models derived from patients with acquired resistance due to secondary EGFR mutations in the kinase domain or cMet amplification and mutation. Currently, EMB-01 is under evaluation in a Phase I/II clinical trial with advanced/metastatic solid tumors. [ClinicalTrials.gov ID: NCT03797391]

The Design of EMB-01



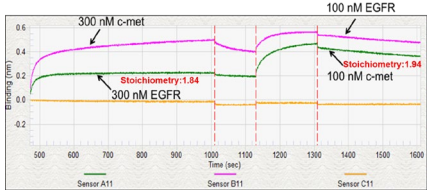
To construct the EMB-01 FIT-Ig molecule, the light chain (VL-CL) domains of parental anti-cMet mAb were directly fused in tandem with the heavy chain (VH-CH1-CH2-CH3) of anti-EGFR mAb at the N terminus. The second construct was VH-CH1 of parental anti-cMet mAb and the third construct was VL-CL of parental anti-EGFR mAb. These three constructs were transfected into cells together for FIT-Ig expression.

Characterization of EMB-01 Binding to Soluble Antigen

EMB-01 Maintains Binding Affinities of Parental mAbs

Captured FIT-Ig or mAb	Antigen	ka (1/Ms)	kd (1/s)	KD (M)
EMB-01	human cMet-His	4.75E+05	5.02E-04	1.06E-09
Anti-cMet mAb	human cMet-His	4.14E+05	5.27E-04	1.27E-09
EMB-01	human EGFR-His	1.09E+05	5.84E-05	5.34E-10
Anti-EGFR mAb	human EGFR-His	8.43E+04	5.10E-05	6.05E-10

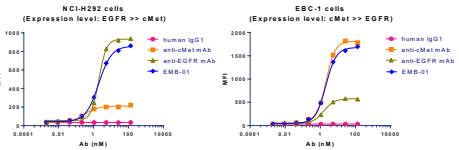
EMB-01 Is Capable of Binding cMet and EGFR Antigen Simultaneously in Solution



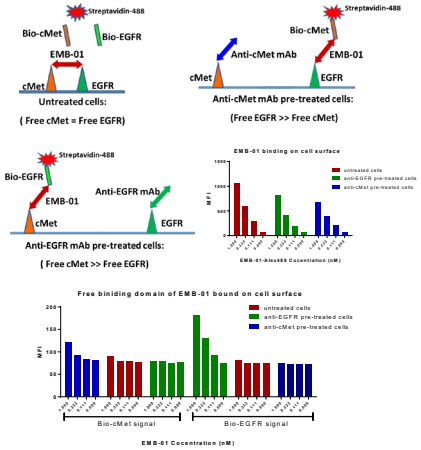
The dual binding of EMB-01 to recombinant human cMet and EGFR antigens was determined by Biacore. EMB-01 was immobilized on the chip, and 300nM cMet or EGFR were injected followed by another 100nM EGFR or cMet injection respectively. The stoichiometry indicated that EMB-01 was capable of binding two cMet antigens and two EGFR antigens simultaneously.

Characterization of EMB-01 Binding to Cell Surface Antigen

EMB-01 Shows Similar Binding to Cell Surface EGFR and cMet Compared to Its Parental mAbs



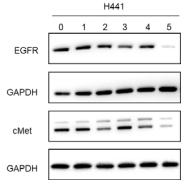
Dual Binding Property of EMB-01 on NCI-H1975 Cell Surface



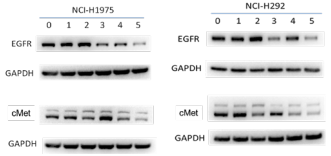
The NCI-H1975 cells were first pre-treated with anti-EGFR or anti-cMet mAb to saturate the EGFR or cMet receptors on the cells, then the free binding domains of bound EMB-01 were stained with either biotinylated soluble EGFR or biotinylated soluble cMet. We demonstrated EMB-01 binding on untreated and pre-treated cells through FACS using Alex488 labeled EMB-01, and at the highest drug concentration, minimal EMB-01 free binding domains on untreated cells were detected. It was therefore demonstrated that EMB-01 can simultaneously bind to both cell surface cMet and EGFR as a tetravalent molecule.

EMB-01 Induces Co-degradation of Cell Surface EGFR and cMet

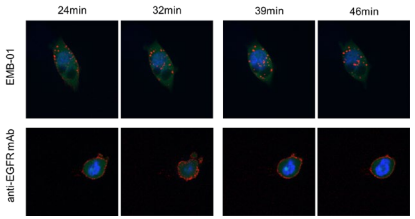
EMB-01 Is Found to Induce Co-degradation of EGFR and cMet in Various Tumor Cells



Western-blot analysis of antibody induced cMet and EGFR degradation in NCI-H1975, NCI-H292, and H441 cell lines. Cells were left untreated (lane 0) or treated with IgG isotype control (lane 1), parental anti-cMet mAb (lane 2), parental anti-EGFR mAb (lane 3), combo of anti-cMet and anti-EGFR mAb (lane 4) or EMB-01 (lane 5) overnight at 37 °C. Cell lysates were analyzed for total levels of cMet, EGFR or GAPDH (loading control).

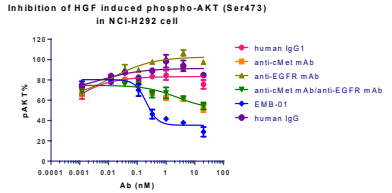


EMB-01 Shows Intense Endocytosis in NCI-H441 Compared to Parental Anti-EGFR mAb



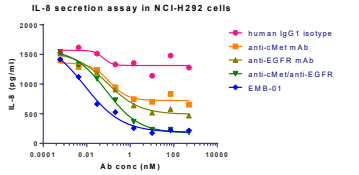
EMB-01 Shows Superior Biology Activity Compared to Its Parental mAbs

EMB-01 Shows Higher Potency in Inhibiting HGF Induced AKT Phosphorylation Compared to Anti-cMet mAb



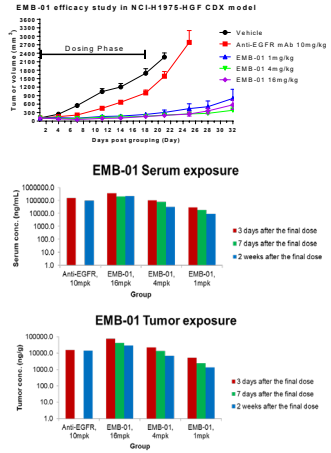
EMB-01 is a more potent inhibitor of AKT phosphorylation compared to the anti-cMet mAb or the anti-cMet/anti-EGFR combo. The increased potency could be driven by the co-degradation of EGFR and cMet receptors induced by EMB-01 binding

Highly Potent Inhibition of IL-8 Secretion From NCI-H292 by EMB-01



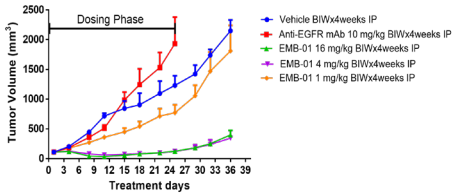
IL-8 secretion from NCI-H292 cells can be partially inhibited by either anti-cMet or anti-EGFR mAb. EMB-01 can more potently inhibit IL-8 secretion compared to each parental mAb alone or in combination.

Highly Potent and Durable Efficacy of EMB-01 In Tumor Models



In this study, animals were injected intraperitoneally twice a week for 3 weeks with test antibodies. EMB-01 exposure in both serum and tumor were evaluated at 3 days, 7 days, and 2 weeks after the final dose. Anti-EGFR mAb exposure was also evaluated 3 days and 2 weeks after final dose as a control. Similar exposure levels in both serum and tumor were detected for EMB-01 and the parental anti-EGFR mAb, while the anti-tumor activity of EMB-01 is significantly improved and lasted several weeks beyond dosing in contrast to the anti-EGFR mAb control in this EGFR double mutation/HGF overexpression model.

EMB-01 efficacy study in LU2503 PDX model



EMB-01 can dose-dependently suppress tumor growth in this EGFR wild type/c-MET amplified/MET Exon14 deletion NSCLC PDX model that is resistance to anti-EGFR mAb treatment.

Conclusion

- EMB-01 is a tetravalent bispecific antibody targeting EGFR and cMet receptors on the tumor cell.
- EMB-01 is found to induce co-degradation of EGFR and cMet in various tumor cells, and such effect is unattainable by each of the parental mAbs alone or in combination.
- EMB-01 is shown to induce more potent inhibition of EGFR and cMet downstream signals than each of the parental mAbs alone or in combination.
- In various tumor models, EMB-01 has a more potent and durable efficacy than anti-EGFR mAb treatment.
- With the unique mechanism of action, EMB-01 can be potentially beneficial to 3rd generation EGFR TKI resistant NSCLC patients or other EGFR and cMet driven cancer types.

EMB-01 is under evaluation in a Phase I/II clinical trial with advanced/metastatic solid tumors. [ClinicalTrials.gov ID: NCT03797391]

Reference: Gong, S., Ren, F., Wu, D., Wu, X. and Wu, C., 2017. Fabs-in-tandem immunoglobulin is a novel and versatile bispecific design for engaging multiple therapeutic targets. mAbs, 9(7), pp.1118-1128.