Abstract LB004

Preclinical discovery and characterization of EO-1022, a site-specific glycan-conjugated anti-HER3 vcMMAE ADC for treating solid tumors

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INTRODUCTION

- HER3 is expressed on the cell surface of multiple solid tumors including breast cancer and non-small cell lung cancer (NSCLC), and is often associated with poor prognosis[1]
- HER3 expression is higher on tumor cells compared with normal healthy cells, thus, HER3 is a promising antibody-drug conjugate (ADC) target[2]
- Patritumab deruxtecan[3], a HER3-targeted ADC, showed encouraging data in phase I/II clinical trials of breast cancer[4] and improvement in PFS versus doublet chemotherapy in patients with EGFR-mutant NSCLC in the HERTHENA-Lung02 Phase III Trial[5]
- To address the need for new therapeutic options to treat HER3-expressing solid tumors, we developed an ADC, EO-1022, that selectively targets and kills HER3-expressing cancer cells
- Development candidate EO-1022 is comprised of the selective and clinically validated HER3 monoclonal antibody (mAb), seribantumab[6], site-specifically glycan-conjugated with a cleavable valine-citrulline linker and monomethyl auristatin E (vcMMAE) payload to give a homogenous drug-to-antibody ratio (DAR) of 4 (**Figure 1**)

EO-1022 is a Differentiated HER3 ADC Designed to Address Significant Unmet Needs Across Multiple Solid Tumors

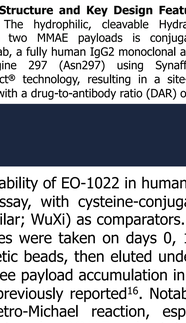

Figure 1. Structure and Key Design Features of EO-1022. The hydrophilic, cleavable HydraSpace® linker with two MMAE payloads is conjugated to seribantumab, a fully human IgG2 monoclonal antibody, at asparagine 297 (Asn297) using Synaffix B.V. GlycoConnect® technology, resulting in a site-specific HER3 ADC with a drug-to-antibody ratio (DAR) of 4.

Target	**HER3**	• Clinically validated oncology and ADC target[7,8] • Overexpressed in a range of solid tumors, including breast cancer, NSCLC, ovarian cancer and pancreatic cancer[1,9,10]
Antibody	**Seribantumab**	• Fully human IgG2 mAb, selective for HER3 and with highly desirable internalization capability • **Demonstrated favorable clinical safety profile** in over 900 patients across multiple studies[11,12,13]
Payload	**MMAE**	• Clinically validated payload, used across multiple ADC programs • **Differentiated from most HER3 ADCs in development** which utilize topoisomerase 1 (TOPO1) inhibitor payloads[3,14,15] • In preclinical study, proof-of-concept MMAE HER3 ADC outperformed benchmark HER3 ADC with deruxtecan payload in inducing tumor regression in PDX model of pancreatic cancer with high HER3 expression[16] • Potential to combat TOPO1 inhibitor resistance, including ADCs[17]
Linker	**HydraSpace®**	• Hydrophilic, cleavable linker to reduce impact of lipophilic payload and to improve ADC stability, PK, tolerability, and efficacy • Valine-citrulline cathepsin-cleavable linker
Site-specific conjugation	**Glycan**	• Designed to **minimize systemic exposure to free MMAE payload** compared to traditional cysteine conjugation, and to increase homogeneity and stability to improve therapeutic index

METHODS

- The stability of EO-1022 in human serum was evaluated using a liquid chromatography-mass spectrometry (LC-MS) assay, with cysteine-conjugated seribantumab-vcMMAE (DAR4)[16] and patritumab-DXd (research-grade biosimilar; WuXi) as comparators. Each ADC (0.5 mg/mL) was incubated in human serum at 37ºC for 14 days. Samples were taken on days 0, 1, 4, 7, and 14, mixed with biotinylated human HER3 bound to streptavidin magnetic beads, then eluted under acidic conditions (0.1M glycine, pH 2.5), neutralized, and analyzed by LC-MS. Free payload accumulation in human serum was measured by LC-MS/MS. The seribantumab-vcMMAE data were previously reported[16]. Notably, ADCs conjugated via maleimide coupling are prone to deconjugation via the retro-Michael reaction, especially those with maleimidocaproyl (MC) moiety-based linkers[17]. Both seribantumab-vcMMAE and patritumab-DXd use MC-based linkers.
- Cell surface binding to BT474 (HER3 high) and SK-BR3 (HER3 high) breast cancer cells was measured by flow cytometry.
- Internalization into BT474 and SK-BR3 cells was quantified by FACS for EO-1022, seribantumab, isotype-MMAE, patritumab-DXd, patritumab, and isotype-DXd. Test articles were incubated with cells for 1 hour at 4ºC, followed by addition of A647 anti-human IgG Fcγ secondary antibody for 30 minutes at 4ºC. Samples were then split into two aliquots and incubated for 3.5 hours at either 4ºC (endocytosis restrictive) or 37ºC (endocytosis permissive). After acid quenching, samples were analyzed by FACS.
- in vitro cytotoxicity was evaluated for EO-1022, isotype-MMAE and free MMAE as well as patritumab-DXd, isotype-DXd and free deruxtecan in BT474 cells, SK-BR3 cells and NCI-H446 lung carcinoma (HER3 low/absent) cells.
- in vivo anti-tumor activity of EO-1022 vs. patritumab-DXd was assessed in BT474 (HER3 high; Charles River Labs) and HCC1569 (HER3 medium; Crown Biosciences) breast carcinoma cell line-derived xenograft (CDX) models and the LU1868 squamous cell lung carcinoma (EGFR L858R/T790M mutation; HER3 low; Crown Biosciences) patient-derived xenograft (PDX) model. Mice were dosed weekly for 4 doses of test article; tumor volume and body weight were measured twice weekly. Percent tumor growth inhibition (TGI) calculations utilized Day 0 and Day 28 tumor volume measurements.

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RESULTS

EO-1022 with Site-Specific Conjugation is Highly Stable with Homogenous DAR 4 and Exhibits Minimal Free Payload in Human Serum

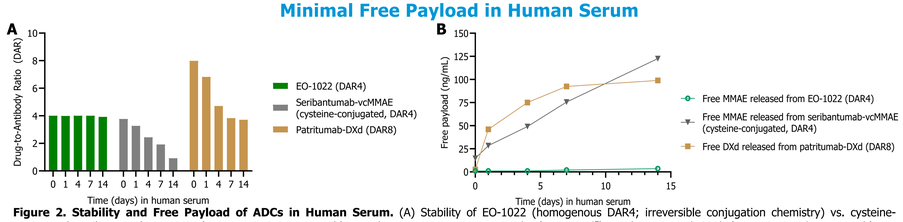
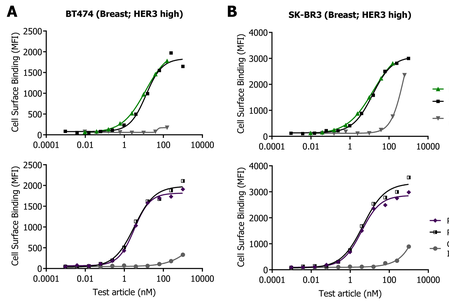


Figure 2. Stability and Free Payload of ADCs in Human Serum. (A) Stability of EO-1022 (homogenous DAR4; irreversible conjugation chemistry) vs. cysteine-conjugated seribantumab-vcMMAE (average DAR4; susceptible to deconjugation via retro-Michael reaction[17]), and patritumab-DXd (average DAR8; susceptible to deconjugation via retro-Michael reaction[17]) in human serum over a 14-day, 37ºC incubation period. (B) Amount of released payload in human serum indicate lower released payload from EO-1022 compared to cysteine-conjugated seribantumab-vcMMAE and patritumab-DXd, respectively.

EO-1022 Retains the Ability to Bind to Cell Surface HER3



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	BT474 EC_{50} (nM)	SK-BR3 EC_{50} (nM)
EO-1022 (DAR4)	12.0	15.5
Seribantumab (mAb)	13.0	14.5
Patritumab-DXd (DAR8)	3.2	3.8
Patritumab (mAb)	3.3	3.6
Control non-binding ADC IgG2-vcMMAE (DAR4)	>1000	>1000
Control non-binding ADC IgG1-DXd (DAR8)	>1000	>1000
Ratio (ADC/mAb)		
EO-1022 EC_{50}/Seribantumab EC_{50}	0.9	1.1
Patritumab-DXd EC_{50}/Patritumab EC_{50}	1.0	1.1

Figure 3. Cell Surface Binding. Both HER3 ADCs (EO-1022 and patritumab-DXd) retain their ability to bind HER3 on the cell surface of endogenously high HER3-expressing (A) BT474 and (B) SK-BR3 breast cancer cell lines. (C) EC_{50} values for mAb, ADC, and non-binding ADC indicate the binding affinity is maintained after conjugation.

EO-1022 is Internalized in HER3-Expressing Cell Lines

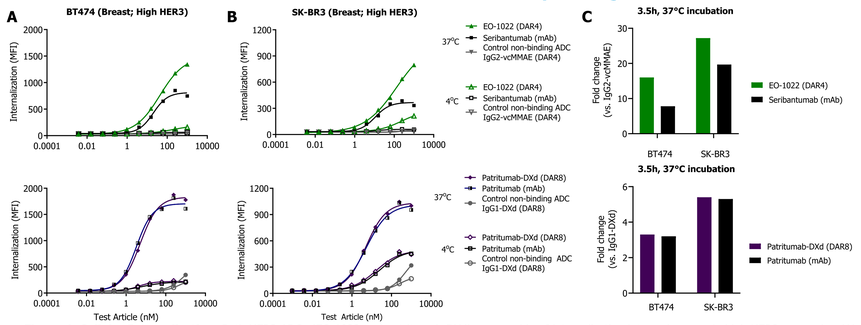
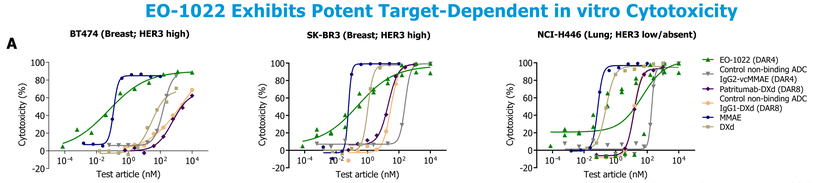


Figure 4. Cellular Internalization. Both HER3 ADCs (EO-1022 and patritumab-DXd) are capable of internalization into endogenously high HER3-expressing (A) BT474 and (B) SK-BR3 breast cancer cell lines. (C) Plot of the fold change in internalization of the ADC and mAb normalized to the respective control non-binding ADC. EO-1022 and its mAb, seribantumab, are internalized with comparable levels.

EO-1022 Exhibits Potent Target-Dependent in vitro Cytotoxicity



B

	BT474 IC_{50} (nM)	SK-BR3 IC_{50} (nM)	NCI-H446 IC_{50} (nM)
EO-1022 (DAR4)	0.1	0.9	100.0
Patritumab-DXd (DAR8)	591.0	21.0	15.0
Control non-binding ADC IgG2-vcMMAE (DAR4)	157.1	247.4	136.0
Control non-binding ADC IgG1-DXd (DAR8)	2509.0	29.0	13.0
MMAE	0.1	0.1	0.1
DXd	29.0	1.1	0.3

Ratio (Control ADC/HER3-ADC)	BT474	SK-BR3	NCI-H446
IgG2-vcMMAE IC_{50}/EO-1022 IC_{50}	2805.4	279.5	1.4
IgG1-DXd IC_{50}/Patritumab-DXd IC_{50}	4.3	1.4	0.9

Figure 5. Cellular Cytotoxicity of ADCs. (A) in vitro cytotoxicity of HER3 ADCs in cell lines expressing varying levels of HER3. EO-1022 cytotoxicity is dependent on target expression. EO-1022 outperforms patritumab-DXd in high HER3-expressing cell lines. (B) IC_{50} values for in vitro cytotoxicity study.

EO-1022 Induces Anti-Tumor Activity and Robust Tumor Growth Inhibition in HER3-Expressing in vivo Models

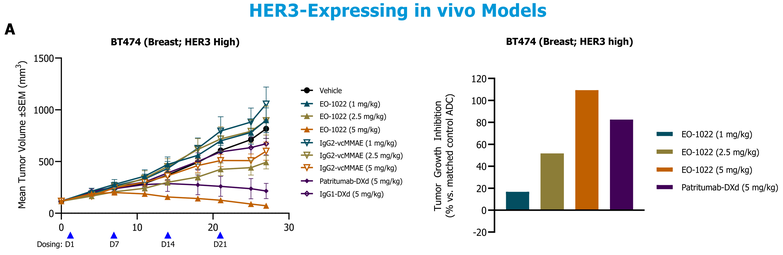


Figure 6. in vivo Anti-Tumor Activity in Models with Varying HER3 Expression. Anti-tumor activity (*left*) and percent (%) tumor growth inhibition (TGI) *(right)* of HER3 ADCs in CDX models (A) BT474 and (B) HCC1569 with high and medium HER3 expression, respectively, and (C) the PDX LU1868 lung cancer model harboring an EGFR L858R activating mutation and EGFR T790M resistance mutation with low HER3 expression. In the HCC1569 model with medium HER3, EO-1022 outperformed patritumab-DXd when normalized to the control non-binding ADC. Based on equivalent payload dose, EO-1022 outperformed patritumab-DXd in the LU1868 PDX model when normalized to the respective control non-binding ADC. Body weight changes were minimal across all groups throughout the studies.

CONCLUSIONS

- EO-1022 is a HER3-targeted ADC with a DAR of 4 that uses the Synaffix B.V. GlycoConnect® site-specific glycan conjugation platform with an MMAE payload
- EO-1022 is highly stable in human serum showing homogenous DAR 4 and minimal free payload compared to seribantumab-vcMMAE and patritumab-DXd, both of which rely on maleimide coupling and are susceptible to the retro-Michael reaction-based deconjugation[17]
  - These findings illustrate that a key feature of EO-1022 is potentially to minimize systemic exposure to free payload, resulting in reduced payload-associated toxicity in patients and an improved safety profile
- EO-1022 exhibited potent in vitro cytotoxicity that is dependent on HER3 expression levels
- EO-1022 elicited anti-tumor activity in models of low, medium, and high HER3 expression level
  - In the in vivo models tested, EO-1022 outperformed patritumab-DXd, including in a low HER3-expressing EGFR-mutant lung cancer PDX model
- These results suggest EO-1022 is a differentiated HER3 ADC designed to address significant unmet needs across solid tumors that express HER3 such as breast cancer and EGFR-mutant NSCLC
- An Investigational New Drug (IND) submission is expected to be filed in 2026

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