MBRC-101: a novel antibody-drug conjugate (ADC) targeting the membrane-associated tyrosine kinase receptor EphA5 in breast cancer

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Introduction

MBRC-101 is a potent EphA5-directed ADC for the treatment of advanced solid tumors

• EphA5 is a member of the receptor tyrosine kinase family of proteins with functional roles in axonal guidance during embryonic development[1,2,3]

• EphA5 expression has been reported in lung, gastric, ovarian and pancreatic cancers, with restricted expression in normal tissues[4,5,6]

• Differential expression in cancers and accessibility through the systemic circulation support the development of antibody-based therapies against EphA5[7,8]

EphA5 is expressed in breast cancer

• Archival breast cancer tissue sections were evaluated by immunohistochemistry (IHC)

• Robust and selective EphA5 expression was detected in > 80% of triple negative breast cancer (TNBC) and > 80% of hormone receptor-positive (HR+) breast cancer tissue samples

• EphA5 expression was not detected in adjacent, non-malignant breast tissue or normal breast

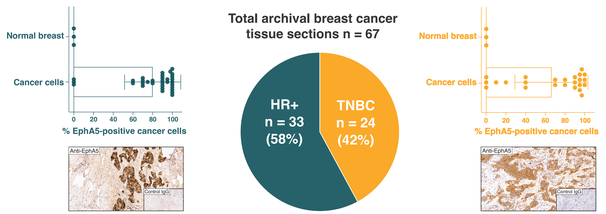

Figure 1. Testing for EphA5 expression by IHC used a commercial rabbit polyclonal antibody. IHC staining was performed on the Leica BOND III automated platform.

MBRC-101

• MBRC-101 is composed of a humanized anti-EphA5 IgG1 antibody conjugated to MMAE

• The anti-EphA5 antibody binds to EphA5 specifically and with high affinity (KD = 2.05 x 10^{-9} M)

• The anti-EphA5 antibody/EphA5 complex internalizes rapidly in EphA5-expressing cells

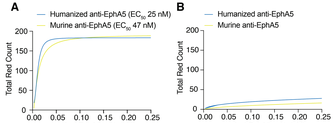

Figure 2. (**A**) Antibody internalization was visualized and quantified using the Incucyte® real-time live cell imaging analysis. (**B**) Antibody internalization was not detected in EphA5-negative cells.

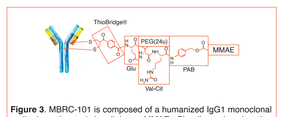

Figure 3. MBRC-101 is composed of a humanized IgG1 monoclonal antibody conjugated via a linker to MMAE. Site-directed conjugation uses the ThioBridge® technology and a protease labile linker, resulting in a linker-payload structure of ThioBridge®-Glu-[Val-Cit-PAB-MMAE] PEG[24u]).

Methods and Results

• MBRC-101 is cytotoxic to EphA5-expressing cells in a concentration-dependent manner

• MBRC-101 binding epitope is conserved in rats and non-human primates

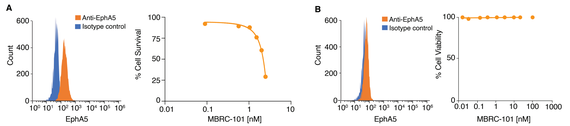

Figure 4. (**A**) Flow cytometry analysis confirming EphA5 expression on cells. MBRC-101 kills EphA5-positive cells in a dose dependent manner. (**B**) Flow cytometry analysis confirming absence of EphA5 on control cells. MBRC-101 does not affect viability of control cells.

Figure 5. Epitope identification by alanine scanning mutagenesis and high throughput flow cytometry. Amino acid residues essential for binding to EphA5 are highlighted in green. Amino acid residues that are not essential but contribute to binding are highlighted in yellow.

MBRC-101 is highly potent *in vivo*

• Weekly administrations of intravenous (IV) MBRC-101 showed dose-dependent, robust, and reproducible anti-tumor activity in patient-derived xenograft models of TNBC

• Partial tumor responses were observed at doses of 2.5 mg/Kg IV and complete tumor responses at 5 mg/Kg IV

• Doses up to 20 mg/Kg IV were well-tolerated with no observable weight loss

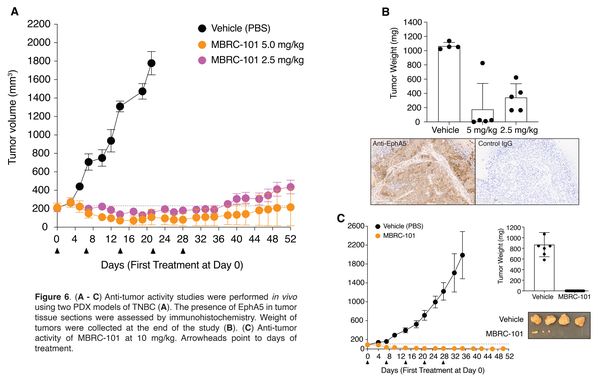

Figure 6. (**A - C**) Anti-tumor activity studies were performed *in vivo* using two PDX models of TNBC (**A**). The presence of EphA5 in tumor tissue sections were assessed by immunohistochemistry. Weight of tumors were collected at the end of the study (**B**). (**C**) Anti-tumor activity of MBRC-101 at 10 mg/kg. Arrowheads point to days of treatment.

Toxicology findings attributed to payload and not target-related

Toxicology study design for MBRC-101

Species	Cynomolgus Monkey		Sprague-Dawley Rat	
Study	DRF	GLP	DRF	GLP
Dosing	2 IV boluses	2 IV boluses	2 IV boluses	2 IV boluses
Interval	3 wk	3 wk/4 wk recovery	3 wk	3 wk/4 wk recovery
Dose (mg/kg)	0, 5, 10, 15	0, 5, 7.5, 10	0, 5, 10, 20, 30	0, 10, 20, 30
HED* (mg/kg)	1.67, 3.2, 5	1.67, 2.5, 3.2	1, 2, 3, 5	2, 3, 5
#/Group/Sex	1F/2M or 1M/2F	3 + 2 recovery	5	10 + 5 recovery

Cynomolgus monkey - toxicology findings

Dose (mg/kg)	HED* (mg/kg)	Finding	Recovery
15	5	**Morbidity:** (n=1) due to sepsis	No
10 - 15	3.2 - 5	**Testicle:** bilateral degeneration/decreased sperm **Ovary:** decreased secondary and tertiary follicles **Neutrophils:** decreased	No No Yes
5 - 15	1.67 - 5	**Lymphocytes:** decreased in blood and spleen **Red blood cell mass:** decreased	Yes Yes
10	3.2	**Cornea:** minimal bilateral degeneration at the limbus	Yes

Sprague-Dawley rat - toxicology findings

Dose (mg/kg)	HED* (mg/kg)	Finding	Recovery
20 - 30	3 - 5	**Liver** • Hepatocellular necrosis, increased ALT, AST • Altered function: increased total bilirubin, ALP, cholesterol and triglycerides (30 mg/kg only)	Yes Yes
		Neutrophils: decreased	Yes
		Red blood cell mass: decreased	Yes
		Cornea: increased mitotic figures and apoptosis	Yes
10 - 30	2 - 5	**Lung:** increased alveolar macrophage and aveolar hyperplasia	No
		Testicle: bilateral degeneration with decreased sperm	No
		Lymphocytes: decreased in blood and thymus	Yes

*Human equivalent dose

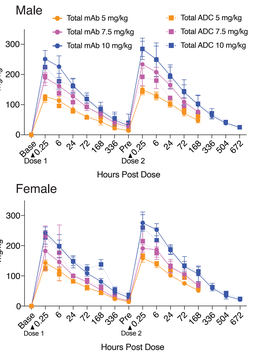

Figure 7. Due to the stability of MBRC-101, exposure for total ADC and total antibody were highly concordant. Both sexes demonstrated similar pharmacokinetics with no accumulation in the second dose; Cmax at 0.25 hours was consistent with IV bolus.

• Half-life of MBRC-101 in cynomolgus monkeys = 7 to 11 days

• Half-life of MBRC-101 in rats = 8 to 20 days

• Highly stable linker leads to equivalent levels of total ADC and total antibody with very low levels of free MMAE (< 0.01 %)

MBRC-101-001 Phase 1/1b: Study Schema

Phase 1 Escalation

Dose Level 1: 0.5 mg/kg
Dose Level 2: 1.0 mg/kg
Dose Level 3: 1.5 mg/kg
Dose Level 4: 2.0 mg/kg

N = 30

Phase 1b Expansion

N = 60

- Breast cancer[1] (N = 20)
- NSCLC (N = 20)
- Tumor agnostic[2] (N = 20)

NSCLC: Non-small cell lung cancer
[1]Triple negative or HR+/HR-
[2]EphA5+ by IHC analysis

Phase 1/1b Trial (Cont.)

Key Eligibility Criteria

• Histologic or cytologic diagnosis of malignant solid tumor

• Refractory to conventional therapy

• No requirements for prior lines of therapy

• Prior ADC therapy is allowed

• Prior MMAE-based ADCs are allowed for Phase 1 but not Phase 1b

• EphA5 expression by IHC required only for Phase 1b tumor agnostic cohort

Objectives	End points
Phase 1 Primary Objectives and Endpoints	
• To identify potential OBRD and dosing regimens • To establish the MTD of MBRC-101 using one or more dose regimens • To identify potential RP2Ds and regimens of MBRC-101	• MTD, DLTs, AEs, SAEs, and clinical laboratory tests
Phase 1b	
• To evaluate the safety of MBRC-101 at potential OBRDs, RP2Ds and dosing regimens • To evaluate preliminary clinical activity of MBRC-101 at potential OBRDs and dosing regimens	• AEs, SAEs, and clinical laboratory tests • Investigator-assessed ORR by response RECIST v1.1
Phase 1 and 1b Secondary Objectives and Endpoints	
• To characterize single and multiple dose PK profiles • To evaluate incidence and persistence of anti-MBRC-101 Ab formation • To evaluate biomarkers of clinical response and resistance, safety, pharmacodynamic activity, and/or mechanism of action	• MBRC-101, Ab, and unconjugated MMAE blood concentrations • Anti-MBRC-101 Ab blood concentration • EphA5 expression as determined by IHC

Ab = total antibody; **AE** = adverse event; **DLT** = dose limiting toxicity; **DOR** = duration of response; **IHC** = immunohistochemistry; **MMAE** = monomethyl auristatin E; **MTD** = maximum tolerated dose; **OBRD** = optimal biologically relevant dose; **ORR** = overall response rate; **PFS** = progression free survival; **PK** = pharmacokinetics; **RECIST** = Response Evaluation Criteria in Solid Tumors; **RP2D** = recommended Phase 2 doses; **SAE** = serious adverse event

Conclusions

• Extensive *in vitro* testing showed that MBRC-101 binds to EphA5 exclusively, it is rapidly internalized and is cytotoxic to cells expressing EphA5

• MBRC-101 is highly potent against PDX models of TNBC

• MBRC-101 was well tolerated at doses up to 30 mg/kg in rats and 10 mg/kg in monkeys. Toxicologic findings in both species were attributed to the MMAE payload and not target-related

• Highest non-severely toxic dose (HNSTD) in monkeys was 10 mg/kg (HED = 3.2 mg/kg). In alignment with ICH S9, 1/6 of the monkey HNSTD was used to determine a human start dose of 0.5 mg/kg

• At 0.5 mg/kg the safety margin was approximated at 15-fold based on projected human exposures

• A Phase 1/1b trial of MBRC-101 is open and currently enrolling patients (NCT06014658)

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