DEBIO 1562M, A NEXT GENERATION ANTIBODY DRUG CONJUGATE (ADC) TARGETING CD37 FOR AML AND MDS TREATMENT

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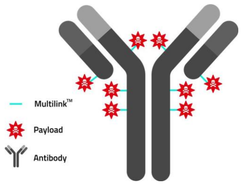
SUMMARY

In this poster we describe Debio 1562M, a new antibody ADC directed against CD37. We first demonstrate that Debio 1562M is stable and specific. Then, we validate that CD37 is expressed and efficiently internalized in acute myeloid leukemia (AML) models, allowing a good anti-tumoral activity of Debio 1562M. Successful AML cells killing is achieved in vitro and in vivo, from cell lines and cell derived xenograft (CDX) models to patient samples and patient derived xenograft (PDX) models. Finally, we compared AML CD37 expression and internalization pattern to healthy and malignant B cells. Interestingly, despite significant higher expression in B cells, total intracellular accumulation is similar, emphasizing CD37 as a target of choice for AML treatment with this ADC.

INTRODUCTION

CD37 is a trans-membrane protein exclusively expressed on hematopoietic tissues such as B cells, neutrophils and macrophages. However, increased expression of CD37 has been observed in various hematological cancers[1-4] and associated with poor patient outcome in AML[5-6].

Debio 1562M is a second-generation ADC directed against CD37, utilizing Debiopharm's proprietary Multilink™ cleavable linker technology. Eight molecules of DM1 tubulin-binding agent are conjugated to naratuximab humanized antibody and despite cleavable linker, we show a sustained in vitro stability in human plasma and in pharmacokinetic study in mice. Additionally, acceptable safety profile in mice was demonstrated[7].



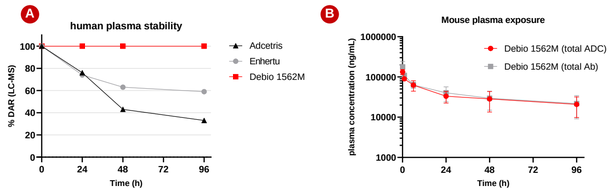


Figure 1. Debio 1562M plasma stability. A. in vitro plasma stability of Debio 1562M and 2 approved ADCs. No drug antibody ratio (DAR) decrease is observed for Debio 1562M over 96h compared to up to 60% for other constructs. B. Debio 1562M was administered at 5 mg/kg in 3 mice. PK profiles of total Ab (Fc part) and total ADC (payload part) were similar demonstrating the stability of the ADC.

METHODS

Human plasma stability (Abzena, UK): 1 mg/mL of ADCs were incubated in human plasma for 96 h and analysis were performed by LC-MS at indicated timepoints

CD37 KO cell lines were generated by CRISPR Cas 9 technology as previously reported[8] and validated by Sanger sequencing at The Ohio State University Comprehensive Cancer Center.

Cell viability assays were read at indicated timepoints by adding either Cell Titer Glo (Promega Cat# G7571) in Figure 6, or MTS in Figure 2.

Mouse AML xenograft models (Crownbio, China). THP-1: NOD/SCID mice were inoculated with $1x10^7$ THP-1 cells in tail vein. Mice were randomized based on body weight in groups of 10 mice, and treatment started seven day after cell inoculation. **MOLM-13:** NOD/SCID mice were inoculated with $2x10^6$ MOLM-13 Luciferase cells in tail vein. Mice were randomized based on total flux value 3 days after inoculation in groups of 8 mice and treatment started 7 days after inoculation. **MV4-11:** NCG mice were inoculated with $2x10^7$ MV4-11 Luciferase cells in tail vein. Mice were randomized based on total flux value 14 days after inoculation in groups of 8 mice and treatment started the same day with azacitidine at 3.5mg/kg for 5 days, venetoclax at 100mg/kg for 14 days and magrolimab at 10mg/kg at days 1, 3 and 6. Tumor growth was imaged twice per week after Luciferin administration. **For all models**, one intravenous injection of 5mg/kg of ADCs was performed. Vehicle is PBS.

PDX mouse model (Champions, US): NOG mice were inoculated with $2x10^6$ AML cells from CTG-2240 in tail vein. Mice were randomized when at least 20% of human CD45 positive cells in the blood and bone marrow was reached. Treatment started the day of randomization.

AML patient samples (Vivia Biotech, Spain): blast cells were seeded in appropriate medium to follow blast viability in proliferative conditions. Staining of the cells with a cocktail of antibodies and annexin-V allowed to discriminate live and proliferative tumor cells with the PharmaFlow platform.

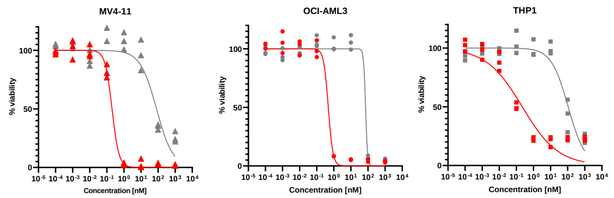
CD37 expression was determined by incubation of a dose range of naratuximab for 30 minutes at 4° C and detection with a AF488-labelled secondary Ab (Figures 4 and 5).

CD37 internalization was measured over 6 hours at 37° C with 5 µg/mL pHrodo dye coupled to Debio 1562M following manufacturer's instructions (Thermo Fisher P35355) (Figure 4). For patient samples, 24h incubation with 10 µg/mL was used. A459 and MOLM13 cell lines were used as negative and positive control, respectively (Figure 5)

Dose range finding was performed in CD1 mice, with 2 administrations on days 1 and 8 of 25, 50 or 100mg/kg of Debio 1562M.

RESULTS

Debio 1562M is highly potent in AML cell lines and specific to CD37 expression



	MV4-11	OCI-AML3	THP-1
Parental IC50 (nM)	0.20	0.47	0.22
CD37 KO IC50 (nM)	81	72	106

Figure 2. Debio 1562M cytotoxicity in parental and CD37 knock-out (KO) AML cell lines. Parental and CD37 KO cells were treated for 72h with increasing concentration of Debio 1562M. IC50 values are presented in the table.

Debio 1562M significantly improves survival in AML CDX models

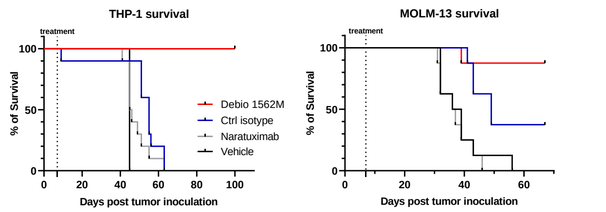


Figure 3. in vivo efficacy of Debio 1562M in THP1 and MOLM-13 models
Debio 1562M, control isotype (Trastuzumab-Multilink™-DM1) or naratuximab were administered once at 5 mg/kg. Debio 1562M treatment allows survival of 10/10 mice in THP1 model (100 days after tumor inoculation) and 7/8 mice in MOLM-13 model (68 days after tumor inoculation).

DLBCL cell line has higher expression of CD37 compared to AML cell line, however internalization of Debio 1562M is equivalent

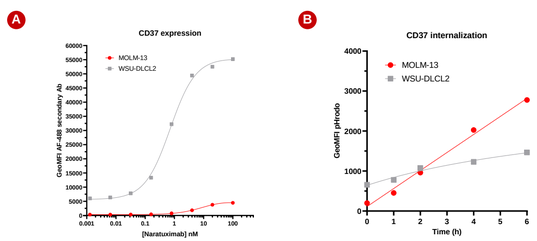


Figure 4. Comparison of CD37 expression and internalization in DLBCL and AML cell lines
A. WSU DLBCL has higher expression of CD37 than MOLM-13 AML cell line. Graph represents the mean fluorescence intensity (MFI) at increasing naratuximab concentrations. B. internalization of Debio 1562M is more efficient in MOLM-13 than in WSU-DLCL2 cell line. Graph represents the MFI of internalized Debio 1562M at different timepoints.

Blasts from AML and MDS patient samples internalize and are sensitive to Debio 1562M, despite lower expression than B cells.

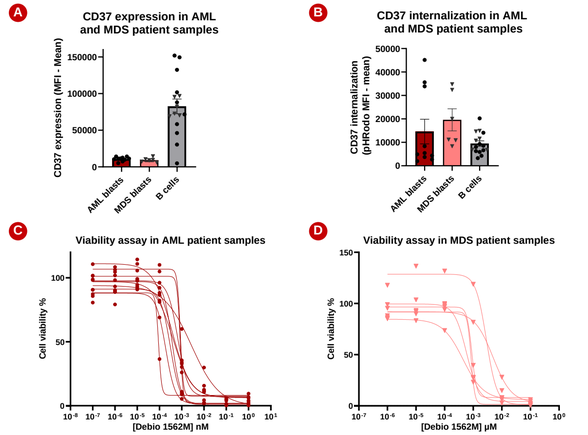


Figure 5. CD37 expression, internalization and activity of Debio 1562M in 10 AML and 6 MDS patient samples. CD37 expression (A) and internalization (B) was measured on cells from each patient sample. Each sample was treated with a dose range of Debio 1562M for 5 days and viability was measured. AML mean EC50 = 230pM (C) and MDS mean EC50 = 1,13nM (E).

Debio 1562M inhibits AML PDX growth both in vitro and in vivo independently of CD37 expression

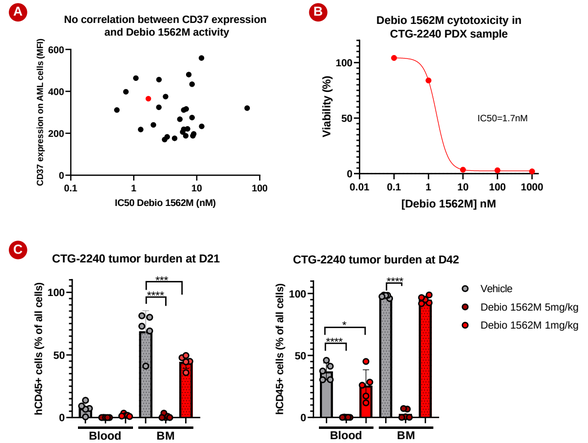


Figure 6. Debio 1562M activity in AML patient derived cells
A. CD37 expression and Debio 1562M cytotoxic activity at 6 days have been determined on 27 patient derived AML cells. No correlation between the 2 parameters is seen. CTG2240 is highlighted in red. B. Dose response curve of Debio 1562M on CTG-2240 sample. C. in vivo activity of Debio 1562M in CTG-2240 PDX. Mice receiving 5mg/kg of Debio 1562M have significant and sustained reduced AML cells in the blood and bone marrow, while 1mg/kg is showing intermediate activity with relapse at day 42.

Debio 1562M activity is superior to standard of care or comparable to drugs in development in preclinical models

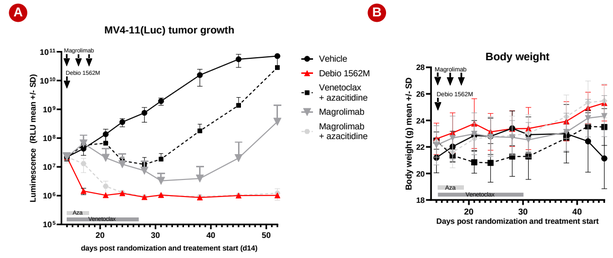


Figure 7. in vivo efficacy of Debio 1562M monotherapy in MV4-11(Luc) model compared to venetoclax + azacytidine, magrolimab or magrolimab + azacitidine
A. Complete and sustained tumor regression was observed both for Debio 1562M and magrolimab +azacytidine. Venetoclax + azacytidine and magrolimab are achieving tumor stasis under treatment and relapse post-treatment. B. Mean body weight shows overall good tolerability with slight decrease for venetoclax + azacytidine group.

Debio 1562M toxicology profile is related to payload's known toxicities but with a significant safety margin

Target organs	Major histopathologic findings
	Hypertrophy, extramedullary hematopoeisis, necrosis
	single cell necrosis
	-
	Extramedullary hematopoesis, degeneration/necrosis
	inflammation at injection site
	-

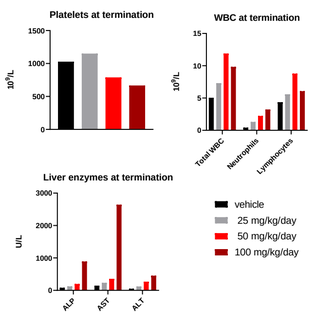


Figure 8. Debio 1562M dose range finding toxicity study in mice
The table summarizes off-target organ toxicities at day 11 after 2 administrations (day 1 and day 8). Severity is increasing with the dose, 100mg/kg not being tolerated. Debio 1562M induces a minor decrease in platelets, increase in white blood cells (WBC) and liver enzymes. Debio 1562M is not cross reactive with mouse CD37 and therefore on-target hematologic toxicity is not evaluated here.

CONCLUSIONS

- **Debio 1562M is a new, potent and stable Multilink™ ADC targeting CD37**
- **AML cells have strong capacity to internalize CD37 bound to Debio 1562M, at the same extent than B cells despite lower expression**
- **Debio 1562M monotherapy improves survival and induces tumor regression in AML preclinical models (CDX and PDX)**
- **Overall Debio 1562M activity and safety in preclinical models is promising for future clinical development in AML**

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