CD37 IS A RELEVANT TARGET FOR AML AND MDS TREATMENT WITH DEBIO 1562M ANTIBODY DRUG CONJUGATE (ADC)

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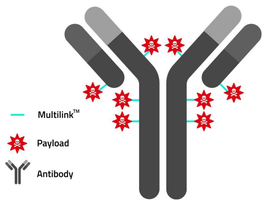
SUMMARY

CD37 relevance as an attractive therapeutic target for immune based therapies has been confirmed in clinical trials for B cell malignancies[1]. In acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS), CD37 expression profile has been less investigated and is more controversial[2]. Here, we demonstrate that CD37 is broadly expressed in diverse AML and MDS models, including patient samples. Debio 1562M, a new CD37-targeting ADC, is efficiently internalized in these models, at a similar extent as in healthy or malignant B cells. With only one administration, Debio 1562M successfully triggers tumor regression of several cell-derived xenograft (CDX) models and strongly improves animal survival compared to venetoclax and azacitidine standard of care (SoC). In patient-derived xenograft (PDX) models, Debio 1562M significantly reduces tumor burden, similarly as venetoclax and azacitidine SoC treatment, or better in a SoC resistant model. In the absence of cross-reactive preclinical species, GLP toxicology study was performed in mice and demonstrated a safe profile related to known payload's toxicities.

BACKGROUND

CD37 is a trans-membrane protein, member of the tetraspanin superfamily, exclusively expressed on hematopoietic tissues. Primarily described on B cells, CD37 plays important roles in their development and modulating their immune function[3]. Increased expression of CD37 has been observed in various hematological cancers[4-7] and associated with poor patient outcome in AML[8-9].

Debio 1562M is a second-generation ADC directed against CD37, utilizing Debiopharm's proprietary Multilink™ cleavable linker technology. Eight molecules of a DM1 (mertansine) derivative, a highly potent anti-tubulin binder inducing mitotic catastrophe and cell death, are conjugated to naratuximab humanized antibody. Cathepsin-B-selective cleavable linker confers to Debio 1562M an excellent plasma stability allowing long exposure in mice and a safe toxicologic profile.



METHODS

Mouse AML xenograft models. THP-1: NOD/SCID mice were inoculated with 1x10^7 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 Luc:** NOD/SCID mice were inoculated with 2x10^6 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 Luc:** NCG mice were inoculated with 2x10^7 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. Tumor growth was imaged twice per week after Luciferin administration. **OCI-AML-3 :** NOD-SCID mice were subcutaneously injected with 2x10^7 cells. Debio 1562M was given once IV at 10mg/kg, azacitidine at 3.5mg/kg for 5 days, venetoclax at 100mg/kg for 14 days.

PDX mouse model: NOG or NOG-EXL mice were inoculated with 2x10^6 AML cells from CTG-2239, CTG-2457, CTG-3930 and CTG-2227 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 and FACS analysis were performed 21 days after treatment start.

AML and MDS patient samples: 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.

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). For patient samples, 24h incubation with 10 µg/mL was used.

GLP toxicity study was performed in CD1 mice, with 2 iv administrations on days 1 and 21 of Debio 1562M at 50 mg/kg.

All in vivo studies were conducted in accordance with institutional guidelines and NCRI Guidelines for the welfare and use of animals in cancer research

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RESULTS

CD37 is expressed on AML and MDS blasts from patient samples

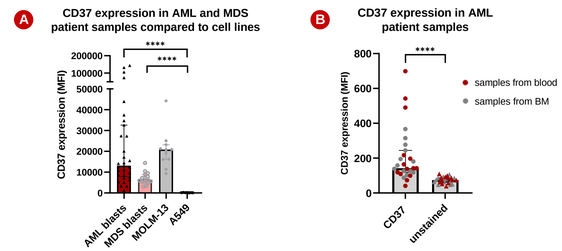

Figure 1. CD37 is detected on AML and MDS patient samples from different sources
A. CD37 mean fluorescence intensity (MFI) on primary bone marrow (BM) samples from 31 AML and 15 MDS patients compared to MOLM-13 AML cell line and A549 (CD37 negative) lung cancer cell line. B. CD37 MFI on primary samples from 13 BM or 14 whole blood of AML patients. Same samples with secondary only staining were used as negative controls (unstained).

Debio 1562M is better internalized in AML and MDS blasts compared to normal and malignant B cells and is highly cytotoxic

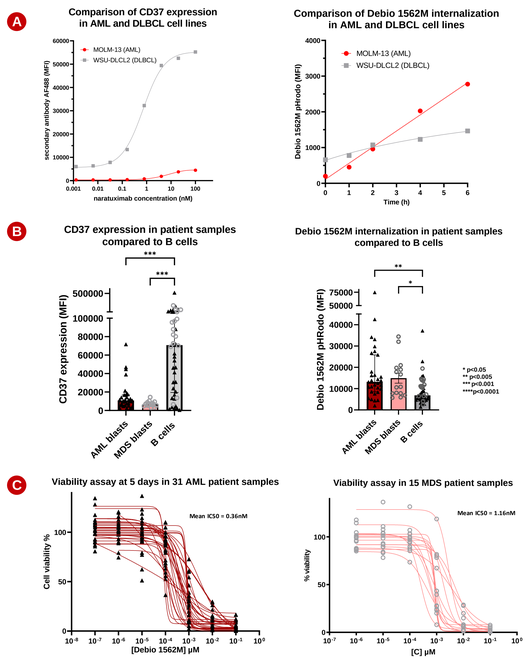

Figure 2. Low CD37 expression in AML/MDS versus B cells is compensated by efficient internalization and thus activity of Debio 1562M
A. WSU-DLCL2 DLBCL cell line has higher expression of CD37 than MOLM-13 AML cell line, however internalization of Debio 1562M is more efficient in MOLM-13 than in WSU-DLCL2 cell line. B. In AML and MDS blasts from patient samples, CD37 expression is lower than on healthy B cells coming from the same samples, however Debio 1562M internalization is more efficient in blasts. C. Efficient internalization is translated by strong cytotoxic activity of Debio 1562M in all AML and MDS blasts.

Debio 1562M significantly improves survival in AML CDX models

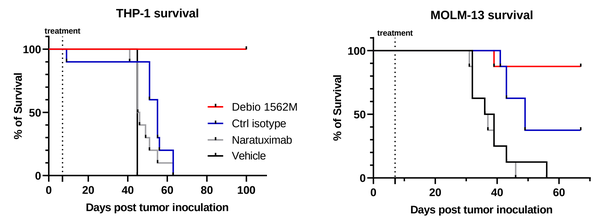

Figure 3. in vivo efficacy of Debio 1562M in THP-1 and MOLM-13 disseminated models
Debio 1562M, control isotype (Trastuzumab-Multilink™-DM1) or naratuximab were administered once at 5 mg/kg. Specific and targeted activity of Debio 1562M is demonstrated in both models compared to naratuximab or control isotype.

Debio 1562M anti-tumoral activity is superior to venetoclax+azacitidine standard of care in preclinical models

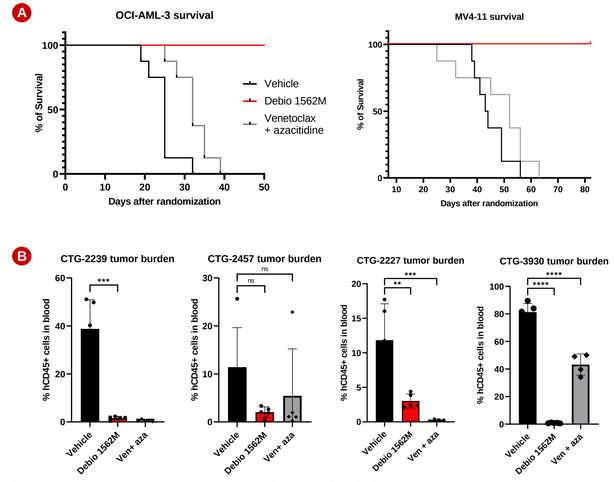

Figure 4. in vivo efficacy of Debio 1562M compared to venetoclax + azacitidine in CDX and PDX models
A. Debio 1562M administered once at 5 mg/kg (MV4-11) or 10mg/kg (OCI-AML-3) significantly improves survival compared to venetoclax + azacitidine (venetoclax QDx15, 100mg/kg and azacitidine QDx5, 3.5mg/kg). B. In PDX models, Debio 1562M administered once at 10 mg/kg is equivalent to venetoclax+ azacitidine (venetoclax QDx21, 100mg/kg and azacitidine 5on2off x3, 2.5mg/kg) to reduce tumor blasts number (measured 21 days after treatment start). Debio 1562M is also very active in CTG-3930 venetoclax+ azacitidine resistant model.

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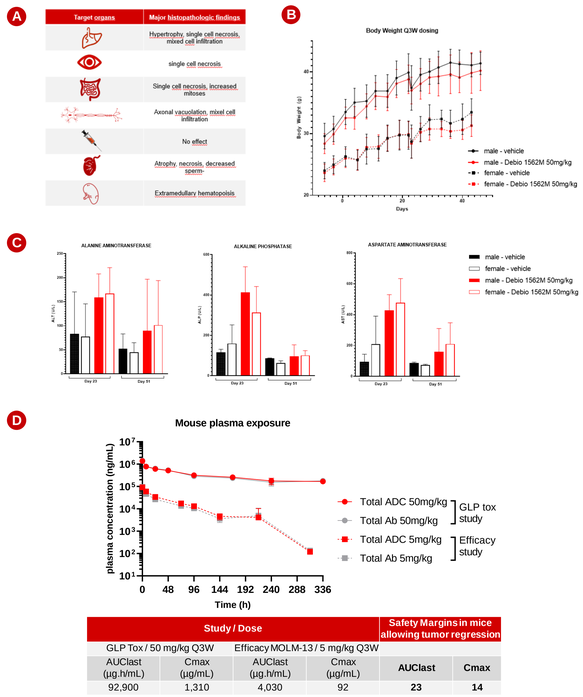
Debio 1562M has a significant safety margin in mice with a toxicology profile related to payload's known toxicities



Study / Dose				Safety Margins in mice allowing tumor regression	
GLP Tox / 50 mg/kg Q3W		Efficacy MOLM-13 / 5 mg/kg Q3W			
AUClast (µg.h/mL)	Cmax (µg/mL)	AUClast (µg.h/mL)	Cmax (µg/mL)	AUClast	Cmax
92,900	1,310	4,030	92	23	14

Figure 5. Debio 1562M is tolerated at 50mg/kg in GLP toxicity study in mice
Debio 1562M is not cross reactive with mouse CD37, therefore on-target toxicity is not evaluated. A. Summary of off-target organ toxicities after 2 administrations (Q3W). B. Debio 1562M has no impact on body weight in males or females compared to control group. C. Liver enzymes were slightly elevated at the end of the treatment phase and were in line with control data at the end of the recovery. D. Comparison of exposure at 50mg/kg in the GLP toxicity study and exposure at 5mg/kg in MOLM-13 tumor bearing mice allows to calculate a safety margin between 14 and 23. PK profile of total ADC and total Ab are overlayed, highlighting the excellent stability of the ADC in plasma.

CONCLUSIONS

- **CD37 is broadly expressed in AML and MDS**
- **Debio 1562M ADC targeting CD37 is efficiently internalized in AML and MDS primary tumor cells and highly cytotoxic**
- **Debio 1562M monotherapy improves survival in several AML CDX models compared to SoC and significantly reduces tumor burden in PDX models**
- **Overall Debio 1562M activity and safety in preclinical models is promising for future clinical development in AML and MDS**

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