THE MULTILINK™ LINKER IS A PROMISING APPROACH TO IMPROVE EFFICACY AND SAFETY OF ADC'S

Josée Hue-Perron[1], Noémie Luong[1], Lisa Ivanschitz[1], Nathalie Bellocq[2], Léo Marx[2], Ulf Andersson[1]

[1]Debiopharm International SA, Chemin Messidor 5-7, CH-1002 Lausanne, Switzerland and [2]Debiopharm Research and Manufacturing, Rue du Levant 146, CH-1920 Martigny, Switzerland

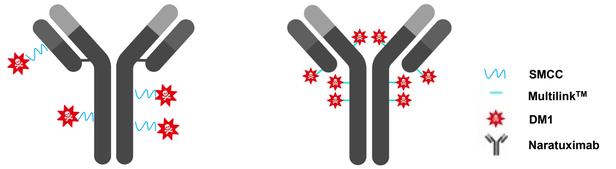
SUMMARY

Antibody drug conjugates (ADCs) represent an attractive method to deliver cytotoxic payloads into tumor cells while reducing systemic toxicities. To improve efficacy, increasing the DAR (drug–antibody ratio) is an appealing approach, although lipophilicity of such constructions can lead to unselective uptake. Non-specific linker cleavage is also associated with off-target toxicity. We have developed the Multilink™ technology, which allows rapid and specific intracellular cleavage of a hydrophilic linker, resulting in efficient payload release from the ADCs.

We investigated the toxicology profiles in mouse of two ADCs targeting CD37. Both ADCs carry the cytotoxic payload DM1; one with a non-cleavable thioether linker (Debio 1562; DAR3.5) and the other with our Multilink™ linker (Debio 1562M; DAR8). Both ADCs demonstrated a toxicity profile expected with DM1, such as hematologic abnormalities, infusion reactions and hepatic lesions. Despite higher DAR, there were fewer toxicities with the Multilink™ ADC, and a trend to lower severity of histopathological lesions. Notably, there were no hemorrhage or degeneration in the sciatic nerve when compared to the thioether conjugated-ADC, which suggests reduced risk of peripheral neuropathy. Unconjugated Multilink™-DM1 linker (quenched with cysteine) was not associated with any signs of toxicity. When evaluated in xenograft efficacy mouse AML models, the Multilink™ ADC demonstrated significantly better tumor regression.

In conclusion, following normalization for dose and difference in DAR in the efficacy and toxicology studies, we have shown that the Multilink™ -conjugated ADC displayed higher therapeutic index (TI) compared to the thioether-conjugated-ADC. This demonstrates the importance of optimizing linker chemistry to improve efficacy and safety, resulting in increased TI.

METHODS



A Debio 1562 (N-DM1)

B Debio 1562M (N-Multilink™-DM1)

Figure 1. (A) N-DM1: Naratuximab Antibody conjugated to DM1 with a non-cleavable thioether linker (DAR 3.5) **(B)** N-Multilink™-DM1: Naratuximab Antibody conjugated to DM1 with our cleavable Multilink™ linker (DAR 8)

In vitro cytotoxicity: in vitro cytotoxicity was evaluated over 72 hours. Naratuximab, N-DM1 and N-Multilink™-DM1 were incubated at concentration from 10^{-14} to 10^{-6} M and the % viability was measured.

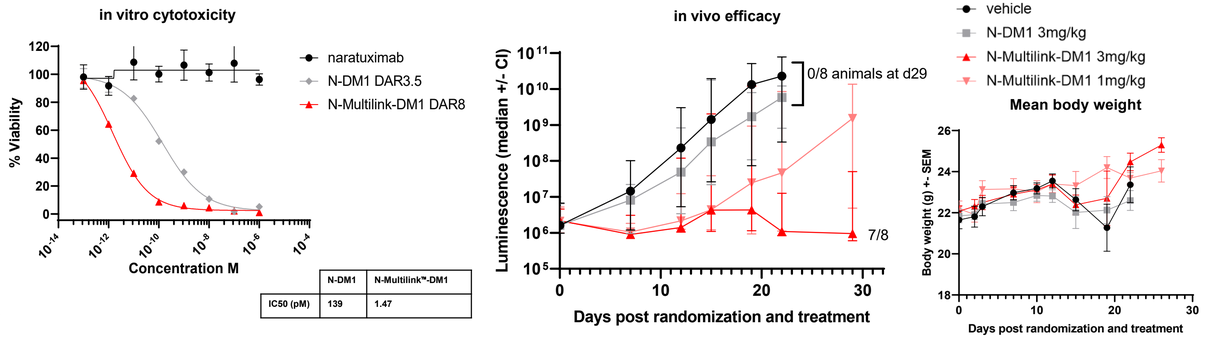
In vivo efficacy: Both N-DM1 and N-Multilink™-DM1 were evaluated in an AML model (MOLM-13 disseminated luciferase model). Eight mice/group were administered either PBS **(vehicle),** N-DM1 at 3 mg/kg, or N-Multilink™-DM1 at 1 and 3 mg/kg. The dosing formulation was injected once in the tail vein of each mouse, 7 days after IV injection of the cancer cells.

Toxicology: The safety profile of N-DM1 and N-Multilink™-DM1 was characterized after a single IV injection to CD-1 mice. 12 mice (N-DM1) and 5 mice (N-Multilink™-DM1) were administered 100 mg/kg and 50 mg/kg of N-DM1 and N-Multilink™-DM1, respectively, at dose levels which are considered DAR-equivalent. Blood was collected at termination for evaluation of clinical pathology parameters. Clinical observation, body weight measurements and ophthalmology examination were conducted. Necropsies were performed 5 days (N-DM1) or 11 days (N-Multilink™-DM1) after treatment.

Stability: Plasma stability of N-Multilink™-DM1 was evaluated in vivo, after one IV injection at 5 mg/kg to mice, and compared to the stability of Naratuximab administered at the same dose level.

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

RESULTS



	N-DM1	N-Multilink™-DM1
IC50 (pM)	139	1.47

Figure 2. In vitro cytotoxicity of the naked antibody (Naratuximab), N-DM1 at DAR 3.5 and N-Multilink™-DM1 at DAR 8 and In vivo efficacy of N-DM1 and N-Multilink™-DM1 in MOLM-13 AML model.

N-Multilink™-DM1 has improved in vitro cytotoxicity compared to N-DM1.

N-Multilink™-DM1 in vivo is well tolerated and has improved anti-tumoral activity in AML model compared to N-DM1.

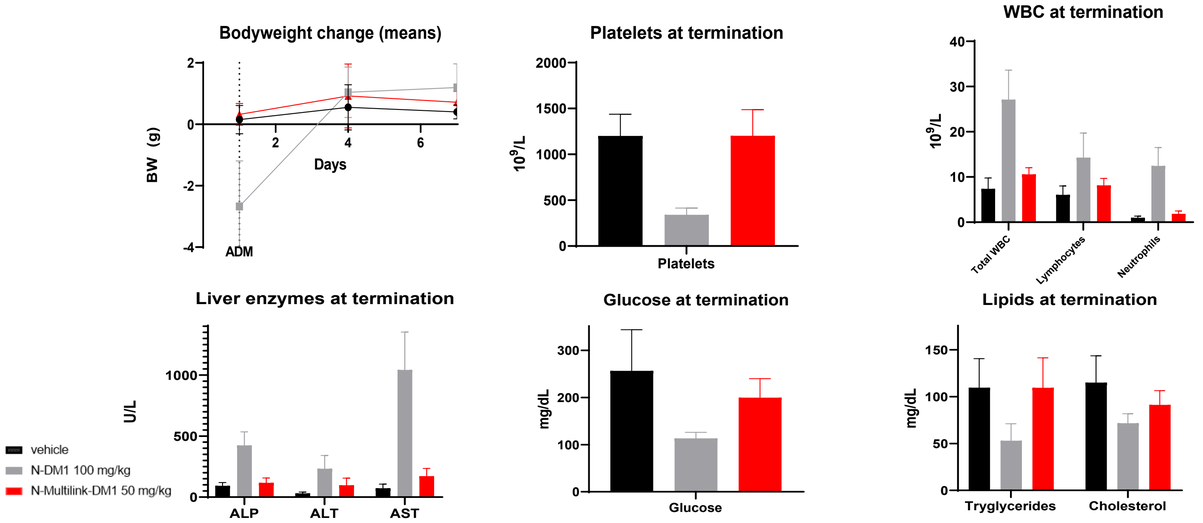


Figure 3. Body weights and clinical pathology parameters after one IV administration of N-Multilink™-DM1 and N-DM1. Clinical pathology parameters were assessed on Day 5 (N-DM1) and on Day 11 (N-Multilink™-DM1)

N-Multilink™-DM1 in vivo is well tolerated and showed minimal effect on clinical pathology parameters. At DAR-equivalent, N-Multilink™-DM1 was noted to affect hematologic and biochemical parameters at a lower severity than N-DM1.

Target organs	N-DM1	N-Multilink™-DM1
	Hypertrophy, extramedullary hematopoeisis, necrosis	Inflammation
	Inflammation single cell necrosis	-
	Hemorrhage, Inflammation	Hemorrhage, Inflammation
	Inflammation, hemorrhage degeneration/necrosis	-
	Degeneration/necrosis inflammation at injection site	Cell infiltration at injection site
	Hemorrhage, axonal/myelin degeneration	-

Figure 5: Histopathology findings after one IV administration of N-Multilink™-DM1 (Day 11) and N-DM1 (Day 5).

At histopathology, N-Multilink™-DM1 has no effect on the eye, heart and sciatic nerve, unlike N-DM1 at DAR equivalent

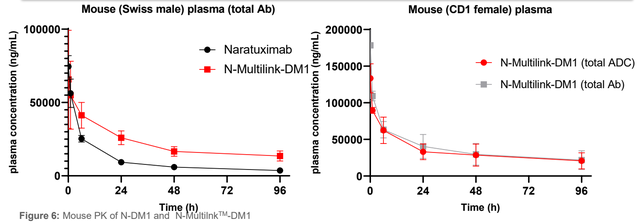


Figure 6: Mouse PK of N-DM1 and N-Multilink™-DM1

PK properties of Naratuximab are not impacted by the conjugation with Multilink™ and N-Multilink™-DM1 is stable in vivo in mouse.

CONCLUSIONS

In vitro, Debio 1562M displayed an improved cytotoxicity profile compared to Debio 1562. In vivo, at doses providing equivalent quantities of payload, Debio 1562M was not only showing more efficacy, it was also safer, by reducing the number of target organs and the severity/incidence of the findings.

By optimizing the linker chemistry, we were able to improve efficacy and safety of an ADC construct, resulting in an increased therapeutic index. As a result, Debio 1562M is currently in the IND-enabling phase, with the aim to progress to clinical phases.

CONTACT

Debiopharm International S.A., Lausanne, Switzerland.
www.debiopharm.com
josee.hueperron@debiopharm.com

DOWNLOAD

This poster is available via:
www.debiopharm.com/medias/publications