[#3129]

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BYON4413, an *in vivo* active CD123-targeting antibody-drug conjugate, combines effectively with azacitidine and venetoclax in acute myeloid leukemia cell lines

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BACKGROUND

CD123, the alpha chain of the interleukin-3 receptor (IL-3Rα), is overexpressed in multiple hematologic malignancies and is particularly highly expressed on the surface of acute myeloid leukemia (AML) cells. Increased CD123 expression on leukemic stem cells relative to non-malignant hematopoietic stem cells makes CD123 an attractive tumor-associated target for an antibody-drug conjugate (ADC). BYON4413 is an ADC that consists of a novel humanized IgG1 anti-CD123 antibody site-specifically conjugated with Byondis' proprietary duocarmazine linker-drug technology, ByonZine® and ByonShieLD®.

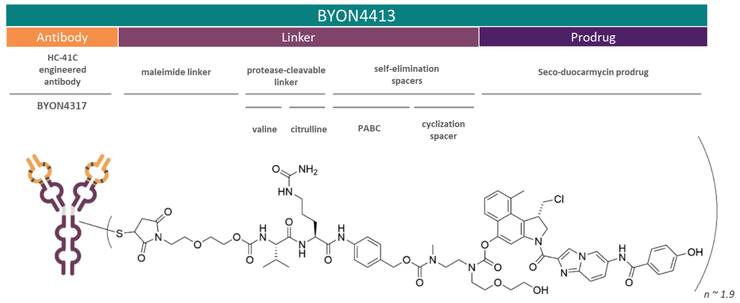


FIGURE 1 The humanized CD123 IgG1 antibody of BYON4413 is site-specifically conjugated to Byondis' proprietary duocarmazine linker-drug (LD) technology, ByonZine® and ByonShieLD®. This LD, when cleaved upon internalization and lysosomal routing, releases a potent duocarmycin payload that alkylates DNA and generates damage that results in replication stress and cell death.

METHODS

The efficacy of BYON4413 is studied as monotherapy and in combination with azacitidine and venetoclax (AZA/VEN), since the AZA/VEN regimen is the standard of care (SOC) for AML patients who are ineligible for intensive chemotherapy. A panel of CD123+ and CD123- hematological cell lines and AML patient-derived bone marrow or peripheral blood mononuclear cells (BMMCs/PBMCs) were utilized in *in vitro* studies to determine the cytotoxic effects of BYON4413. The induction of apoptosis and cell killing by BYON4413 in a triple combination with AZA/VEN was studied *in vitro* using the CD123+ AML cell lines MOLM-13 and MV4-11. AML patient and cell line-derived xenograft models were used to investigate the *in vivo* efficacy of BYON4413.

IN VITRO RESULTS

BYON4413 POTENTLY KILLS CD123-EXPRESSING AML CELL LINES *IN VITRO*

TABLE 1 Summary of the *in vitro* cytotoxicity of BYON4413 and isotype control ADC on a panel of leukemic cell lines. IC50 and % efficacy were determined after 6 days of treatment with the ADC using Cell-TiterGlo™ as a read-out.

Cell line	KASUMI-3	MV4-11	HNT-34	UCSD-AML1	EOL-1	MOLM-13	THP-1	MOLM-14	SHI-1	NOMO-1	SKM-1	Jurkat Clone E6-1	HL-60
CD123 (SABC)	15209	14063	13054	10670	10207	9917	9259	6911	6212	5800	3291	0	0
BYON4413													
IC50 (ng/mL)	9.11	4.93	1.07	7.49	1.47	4.06	~8286*	5.93	~6078*	~300*	~2218*	~2615*	>10000*
Efficacy (%)	66	98	94	89	98	98	62#	97	71#	95#	89#	96#	25#
Isotype control ADC													
IC50 (ng/mL)	>10000*	~3228*	~2346*	~3596*	~1918*	~2138*	~9486*	~3549*	~9979*	~3743*	~1849*	~3932*	>10000*
Efficacy (%)	29#	94#	95#	90#	98#	98#	73#	99#	51#	93#	96#	80#	38#

*The IC50 and efficacy values were calculated based on data from two experiments performed in duplicate. CD123 expression, indicated as Specific Antibody Binding Capacity (SABC), was analyzed using the DAKO QIFIKIT®. Cytotoxicity was assessed in the presence of 100 µg/mL pooled human IgGs (IVIg, Intravenous Immunoglobulin). The efficacy was calculated using the following formula: Efficacy (%) = 100 – (lowest value of the % survival at the highest concentration tested). *IC50 calculated by GraphPad Prism is an estimation of concentration at 50% cell death due to incomplete curve (no sigmoidal curve fitting). #Efficacy calculated by GraphPad Prism was determined to be irrelevant due to incomplete curves.*

BYON4413 SELECTIVELY KILLS HUMAN AML PATIENT-DERIVED BMMCs/PBMCs *EX VIVO*

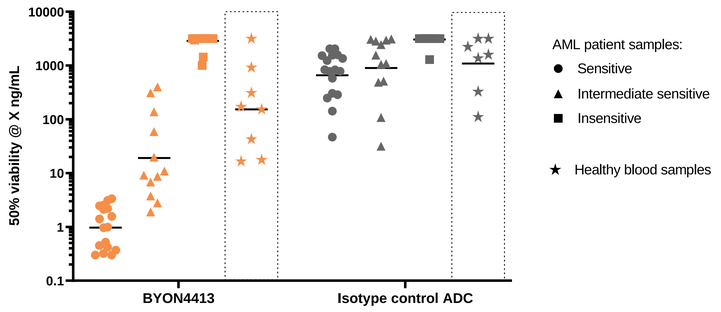


FIGURE 2 Overview of *ex vivo* activity of BYON4413 and isotype control ADC on individual CD123-positive AML patient-derived BMMCs/PBMCs and healthy blood hematopoietic stem/progenitor cells. Dot plot depicts the ADC concentration in ng/mL which induced 50% cell killing (50% viability). Response groups were defined based on BYON4413 activity as follows: Sensitive AML samples = Efficacy ≥ 75% and potency (is 50% effect value) < 5 ng/mL. Intermediate sensitive AML samples = Efficacy between 50% to 95% and potency < 500 ng/mL. Insensitive AML samples = Efficacy < 65% and potency > 1000 ng/mL.

BYON4413 INDUCES APOPTOSIS AND CELL KILLING UPON INTERNALIZATION

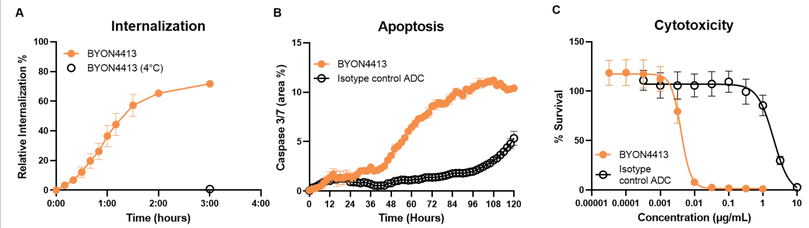


FIGURE 3 (A) Internalization of AF488-labeled BYON4413 in MOLM-13 cells over time measured using flow cytometry analysis. Extracellular fluorescence was quenched to quantify the % of internalized BYON4413. (B) Caspase 3/7 activation induced by 100 ng/mL BYON4413 and an isotype control ADC in MOLM-13 cells measured over time using the IncuCyte® S3. (C) Cytotoxicity of BYON4413 and the isotype control ADC in MOLM-13 cells after 6 days of treatment using CellTiter-Glo™ as a read-out. Data show average ± SD of two experiments.

BYON4413 SYNERGIZES WITH AZACITIDINE AND VENETOCLAX *IN VITRO*

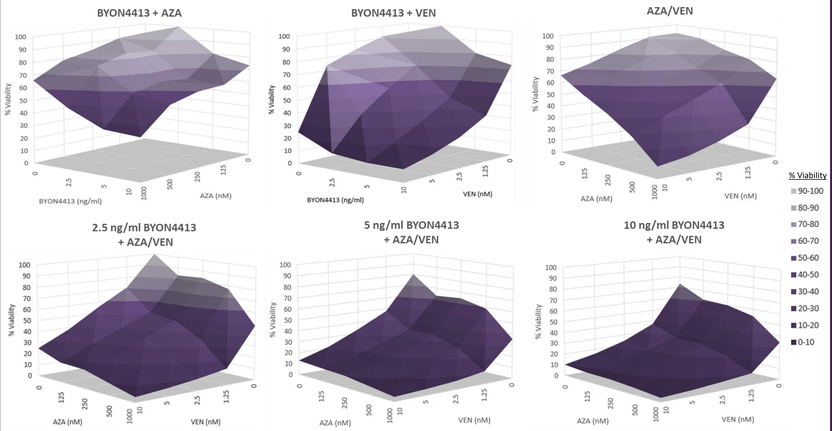


FIGURE 4 Effects of BYON4413 combined with AZA and VEN on cell viability of the human MOLM-13 AML cell line. Cells were treated with either 2.5, 5, or 10 ng/mL BYON4413 for 24 hours and subsequently treated with azacitidine (AZA) and venetoclax (VEN) concentrations either individually or combined, for an additional 24 hours. Cell growth was assessed with the CellTiter-Glo™ assay.

TABLE 2 The effect of synergistic interactions between BYON4413 combined with AZA and VEN on cell viability of the MOLM-13 and MV4-11 AML cell lines. Synergistic interactions were calculated for each combination against BYON4413 alone using the Excess over Bliss (EOB=(Fa1+2-[(Fa1+Fa2) – (Fa1*Fa2)])*100)[1,2]. EOB values for each dose combination >10 represents synergy (aqua).

5 ng/mL BYON4413 combined with AZA/VEN

	MOLM-13						MV4-11					
Ven (nM)	AZA (nM)					Ven (nM)	AZA (nM)					
	1000	500	250	125	0		1000	500	250	125	0
10	3.9	15.8	23.7	31.7	40.3	10	6.7	10.3	10.8	16.2	12.4
5	5.8	21.7	28.4	36.8	37.9	5	8.1	10.3	10.3	14.4	12.5
2.5	9.8	23.3	28.5	37.4	33.2	2.5	6.5	9.5	12.8	15.9	10.4
1.25	13.9	22.5	23.3	31.6	25.9	1.25	9.6	8.1	6.4	13.3	9.0
0	17.2	4.2	2.7	9.9	-6.0	0	6.7	-1.8	0.3	1.7	-5.1

[1] *Amirouchene-Angelozzi N, et al. Oncotarget. 2016 Apr 26;7(17):23633-46.*
[2] *https://synergyfinder.fimm.fi/synergy/synfin_docs*

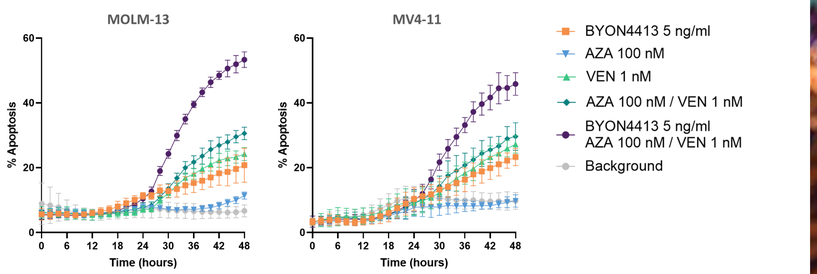


FIGURE 5 Effects of BYON4413 combined with AZA and VEN on apoptosis of human MOLM-13 and MV4-11 cell lines. Caspase 3/7 activation induced by 5 ng/mL BYON4413, 100 nM AZA, or 1 nM VEN alone or in different combinations were measured over time using the IncuCyte® S3. Data show average ± SD of two experiments.

IN VIVO RESULTS

BYON4413 DOSE DEPENDENTLY REDUCES AML PATIENT-DERIVED HUMAN BLASTS AFTER MULTIPLE DOSING IN MICE

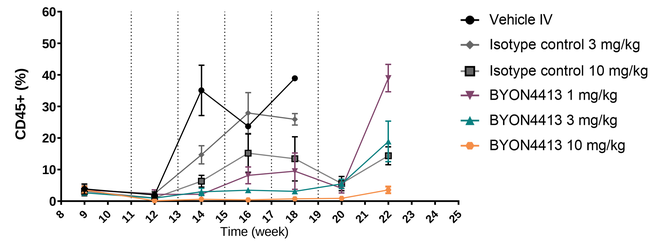


FIGURE 6 BYON4413 *in vivo* killing of CD3-depleted human AML mononuclear cells IV-injected in NSG mice. Mice were randomized between 1 and 8% of human CD45⁺ cells. For treatment, mice were IV-injected (retro-orbital) 5 times at two-week intervals (Q2WX5) with 1, 3, or 10 mg/kg BYON4413, and 3 or 10 mg/kg of the isotype control ADC or vehicle (5 mice/group). Blood was collected one week after each treatment and human CD45⁺ cells were quantified.

DOSE-DEPENDENT AND CD123-MEDIATED ANTI-TUMOR ACTIVITY OF BYON4413 IN MOLM-13-LUC DISSEMINATED XENOGRAFT

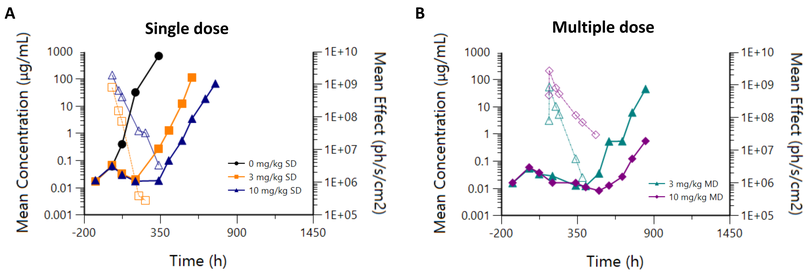
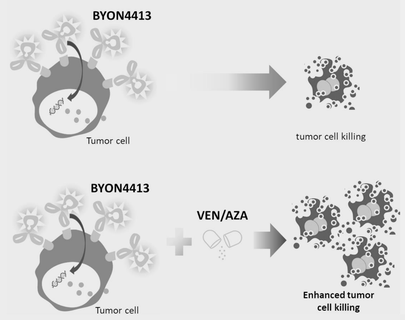


FIGURE 7 Summary of PK and PD of BYON4413 in MOLM-13 NOD-SCID mice. Prior to IV MOLM-13-Luc cell injection on day 0, mice were treated with 100 mg/kg cyclophosphamide to reduce the endogenous bone marrow cell population. Eight days after cell injection, mice were randomized and received a single IV injection with vehicle, (A) a single dose (SD) or (B) a multiple dose (MD) (Q3Dx3) of BYON4413 (9 mice/group). Twice weekly, bioluminescence (BLI) was measured to determine whole body tumor cell growth. Blood was collected to determine exposure in plasma. Figures show whole body tumor growth inhibition (thick lines) and pharmacokinetics (dotted lines) of BYON4413.

SUMMARY RESULTS AND CONCLUSIONS

- *In vitro* studies with human AML cell lines demonstrate that BYON4413 is highly effective at eradicating CD123-positive cells while having little impact on CD123-negative cells.
- BYON4413 kills human AML patient-derived BMMCs/PBMCs *ex vivo* with a stronger potency towards AML patient-derived blasts than to healthy hematopoietic cells.
- The triple combination of BYON4413 with AZA/VEN enhanced both cell killing and caspase-induced apoptosis of AML cell lines compared to BYON4413 single agent treatment.
- *In vivo* PK/PD studies demonstrate that BYON4413 is very efficient at reducing the tumor burden in multiple AML patient- and cell line-derived xenograft models.



- Together, these preclinical studies show that BYON4413 has potential to be an effective targeted therapy against CD123+ hematological malignancies such as AML.
- The enhanced efficacy of BYON4413 together with AZA/VEN suggests that a clinical benefit from the triple combination may be achievable and is worth further exploration.

FUTURE DIRECTIONS

- A first-in-human trial with BYON4413 to evaluate safety, pharmacokinetics, and preliminary efficacy in patients with R/R AML or MDS patients, is scheduled to start in Q2 2024. EU clinical trial number: 2023-507781-13-00.

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