Preclinical Characterization Of NaPi2b-PL2202, A Novel Exatecan-Based Antibody Drug Conjugate

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In vivo studies: Champions Oncology (USA) (patient derived xenograft study)

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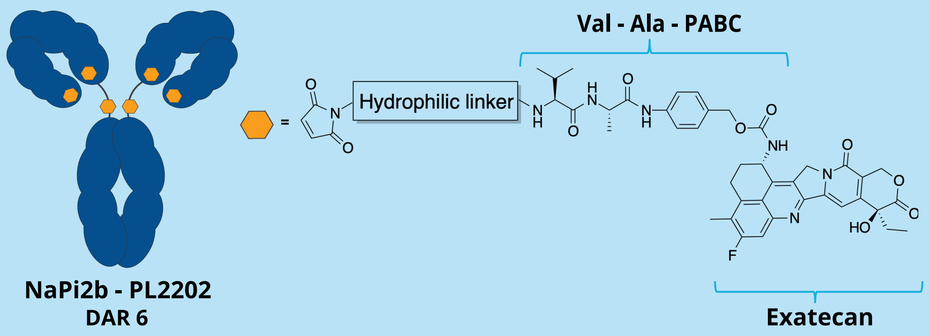
Introduction

- NaPi2b, a cell surface type-2 sodium-dependent phosphate transporter, is highly expressed in ovarian and lung cancer with limited expression in normal tissues. Based on its differential expression, NaPi2b is a promising target for ADC therapy.
- NaPi2b-PL2202, an ADC composed of a humanized, Fc silenced IgG1 monoclonal antibody (mAb) directed against human NaPi2b conjugated to PL2202, a novel payload containing a valine-alanine cleavable linker and exatecan, a potent camptothecin warhead. NaPi2b-PL2202 is site-specifically conjugated with a drug to antibody ratio of six. (Figure 1).
- Previously, we have shown that NaPi2b-PL2202 demonstrated potent and specific *in vitro* and *in vivo* anti-tumor activity in xenograft models, *in vitro* bystander activity and was stable and well tolerated in both rats and cynomolgus monkeys[1].

Figure 1. Napi2b-PL2202



Aim of the study

Aim of this study was to characterise the specificity, internalization, efficacy and potential for combination therapy of NaPi2b-PL2202. In addition, the level of NaPi2b and Folate Receptor 1 (FOLR1) expression in human ovarian tumor specimens was evaluated by immunohistochemistry (IHC).

Materials & methods

- NaPi2b mAb binding specificity was determined by using CHO cells expressing NaPi2a, NaPi2b, NaPi2c and untransfected cells. Antibody cell binding data was analysed by flow cytometry.
- Binding of NaPi2b mAb and Fc silenced NaPi2b mAb, to FcγRIIIa (CD16a) and FcγRIIa (CD32a) was evaluated using Octet, whereas binding to FcγRI (CD64) was analysed using KinExA.
- Internalization of NaPi2b-PL2202 and IgG isotype control ADC was visualized by standard immunofluorescence techniques.
- The combination of NaPi2b-PL2202 and olaparib was determined by *in vitro* cytotoxicity in ovarian cancer cell lines using the CellTiterGlo® assay (Promega).
- In *in vivo* studies, NaPi2b-PL2202, or B12-VA-PL2202 was administered intravenously (i.v.) as single dose to immunocompromised mice containing:
  - A. Human non-small cell lung cancer patient derived xenograft (PDX) CTG-0860.
  - B. Human ovarian PDXs CTG-1301, CTG-0958, CTG-0703, CTG-3718 and CTG-1703.
- Analysis of NaPi2b and FOLR1 membranous expression on human TMAs from patients diagnosed with ovarian cancer was performed by IHC using NaPi2b antibody, MERS67[2] and FOLR1 antibody BN3.2[3] (CST).

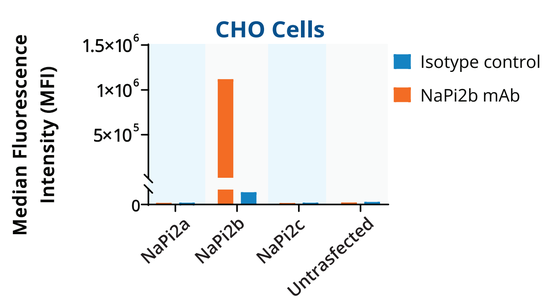
Conclusions

- The NaPi2b mAb showed specific binding to NaPi2b expressing CHO cells but no binding to NaPi2a and NaPi2c expressing cells.
- The Fc silenced NaPi2b mAb did not show binding to FcγRIIIa (CD16a) and FcγRIIa (CD32a) and displayed >1000x reduced affinity to FcγRI (CD64) *in vitro*.
- NaPi2b-PL2202 internalization demonstrated target specific uptake and localisation in lysosomes.
- The combination of NaPi2b-PL2202 with olaparib showed evidence of synergistic activity in the ovarian cancer cell lines.
- *In vivo*, NaPi2b-PL2202 showed dose-dependent tumor regression in a lung PDX model.
- In ovarian cancer PDX models, a single dose of NaPi2b-PL2202 was associated with durable antitumor activity. Analysis of Napi2b expression by IHC of these models showed efficacy in PDX models with H-scores > 100, while no efficacy was observed in PDX models with H-score < 100, indicating its target-mediated antitumor activity.
- Medium or high NaPi2b expression was detected in a significant proportion of ovarian cancer patient derived samples which do not express high levels of FOLR1.

In conclusion, NaPi2b-PL2202 demonstrated specific NaPi2b binding, efficient internalization, potent *in vivo* anti-tumor activity in PDX models representing the indications of interest, and synergistic *in vitro* combination activity with Olaparib, warranting future clinical development of NaPi2b-PL2202 for the treatment of NaPi2b-expressing cancers as a single agent and in combination settings.

Results

Figure 2. NaPi2b mAb is not cross-reactive to NaPi2a & NaPi2c



NaPi2b mAb binds to NaPi2b expressing CHO cells but does not bind CHO cells expressing NaPi2a or NaPi2c, nor untransfected CHO cells.

Table 1. Fc gamma receptor binding by NaPi2b mAb and Fc silenced NaPi2b mAb

Antibody	FcγRIIIa Binding (K_D, M)	FcγRIIa Binding (K_D, M)
NaPi2b mAb	2.07E-07 ± 1.15E-08	6.03E-07 ± 3.51E-08
Fc silenced NaPi2b mAb	No binding	No binding

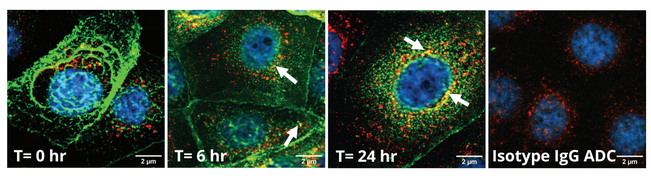
Data are given as mean ± standard deviation; experiment was run in triplicate.

1.A. Octet data. Binding of NaPi2b mAb and Fc silenced NaPi2b mAb, to FcγRIIIa (CD16a) and FcγRIIa (CD32a) evaluated using Octet. NaPi2b mAb exhibited binding to FcγRIIIa (CD16a) and FcγRIIa (CD32a) while Fc silenced NaPi2b mAb did not exhibit any binding.

	NaPi2b mAb	Fc silenced NaPi2b mAb
Ligand	**FcγRI**	**FcγRI**
K_D (nM)	0.017	24.3
K_D confidence intervals (nM)	0.010 - 0.026	11.7 - 78.6
K_{on} (1/Ms)	7.84E06	N/A[$]
K_{on} confidence intervals (1/Ms)	7.47E06 - 8.23E06	N/A[$]
K_{off} (1/s)	1.30E-04	N/A[$]

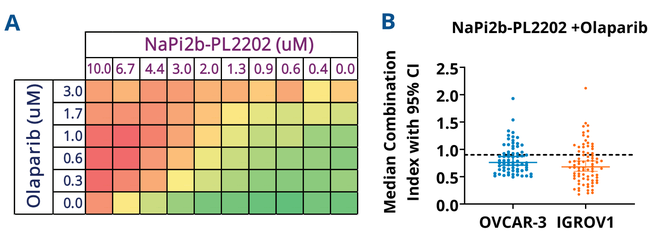
1.B. KinExa data. Binding of NaPi2b mAb and Fc silenced NaPi2b mAb to FcγRI (CD64) was analysed using KinExA. The Fc silenced NaPi2b mAb displayed >1000x reduced affinity to FcγRI in vitro. [$]$K_{on}$ & K_{off} for Fc silenced mAb are too fast to measure using the standard kinetics direct method due to low measured activity.

Figure 3. Internalization of NaPi2b-PL2202 ADC in OVCAR-3



A. Immunofluorescence images of OVCAR-3 cells stained for nuclei (blue), LAMP-1 (red) and human IgG antibody (green). Solid white arrows show colocalised signal (yellow) of NaPi2b-PL2202 ADC with LAMP1 protein. Length of ADC incubation with cells at 37°C (T) in hours. Isotype IgG ADC did not bind to the cells. **B.** *In vitro* cytotoxic activity of NaPi2b-PL2202 ADC on OVCAR-3 cells.

Figure 4. NaPi2b-PL2202 ADC shows *in vitro* synergistic efficacy with Olaparib

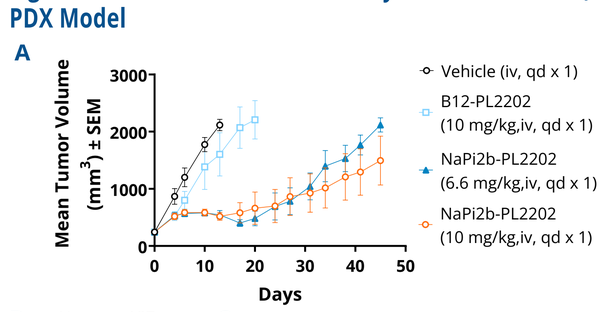


C

	NaPi2b-PL2202 + Olaparib	
	Median Combination index	95%, confidence interval
OVCAR-3	0.76	0.71-0.87
IGROV-1	0.67	0.59-0.78

A. NaPi2b-PL2202 and olaparib combination matrix design. Single drugs and 45 dose combinations were tested on each cell line. **B.** Distribution of Chou-Talalay Combination Index (C.I.) values obtained combining NaPi2b-PL2202 and olaparib in OVCAR-3 and IGROV1 cell lines. In each plot, the horizontal line indicates median CI and the whiskers represent 95% confidence interval values. Dotted horizontal line indicates threshold for synergy. **C.** Table summarizing median CI values with 95% confidence interval values.

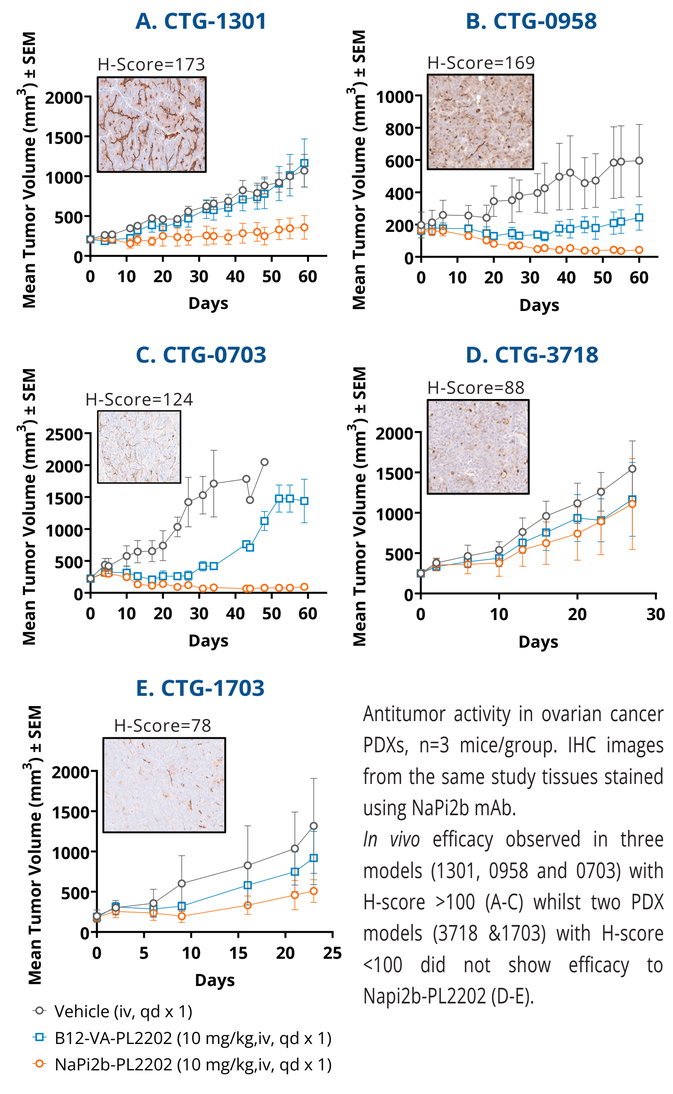
Figure 5. *In vivo* anti-tumor activity in the CTG-0860 (NSCLC) PDX Model



ADCs	REGRESSION		
	PR	CR	TFS
NaPi2b-PL2202, 10 mg/kg	0	0	1
NaPi2b-PL2202, 6.6mg/kg	0	0	0
B12-PL2202, 10 mg/kg	0	0	0

A. NaPi2b-PL2202 and B12-PL2202 isotype-control ADCs were administered i.v. (day 1) to treatment groups of 8 mice. A vehicle-treated group served as control. **B.** Representative scan of FFPE CTG-0860 tumor section stained for NaPi2b by IHC. **C.** Response summary. PR, partial response; CR, complete response; TFS, tumor-free survivors.

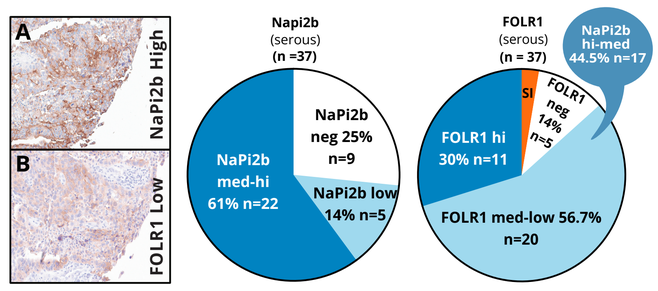
Figure 6. *In vivo* anti-tumor activity in ovarian carcinoma PDX Models



Antitumor activity in ovarian cancer PDXs, n=3 mice/group. IHC images from the same study tissues stained using NaPi2b mAb.

In vivo efficacy observed in three models (1301, 0958 and 0703) with H-score >100 (A-C) whilst two PDX models (3718 &1703) with H-score <100 did not show efficacy to Napi2b-PL2202 (D-E).

Figure 7. Overlap of NaPi2b and FOLR1 membranous expression in human ovarian tumor specimens



Representative images showing NaPi2b and FOLR1 membranous expression in human ovarian tumor specimens. High expression of Napi2b in a tumor core from serous adenocarcinoma (A) whilst low expression of FOLR1 (B) scored in the same tumor specimen.

Pie charts indicate % positivity for each IHC-score across ovarian cancer patients (n=37). Outside the FOLR1 high group, 44.5% (n=37) cases were found with medium or high NaPi2b expression. SI (core insufficient for analysis).

References

1. Horsley, L., et al., Preclinical development of NaPi2b-PL2202, a novel camptothecin-based antibody-drug conjugate targeting solid tumors expressing NaPi2b. AACR 2024.
2. Heynemann, S., et al., NaPi2b expression in a large surgical Non-Small Cell Lung Cancer (NSCLC) cohort. Clin Lung Cancer. 2022 Mar;23(2):e90-e98.
3. Bax, H.J. et al. Folate receptor alpha in ovarian cancer tissue and patient serum is associated with disease burden and treatment outcomes. Br J Cancer.2023, 23 (128): 342–353.

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