Oxidized anthracycline payloads induce anti-tumor immunogenic cell-death and show linker-dependent tolerability when delivered as ADCs

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Background

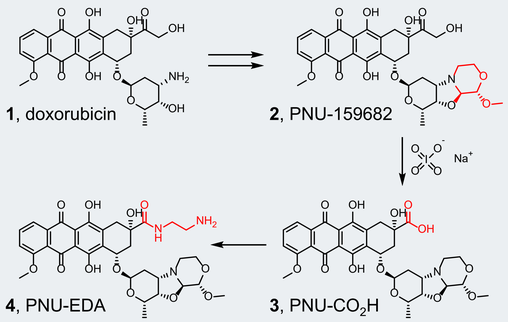
Anthracyclines in Oncology

- Systemic anthracyclines remain an important component of chemotherapy regimens
  - **A**BVD, BE**A**COPP – **A**driamycin (doxorubicin (**1**))
  - C**H**OP – **H**ydroxydaunorubicin (doxorubicin (**1**))
- Treatment is limited by a cumulative dose-dependent cardiotoxicity
  - Liposomal delivery strategies can partially alleviate this toxicity
- Anthracyclines display a unique MOA and are canonical inducers of ICD
  - Diverse MOA includes Topo I/II inhibition, ROS generation, DNA intercalation/alkylation

ADCs and Anthracyclines

- ADCs (antibody-drug conjugates) continue to expand in clinical importance
  - There is an ongoing need for new payloads and MOAs orthogonal to established technologies
  - ICD induction and combinatorial activity with immune checkpoint inhibitors are desirable properties for new technologies in the clinic
- BR96-Doxorubicin was one of the earliest clinical ADCs
  - Drug was attached via an unstable hydrazone linker
  - Program discontinued due to dose limiting GI toxicities
    - Modest activity of the drug necessitated high ADC doses

Oxidized Analogues and Free Drug Activity

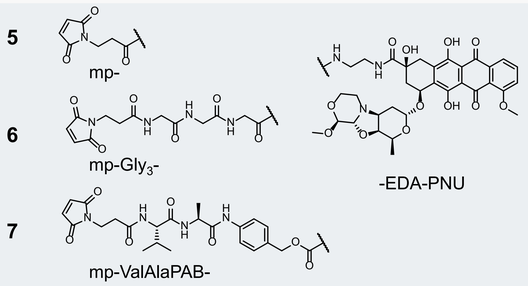


- PNU-159682 (**2**) is a highly potent metabolite of nemorubicin
- This free drug can be modified via an oxidative cleavage of the hydroxy ketone
  - Subsequent amidation of the revealed acid results in a range of free drug properties (Table 1)

Drug IC_{50} in nM	L540cy HL	SK-MEL-5 MEL	HL-60 AML	HL60/RV AML	786-O RCC
Doxorubicin, **1**	13	24	16	388	29
PNU-159682, **2**	0.01	<.0038	0.01	0.01	<.0038
PNU-CO_2H, **3**	9	8	19	18	5
PNU-EDA, **4**	2	1	1	139	7

Table 1. Free Drug Potency, MDR+ cell lines

Drug Linkers and ADC Activity

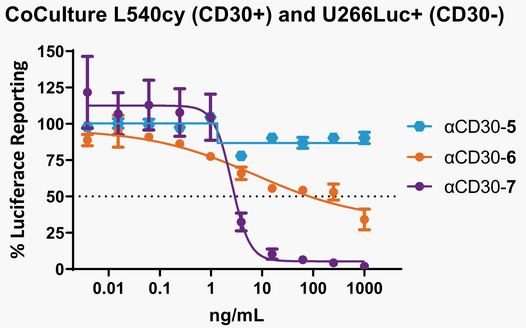


DAR 4 ADCs (ng/mL)	L540cy HL	DEL ALCL	DEL/BVR ALCL	L-428 HL	Ramos BL (CD30-)
αCD30-5	0.8	0.6	1	3	>1K
αCD30-6	1	0.2	2	386	313
αCD30-7	4	0.9	>1K	>1K	320

Table 2. ADC Potency, , MDR+ cell lines

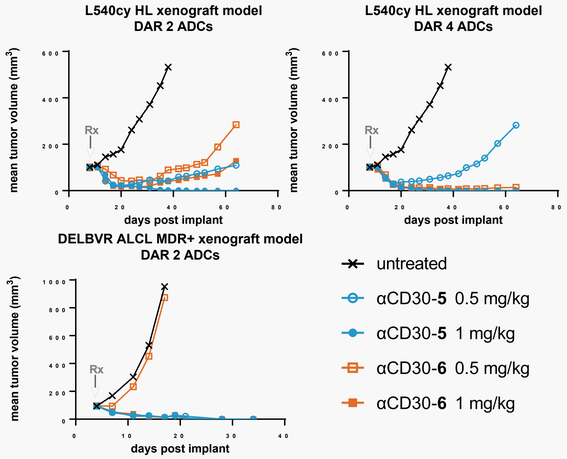
- Non-cleavable (**5**) and cleavable (**6**, **7**) drug-linkers were synthesized and evaluated as αCD30 ADCs
  - Non-cleavable linker **5** outperforms cleavable analogues in multi-drug resistant cell lines (Table 2)
  - Modest off-target activity in CD30- cell lines observed with cleavable linkers **6** and **7**

Bystander Activity



- An admixed Ag-/Ag+ luciferase coculture model shows strong bystander activity for cleavable linker **7**, modest bystander for **6**, and minimal bystander for non-cleavable **5**

In Vivo Activity



- Anthracycline drug linkers were evaluated as drug-antibody ratio (DAR) 2 and DAR 4 ADCs with activity at 0.5 mg/kg
- ADC-**5** showed superior drug-matched activity when DAR 2
- ADC-**5** outperformed ADC-**6** in the MDR+ DELBVR model, consistent with *in vitro* results

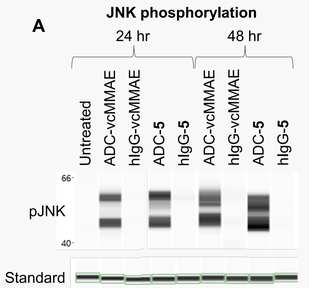
Rat Tolerability

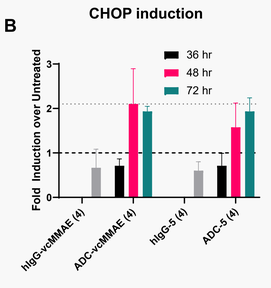
- The highest tested dose of 10 mg/kg of ADC-**5** at DAR 4 was tolerated
- Target organs common to all drug linkers included kidney (tubule degeneration), liver (hepatocellular necrosis and/or increased liver enzymes), bone marrow (depletion).
- No microscopic cardiac toxicity
- Improved MDR+ activity and a broader therapeutic window led to prioritizing compound **5**

ADC	DAR	MTD
hIgG-5	2	10 mg/kg
	4	**≥10 mg/kg**
hIgG-6	2	10 mg/kg
	4	6 mg/kg
hIgG-7	2	10 mg/kg
	4	6 mg/kg

Table 3. ADC single maximum tolerated doses (MTDs) in Sprague-Dawley rats (n=1)

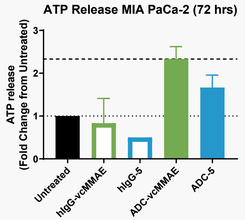
ICD induction similar to vcMMAE

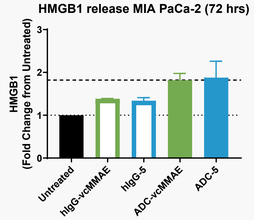




(A) Simple Western (Wes™ Protein Simple) analysis of MIA PaCa-2 cells treated with ADC at 1 ug/mL (B) MIA-PaCa-2 cells expressing CHOP-driven luciferase reporter (Signosis, Inc.) were treated with ADCs at IC50 dose

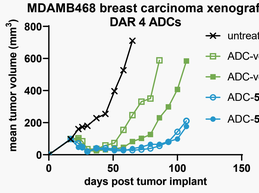
- Endoplasmic reticulum (ER) stress is important for ICD and can be monitored by JNK phosphorylation and CHOP induction

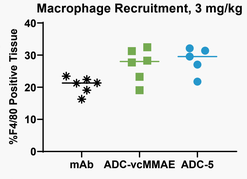




Supernatants were collected from MIA PaCa-2 pancreatic tumor cells treated with ADCs at IC50 doses for 72hr and ATP release determined by Cell Titer Glo and HMGB1 secretion by ELISA was performed.

- Release of damage associated molecular patterns (DAMPs) is a hallmark of ICD





Tissues from an MDAMB468 breast carcinoma xenograft were harvest on day 7 post-treated and analyzed by IHC for increased F4/80+ infiltrating immune cells

- ADC-**5** shows superior activity to vcMMAE at 1 and 3 mg/kg
- Increased macrophage infiltration demonstrates an immunomodulatory impact to the tumor microenvironment

Conclusions

- Novel ADCs bearing potent anthracycline payloads were active *in vitro* and *in vivo* with a broad rodent therapeutic window
- Non-cleavable analogue **5** was prioritized due to improved activity and tolerability relative to cleavable analogues
  - Activity improvements were most pronounced in MDR+ settings
  - These improvements correlated to reduced membrane permeability of the released payload (bystander activity)
- Anthracycline drug linkers demonstrate *in vitro* and *in vivo* hallmarks of immunogenic cell death on par with canonical inducer vcMMAE
  - Further studies into immune memory and immune checkpoint inhibitor combinations are ongoing

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