Discovery of AN4035: A Novel CEACAM5-Targeting Antibody Drug Conjugate (ADC) Armed with a Proprietary Pan-RAS(ON) Inhibitor Payload, Designed to Broaden the Therapeutic Window

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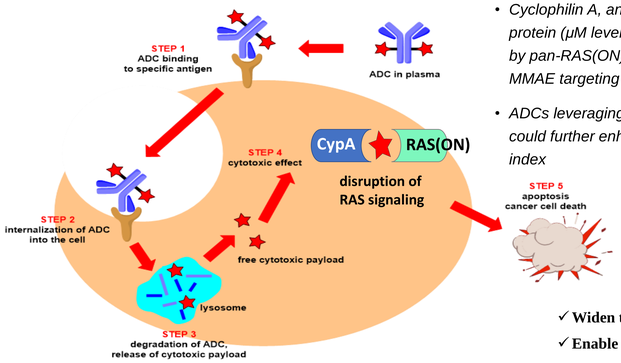
Introduction

- **Background**: Tricomplex pan-RAS(ON) inhibitors (e.g. RMC-6236) are a promising class of therapeutics against RAS-driven cancers
- **Potential limitations**: On-target, off-tumor toxicities may restrict dose and limit safety in combinations
- **Our Hypothesis**: Targeted delivery via an ADC could localize pan-RAS(ON) inhibitor activity to tumors while minimizing systemic RAS pathway inhibition



- *Cyclophilin A, an abundant intracellular protein (μM levels), is the target engaged by pan-RAS(ON) inhibitors, much like MMAE targeting tubulin*
- *ADCs leveraging pan-RAS(ON) inhibitors could further enhance the therapeutic index*

✓ **Widen therapeutic window**

✓ **Enable rational combinations**

This led to the discovery of AN4035, a CEACAM5-directed ADC, leveraging CEACAM5's high expression in RAS-driven malignancies (e.g., colorectal, pancreatic, lung that frequently harbor RAS mutations).

AN4035 Payload is a proprietary highly potent pan-RAS(ON) inhibitor

Compound	Binary (CYPA) K_{D1} (nM)	Tri-complex (KRAS) K_{D2} (nM)			
		G12C	G12D	G12V	WT
Payload	14.9	4.7	30.5	14.0	18.6
RMC-6236	12.5	19.1	174	45.9	52.8

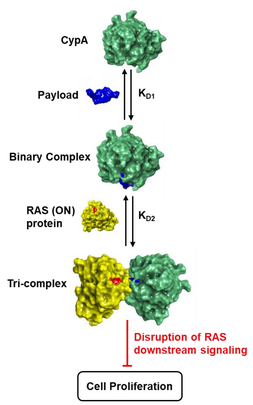
Table 1. Binding affinity of the payload of AN4035 and RMC-6236 with various RAS mutant proteins. Our payload exhibits superior binding affinity, forming the critical tricomplex with RAS(ON) proteins compared to the benchmark RMC-6236 across multiple KRAS mutants.

Cancer Cell Line	Indication	RAS Mutation Type	Payload 72h CTG	
			IC_{50} (nM)	Max inhibition %
NCI-H2009	NSCLC	Kras G12A	0.02	65
NCI-H358		Kras G12C	0.06	93
NCI-H2030		Kras G12C	0.04	22
NCI-H2122		Kras G12C	0.01	93
NCI-H441		Kras G12V	<0.01	44
SW900		Kras G12V	0.12	80
ASPC1	PDAC	Kras G12D	0.06	63
HPAC		Kras G12D	0.07	63
KP4		Kras G12D	0.75	56
Capan1		Kras G12V	0.02	57
Capan2		Kras G12V	0.09	61
SW620	CRC	Kras G12V	0.01	80
HCT116		Kras G13D	0.41	93
LoVo		Kras G13D	0.34	67
T84		Kras G13D	2.59	74
LS1034		Kras A146T	1.0	80

Table 2. *In vitro* antitumor activity of AN4035 payload. Our payload exhibits potent anti-tumor activity across a broad panel of NSCLC, PDAC, and CRC cell lines harboring diverse RAS mutations, with picomolar to low-nanomolar potency.

MOA of Payload

Pan-RAS(ON) molecular glue



AN4035 demonstrated favorable drug-like characteristics and strong intracellular payload retention

- AN4035 is a homogeneous DAR8 ADC constructed via conventional disulfide bonds and equipped with a hydrophilic, enzyme-cleavable linker, which confers favorable thermal and plasma stability as well as desirable pharmacokinetic properties.
- The molecular glue mechanism of AN4035 payload promotes significant intracellular retention due to μM level of Cyclophilin A (an abundant intracellular protein).

Compound	Human Plasma Incubation Time	LC-MS DAR
AN4035	Day 0	8.04
	Day 7	7.85

Table 3. Human plasma stability of AN4035 (DAR)

Compound	Plasma Incubation Time	Payload Released Rate %			
		Human	Mouse	Rat	Monkey
AN4035	Day 0	<0.05	<0.05	0.02	<0.05
	Day 7	<0.05	<0.05	0.01	<0.05
	Day 14	0.05	<0.05	0.01	0.06

Table 4. Plasma stability of AN4035 (Free drug release)

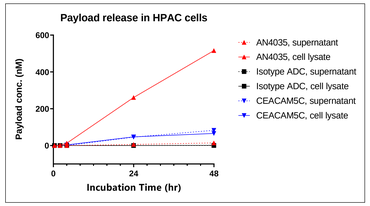


Figure 1. Dynamic changes in payload levels in the supernatant and cell lysate of AN4035 *v.s.* CEACAM5C treated HPAC cells. All ADCs were incubation in 10 μg/mL. For AN4035, the cell lysate/supernatant ratio was 44-fold (1.0-fold for CEACAM5C) at 24 hours, indicating highly efficient trapping of the payload inside the cancer cell due to the molecular glue mechanism.

AN4035 demonstrated potent cytotoxicity in CEACAM5-positive, RAS-addicted cancer cells and a robust bystander killing effect

Cancer Cell Line	Cancer Type	CEACAM5 mRNA nTPM	RAS Mutation Type	6-day CTG		
				AN4035		Isotype ADC
				IC_{50} (nM)	Max Inhibition %	IC_{50} (nM)
CL-40	CRC	1821	Kras G12D	0.77	98	-
SK-CO-1		784	Kras G12V	0.02	99	-
LS513		208	Kras G12D	0.15	100	-
SW403		443	Kras G12V	2.8	95	-
SW1463		198	Kras G12C	2.3	99	-
LoVo		122	Kras G13D	4.4	48	-
GP2D		42	Kras G12D	1.4	84	-
QGP-1	PDAC	1360	Kras G12V	0.05	91	-
HPAC		714	Kras G12D	0.10	73	>100
ASPC1		499	Kras G12D	0.31	88	-
Capan1		97	Kras G12V	0.23	76	-
NCI-H2122	NSCLC	616	Kras G12C	0.30	94	-
NCI-H727		332	Kras G12V	0.09	93	-
MKN45	Gastric cancer	1087	Kras WT	0.13	95	>100

Table 5. *In vitro* antitumor activity of AN4035. AN4035 demonstrated nanomolar to picomolar cytotoxicity in CEACAM5-positive/RAS-addicted cancer cell lines, even in those exhibiting low CEACAM5 expression levels.

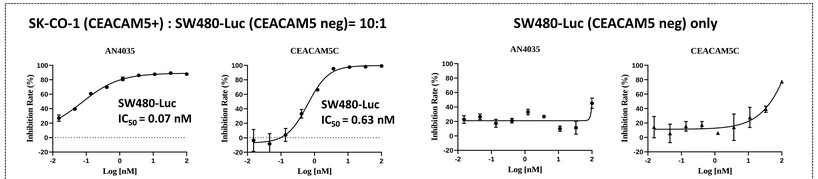
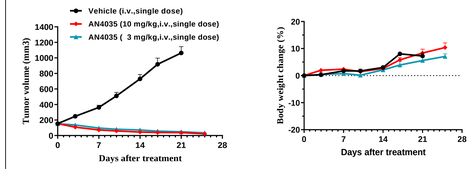


Figure 2. Bystander killing effect of AN4035 vs CEACAM5C in SK-CO-1 (CEACAM5+) / SW480-Luc (CEACAM5 neg) model. AN4035 showed a superior bystander killing effect compared to CEACAM5 Top1-based ADC.

AN4035 demonstrated robust anti-tumor activity in CDX models

HPAC$^{KRAS\ G12D}$ model (PDAC, CEACAM5 mRNA 714 nTPM):

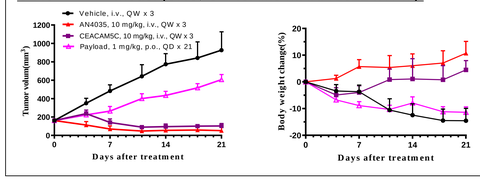


Compd	CTG (IC_{50}, nM)	Dose (mg/kg)	ΔRTV (%) on Day 25
AN4035	0.10	3.0, single dose	-78%
		10, single dose	-85%

ΔRTV = relative to the tumor volume at the start of treatment, ΔRTV = (Vt-V0) / V0 × 100

- Durable tumor regression with single-dose of 3 mg/kg

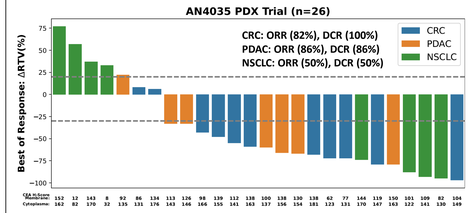
CL40$^{KRAS\ G12D}$ model (CRC, CEACAM5 mRNA 1821 nTPM):



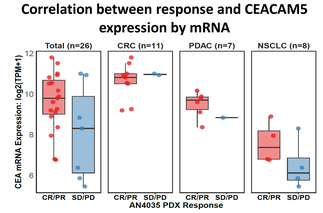
Compd	CTG (IC_{50}, nM)	Dose (mg/kg)	ΔRTV (%) on Day 21
AN4035	0.38	10, QW	-89%
CEACAM5C	1.2	10, QW	-36%
payload	0.08	1.0, QD	276%

- Superior or comparable efficacy to CEACAM5C, a CEACAM5 Topo I-based ADC or naked pan-RAS(ON) payload at tolerable doses

AN4035 exhibited compelling activity in single-mouse PDX trials

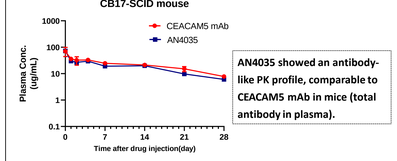


Correlation between response and CEACAM5 expression by mRNA



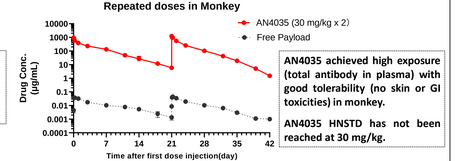
All PDX models were treated with AN4035 (10 mg/kg, QW, IV).The response was determined by comparing tumor volume change at time t to its baseline with ΔRTV = (Vt-V0) / V0 × 100; Criteria for response were adapted from RECIST clinical criteria; **CR**: at least a 85% decrease in the tumor volume compared to baseline; **PR**: At least a 30% decrease in the tumor volume compared to baseline; **PD**: At least a 20% increase in the tumor volume compared to baseline; **SD**: Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD

AN4035 demonstrated favorable PK and tolerability in monkeys



AN4035 showed an antibody-like PK profile, comparable to CEACAM5 mAb in mice (total antibody in plasma).

Compound	Dose	C_0 (μg/mL)	AUC (h*μg/mL)	T1/2 (day)
AN4035	3 mg/kg, IV	72	13832	8.2
CEACAM5 mAb	3 mg/kg, IV	71	17218	9.7



AN4035 achieved high exposure (total antibody in plasma) with good tolerability (no skin or GI toxicities) in monkey.

AN4035 HNSTD has not been reached at 30 mg/kg.

Compound	Dose	C_0 (μg/mL)	AUC (h*μg/mL)	T1/2 (day)
AN4035	30 mg/kg, IV	942	60377	3.3
Free Payload		0.038	5.40	4.3

Conclusions

- ✓ AN4035 is a first-in-class CEACAM5-targeting ADC armed with a highly potent pan-RAS(ON) inhibitor payload.
- ✓ AN4035 has favorable thermal and plasma stability with desirable pharmacokinetic properties.
- ✓ AN4035 exhibits strong intracellular payload retention, resulting in nanomolar to picomolar cytotoxicity in CEACAM5-positive, RAS-addicted cancer cell lines, and demonstrates a potent bystander-killing effect.
- ✓ AN4035 shows robust anti-tumor activity with deep regression in CEACAM5-positive/RAS-addicted CDX and PDX models.
- ✓ AN4035 shows favorable preliminary toxicology profile in cynomolgus monkeys.
- ✓ IND-enabling studies are currently ongoing.

- We are forging strategic collaborations to accelerate the development of AN4035 and unlock the potential of the pan-RAS(ON) ADC platform. Contact information: alex.ye@adlainortye.com