A pan-RASi antibody-drug conjugate platform with high activity in RAS-mutant cancers

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Introduction

Tricomplex RAS inhibitors (RASi) are an emerging class of drugs that disrupt RAS signaling by forming a ternary complex with cyclophilin A (CypA) and the active, GTP-bound conformation of RAS [RAS(ON)], thereby blocking downstream signaling through the MAPK and PI3K pathways. Despite promising initial results in the clinic with RMC-6236, the prototypical molecule of this class, treatment-related adverse events such as skin and gastrointestinal toxicities have emerged, likely as a result of on-target, off-tumor inhibition of RAS in normal tissues. We hypothesized that targeted delivery of a potent pan-RASi as an antibody-drug conjugate (ADC) payload could both enhance anti-tumor efficacy and improve tolerability, thereby overcoming the limitations of orally administered RASi small molecules.

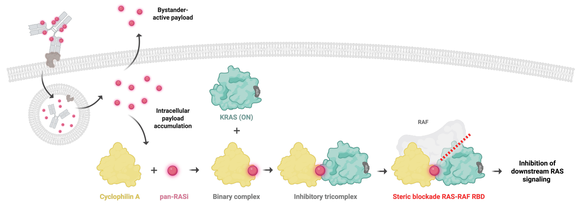


Figure 1. Mechanism of action of a RASi ADC. RASi payload is delivered into target-expressing cancer cells via target-mediated endocytosis. Once delivered, the RASi payload operates through a tricomplex mechanism, first binding to cyclophilin A to form a binary complex which subsequently forms a tricomplex with RAS, thereby disrupting downstream signaling[1]. Figure created with Biorender.com

RASi ADC proof-of-concept established with clinically evaluated small molecules as payloads

***In vitro* proof-of-concept** (POC) with clinical agents RMC-6291, RMC-6236, and divarasib. Small molecules and ADCs were screened against cancer cell lines in spheroid cytotoxicity assays.

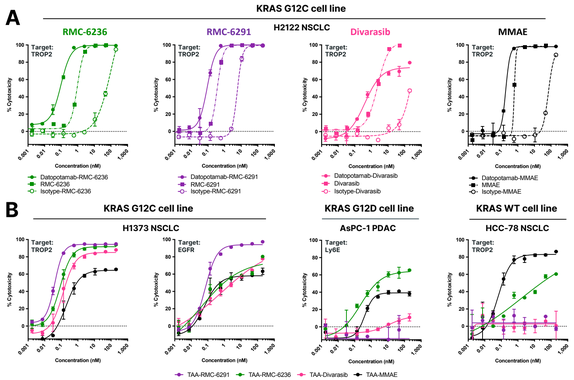


Figure 2. RASi ADCs demonstrate target and RAS-mutation dependent cytotoxicity *in vitro*. **(A)** ADCs using clinical RASi as payloads demonstrate target-specific activity *in vitro* against RAS-mutant cell lines (spheroid format). **(B)** RASi ADCs exhibit selective cytotoxicity against RAS-mutant cell lines. RASi ADCs were generated with a drug-to-antibody ratio (DAR) of 8. MMAE ADCs had a DAR of 4. TAA = tumor associated antigen. Isotype antibody = palivizumab.

***In vivo* proof-of-concept** using clinical RASi as ADCs payloads.

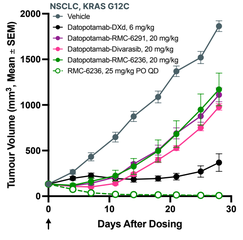


Figure 3. Proof-of-concept RASi ADCs achieved transient tumour stasis in an H1373 cell line-derived xenograft (CDX) model in BALB/c nude mice, n = 4 per treatment group. All ADCs were conjugated to DAR8 using stochastic cysteine conjugation and singly dosed on day 0. Datopotamab was used as a model target system.

RMC-6236 dose across treatment groups

Drug, dose	Total dose over 28 days	%F	ADC %RMC-6236 by weight	RMC-6236 normalized dose
RMC-6236 25 mg/kg, PO, QD	700 mg/kg	33%[2]	-	**230 mg/kg**
RMC-6236 ADC 20 mg/kg, IV, d0	20 mg/kg	100%	5%	**1 mg/kg**

%F = % bioavailability

Novel RASi payloads were screened for activity and biophysical characteristics

>170 novel small molecule RASi were generated. RASi were optimized for potency and linkability to provide high-quality ADC payloads.

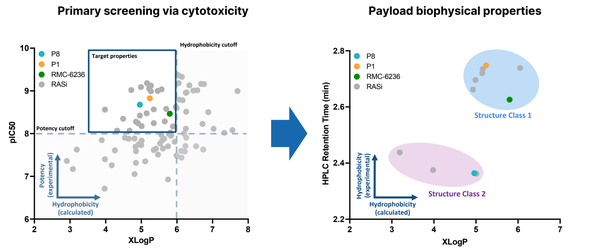


Figure 4. Evaluation of RASi payloads. Novel RASi payloads were screened for cytotoxicity against RAS-mutant cell lines (monolayer format, H1373 shown, n = 2). A subset of compounds selected for evaluation as ADC payloads had increased potency and polarity as compared to RMC-6236. RASi = *de novo* generated RASi payload, P1= prototype payload, P8 = optimized payload. pIC50 = -log(IC50 in M). XLogP calculated with Dotmatics.

Novel RASi payloads were conjugated to produce RASi ADCs

>30 RASi ADCs were generated from novel RASi payloads. A hydrophilic linker system was employed to improve ADC biophysical properties.

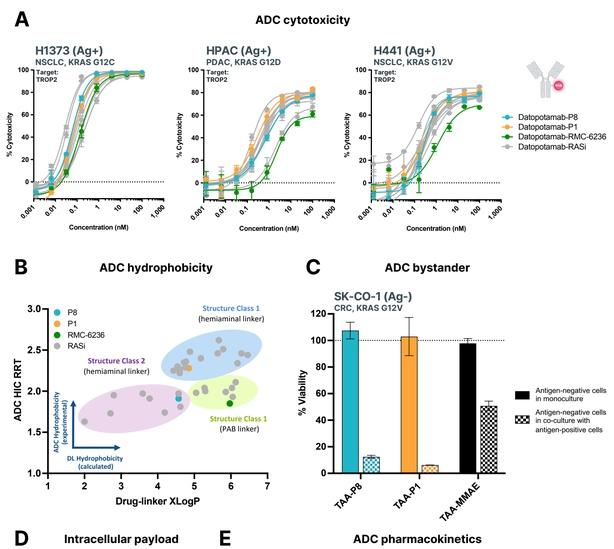


Figure 5. Evaluation of RASi ADCs. **(A)** *In vitro* cytotoxicity of RASi ADCs generated using a hydrophilic linker system. **(B)** Increased drug-linker hydrophilicity translated into increased ADC hydrophilicity. DL= drug-linker, HIC = hydrophobic interaction chromatography. RRT = relative retention time of DAR8 to DAR0 species via HIC. **(C)** Bystander activity of TAA-P8, TAA-P1 and TAA-MMAE assessed by measuring the viable cell population of fluorescently-labelled antigen-negative cells (SK-CO-1 mCherry) in monoculture and in co-culture with antigen-positive cells (PA-TU-8988s) via flow cytometry following treatment at 10 nM for 4 days. **(D)** Intracellular payload accumulation as delivered by RASi ADCs (DAR8) compared to an MMAE ADC (DAR4) in antigen-positive cells (PA-TU-8988s). Cells were incubated with 10 nM of the indicated ADC for 24 hours prior to quantification of intracellular payload via mass spectrometry. **(E)** ADC improvements translated to higher total antibody exposures for optimized RASi ADCs in CDX models.

RASi ADCs were highly active in G12C and G12D CDX models at a single dose of 1 mg/kg

RASi ADCs exhibit strong anti-tumor activity in H1373 and HPAC CDX models.

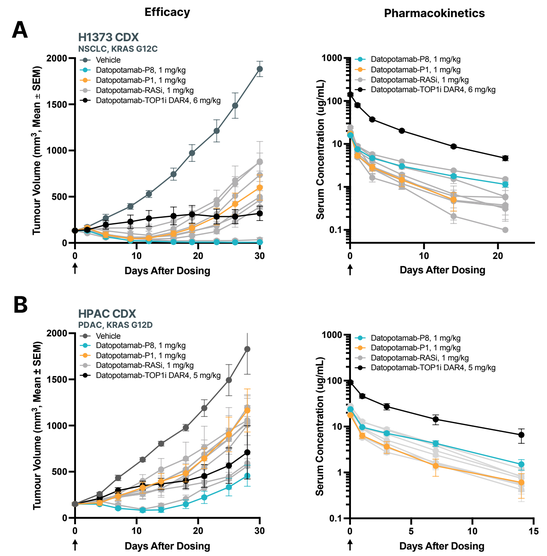
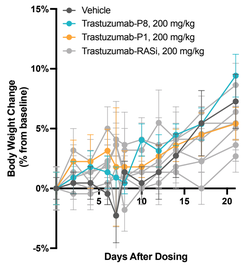


Figure 6. *In vivo* efficacy and PK of novel RASi ADCs in H1373 **(A)** and HPAC **(B)** CDX models in BALB/c nude mice, n = 4 per group. TOP1i drug-linker = ZD007-06519.

Tolerability of RASi ADCs was evaluated in mice with no adverse effects at 200 mg/kg

RASi ADCs were compared at a single dose of 200 mg/kg in mice.



- No body weight loss or abnormal findings (hematology, clinical chemistry, histopathology) noted
- MTD ≥200 mg/kg

Murine tolerability comparison to RMC-6236

Drug	Efficacious dose across majority of CDX models	Highest tolerated dose in mice	Murine therapeutic index
RMC-6236, PO, QD	25 mg/kg	≤100 mg/kg*	**≤4**
RASi ADC, IV, d1	<10 mg/kg	≥200 mg/kg	**>20**

*Model dependent. WO2025/255438: Doses of 100 mg/kg Q2D induce >10% BWL

Figure 7. *In vivo* tolerability of RASi ADCs administered as a single 200 mg/kg dose to non-tumor-bearing BALB/c mice, n = 6 per treatment group. RASi payloads were conjugated to trastuzumab as a model antibody at a DAR of 8. In-life measurements included serum chemistry, hematology, gross necropsy and histopathology assessment at 1 and 3 weeks post dose.

RASi ADCs well tolerated in NHP at 120 mg/kg

Non-human primate platform tolerability demonstrated for two ADCs bearing distinct payloads.

ADC (DAR8)	Dose (mg/kg); schedule	Clinical Signs & Body Weights	Clinical Pathology	MTD
TAA-RASi ADC 1	90; single dose 120; single dose	No treatment related signs or effects	Effects consistent with a transient inflammatory response	**≥120 mg/kg**
TAA-RASi ADC 2	90; q3wx2 120; q3wx2			

Non-human primate (NHP) study design: ADCs dosed single dose or every 3 weeks for 2 doses (2M and 1F cynomolgus monkey per group) with termination 7 days post-second dose; histopathology pending.

In vivo studies reveal distinct PK/PD profile between RASi ADC and oral RMC-6236

In vivo studies were conducted to explore the pharmacological basis of RASi ADC activity using a prototype RASi ADC (TAA-P1).

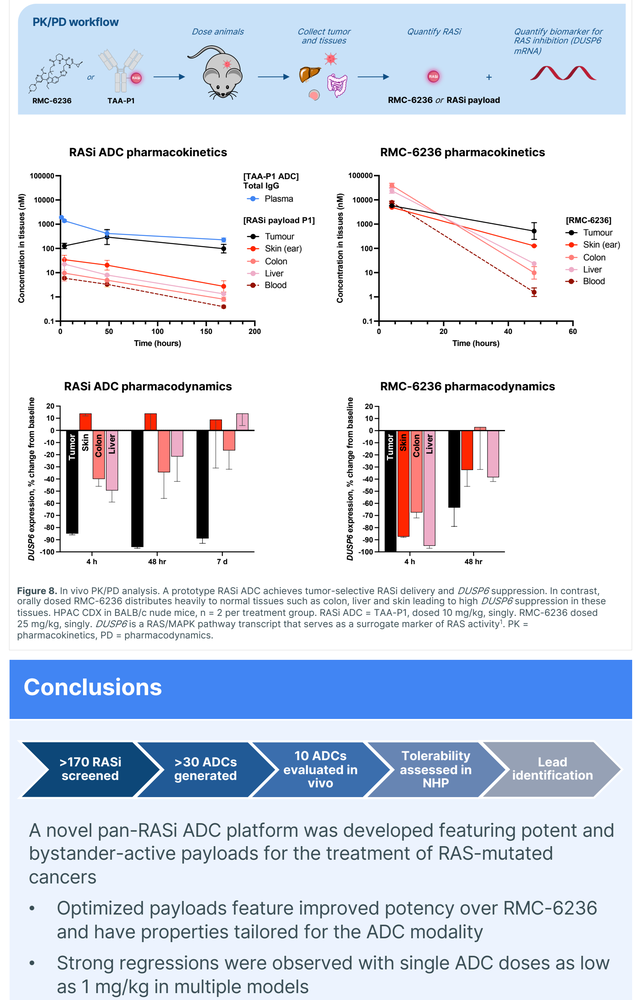


Figure 8. In vivo PK/PD analysis. A prototype RASi ADC achieves tumor-selective RASi delivery and *DUSP6* suppression. In contrast, orally dosed RMC-6236 distributes heavily to normal tissues such as colon, liver and skin leading to high *DUSP6* suppression in these tissues. HPAC CDX in BALB/c nude mice, n = 2 per treatment group. RASi ADC = TAA-P1, dosed 10 mg/kg, singly. RMC-6236 dosed 25 mg/kg, singly. *DUSP6* is a RAS/MAPK pathway transcript that serves as a surrogate marker of RAS activity[1]. PK = pharmacokinetics, PD = pharmacodynamics.

Conclusions

>170 RASi screened → >30 ADCs generated → 10 ADCs evaluated in vivo → Tolerability assessed in NHP → Lead identification

A novel pan-RASi ADC platform was developed featuring potent and bystander-active payloads for the treatment of RAS-mutated cancers

- Optimized payloads feature improved potency over RMC-6236 and have properties tailored for the ADC modality
- Strong regressions were observed with single ADC doses as low as 1 mg/kg in multiple models
- PK/PD studies illustrate high tumor vs normal tissue selectivity of ADC as compared to orally administered RMC-6236
- Excellent tolerability evident in both rodents (MTD ≥200 mg/kg) and non-human primates (MTD ≥120 mg/kg)

The highly encouraging efficacy, PK and nonclinical safety data, support doses and drug exposures that are expected to provide clinical benefit in RAS-mutant cancers such as NSCLC, PDAC, and CRC.

References
1. J. Jiang et al. *Cancer Discov.* **2024**, *14*, 994-1017
2. J. Cregg et al. *J. Med. Chem.* **2025**, *68*, 6064-6083

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