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VLS-101 is a Novel Therapeutic Antibody-Drug Conjugate (ADC) Targeting Receptor Tyrosine Kinase-Like Orphan Receptor 1 (ROR1) in Richter Syndrome (RS)

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BACKGROUND AND AIMS

- ROR1 is a transmembrane receptor with tightly controlled expression during development. It is present on multiple tumor types but not on normal adult tissues. Hematological malignancies are often ROR1-positive (ROR1+), including chronic lymphocytic leukemia (CLL) and diffuse large B-cell lymphoma (DLBCL) (Daneshmanesh, AH et al., *Leuk Lymphoma.*54:843, 2013).
- Richter syndrome (RS) is an aggressive lymphoma, typically of DLBCL type, arising as a transformation of CLL. Despite progressive improvements in the therapy of CLL, very few effective treatment options exist for patients with RS.
- Given its unique pattern of expression, ROR1 represents a tumor-specific therapeutic target. The anti-ROR1 antibody, UC-961, is a humanized IgG1 monoclonal antibody (mAb) that binds with high affinity to a specific extracellular epitope of human ROR1 receptor and can block Wnt5a-induced ROR1 signaling (Hasan, K et al., *Blood* 132: 170, 2018). Nonclinical studies document that UC-961 does not react with normal adult human tissues and selectively binds to tumor cells. Because of its high specificity, rapid internalization, and trafficking to lysosomes, UC-961 appears ideally suited to serve as the targeting moiety for an anti-ROR1 antibody-drug conjugate (ADC) (Mian, Y et al., *Blood* 132:1862, 2018).
- Using our recently established RS patient-derived xenografts (RS-PDXs), we explored the expression and signaling properties of ROR1 in RS and investigated the *ex vivo* and *in vivo* effects of targeting ROR1 with UC-961 and the ADC, VLS-101.

Main characteristics of the available RS-PDXs

- Four different RS-PDX models were used for these studies: RS9737 and RS1316 were previously genetically characterized and reported, while RS1050 and IP867/17 were more recently established (Vaisitti, T et al., *Cancer Research*. 78:3413, 2018).
- When evaluating immunoglobulin heavy chain (IgVH) rearrangements, RS9737 and RS1316 are characterized by a BCR-unmutated profile with IgVH3-7 and IgVH3-21 usage, while RS1050 and IP867/17 present mutated sequences, IgVH4-34 and IgVH3-49, respectively, all consistent with the primary samples (Table 1).
- Genetic characterization indicates these RS-PDXs have heterogeneous profiles carrying different mutations (Table 2).
- The RS-PDXs were obtained from patients who underwent extensive prior therapy and several (RS9737, RS1050, and RS1316) were known to be resistant to conventional chemotherapy.

Table 1. IgVH Rearrangements in RS-PDX Models

Model ID	IgVh	% of IgVh mutations
IP867/17 Primary	IGHV3-49*04; IGHJ6*02	2.7
IP867/17 PDX	IGHV3-49*04; IGHJ6*02	2.7
RS1316 Primary	IGHV3-21*01; IGHJ3*02	0
RS1316 PDX	IGHV3-21*01; IGHJ3*02	0
RS1050 Primary	IGHV4-34*02; IGHJ4*02	2.1
RS1050 PDX	IGHV4-34*02; IGHJ4*02	2.1
RS9737 Primary	IGHV3-7*01; IGHJ4*02	1.0
RS9737 PDX	IGHV3-7*01; IGHJ4*02	1.0

Table 2. Genetic Characterization of RS-PDX Models

Gene ID and mutation	IP867/17	RS1316	RS1050	RS9737
TP53 (c.673-2A>G; SAV)				
TP53 (c.254delC; fs)				
TP53 (p.H214fs)				
NOTCH1 (p.2514fs4)				
NOTCH2 (p.N1516S)				
BTK (p.E96G)				
BTK (p.C481S)				
MYC (p.P60T)				
PIK3C2G (c.3263delA; fs)				
KRAS (p.G13C)				
KRAS (p.L19F)				
KRAS (p.T58I)				
EGR2 (p.H384N)				
EGR2 (p.D411Y)				
SETD2 (p.G889V)				
TBL1XR1 (p.H127p)				
TRAF3 (c.1230_1231delGA; fs)				
TRAF3 (p.T411S)				
MED12 (p.G44R)				
TBL1XR1 (p.H127p)				
ERBB3 (p.V606I)				
CYLD (p.L475F)				
SMAD5 (p.S351T)				
PTPRK (p.W1055L)				
PAX5 (p.K196M)				
VCAM1 (p.P254A)				
STAT3 (p.K283T)				
NFKBIZ (p.L399P)				

Legend

0 < VAF ≤ 0.30
0.31 ≤ VAF ≤ 0.60
0.61 ≤ VAF ≤ 1.00

VAF=variant allele fraction

RESULTS

Figure 1. ROR1 is Expressed at Variable Levels in RS

- ROR1 is expressed at variable levels in RS-PDXs as inferred by flow cytometry analysis (Figure 1A); IP867/17 and RS1316 show the highest surface expression, RS1050 is characterized by lower levels of ROR1, and RS9737 shows almost no expression.
- Expression is confirmed by IHC staining on RS-PDX tumor masses, varying from universal (IP867/17) to absent (RS9737) (Figure 1B).

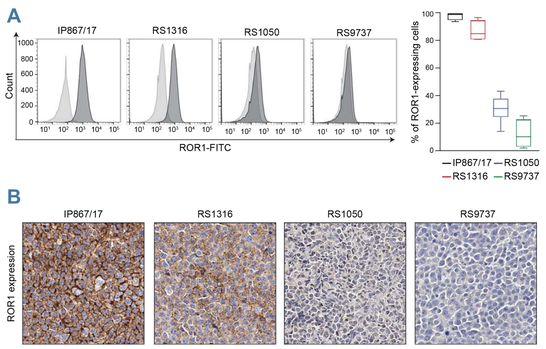


Figure 2. ROR1 Pathway is not Active in RS but Represents a Good ADC Target

- Binding of Wnt5a to ROR1 and downstream activation of the GTP/GDP exchange factors, Rac1 and RhoA, was evaluated in ROR1+ RS-PDXs to determine ROR1 signaling capability in RS (Figure 2A). The addition of Wnt5a does not result in activation of the pathway in RS cells.
- Targeting of ROR1 in RS cells by the UC-961 antibody, known to efficiently inhibit ROR1 signaling in CLL, does not affect RS-PDX growth. In an *in vivo* sub-cutaneous model of RS-PDX, UC-961 is unable to block tumor proliferation; there is a complete overlap with vehicle-treated mice in terms of growth kinetics (Figure 2B).

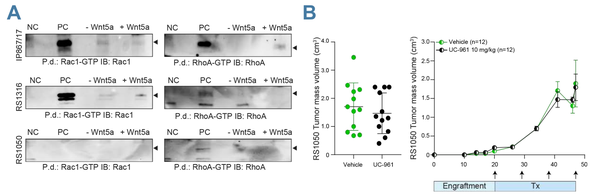
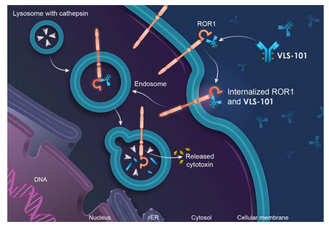


Figure 3. VLS-101 is an ADC that Targets ROR1 to Deliver MMAE



- VLS-101 comprises UC-961, a proteolytically cleavable linker, and the anti-microtubule cytotoxic agent monomethyl auristatin E (MMAE).
- The primary mechanism of action of VLS-101 is targeted delivery of a cytotoxic agent into the cancer cell through the ROR1 receptor.

Figure 4. VLS-101 Shows *Ex Vivo* Efficacy in RS Cells

- Exposure of ROR1+ RS cells to VLS-101 induces apoptosis in a dose- and time-dependent manner. No statistically significant difference is evident at 24h, but the drug shows efficacy by 48h, and is maximal at 72h (Figure 4A).
- These results are confirmed at the biochemical level, as shown by the cleavage of Caspase-3 and PARP in all ROR1+ models analyzed, with a more pronounced effect at 48h of treatment and at the highest dose (Figure 4B).

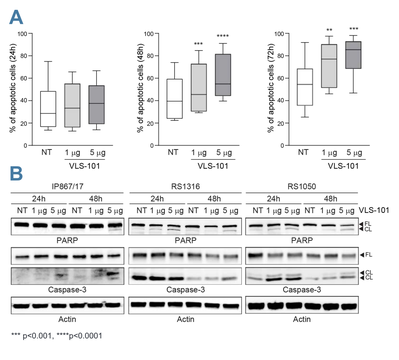


Figure 5. VLS-101 Inhibits Tumor Growth and Prolongs Survival in Subcutaneous Models of ROR1+ RS-PDXs

- Treatment of mice with palpable tumor masses induces rapid and complete tumor regression by the end of therapy in all 3 ROR1+ models by the end of therapy at the 5 mg/kg dose, with a significant and prolonged therapeutic response. The lower dose induces a significant response, but signs of tumor regrowth are observed ~30 days after discontinuing treatment (Figure 5A-C).
- Notable is that complete regressions are observed in RS1316 and RS1050 models, even though these PDXs are not universally ROR1+ (Figure 5A-C).
- In contrast, in the ROR1- RS9737 model, VLS-101 efficacy is not observed; tumor masses in VLS-101-treated animals grow at a similar rate as those in vehicle-treated mice, with no differences in terms of tumor growth kinetics or survival (Figure 5D).
- Animals showed no adverse changes in body weight at either VLS-101 dose level (data not shown).

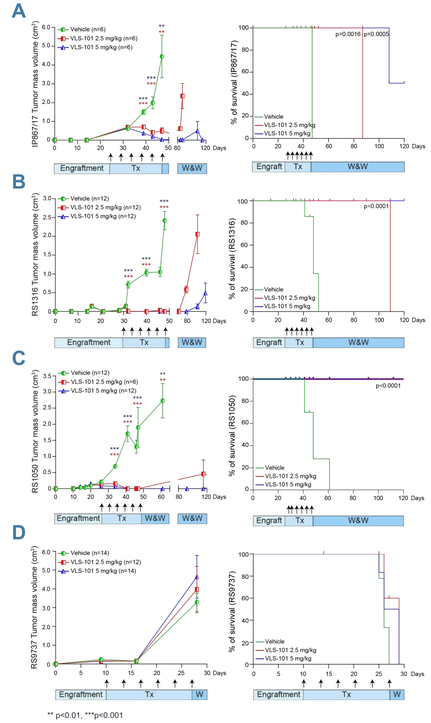
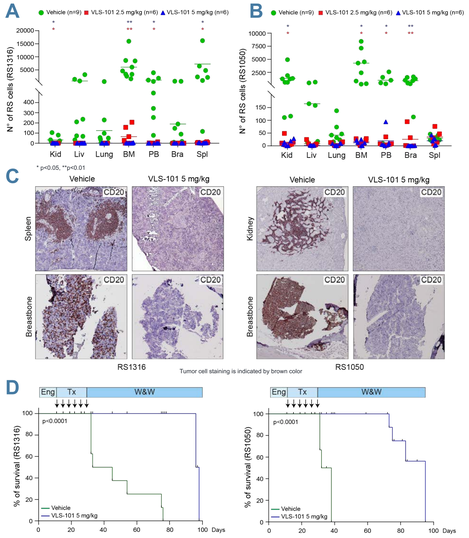


Figure 6. VLS-101 Inhibits Tumor Growth and Prolongs Survival in Systemic RS-PDX models

- When evaluated by flow cytometry and by IHC in systemic models of ROR1+ RS, VLS-101-treated mice show a dramatic decrease, to almost undetectable levels, of tumor cells in all compartments compared to vehicle-treated mice, independent of the dose level administered (Figure 6A-C).
- Kaplan-Meier curves indicate that VLS-101 significantly extends life; ADC-treated groups have median survival values that are >50 days longer than those in the vehicle-treated groups (Figure 6D).



CONCLUSIONS

- The oncofetal transmembrane ROR1 receptor can be expressed on aggressive, chemotherapy-resistant, RS lymphomas, making it an attractive target for therapy with anti-ROR1 antibodies or ADCs.
- In RS cells, ROR1 does not appear to be functionally active; its ligand, Wnt5a, does not enhance ROR1-mediated signaling and the naked ROR1-targeting antibody, UC-961, does not inhibit RS-PDX tumor growth *in vivo*.
- However, VLS-101, a ROR1-targeting ADC (the UC-961 antibody linked to the cytotoxin, MMAE) is highly active in ROR1+ RS-PDXs, even in models with only partial ROR1 expression:
  - Induces dose- and time-dependent apoptosis
  - Causes complete tumor regressions and prolongs survival in ROR1+ subcutaneous and systemic RS-PDX models
- VLS-101 is well tolerated, causing no animal weight loss.
- A Phase 1 clinical trial of VLS-101 (NCT03833180) is ongoing in patients with lymphoid cancers.

DISCLOSURES

- This study and poster are sponsored by VelosBio Inc.
- KJ, TTL, MK, LLM, and BJL are employees of VelosBio Inc.

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