828

ANS03, a novel, orally bioavailable small-molecule type II ROS1/NTRK inhibitor, effectively overcomes clinically relevant ROS1/NTRK resistance mutations and exhibits potent antitumor activity in preclinical tumor models

Gong Li[1], Alexander Drilon[2], Shengxiang Ren[3], Peilong Zhang[1], Xiangqiu Li[1], Linna Li[1], Ruiya Zhang[1], Wenli Lan[1], Di Han[1], Weizhe Xue[1], Peipei Yang[1], Shutan Liao[1], Kevin Schaab[4], Brendan Putz[4], Hepeng Shi[1]

[1]Avistone Biotechnology Ltd., Beijing, China; [2]Memorial Sloan Kettering Cancer Center and Weill Cornell Medical College, NY, NY USA; [3]Shanghai Pulmonary Hospital, Shanghai, China; [4]Avistone Biotechnology Inc., USA

INTRODUCTION

***ROS1* and *NTRK* fusions are validated oncogenic drivers across multiple tumor types, yet the clinical utility of current Type I tyrosine kinase inhibitors (TKIs) is limited by acquired resistance mutations, including:**

- *ROS1* solvent front (SF) mutations → steric hindrance blocking drug binding
- *ROS1* Cβ6 mutation → structural rearrangement destabilizing Type I TKI interactions
- *NTRK* xDFG mutations → disruption of ATP pocket geometry

These mutations drive >60% of relapses, demanding next-generation Type II TKIs capable of:

- ✓ Targeting the "DFG-out" kinase conformation to evade steric hindrance
- ✓ Engaging both ATP-binding and allosteric pockets for broad mutation coverage

Table 1 Comparison of Type I vs Type II TKIs targeted ROS1/NTRK

Feature	Type I TKIs	Type II TKIs
Binding Conformation	active "DFG-in" conformation	inactive "DFG-out" conformation
Binding Site	ATP-binding pocket only	ATP pocket + adjacent allosteric pocket
Resistance Coverage	SF and GK mutations	GK, SF, Cβ6, xDFG and compound mutations

OBJECTIVES

To develop an effective type II ROS1/NTRK inhibitor to treat patients with locally advanced or metastatic solid tumors harboring ROS1 /NTRK alterations, especially those with ROS1/NTRK-resistant mutations against currently approved type I ROS1/NTRK TKIs, thereby addressing a clear unmet medical need.

RESULTS

In vitro pharmacology

ANS03 broadly and potently inhibits a spectrum of ROS1/TRK WT/Mt proteins

Table 2 *In Vitro* Kinases Inhibitory Activity of ANS03

Kinases	Mutations	IC_{50} (nM, Mean±SD)	
		ANS03 (Type II)	Repotrectinib (Type I)
ROS1	WT	0.846±0.308	0.274±0.180
ROS1 G2032R	SF	1.61±0.221	2.32±1.19
ROS1 L2086F	**Cβ6**	**3.35±0.64**	**2387±748**
TRKA	WT	1.10±0.045	0.158±0.001
TRKB	WT	0.733±0.049	0.261±0.002
TRKC	WT	1.60±0.390	0.916±0.288
TRKA G595R	SF	4.88±1.46	0.594±0.069
TRKA G667C	xDFG	0.324±0.045	1.11±0.675

Table 3 *In Vitro* Anti-Tumor Activity of ANS03 in Ba/F3 Cell Lines

Targets	Mutation types	Ba/F3 Cell lines	IC_{50} (nM, Mean±SD)		IC_{50} ratio (Repotrectinib / ANS03)
			ANS03 (Type II)	Repotrectinib (Type I)	
ROS1	WT fusion	CD74-ROS1	0.975±0.206	1.126±0.008	1.2
	SF Mutation	**CD74-ROS1-G2032R**	**2.740±0.820**	**60.710±12.148**	**22.2**
		CD74-ROS1-D2033N	**0.192±0.018**	**5.869±0.723**	**30.6**
	Cβ6 mutation	**CD74-ROS1-L1982W-L2086F**	**0.123±0.008**	**>1000**	**>8130**
NTRK	WT fusion	LMNA-NTRK1	0.832±0.164	1.534±0.334	1.8
		TEL-NTRK2	1.343±0.094	1.412±0.213	1.1
		TEL-NTRK3	3.971±0.057	3.061±0.094	0.8
	GK mutation	LMNA-NTRK1-F589L	1.691±0.180	2.403±0.720	1.4
	SF mutation	LMNA-NTRK1-G595R	4.778±0.288	13.195±0.134	2.8
	xDFG mutation	**LMNA-NTRK1-G667C**	**0.998±0.022**	**52.395±5.523**	**52.5**
	Compound mutation	**LMNA-NTRK1-G595R-G667C**	**9.576±1.109**	**>1000**	**>104.4**
		LMNA-NTRK1-G595R-F589L	26.045±1.365	98.155±18.166	3.8

Table 4 *In Vitro* Anti-Tumor Activity of ANS03 in a Ba/F3-CD74-ROS1-L1982W-L2086F (Cβ6 mutation) mutant cell line

Compound	TKI types	IC_{50} (nM)	IC_{50} ratio (compared to ANS03)
ANS03	**Type II**	**0.1**	**1**
Foretinib	Type II	1.6	16
Cabozantinib	Type II	4.1	41
Repotrectinib	Type I	~1005.0	~10050
NVL-520	Type I	>10000.0	>100000
Crizotinib	Type I	~964.5	~9645
Entrectinib	Type I	1493.0	14930
Lorlatinb	Type I	>10000.0	>100000
Ceritinib	Type I	1302.0	13020

Table 5 Next-Generation Type II ROS1/NTRK Inhibitor ANS03 Activity Against Other Tumor-Related Kinases *in vitro*

Top 5 kinases most sensitive to ANS03				
		ANS03 IC_{50} (nM)	Reference	IC_{50} (nM)
1	AXL	1.436	Cabozantinib	4.556
2	TYRO3	1.907	BMS777607	1.749
3	**MET**	**2.684**	**Cabozantinib**	**1.859**
4	RON	2.858	Staurosporine	259.9
5	MER	2.86	Sitravatinib	2.536

Type II ROS1/NTRK -TKIs are multitargeted inhibitors that bind the inactive state of the ROS1/TRK (DFG-out) in the ATP pocket.

Inhibitory activity ($IC_{50's}$) for the top 5 inhibited kinases were determined and are shown in Table 5. The results indicate that ANS03 is comparable in wild-type c-Met potency to Cabozantinib. Importantly, ANS03 did not substantially inhibit VEGFR2 (IC_{50} >100 nM) unlike Cabozantinib (VEGFR2 IC_{50}= ~1nM).

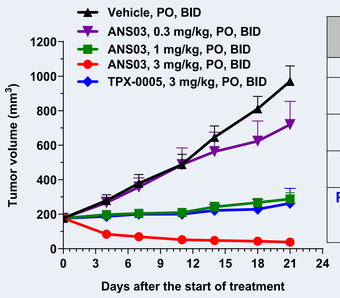
In vivo PD-PK Studies

The *in vivo* antitumor effects of ANS03 were evaluated using subcutaneously and intracranially transplanted tumor models in Balb/c nude mice.

Figure 1: NSCLC patient-derived xenograft model demonstrates the superior efficacy of ANS03 in a ROS1 fusion-positive model.

NSCLC PDX LU-01-0414 (ROS1 fusion) subcutaneous tumor model

ANS03 showed a dose-dependent inhibitory effect and 3 mg/kg of ANS03 was sufficient to induce tumor regression.



Group	Tumor volume (mm^3) at Day 21	TGI (%)
Vehicle	970 ± 89	--
ANS03 (0.3 mg/kg)	719 ± 135	31.59
ANS03 (1 mg/kg)	289 ± 36	85.87
ANS03 (3 mg/kg)	39 ± 15	117.43
Repotrectinib (TPX-0005) (3 mg/kg)	264 ± 86	89.07

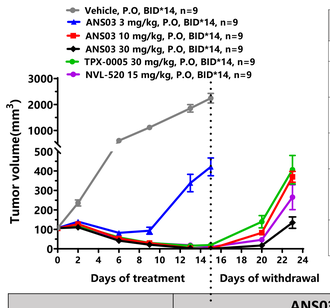
TGI(%) = (1-ΔT/ΔC) × 100, ΔT/ΔC (%) = 100 × (TV treated final-TV treated initial)/ (TV Vehicle final- TV Vehicle initial)

PK parameters	ANS03			TPX-0005
	0.3 mg/kg	1 mg/kg	3 mg/kg	3 mg/kg
$T_{1/2}$ (h)	1.55	1.95	1.94	3.79
T_{max} (h)	1	1	1	0.5
C_{max} (ng*/mL)	68	220	801	145
AUC_{0-t} (hr*ng/mL)	191	665	2969	588
MRT_{0-t} (h)	2.22	2.45	2.78	3.81

Figure 2: ANS03 demonstrates superior potential to overcome on-target acquired resistance in ROS1.

Ba/F3-CD-ROS1-G2032R (SF Mutation) subcutaneous xenograft tumor model

ANS03 achieved complete tumor regression during treatment. Its post-withdrawal tumor recurrence was delayed relative to Repotrectinib (TPX-0005) and Zidesamtinib (NVL-520) which relapse occurred earlier.



Treatment	Tumor Size (mm^3) at Day 15	TGI (%)
Vehicle	2244±185	-
ANS03 (3mg/kg)	421±46	87
ANS03 (10 mg/kg)	0±0	106
ANS03 (30 mg/kg)	0±0	106
Repotrectinib (TPX-0005) (30 mg/kg)	19±8	105
Zidesamtinib (15 mg/kg)	10±5	105

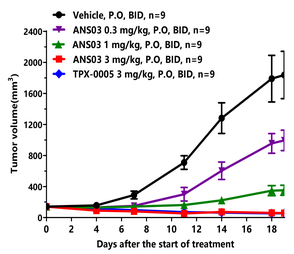
TGI(%) = (1-ΔT/ΔC) × 100, ΔT/ΔC (%) = 100 × (TV treated final-TV treated initial)/ (TV Vehicle final- TV Vehicle initial)

PK parameters	ANS03			TPX-0005	NVL-520
	3 mg/kg	10 mg/kg	30 mg/kg	30 mg/kg	15 mg/kg
$T_{1/2}$ (hr)	1.94	2.47	5.48	1.26	1.65
T_{max} (hr)	1.0	1.0	4.0	0.5	0.5
C_{max} (ng/mL)	1277	5103	7817	5623	7050
AUC_{0-t} (ng*hr/mL)	5460	22288	74670	23449	25639
MRT (hr)	2.99	3.63	5.35	3.14	3.11

Figure 3: ANS03's inhibitory effect on NTRK fusion–positive tumors is comparable to Repotrectinib.

Human colon tumor cell line KM12-Luc (NTRK1 fusion) subcutaneous xenograft tumor model

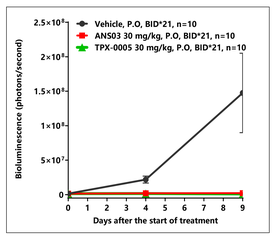
ANS03 showed a dose-dependent inhibitory effect and 3 mg/kg of ANS03 was sufficient to induce tumor regression.



Group	Tumor volume (mm^3)[a]	TGI (%)
Vehicle	1838.45±305.64	--
ANS03 (0.3 mg/kg)	995.24±131.96	50
ANS03 (1 mg/kg)	352.16±64.73	88
ANS03 (3 mg/kg)	58.45±8.84	105
Repotrectinib (TPX-0005) (3 mg/kg)	55.19±6.11	105

TGI(%) = (1-ΔT/ΔC) x 100, ΔT/ΔC (%) = 100 × (TV treated final-TV treated initial)/ (TV Vehicle final- TV Vehicle initial)

KM12-Luc (NTRK1 fusion) Intracranial xenograft tumor model



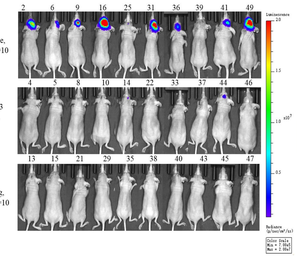
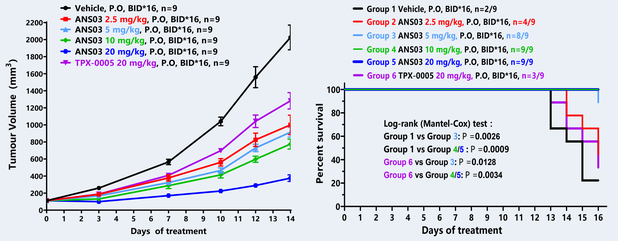


Figure 4: ANS03 effectively overcomes on-target acquired resistance caused by Type I TRK inhibitors

Ba/F3-LMNA-NTRK1-G595R-G667C (NTRK1 compound mutation) subcutaneous xenograft tumor model

ANS03 could effectively inhibit the growth of TRK on-target resistant tumors and significantly improve the survival rate of tumor bearing mice in a dose-dependent manner.



Treatment	Tumor Size (mm^3) at D12	TGI (%)	Number of animals at D0	Number of surviving animals at D16
Vehicle	1559±123	-	9	2
ANS03 (2.5 mg/kg)	825±79	51	9	4
ANS03 (5 mg/kg)	730±44	57	9	8
ANS03 (10 mg/kg)	597±37	66	9	9
ANS03 (20 mg/kg)	290±23	88	9	9
Repotrectinib (TPX-0005, 20 mg/kg)	1042±73	36	9	3

TGI(%) = (1-ΔT/ΔC) x 100, ΔT/ΔC (%) = 100 × (TV treated final-TV treated initial)/ (TV Vehicle final- TV Vehicle initial)

CONCLUSIONS

Non-clinical studies of ANS03 have demonstrated that it is a potent, orally bioavailable Type II ROS1/TRK inhibitor with a groundbreaking preclinical profile:

1. **Broad-Spectrum Resistance Mutation Coverage**
   - Targets ROS1/TRK fusions, acquired mutations (*ROS1*: G2032R, D2033N, L2086F; *NTRK1*: G595R, F589L, G667C), and compound mutations.
   - IC_{50} range: 0.324–4.88 nM (kinase level), 0.1–26.045 nM (cellular level).
2. **Substantial Potency Against Select Resistance Mutations**
   - >10,000× improvement vs. Type I TKIs for ROS1 Cβ6 (L2086F)
   - 52.5× stronger inhibition of TRK1 xDFG (G667C) and >104.4× activity against *NTRK1* compound mutations.
3. **Robust *In Vivo* Efficacy**
   - Achieved complete tumor regression in ROS1-G2032R models at 10 mg/kg BID.
   - Induced tumor regression in *ROS1/NTRK* fusion-positive models at 3 mg/kg BID.
   - Dose-dependent survival improvement in NTRK1-G595R-G667C tumor-bearing mice.
4. **Optimized Pharmacokinetic Profile**
   - Linear dose-exposure correlation with favorable tumor distribution.
   - Demonstrated brain penetration, critical for treating CNS metastases.

ANS03, a novel Type II ROS1/NTRK inhibitor, is advancing into a global Phase 1 clinical trial (NCT06716138) in adult patients with locally advanced or metastatic solid tumors harboring *ROS1* alterations, and in adult and pediatric patients aged 12 and older with *NTRK* alterations.

CONTACT

To contact author: ligong@avistonebio.com
Make general inquiry to Avistone: contact@avistonebio.com
Learn collaboration opportunities: BD@avistonebio.com

Scan to visit website