Abstract #2559

FORTITUDE: Results of a phase 1a study of the novel transgene-armed and tumor-selective vector NG-350A with and without pembrolizumab

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Background

- Tumor-Specific Immuno Gene [T-SIGn] vectors are transgene-armed variants of the epithelial tumor-selective adenovirus enadenotucirev
  - T-SIGn vectors are designed to re-programme immunosuppressive tumors by selectively expressing immunostimulatory payloads within tumor microenvironments
  - The vector is blood stable and has low immunogenicity, allowing successful intravenous (IV) delivery to primary and metastatic tumors sites[1]
  - Vector persistence in resections taken up to ~50 days post dosing has been observed following a single cycle of dosing[1]
- NG-350A is a T-SIGn vector armed with a fully human IgG agonistic anti-CD40 Ab to drive tumor-specific T cell responses
  - Anti-CD40 agonists are a promising strategy to drive functional anti-cancer immune responses;[2] however, efficacy has been limited at tolerable dose levels, with on-target immune-related AEs observed[3,4]
- We hypothesized that NG-350A could drive re-programming of immunosuppressive TMEs to 'hot' immune-inflamed environments, without the systemic exposure of standard anti-CD40 dosing
  - This mechanism of action is likely to be complementary to checkpoint inhibitors

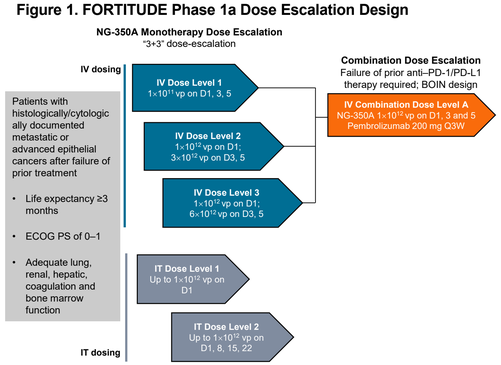
Objectives

- Primary: to assess the safety and tolerability of NG-350A as a monotherapy and with pembrolizumab (pembro)
- Additional: determining a recommended dose of NG-350A ± pembrolizumab and assessing preliminary anti-tumour activity

Methods

- FORTITUDE (NCT03852511) is a phase 1a/1b open-label dose-escalation study of NG-350A with or without pembrolizumab (**Fig 1**)

Figure 1. FORTITUDE Phase 1a Dose Escalation Design



BOIN = Bayesian Optimal Interval; ECOG PS = Eastern Cooperative Oncology Group performance score; IT = intratumoural; IV = intravenous; PD-1 = programmed death 1; PD-L1 = programmed death ligand 1; Q3W = every three weeks; vp = viral particles

- Tumour imaging was performed every 8 weeks and as clinically indicated
  - Images were evaluated using RECIST v1.1
- Frequent blood samples were taken and serum cytokine levels were determined using a 17-analyte multiplex Luminex assay for the following analytes: IL-2, IL-5, IL-6, IL-10, IL-17A, MCP-1, TNF-α, IFNγ, IL-13, IL-15, CXCL9 (MIG), CXCL10 (IP-10), CXCL11 (I-TAC), IFNα2 , MIP1α, IL-8 and IL12p70
  - Comparative cytokine data for enadenotucirev (a structurally identical empty vector) were taken from the EVOLVE trial[5]
- For T cell receptor (TCR) repertoire analyses, genomic DNA was extracted from patient whole blood and TCR beta sequences were determined using the ImmunoSEQ assay (Adaptive Biotech)

Results

Exposure and demographics

- In total, 25 heavily pre-treated patients received NG-350A monotherapy and 3 patients received NG-350A + pembro (**Table 1**)
- The most common cancers were colorectal cancer (n=8, 29%), pancreatic cancer (n=4, 14%), cholangiocarcinoma, oesophageal cancer, and oral and oropharyngeal cancer (all n=3, 11%)

Table 1. Patient demographics

	IT	IV monotherapy			IV + pembro	Total N = 28
	Dose Level 1 and 2 n = 9	Dose Level 1 n = 6	Dose Level 2 n = 4	Dose Level 3 n = 6	Dose Level A n=3	
Median age (range)	54 (36-72)	56 (47-63)	53 (32-75)	55 (50-78)	64 (57-77)	56 (32-78)
Sex (female/male, n)	3 / 6	2 / 4	3 / 1	5 / 1	0 / 3	13 / 15
ECOG PS						
0	2 (22%)	1 (17%)	0	2 (33%)	0	5 (18%)
1	7 (78%)	5 (83%)	4 (100%)	4 (67%)	3 (100%)	23 (82%)
Median prior lines of therapy (range)	3 (2-5)	3 (2-11)	3 (2-5)	3.5 (2-14)	3 (2-8)	3 (2-14)
Median time from initial diagnosis, months (range)	22 (13-47)	53 (21-178)	35 (23-47)	48 (16-88)	29 (19-39)	32 (13-178)

Data are n (%) unless stated otherwise. Dose Levels are shown in Figure 1.

Safety and tolerability

- The MTD of NG-350A given either IT or IV was not reached
  - No dose-limiting toxicities (DLTs) occurred IT Dose Level 1 or 2
  - The only DLT with IV dosing occurred at NG-350A IV Dose Level 1 (blood creatinine phosphokinase increased, Grade 4)
- No DLTs or MTD were observed with combination dose-escalation; however, the study was closed after one cohort to allow dose-escalation to continue in a new study in anti-PD-(L)1 resistant cancer
- The most frequently observed AEs were consistent with acute reactions to infused viral particles (**Table 2**)

Table 2. Safety summary

Number (%) of patients with adverse events	IT NG-350A monotherapy (n=9)	IV NG-350A monotherapy (n=16)	IV NG-350A + pembro (n=3)
Most common TEAEs (any cause)			
aPTT prolongation	6 (67)	5 (31)	2 (67)
Pyrexia	4 (44)	6 (38)	0
Nausea	3 (33)	6 (38)	0
Chills	2 (22)	7 (44)	0
Fatigue	3 (33)	4 (25)	1 (33)
Hypokalaemia	1 (11)	6 (38)	0
Anaemia	3 (33)	3 (33)	1 (33)
Any Grade ≥3 TEAE	4 (44)	6 (38)	2 (67)
TE-SAEs	3 (33)	8 (50)	1 (33)
Pneumonia	0	4 (25)	0
Any treatment-related SAE	1 (11)	3 (19)	1 (33)
DLTs	0	1 (Dose Level 1)	0

aPTT; activated partial thromboplastin time; TEAE=treatment emergent adverse event; TE-SAE=treatment emergent serious adverse event; DLT=dose-limiting toxicity

- The only SAE to occur in more than one patient was pneumonia
- One SAE of CTCAE Grade 2 CRS occurred with IV monotherapy; this case occurred shortly after dosing and appeared not to be associated with transgene expression
- Unlike systemically dosed CD40 agonists, no evidence of raised liver enzymes was seen; hepatobiliary disorder TEAEs occurred in 2 patients (7%), both were Grade 1 and considered unrelated to the study drug
- aPTT prolongation was frequently observed; however, these events appear to be due to induction of transient non-thrombotic antiphospholipid antibodies (APLAs) with no apparent clinical sequelae

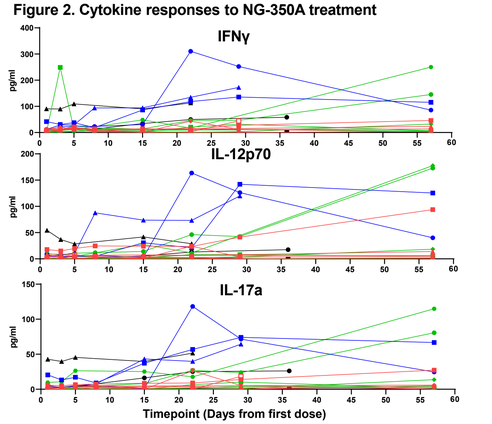
Efficacy

- No objective responses occurred with IV or IT dosing
- Overall, 7/15 evaluable patients treated with IV monotherapy and 2/8 patients evaluable treated with IT monotherapy achieved stable disease
  - This included 3/6 patients treated at the highest dose of NG-350A tested in this protocol (Dose Level 3)
  - This dose was not tested in combination with pembrolizumab, and will be assessed in a newly initiated study
  - At the lower dose of NG-350A tested with pembrolizumab, 1 of 3 patients treated achieved SD
- The durability of SD was not able to be ascertained in most patients, but will be assessed in future studies with extended imaging follow-up

Pharmacodynamics and serum cytokine profile

- Dose-dependent and specific increases in IL-12, IFNγ and IL-17A were detected in serum from evaluable patients (**Fig 2**)

Figure 2. Cytokine responses to NG-350A treatment



- These sustained cytokine increases from ~Day 15 onward were restricted to a subset of inflammatory cytokines (**Table 3**)
  - Elevations were detected at IV Dose Level 2 and beyond

Table 3. Specific cytokine/chemokine responses following NG-350A

Th1 and related cytokines	IL-12	IFNγ	IL-17A	IL-2	TNF-α	
Th2/regulatory cytokines	IL-5	IL-10	IL-13			
Other cytokines	IFNα2	IL-6	IL-15			
Chemokines	CXCL9	CXCL10	CXCL11	MIP1α	MCP-1	IL-8

Cytokines/chemokines that followed a pattern of late increases following NG-350A are shown in blue.

- Similar increases in these cytokines were not observed with IT dosing or with the empty vector (enadenotucirev [EnAd]) that does not express anti-CD40 Ab
- Notably, no anti-CD40 transgene protein was detected in serum samples (limit of detection <1 ng/mL), suggesting a lack of spill over from the TME

TCR repertoire analysis

- TCR repertoire in blood was assessed for 4 patients following NG-350A treatment (IV Dose Level 2 [n=3] and IV Dose Level 3 [n=1])
- Emergence of new T cell clones and expansion of pre-existing clones occurred following NG-350A treatment (**Fig 3** and **Fig 4)**

Figure 3. A high proportion of expanded T cell clones detected following treatment were not present prior to NG-350A treatment

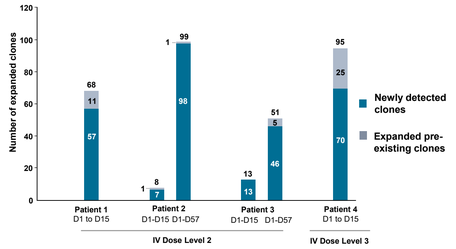
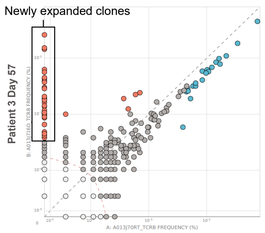


Figure 4. Emergence of new T cell clones following treatment



- Expansion of T cell clones was observed following NG-350A treatment, a high proportion of which were newly detected

Abundance plot for one representative patient treated with NG-350A (IV Dose Level 2). Clones expanding between Day 1 and Day 57 are shown in orange.

Discussion

- In this first-in-human phase 1a trial, NG-350A was well-tolerated with most AEs consistent with reactions to infused viral particles
  - No pattern of toxicity consistent with the side-effects of systemically dosed anti-CD40 Abs were observed
- Stable disease was seen with monotherapy dossing and dose-escalation with pembro continues in a new combination study (FORTIFY) to establish the effect of combination therapy incorporating higher NG-350A doses
- IV dosing of NG-350A led to higher and more sustained elevations in IL-12, IFNγ and IL-17 than are typically observed with systemic anti-CD40 Abs
- Cytokine levels were higher following IV vs IT dosing, suggesting IV dosing leads to more extensive viral replication in the tumour, and higher levels of transgene expression
- NG-350A also led to the expansion of T cell clones in blood
  - The majority of expanded clones were newly detected, and further increases were seen between D15 and D57, consistent with the timing of cytokine elevations

References

1. Garcia-Carbonero R et al. *J Immunother Cancer* 2017;5:71 **2.** Vonderheide RH. *Clinical Cancer Res.* 2007;13:1083–8. **3.** Li DK and Wang W. *Oncol Lett.* 2020;20:176. **4.** Filbert EL et al. *Cancer Immunol Immunother.* 2021;70:1853–65. **5.** Machiels J-P, Salazar R, Rottey S et al. *J Immunother Cancer* 2019;7:20.

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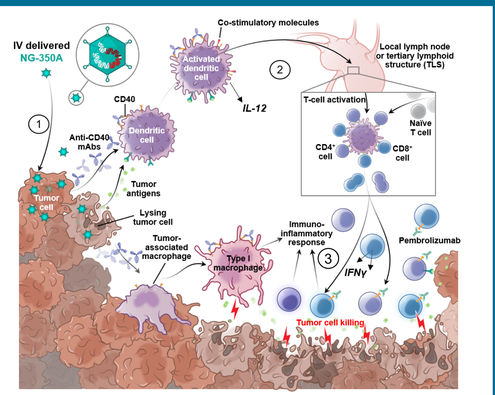
Disclosures

Presenter: Institution receives research funding from PsiOxus Therapeutics Ltd.

- NG-350A is a novel tumor-selective T-SIGn vector that that expresses an agonistic anti-CD40 antibody
- In this phase 1 trial, NG-350A was well-tolerated, with no ≥G3 CRS or liver toxicity
- Stable disease was seen in 3/6 patients at the highest monotherapy IV dose level
  - Although not tested at this dose level yet, efficacy is expected to be enhanced with checkpoint inhibitor combination
- IV delivery of NG-350A drove sustained cytokine elevations without systemic spill over of anti-CD40 transgene from the TME
  - These data suggest innate immune cell stimulation and Th1-/Th17-type T-cell activation, consistent with the MoA of CD40 agonists
  - Expansion of T cell clones, including the emergence of new clones, was observed
- NG-350A may contribute to TME re-programming through localized production of anti-CD40, while avoiding the associated toxicity of systemic dosing
- Dose-escalation of NG-350A plus pembro will continue in a follow-on study to identify a dose level for efficacy assessments (FORTIFY; NCT05165433)

Proposed mechanism of tumour re-programming by NG-350A



1. NG-350A replicates only in tumor cells; anti-CD40 produced in the TME
2. Anti-CD40 activates dendritic cells and macrophages, leading to IL-12 production and anti-tumor CD4+ and CD8+ T cell activation
3. Activated and functional T-cells secrete IFNγ and drive immuno-inflammatory responses and tumor cell killing

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