Presentation #1011P

FORTITUDE – A phase 1 study of NG-350A, a novel tumour-selective adenoviral vector expressing an anti-CD40 agonist antibody: monotherapy dose escalation results

Aung Naing,[1] Lee Rosen,[2] D. Ross Camidge,[3] Danny Khalil,[4] Jessica Davies,[5] David Miles,[5] Minesh Patel,[5] Paul Cockle,[5*] Jenny Lee,[5] Jo Carter,[5] Brian Champion,[5] David Krige,[5] Tom Lillie[5]

[1]The University of Texas MD Anderson Cancer Center, Houston, TX, USA ; [2] University of California, Los Angeles, CA, USA; [3]University of Colorado Denver, Aurora, CO, USA; [4]Memorial Sloan Kettering Cancer Center, New York, NY, USA; [5]PsiOxus Therapeutics Ltd, Abingdon, Oxford, UK.

*Affiliation at the time of study

Background

- Stimulating CD40 has been considered a promising mechanism to drive functional anti-cancer immune responses for over a decade[1]
- Although multiple anti-CD40 antibodies (Abs) have been evaluated, monotherapy efficacy has been limited at tolerable dose levels, with on-target immune-related adverse events (AEs) including cytokine release syndrome (CRS) and liver enzyme elevations observed[2,3]
- NG-350A is a novel transgene 'armed' adenoviral vector (Tumour-Specific Immuno Gene Therapy [T-SIGn]) expressing a fully human IgG agonistic anti-CD40 Ab
  - NG-350A selectively replicates in tumour cells, so the anti-CD40 Ab is selectively expressed in the tumour microenvironment (TME)
  - Unlike many viral vectors, NG-350A is stable in blood allowing intravenous (IV) delivery to multiple tumor sites
- We hypothesized that NG-350A could drive re-programming of immunosuppressive 'cold' TMEs to 'hot' immune-inflamed environments, without the systemic exposure of standard anti-CD40 Ab dosing
- Here we describe safety and translational data for NG-350A monotherapy in a first-in-human phase 1 study (FORTITUDE)

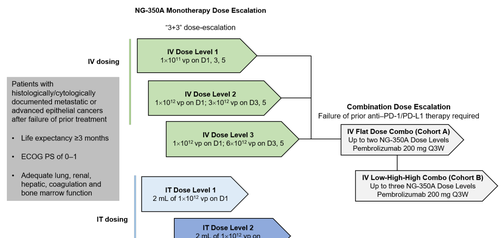
Objective

- To assess the safety and pharmacodynamic effects of NG-350A, including markers of immune activation

Methods

- FORTITUDE (NCT03852511) is a phase 1a/1b open-label dose-escalation study of NG-350A with or without pembrolizumab in patients with metastatic or advanced epithelial tumours
- In phase 1a monotherapy, patients received NG-350A either by intratumoural (IT) or intravenous (IV) delivery, according to the design in **Figure 1**

Figure 1. FORTITUDE Phase 1a Dose Escalation Design



Cohorts not utilized are not shown. 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

- In patients administered NG-350A IV, blood samples were taken for cytokine assessments at D1, 3, 5, 8, 15, 22, 29 and 57
- 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[4]
- 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 – NG-350A monotherapy

Exposure and demographics

- At data cut-off (May 2021), 25 heavily pre-treated patients had received NG-350A monotherapy (IT, n=9; IV, n=16; **Table 1**)
- The most common cancer types were colorectal cancer (n=8, 32%), pancreatic cancer (n=4, 16%), cholangiocarcinoma (n=3, 12%), and oral and oropharyngeal cancer (n=3, 12%)

Table 1. Patient demographics

	IT Dosing		IV Dosing			Total
	Dose Level 1 n = 6	Dose Level 2 n = 3	Dose Level 1 n = 6	Dose Level 2 n = 4	Dose Level 3 n = 6	N = 25
Median age (range)	58 (36-69)	54 (51-72)	56 (47-63)	53 (32-75)	55 (50-78)	55 (32-78)
Female sex	3 (50%)	0	2 (33%)	3 (75%)	5 (83%)	13 (52%)
ECOG PS						
0	2 (33%)	0	1 (17%)	0	2 (33%)	5 (20%)
1	4 (67%)	3 (100%)	5 (83%)	4 (100%)	4 (67%)	20 (80%)
Median prior lines of therapy (range)	3.5 (2-5)	3 (2-4)	3 (2-11)	3 (2-5)	3.5 (2-14)	3 (2-14)
Median time from initial diagnosis, months (range)	22 (13-30)	37 (17-47)	53 (21-178)	35 (23-47)	48 (16-88)	34 (13-178)

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

Safety and tolerability

- No dose-limiting toxicities (DLTs) occurred at either IT Dose Level
- One DLT (blood creatinine phosphokinase increased, Grade 4) occurred at NG-350A IV Dose Level 1
- No DLTs occurred at IV Dose Level 2 or Dose Level 3, both of which utilized "low-high-high" dosing regimens (fixed low Day 1 dose)
  - IV Dose Level 3 was expanded to 6 patients to generate further data
- Overall, the most frequent treatment-emergent AEs (TEAEs) were pyrexia, chills, nausea and prolonged activated partial thromboplastin time (aPTT) (**Table 2**)
  - Prolonged aPTT was not associated with clinical consequences and appears to be driven by interference in laboratory assays by an acquired non-specific lupus-like anticoagulant following viral infection
- The only SAE to occur in >1 patient was pneumonia; no Grade 5 AEs occurred
  - The three cases of pneumonia observed occurred in patients with existing lung metastases

Table 2. Most frequent treatment-emergent AEs and serious AEs

	IT administration	IV administration	Total N = 25	
	n=9	n=16	Any grade	Grade 3–4
Patients with ≥1 AE	9 (100%)	16 (100%)	25 (100%)	10 (40%)
AEs occurring in >20% of patients				
Pyrexia	4 (44%)	7 (44%)	11 (44%)	0
Chills	2 (22%)	8 (50%)	10 (40%)	0
Nausea	3 (33%)	6 (38%)	9 (36%)	0
Prolonged aPTT	5 (56%)	4 (25%)	9 (36%)	3 (12%)
Fatigue	3 (33%)	4 (25%)	7 (28%)	1 (4%)
Hypokalaemia	1 (11%)	6 (38%)	7 (28%)	0
Patients with ≥1 SAE	3 (33%)	8 (50%)	11 (44%)	5 (20%)
SAEs occurring in >1 patient				
Pneumonia	0	3 (19%)	3 (12%)	2 (8%)

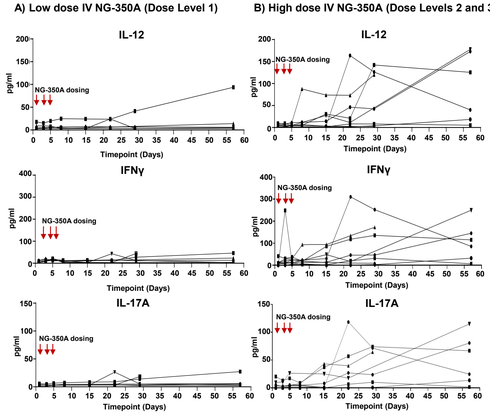
Data are n (%) patients. Data shown for any-cause treatment-emergent AEs (in >20% of patients) and SAEs (occurring in >1 patient). aPTT = activated partial thromboplastin time.

- CRS occurred in one patient (Grade 2)
- No evidence of raised liver enzymes was observed; hepatobiliary disorder TEAEs occurred in 2 patients (8%), both were Grade 1 and considered unrelated to the study drug

Pharmacodynamics and serum cytokine profile

- Dose-dependent increases in IL-12, IFNγ and IL-17A were detected in serum from evaluable patients (**Figure 2**)
  - In the majority of patients treated at IV Dose Level 2 and 3, IL-12, IFNγ and IL-17A levels remained at ≥40pg/ml and 5x baseline levels at 7 weeks after dosing

Figure 2. Prolonged increases in Th1 and related cytokines with NG-350A



Data from evaluable patients with available matching samples. Two patients were excluded (one withdrew at D8, one received steroids following treatment).

- This pattern of sustained cytokine increases from ~Day 15 onward was restricted to a subset of inflammatory cytokines shown in green in **Table 3**

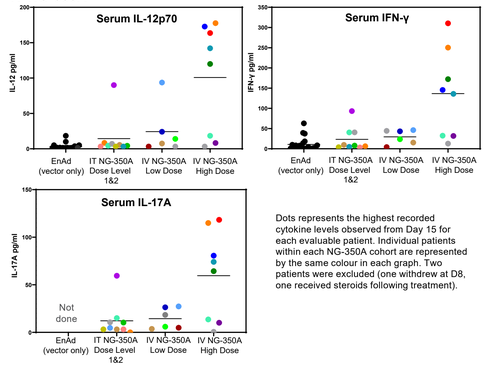
Table 3. Specific cytokine/chemokine responses following NG-350A

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 measured that followed a pattern of late increases following NG-350A are shown in green

- As shown in **Figure 3**, similar increases in these cytokines were not observed with IT dosing or with the empty vector (enadenotucirev [EnAd]) that does not express anti-CD40Ab

Figure 3. Late increases in Th1 and related cytokines are specific to IV NG-350A



Dots represents the highest recorded cytokine levels observed from Day 15 for each evaluable patient. Individual patients within each NG-350A cohort are represented by the same colour in each graph. Two patients were excluded (one withdrew at D8, one received steroids following treatment).

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 occurred following NG-350A treatment (**Figure 4** and **Figure 5)**

Figure 4. A high proportion of expanded T cell clones detected following treatment were not present prior to NG-350A treatment

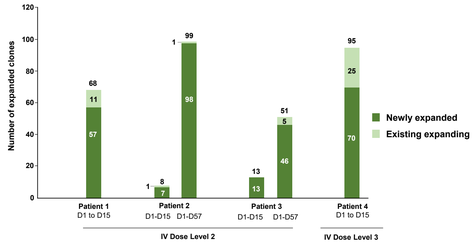
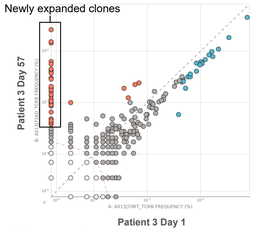


Figure 5. Emergence of new T cell clones following NG-350A treatment



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 phase 1a trial, NG-350A was well-tolerated with most AEs consistent with acute reactions to infused viral particles
  - Few AEs consistent with the known key side-effects of systemically dosed anti-CD40 Abs were observed
- IV dosing of NG-350A led to higher and more sustained elevations in IL-12, IFNγ and IL-17 than typically observed with systemic anti-CD40 Abs
- Cytokine levels were notably higher following IV vs IT dosing, suggesting that IV dosing leads to more extensive viral replication in the tumour, and higher levels of transgene expression
  - The pattern of elevations observed suggest innate immune cell stimulation and Th1/Th17-type T-cell activation, consistent with the mechanism of action of CD40 agonists
- 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
  - Further experiments will assess the specificity of new clones to tumour and viral vector antigens

References

1. Vonderheide RH. *Clinical Cancer Res.* 2007;13:1083–8. **2.** Li DK and Wang W. *Oncol Lett.* 2020;20:176. **3.** Filbert EL, Björck PK, Srivastava MK et al. *Cancer Immunol Immunother.* **4.** Machiels J-P, Salazar R, Rottey S et al. *J Immunother Cancer* 2019;7:20.

Acknowledgments

- This study was sponsored by PsiOxus Therapeutics Ltd. Medical writing support was provided by Matthew Thomas of PsiOxus Therapeutics Ltd.

Disclosures

Dr Aung Naing. Research funding: NCI, EMD Serono, MedImmune, Healios Onc. Nutrition, Atterocor/Millendo, Amplimmune, ARMO BioSciences, Karyopharm Therapeutics, Incyte, Novartis, Regeneron, Merck, Bristol-Myers Squibb, Pfizer, CytomX Therapeutics, Neon Therapeutics, Calithera Biosciences, TopAlliance Biosciences, Eli Lilly, Kymab, PsiOxus, Arcus Biosciences, NeoImmuneTech, ImmuneOncia, and Surface Oncology. Advisory boards for: CytomX Therapeutics, Novartis, Genome & Company, OncoSec KEYNOTE-695, Kymab, and STCube Pharmaceuticals. Travel/expenses: ARMO BioSciences.

- NG-350A is a novel transgene-armed T-SIGn vector that selectively replicates in tumour cells and expresses an agonistic anti-CD40 antibody
- In a phase 1 clinical trial, IV delivered NG-350A monotherapy drove sustained elevations in IL-12, IFNγ and IL-17
  - The elevations observed suggest innate immune cell stimulation and Th1- and Th17-type T-cell activation, consistent with the mechanism of action of CD40 agonists
- Expansion of T cell clones, including the emergence of new clones, was also observed following NG-350A treatment
- NG-350A was well-tolerated in an advanced cancer population, with no Grade ≥3 CRS or liver toxicity
- NG-350A may contribute to TME re-programming through localized production of anti-CD40, while avoiding the associated toxicity of systemic dosing
- Based on these data, NG-350A will now be assessed in combination with pembrolizumab in patients resistant to prior anti–PD-1 therapy

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