Asian Journal of Current Research in Clinical Cancer
ISSN: 3062-4444
2024, Volume 4, Issue 1, Page No: 164-175

Available online at: www.galaxypub.co/page/journals

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Clinical Characterization of NG-350A: A Blood-Stable Oncolytic Adenoviral Vector Encoding a CD40 Agonist

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Received: 12 August 2024; **Revised:** 28 October 2024; **Accepted:** 05 November 2024

ABSTRACT

Oncolytic viruses engineered for tumor-specific replication hold significant promise as cancer therapies, yet achieving adequate dosing and potency in patients with metastatic or advanced solid tumors has proven difficult, thereby restricting broader adoption. NG-350A is an innovative, plasma-stable adenovirus modified to encode a full-length agonist antibody against CD40, without compromising its inherent tumor-selective replication or cytotoxic capabilities. The safety and activity of NG-350A delivered either intravenously or intratumorally (IT) were investigated in a first-in-human phase Ia/Ib trial involving individuals with advanced/metastatic epithelial malignancies (NCT03852511). Separate dose-escalation cohorts were employed: intravenous dosing utilized four escalating levels with infusions scheduled on Days 1, 3, and 5 within a 57-day cycle, whereas IT dosing involved either a single injection on Day 1 or repeated injections on Days 1, 8, 15, and 22. The main goal was to establish safety and tolerability; additional aims included defining a recommended phase 2 dose, characterizing pharmacokinetics, and evaluating anti-vector immune responses. Twenty-five extensively pretreated participants were enrolled and treated (16 intravenous, 9 IT). Both administration routes proved safe and tolerable, showing no signs of toxicity linked to the transgene product or unintended viral replication outside tumors. Peak plasma concentrations (Cmax) of NG-350A rose proportionally with dose irrespective of route. Although neutralizing anti-adenoviral antibodies developed in nearly all patients, circulating vector genomes persisted detectably for up to 7 weeks post-final dose, most prominently at higher intravenous doses. Successful vector delivery into tumor tissue was confirmed by PCR in post-treatment biopsies from both cohorts; intravenous administration exhibited clear dose-dependency, with four individuals still harboring detectable vector DNA on Day 57. Evidence of active viral replication and transgene transcription (detection of transgene mRNA) occurred in 5/12 intravenous and 1/9 IT patients. Dosing triggered prolonged elevation of multiple inflammatory cytokines, with the most pronounced and durable responses seen at higher intravenous dose levels. This early-phase clinical evaluation delivered clear proof-of-mechanism for NG-350A, confirming effective tumor transduction, intra-tumoral replication, and functional transgene expression — especially via the intravenous route. The favorable safety profile, devoid of transgene- or off-target-related adverse events, underscores the vector's stringent tumor selectivity even when administered systemically. Further assessment of intravenously delivered NG-350A is ongoing in combination with pembrolizumab (NCT05165433) and with concurrent chemoradiotherapy (NCT06459869).

Keywords: NG-350A, Blood-Stable, Oncolytic, Adenoviral vector, D40 Agonist

How to Cite This Article: Li KD, Singh RY, Rossi L, Martinez M. Clinical Characterization of NG-350A: A Blood-Stable Oncolytic Adenoviral Vector Encoding a CD40 Agonist. Asian J Curr Res Clin Cancer. 2024;4(1):164-75. https://doi.org/10.51847/FzCv8ONl6v

Introduction

Viral vectors have been investigated as anticancer agents owing to their capacity for tumor-specific oncolytic effects. Traditionally, efforts have centered on intratumoral (IT) injection to ensure direct viral delivery to tumor sites while minimizing host antiviral immunity [1]. While IT administration can enhance the therapeutic window of immunotherapies, it presents significant practical and procedural difficulties. Moreover, since IT injection is restricted to accessible and injectable lesions, broader systemic antitumor responses often rely on abscopal phenomena, which can constrain overall effectiveness [1]. To date, among virally based therapies given

intratumorally, only talimogene laherparepvec has received approval from the US Food and Drug Administration (for unresectable melanoma) [2, 3].

The ability to address these constraints has been demonstrated with viral vectors suitable for intravenous delivery that maintain tumor selectivity [4]. This strategy becomes especially compelling when oncolytic potency is paired with targeted transgene-mediated immunostimulation across all diseased sites.

Tumor-specific immuno gene (T-SIGn) therapeutics represent an advanced class of tumor-targeted viral vectors built on enadenotucirev, a non-armed, blood-stable chimeric group B adenovirus (Ad11p/Ad3) engineered through directed evolution for selective replication in primary and metastatic epithelial carcinomas, resulting in strong cell killing after systemic administration [5]. Multiple phase 1 trials of enadenotucirev revealed favorable viral pharmacokinetics, tumor trafficking, and safety profiles [4, 6, 7], paving the way for the creation of the T-SIGn vector NG-350A. This vector incorporates a full-length encoding sequence for an agonist anti-CD40 antibody placed under control of the enadenotucirev major late promoter. Such positioning links antibody production directly to viral replication (confining transgene expression to tumor cells actively supporting NG-350A propagation), thereby merging enadenotucirev's tumor-restricted oncolysis with localized production of a powerful immunostimulatory monoclonal antibody inside the tumor microenvironment. The inherent tumor-homing property of NG-350A, together with its CD40-agonist transgene, could enable localized multimodality therapy and selective remodeling of the tumor milieu. This design fulfills a primary goal of IT approaches (namely, attaining elevated intratumoral concentrations of active agent) without incurring systemic toxicity risks [1] while simultaneously addressing all tumor deposits for potentially superior outcomes. Furthermore, the ease of intravenous dosing facilitates repeated administration.

Here, we report findings from the first-in-human trial of NG-350A. The primary aims were to evaluate safety and tolerability, and to compare the effects of IT versus intravenous routes on vector delivery, persistence, and pharmacodynamic activity. Dose selection was informed by prior experience with the unarmed tumor-selective vector enadenotucirev, where 3×1012 viral particles (vp) was initially identified as the maximum tolerated dose [6]. Efforts to intensify dosing per cycle prompted evaluation of "low-high-high" schedules, confirming the tolerability of 3×1012 and 6×1012 vp on Days 3 and 5 after an initial 1×1012 vp on Day 1 [7].

Materials and Methods

FORTITUDE clinical study design

FORTITUDE was a multicenter, open-label, phase Ia/Ib, non-comparative trial conducted in patients with advanced or metastatic epithelial malignancies (NCT03852511). The trial featured independent arms evaluating monotherapy NG-350A administered either intratumorally or intravenously. Dose escalation proceeded in parallel across both arms using a conventional 3+3 design. A Safety Review Committee assessed data (including dose-limiting toxicities (DLTs)) after each cohort and made all escalation decisions. Following the 57-day treatment phase, patients underwent imaging follow-up until disease progression (PD); thereafter, survival and subsequent treatments were monitored.

Dosing regimen

The volume and dose for IT injection varied according to the dimensions of the target lesion(s). Injections involved multiple needle passes spaced roughly 0.5–1.0 cm apart, delivering 100–200 µL per pass. Two IT dose levels were implemented: patients at IT Dose Level 1 received a single injection on Day 1, while those at IT Dose Level 2 were injected on Days 1, 8, 15, and 22. Each eligible lesion was treated individually, with a cumulative maximum total dose (summed across all lesions) of 1×1012 vp for both levels.

Intravenous dosing

The intravenous monotherapy escalation included four planned dose levels (with provision for a lower de-escalation level), each comprising three infusions given within the initial 9 days (on Days 1, 3, and 5). The initial level (intravenous Dose Level 1) consisted of 1×1011 vp on Days 1, 3, and 5, selected based on enadenotucirev data [6]. Higher levels employed a "low-high-high" schedule previously validated for better tolerance at doses exceeding 1×1012 vp:[7] 1×1012 vp on Day 1 followed by 3×1012 vp on Days 3 and 5 (Dose Level 2; 1-3−3×1012 vp), 1×1012 vp on Day 1 followed by 6×1012 vp on Days 3 and 5 (Dose Level 3; 1-6−6×1012 vp), and 1×1012 vp on Day 1 followed by 10×1012 vp on Days 3 and 5 (Dose Level 4; 1-10−10×1012 vp).

Participants

Qualified patients presented with advanced or metastatic epithelial malignancies refractory to at least one prior systemic regimen and not curable by locoregional interventions. Other key inclusion requirements were age ≥18 years, Eastern Cooperative Oncology Group performance status 0–1, satisfactory pulmonary capacity (oxygen saturation ≥95% on room air at sea level), and preserved liver, bone marrow, and clotting function. Renal adequacy was mandated, with criteria of creatinine ≤1.5 mg/dL and estimated glomerular filtration rate ≥60 mL/min/1.73 m^2, plus minimal or no proteinuria (spot albumin:creatinine ratio ≤30 mg/g or 24-hour urine protein <1 g/24 hours). Individuals with substantial immunodeficiency, severe renal, autoimmune, or pulmonary disorders (including lymphangitic carcinomatosis), or elevated hemorrhage predisposition were excluded.

Objectives, endpoints and assessments

Primary objective

The chief aim was to profile the safety and tolerability profile of NG-350A, both alone and combined with pembrolizumab, in individuals bearing advanced or metastatic epithelial neoplasms.

Monitoring encompassed rates of adverse events (AEs), serious adverse events (SAEs), dose-limiting toxicities (DLTs), and AEs prompting withdrawal or fatal outcomes. Event grading followed National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) version 5.0. DLT evaluation occurred over the first 28 days (applicable to all intravenous levels and IT Dose Level 1) or 35 days (IT Dose Level 2).

Secondary objectives

Principal secondary goals comprised selection of an optimal NG-350A dose for future trials, evaluation of its pharmacokinetic (PK) behavior and immunogenic potential, and initial appraisal of anticancer effects.

PK profiling of NG-350A involved whole blood sampling on dosing days (before and right after infusion), Day 15, and the end-of-study-treatment (EOST) assessment at Day 57. An additional draw at Day 36 applied to IT Dose Level 2 recipients. Genomic viral DNA was detected via quantitative PCR (qPCR) with primers supplied by Eurofins Genomics (Ebersberg, Germany). Serum for antiviral antibody measurement was collected before dosing on Day 1 and subsequently on Days 8, 15, 22, 29, and at EOST. Detection relied on an electrochemiluminescence ligand-binding assay as outlined earlier [4].

Early signals of efficacy—encompassing objective response rate per Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1, progression-free survival (PFS), and overall survival (OS)—were recorded. Imaging occurred pretreatment, at the Day 57 EOST (or sooner if indicated), and every 8 weeks in follow-up.

Exploratory objectives

Supplementary exploratory analyses addressed cytokine/chemokine induction by NG-350A, vector trafficking to tumor sites, environmental shedding, and evidence of biologic engagement, such as transgene transcription.

Cytokine sampling from serum took place on dosing days (pre-dose and 6–8 hours afterward), Days 8, 15, 22, 29, and EOST. Multiplex Luminex technology measured these markers: IL-2, IL-5, IL-6, IL-10, IL-17A, MCP-1, TNF-α, IFN-γ, IL-13, IL-15, CXCL9 (MIG), CXCL10 (IP-10), CXCL11 (I-TAC), IFN-α2, MIP-1α, IL-8, and IL-12p70. Blood levels of NG-350A-encoded CD40 antibody mRNA were quantified in serum at Days 1, 8, 15, 22, 29, and EOST via reverse transcription-quantitative PCR (RT-qPCR) (Eurofins Genomics, Ebersberg, Germany). Vector presence within tumors was investigated through core needle biopsies taken at baseline, Days 15 and 29, and EOST. Shedding specimens (buccal, rectal, urine) were gathered predose, on Days 8, 15, 29, and EOST. NG-350A DNA quantification in biopsies and shedding material used the identical qPCR protocol applied for PK.

Statistical analyses

Findings reported herein are purely descriptive, without predefined sample size estimates or formal statistical testing. The safety population consisted of every patient receiving at least one treatment dose. Efficacy and PK datasets comprised safety population members with assessable pretreatment and post-treatment data. Kaplan-Meier estimation was applied for PFS and OS, with 95% confidence intervals computed by the Brookmeyer-Crowley approach.

Results and Discussion

Patient disposition and demographics

From an initial screening of 48 individuals, 25 proceeded to receive NG-350A as single-agent therapy: 9 assigned to the intratumoral (IT) group and 16 to the intravenous group **(Figure 1).** Primary factors leading to exclusion during screening included voluntary withdrawal of consent (five instances), suboptimal pulmonary capacity (three instances), and compromised kidney function (three instances). Prevalent malignancies comprised colorectal carcinoma (eight cases) and head and neck squamous cell carcinoma (four cases). Patient profiles showed broad consistency across the IT and intravenous groups, matching typical features of a diverse cohort with progressive metastatic illness **(Table 1).** Every participant had undergone previous treatments, yielding a median of three antecedent systemic regimens (ranging from 1–14 in the intravenous group and 2–5 in the IT group). Metastatic involvement was universal at enrollment, with 60% exhibiting at least three distinct metastatic locations.

Table 1. Baseline demographics and disease characteristics

Parameter	IT DL1D1 only(n=6)	IT DL2D1, 8, 15, 22(n=3)	IT total(n=9)	IV DL1 1×10^{11}(n=6)	IV DL2 $1–3\times10^{12}$(n=4)	IV DL3 $1–6\times10^{12}$(n=6)	IV total(n=16)
Age, median years (range)	57.5 (36, 69)	54 (51, 72)	54 (36, 72)	56 (47, 63)	53 (32, 75)	55 (50, 78)	55 (32, 78)
Male sex, n (%)	3 (50.0)	3 (100)	6 (66.7)	4 (66.7)	1 (25.0)	1 (16.7)	6 (37.5)
Race, n (%)							
White	4 (66.7)	1 (33.3)	5 (55.6)	3 (50.0)	4 (100)	5 (83.3)	12 (75.0)
Asian	2 (33.3)	1 (33.3)	3 (33.3)	1 (16.7)	0	1 (16.7)	2 (12.5)
Other	0	1 (33.3)	1 (11.1)	2 (33.3)	0	0	2 (12.5)
Cancer indication, n (%)							
Colorectal cancer (CRC)	3 (50.0)	1 (33.3)	4 (44.4)	0	1 (25.0)	3 (50.0)	4 (25.0)
SCCHN	0	0	0	2 (33.3)	1 (25.0)	1 (16.7)	4 (25.0)
Pancreatic malignancy	0	1 (33.3)	1 (11.1)	1 (16.7)	2 (50.0)	0	3 (18.8)
Esophageal/GEJ carcinoma	0	1 (33.3)	1 (11.1)	1 (16.7)	0	1 (16.7)	2 (12.5)
Cholangiocarcinoma	2 (33.3)	0	2 (22.2)	1 (16.7)	0	0	1 (6.3)
Other*	1 (16.7)	0	1 (11.1)	1 (16.7)	0	1 (16.7)	2 (12.5)
Time since diagnosis, median months (range)	22 (13, 30)	37 (16, 46)	22 (13, 46)	52 (21, 178)	35 (23, 47)	47 (16, 87)	42 (16, 178)
Any prior anticancer treatment, n (%)	6 (100)	3 (100)	9 (100)	6 (100)	4 (100)	6 (100)	16 (100)
1–2 previous lines	2 (33.3)	1 (33.3)	3 (33.3)	3 (50.0)	2 (50.0)	2 (33.3)	7 (43.8)
≥3 previous lines	4 (66.7)	2 (66.7)	6 (66.7)	3 (50.0)	2 (50.0)	4 (66.7)	9 (56.7)
Previous antineoplastic drugs	6 (100)	2 (66.7)	8 (88.9)	6 (100)	4 (100)	6 (100)	16 (100)
Prior radiotherapy exposure	1 (16.7)	2 (66.7)	3 (33.3)	6 (100)	1 (25.0)	3 (50.0)	10 (62.5)
History of cancer-related surgery	4 (66.7)	3 (100)	7 (77.8)	6 (100)	4 (100)	4 (66.7)	14 (87.5)

*Includes breast cancer, cervical cancer, and prostate cancer.

†CRC, colorectal cancer; SCCHN, squamous cell carcinoma of head and neck; GEJ, gastroesophageal junction.

DL1, Dose Level 1; DL2, Dose Level 2; DL3, Dose Level 3; IT, intratumoral.

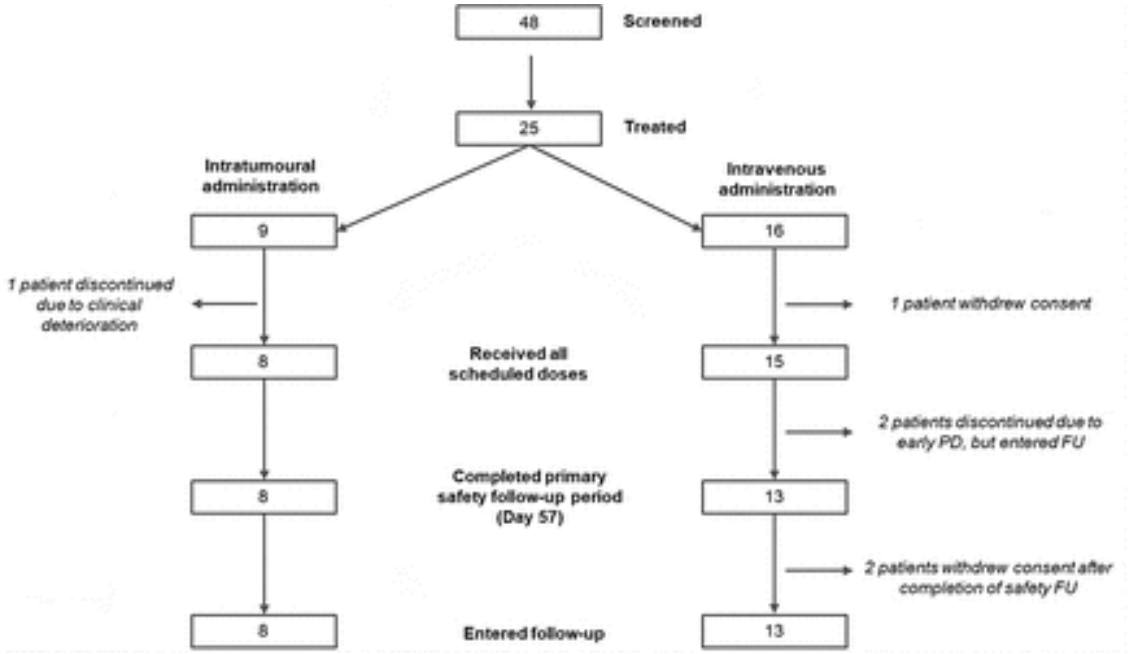


Figure 1. Patient disposition. FU, follow-up; PD, progressive disease.

A substantial proportion, 21 of 25 participants (84%), finished the designated 57-day treatment phase **(Figure 1)**. Although many advanced to surveillance, actual imaging continuation involved fewer cases (n=8 initiated imaging surveillance, while n=5 underwent additional scans).

Dose-escalation

Among IT participants, six received IT Dose Level 1 without any dose-limiting toxicities (DLTs). Following protocol guidelines, three more were enrolled at IT Dose Level 2, also without DLTs. Accordingly, the two planned IT levels were judged acceptable.

For intravenous administration, enrollment of six patients at intravenous Dose Level 1 revealed one DLT (elevated serum creatine kinase). Deemed acceptable overall, progression occurred to the "low-high-high" schedules intended to elevate cumulative viral load across the three-dose sequence. Four individuals were dosed at intravenous Dose Level 2 (1-3−3×1012 vp schedule) without DLTs. This facilitated advancement to intravenous Dose Level 3 (1-6−6×1012 vp), involving six patients and yielding no DLTs. Level 3 was confirmed acceptable, yet additional upward escalation was withheld, drawing on concurrent findings from related T-SIGn programs. A protocol modification instead incorporated a cohort assessing intravenous NG-350A co-administered with pembrolizumab for safety. After evaluating one such combination level, an independent phase 1a/b investigation was launched for more extensive pairing evaluation. Combination data will thus appear in an upcoming dedicated manuscript addressing NG-350A alongside pembrolizumab.

Patient exposure

Exceptions included one individual at intravenous Dose Level 2 who discontinued consent post-initial infusion, and another at IT Dose Level 2 who skipped a single planned dose; otherwise, participants completed all prescribed NG-350A deliveries **(Table 2).**

Table 2. Exposure

Measure	IT DL1D1 only(n=6)	IT DL2D1, 8, 15, 22(n=3)	IT total(n=9)	IV DL1 1×10^{11}(n=6)	IV DL2 3×10^{12}(n=4)	IV DL3 6×10^{12}(n=6)	IV total(n=16)
Participants completing scheduled dosing, n (%)	6 (100)	2 (66.7)	8 (88.9)	6 (100)	3 (75)	6 (100)	15 (93)
Total doses administered, n (%)			NA				NA

1 dose	6 (100)	0	0	1	0	
2 doses	–	0	0	0	0	
3 doses	–	1	6	3	6	
4 doses	–	2	–	–	–	
Total viral particles delivered (mean (range); ×10¹² vp)			NA			NA
Planned exposure	1.00	4.00	0.30	7.00	13.00	
Observed exposure	0.47 (0.2, 0.8)	2.67 (1.0, 4.0)	0.30 (0.30, 0.30)	5.50 (1.0, 7.0)	13.00 (13.0, 13.0)	

DL1, Dose Level 1; DL2, Dose Level 2; DL3, Dose Level 3; IT, intratumoral; vp, viral particles.
Procedural hurdles in IT delivery meant that the intended peak daily dose of 1×1012 vp—requiring a minimum of five discrete needle paths to instill 2 mL total volume into an appropriately voluminous lesion—was realized in few instances. Conversely, intravenous recipients uniformly obtained their full targeted quantities.

Safety and tolerability

Treatment-emergent (TE) adverse events (AEs) were universal, affecting all participants, while Grade 3–4 TEAEs impacted 13 individuals (52%) **(Table 3)**. The bulk of these TEAEs remained low-grade (1–2) and clustered around the first dosing window (within 8 days). Just one instance led to NG-350A cessation (Clostridium difficile colitis in an intravenous monotherapy patient from cohort 2; not treatment-related).

Table 3. Safety summary

Safety outcome	IT DL1D1 only(n=6)	IT DL2D1, 8, 15, 22(n=3)	IT total(n=9)	IV DL11–1×10¹¹(n=6)	IV DL21–3×10¹²(n=4)	IV DL31–6×10¹²(n=6)	IV total(n=16)
Participants experiencing ≥1 TEAE, n (%)	6 (100)	3 (100)	9 (100)	6 (100)	4 (100)	6 (100)	16 (100)
Most frequently reported TEAEs (>20% of participants)							
Prolonged aPTT	4 (67)	2 (67)	6 (67)	3 (50)	0	2 (33)	5 (31)
Pyrexia	3 (50)	1 (33)	4 (44)	3 (50)	1 (25)	2 (33)	6 (38)
Chills	2 (33)	0	2 (22)	1 (17)	3 (75)	3 (50)	7 (44)
Nausea	2 (33)	1 (33)	3 (33)	3 (50)	2 (50)	1 (17)	6 (38)
Fatigue	3 (50)	0	3 (33)	0	2 (50)	2 (33)	4 (25)
Hypokalaemia	1 (17)	0	1 (11)	3 (50)	1 (25)	2 (33)	6 (38)
Body weight loss	3 (50)	0	3 (33)	2 (33)	1 (25)	0	3 (19)
Anaemia	3 (50)	0	3 (33)	1 (17)	0	2 (33)	3 (19)
Participants with ≥1 TE-SAE, n (%)	2 (33.3)	1 (33.3)	3 (33.3)	3 (50.0)	2 (50.0)	3 (50.0)	8 (50)
Participants with ≥1 NG-350A-related TE-SAE, n (%)	1 (16.7)	0	1 (11.1)	1 (16.7)	0	2 (33.3)	3 (18.8)
Participants with ≥1 Grade 3–4 TEAE, n (%)	4 (66.7)	0	4 (44.4)	4 (66.7)	1 (25.0)	4 (66.7)	9 (56.3)
Participants with ≥1 Grade 3–4 NG-350A-related TEAE, n (%)	2 (33.3)	0	2 (22.2)	2 (33.3)	0	1 (50.0)	3 (18.8)
Prolonged aPTT	2 (33.3)	0	2 (22.2)	1 (16.7)	0	0	1 (6.3)
Elevated blood creatinine	0	0	0	1 (16.7)	0	0	1 (6.3)
Reduced appetite	0	0	0	1 (16.7)	0	0	1 (6.3)
Acute renal injury	0	0	0	0	0	1 (16.7)	1 (6.3)
Participants with ≥1 TEAE meeting DLT criteria, n (%)	0	0	0	1 (16.7)	0	0	1 (6.3)
Participants with ≥1 TEAE resulting in NG-350A discontinuation, n (%)	0	0	0	0	1 (25.0)	0	1 (6.3)

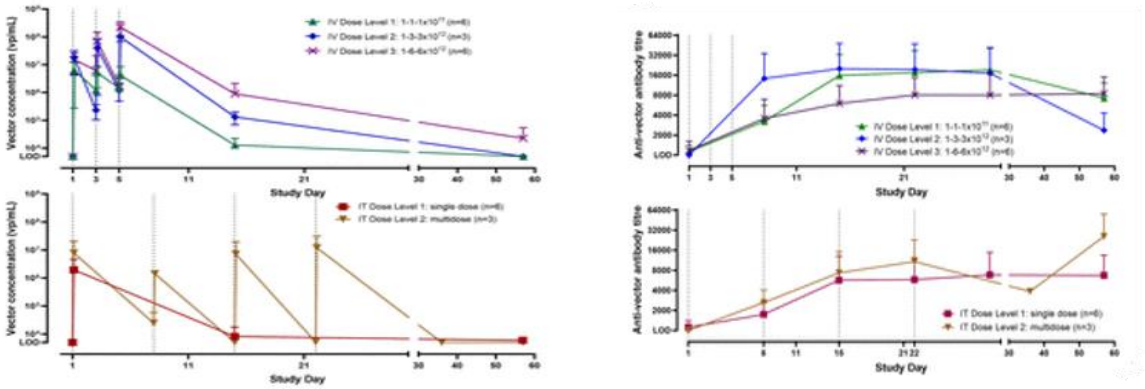
AE, adverse event; aPTT, activated partial thromboplastin time; DL1, Dose Level 1; DL2, Dose Level 2; DL3, Dose Level 3; IT, intratumoral; SAE, Serious Adverse Event; TE, treatment-emergent.

Among Grade 3–4 TEAEs, the leading ones included pneumonia (4/25 patients; 16.0%), extended activated partial thromboplastin time (aPTT) (3/25; 12%), raised blood bilirubin (2/25; 8%), diarrhea (2/25; 8.0%), and acute kidney injury (2/25; 8%). Grade 3 aPTT extension—free of any bleeding or thrombosis—was the only drug-related Grade 3–4 TEAE in multiple cases (two IT patients, one intravenous patient). Serious TEAEs arose in 11/25 participants total, affecting 3/9 (33%) in the IT group and 8/16 (50%) in the intravenous group. Pneumonia stood as the sole serious TEAE in over one patient (4/25; 16%), all deemed unrelated and linked to lung metastases or predisposing conditions. Other serious events involved one Grade 4 acute kidney injury (acute tubular necrosis) appearing 10 weeks post-final dose and one Grade 2 cytokine release syndrome (CRS), both at intravenous Dose Level 3. The kidney case was deemed related by the site investigator but unrelated by the sponsor, citing delayed onset, no on-treatment proteinuria, and recent exposure to nephrotoxic antibiotics or checkpoint inhibitors. Biopsy revealed acute tubular necrosis, resolving quickly with prednisone. The CRS case emerged promptly after infusion, typical of a reaction to viral particles, and cleared the next day.

Urine dipstick tests frequently revealed proteinuria in 13/25 cases (52.0%), showing ≥1+ protein during treatment. It was mostly short-lived and minor (highest 1+ in 9/13), clearing to <1+ by EOST in 6/13 (46%). No proteinuria-positive patient developed major kidney problems. Prolonged aPTT (≥Grade 2) appeared in 13/25 participants (52.0%; 4/9 IT, 9/16 intravenous), with three having baseline elevations. By EOST, 10/13 (77%) improved by ≥1 grade, without bleeding or clotting complications. Hepatobiliary issues affected 2/25 patients (8.0%; one Grade 1 hepatitis, one jaundice), both with tumor involvement in liver or pancreas.

NG-350A pharmacokinetics

Higher intravenous doses produced correspondingly greater peak NG-350A levels (Cmax) **(Figure 2a).** Circulating vector DNA lingered notably, remaining detectable beyond assay limits at Day 57 EOST for most patients on the top intravenous dose (three of four). IT delivery yielded strong yet brief serum spikes **(Figure 2a).** Weekly IT injections briefly boosted levels to roughly 1×107 vp each time, but sustained circulation was rarer, with just one of three patients detectable at Day 57.



a) b)

Figure 2. NG-350A pharmacokinetics and immunogenicity. (a) Mean NG-350A concentration in blood over time, according to dose level and route of administration. (b) Mean anti-vector antibody titer over time, according to dose level and route of administration. Dashed lines represent scheduled dosing days (first line only for IT Dose Level 1). IT, intratumoral; IV, intravenous; LOD, limit of detection; PK, pharmacokinetics.

Vector immunogenicity

Pretreatment anti-vector antibodies were absent in all but four participants **(Figure 2b).** Following administration, titers climbed from baseline in 88% of cases, showing near-identical conversion rates between IT (7/9) and intravenous (14/15) routes. Low baseline antibodies had no obvious PK influence versus antibody-free patients, and titer heights lacked a clear dose link, displaying high patient-to-patient variation. Overall, levels rose gradually then leveled off near Day 22.

Viral shedding

Shedding remained scarce and followed similar patterns for both delivery methods. Peaks typically hit at Day 8 or 15, then dropped sharply. Quantifiable DNA post-treatment was uncommon: only 1/75 (1%) urine, 7/81 (8%) buccal, and 8/79 (10%) rectal samples surpassed the quantification threshold.

Detection of NG-350A in tumor biopsies

qPCR analysis confirmed the presence of NG-350A vector DNA in post-treatment tumor biopsies for both administration routes. In intravenous recipients, positivity rates climbed with dose escalation: two of six assessable cases at Dose Level 1 showed detectable vector (≥1 reading above quantification threshold) versus six of seven at combined Dose Levels 2 and 3. Of particular note, vector DNA persisted in Day 57 (EOST) biopsies from four patients in the intravenous group.

Anti-CD40 transgene mRNA detection

Core needle biopsies yielded no detectable anti-CD40 transgene protein; in contrast, circulating transgene mRNA emerged at Day 8 or 15 in serum from 1/9 assessable IT patients (11%; one at Dose Level 1) and 5/12 assessable intravenous patients (42%; confined to higher-dose subgroups) **(Figure 3).**

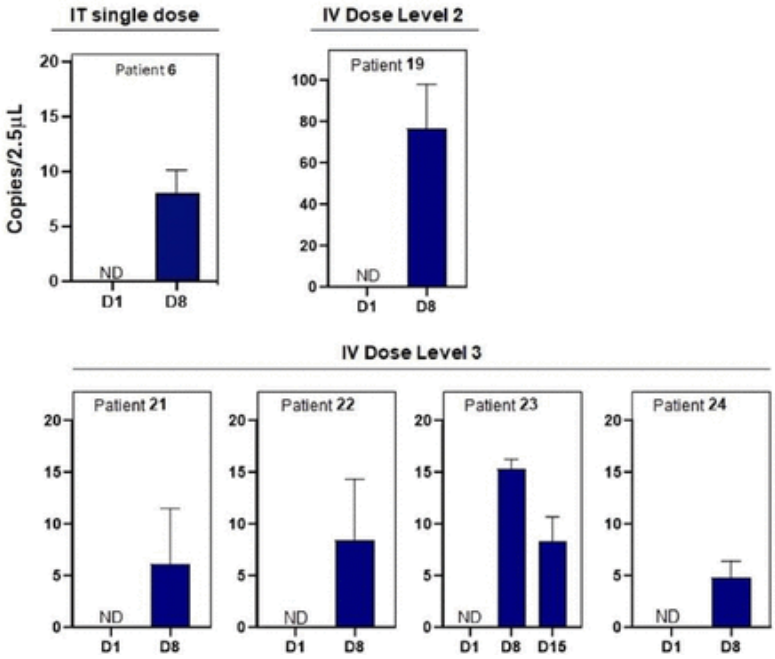


Figure 3. Detection of CD40 transgene in blood. Copies of CD40 agonist transgene detected in blood for patients positive at one or more time points (not normalized to sample volumes). IT, intratumoral; IV, intravenous; ND, not detected.

Serum cytokines

Treatment triggered serum rises in IL-12p70, IFN-α2, and IL-17a. Such elevations proved most reliable and pronounced among patients on higher intravenous regimens (Dose Levels 2 and 3) **(Figure 4).** Additional upticks in IFN-γ and IL-2 were recorded. These specific inflammatory cytokine surges generally started near Day 12, remained elevated over time, and involved only a limited panel of mediators.

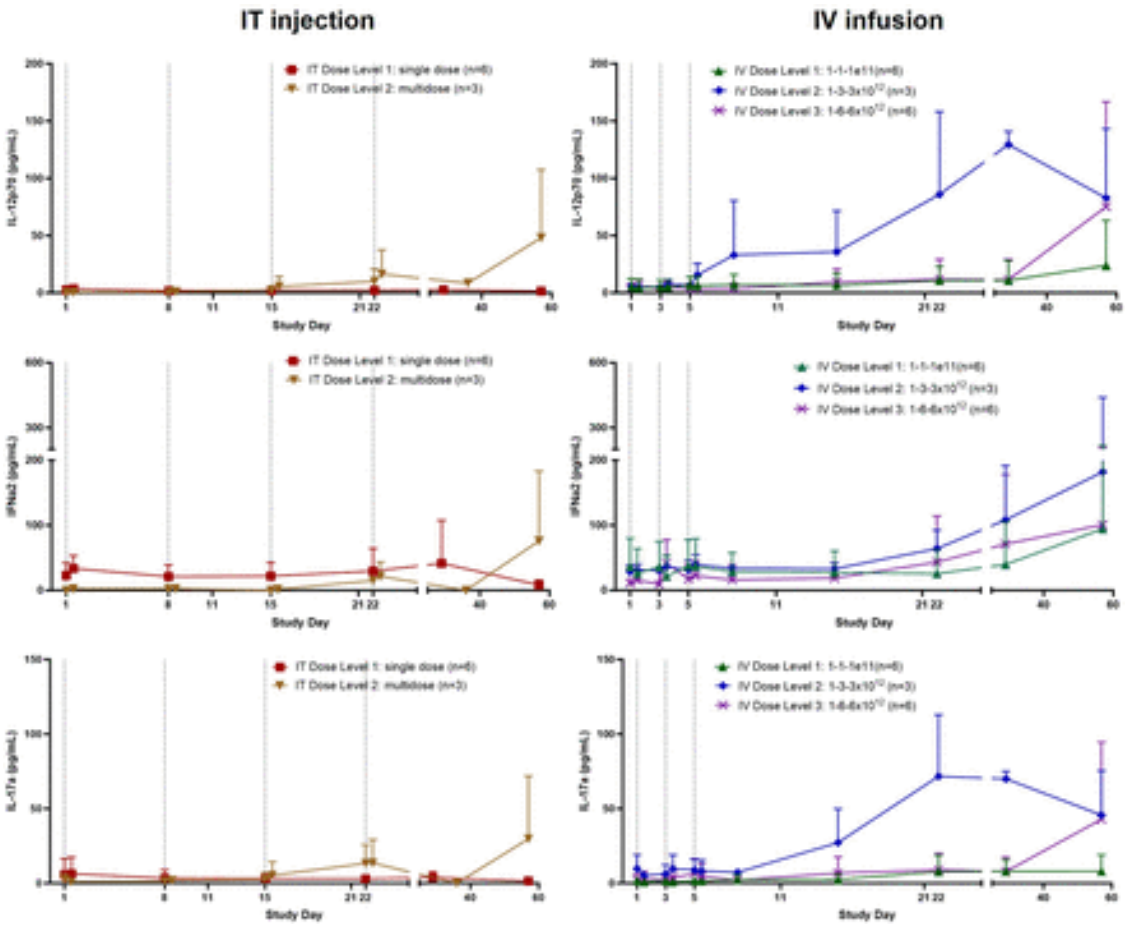


Figure 4.

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NG-350A pharmacodynamics—serum cytokines. Dose-dependent and sustained increases in IL-12p70, IFN-α2, and IL-17a were detected in serum from evaluable patients treated with higher intravenous dose levels. Dashed lines represent scheduled dosing days (first line only for IT Dose Level 1). IT, intratumoral; IV, intravenous.

Efficacy

No confirmed objective responses were recorded across either route. Stable disease represented the best response in 2/9 IT cases (22.2%) and 7/15 intravenous cases (46.7%). A cholangiocarcinoma patient (Royal Marsden Hospital prognostic score [8] of 2) previously refractory to three regimens displayed durable target lesion stability exceeding 36 weeks after intravenous monotherapy (Dose Level 1), delaying further therapy beyond 10 months (despite RECIST progression from a non-target site at 18 weeks). Post-treatment biopsies in this case revealed a shift from “Desert” to “Inflamed” tumor phenotype, with marked infiltration by CD8+ cells (>10-fold increase) and granzyme B+ cells (>8-fold increase).

Median PFS by RECIST v1.1 (95% CI) stood at 1.8 months (1.7, 3.0 months) for IT administration and 1.9 months (1.7, 4.1 months) for intravenous. Median OS (95% CI) reached 8.2 months (4.9, 14.4 months) with IT and 6.9 months (2.9, 17.1 months) with intravenous delivery.

This report presents findings from the inaugural clinical trial of NG-350A, an advanced tumor-targeted T-SIGn vector encoding a CD40-agonist monoclonal antibody. Effective trafficking of NG-350A to tumor lesions was achieved via both intravenous and intratumoral (IT) routes. Intravenous administration produced dose-proportional systemic pharmacokinetics, whereas IT injection also generated significant transient peaks in circulating vector. Prolonged detection of NG-350A occurred in blood and tumor tissue, remaining identifiable at the final study evaluation around 7 weeks post-final dose, even amid anti-viral antibody responses. Moreover, intravenous delivery specifically enabled detection of replicating-virus-derived anti-CD40 antibody mRNA.

Direct comparison of intravenous and IT approaches revealed comparable peak blood levels after dosing (accounting for variable total IT doses), similar rates of anti-drug antibody induction, and overlapping virus-

associated safety features (such as prolonged aPTT and infusion-related inflammatory responses). These observations indicate that IT administration offered no clear advantage in tumor restriction or reduction of systemic exposure compared to intravenous. In contrast, "low-high-high" intravenous schedules yielded stronger signs of extended vector persistence and higher rates of replication-linked transgene mRNA positivity (56% at elevated intravenous doses versus 11% with IT).

Systemic PK measurements for a replication-competent, highly tumor-selective agent demand cautious interpretation, as circulating vector after initial clearance likely reflects tumor spillover alone. Results aligned with rapid early elimination of NG-350A from circulation after either route, consistent with the <20 min half-life previously reported for the unarmed precursor enadenotucirev [4, 6] and the brief persistence of free DNA [9]. Critically, however, intravenous—but not IT—dosing produced extended late-phase vector DNA in blood. Considering the vector's established tumor specificity from enadenotucirev studies [4] and the absence of off-tumor toxicity here, this strongly supports continued intra-tumoral replication driving ongoing release into circulation. Transgene mRNA detection, predominant with intravenous administration, further corroborates active replication, since T-SIGn transgene output is replication-dependent [10]. Circulating transgene mRNA is postulated to originate from tumor-cell expression with subsequent exosomal spillover into blood; additional work is underway to validate this mechanism. Failure to detect anti-CD40 protein may stem from limitations of core needle sampling (low tissue yield and assay sensitivity) and minimal release of free antibody into systemic circulation. Additionally, only intravenous NG-350A induced prolonged, selective inflammatory cytokine increases aligned with CD40 stimulation, potentially representing another spillover phenomenon—systemic dissemination of tumor-produced cytokines reflecting local immune engagement after replication and transgene activity.

NG-350A exhibited favorable tolerability regardless of route, without treatment-related serious adverse events in more than one patient and no dose-limiting toxicities at the top levels evaluated. Notably, the "low-high-high" schedules rendered Dose Level 2 (1-3−3×1012 vp) and Dose Level 3 (1-6−6×1012 vp) safe, showing minimal dose-related toxicity escalation. This reinforces earlier evidence that a priming low dose before two higher doses attenuates cytokine-mediated reactions otherwise seen at ≥1×1012 vp, permitting greater total viral exposure than single high-dose approaches [6, 7]. No level proved intolerable here, though Dose Level 4 went untested, informed by parallel enadenotucirev and T-SIGn trials [6, 11]. Those studies documented severe events (hypoxia, dyspnea, cytokine release syndrome) after 10×1012 vp infusions (matching Dose Level 4), sometimes despite priming, attributed to acute innate responses to massive particle loads rather than transgene effects. A maximum tolerated intravenous regimen of 1-6−6×1012 vp is therefore proposed across T-SIGn platforms, including NG-350A. Intravenous Dose Levels 2 and 3 displayed encouraging pharmacokinetic, pharmacodynamic, and safety characteristics, yet limited patient exposure necessitates additional evaluation to establish a recommended phase 2 dose. Based on the tolerability and exposure achieved in this first-in-human trial, upcoming investigations will examine multicycle intravenous regimens to determine whether repeated dosing can augment and sustain intra-tumoral viral burdens, thereby elevating transgene expression and antitumor immunity.

In general, NG-350A exhibited a safety pattern comparable to that of the non-transgene-bearing vector enadenotucirev [4, 6, 7], indicating that integrating the anti-CD40 transgene did not introduce additional safety concerns. The bulk of adverse events (AEs) were mild in severity and emerged soon after administration, consistent with the typical profile expected from an adenoviral-based therapy, while no marked variations in patient tolerance were noted across the two delivery methods (intravenous versus intratumoral [IT]). Events linked to inflammation or cytokine release after virus particle (vp) administration were common regardless of route; yet, these were predominantly minor, with just a single AE prompting treatment cessation (an instance of Clostridium difficile colitis judged unrelated to the investigational agent). Aligning with the tumor-restricted expression of the CD40 agonist driven by NG-350A, neither intravenous nor IT administration showed indications of anti-CD40-associated adverse effects: notably, the hepatic damage and cytokine release syndrome (CRS) documented for earlier systemic anti-CD40 agonist antibodies [1, 12–16] were absent here, as the trial reported minimal liver-related AEs and no CRS events of Grade ≥3. These results bolster the core concept behind NG-350A, which involves confined, tumor-specific delivery of a CD40 agonist within the tumor microenvironment.

Extended activated partial thromboplastin time (aPTT) appeared following either delivery method but lacked obvious clinical impact; prior work had connected this to short-lived antiphospholipid antibody induction common to enadenotucirev and all T-SIGn vectors. More precisely, a lupus anticoagulant is transiently produced (distinct from the thrombosis-linked anticardiolipin or anti-β2-glycoprotein I antibodies seen in antiphospholipid

syndrome), altering aPTT lab results, as noted previously [17]. An earlier hint of acute kidney injury had emerged with the precursor virus enadenotucirev [7]. As a result, individuals with compromised kidney function or prior significant renal events were barred from enrollment, and kidney parameters were vigilantly tracked across participants. About 50% of NG-350A recipients experienced proteinuria; this was generally minor and short-lived, never advancing to serious kidney damage. A single Grade 4 acute tubular necrosis case arose 10 weeks post-final dose, deemed possibly linked to treatment by the investigator. Occurring absent prior proteinuria in a patient lately exposed to nephrotoxic antibiotics and a checkpoint inhibitor, this delayed occurrence implied potential alternate explanations. Although ongoing vigilance persists regarding adenovirus or T-SIGn vector potential to provoke infection-related or post-infectious glomerulonephritis, no pattern of meaningful kidney harm emerged as prevalent with NG-350A in this trial, justifying broader patient inclusion in upcoming investigations.

Designed as a phase 1a trial without primary efficacy endpoints, this study yielded only modest indications of standalone NG-350A antitumor effects in a cohort of heavily pretreated, late-stage cancer patients. Median progression-free survival (PFS) hovered near 2 months, presumably attributable to the refractory characteristics of the participants and comparable to PFS figures from checkpoint inhibitors in the immunotherapy-refractory tumor types included [18–24]. Upcoming trials will explore intravenously delivered NG-350A alongside other therapeutic agents to better gauge its potential benefits.

Conclusion

The inaugural human trial of NG-350A, an advanced transgene-equipped T-SIGn vector, established preliminary evidence of its intended biological mechanism. Intravenous administration succeeded in tumor targeting despite preexisting antiviral immunity, yielding prolonged viral presence within tumors, production of the anti-CD40 monoclonal antibody transgene, and persistent elevation of proinflammatory cytokines. Intratumoral administration, in contrast, conferred no evident gains in targeting, immune response, or tolerability, whereas intravenous dosing provided clearer signs of sustained viral propagation and transgene activity. These outcomes highlight the tumor-specific and circulation-stable properties of NG-350A as a notable edge over alternative oncolytic platforms, facilitating superior biological effects in both primary tumors and distant metastases via systemic delivery. Crucially, NG-350A proved tolerable by either route, devoid of the adverse patterns typical of broad systemic anti-CD40 agonist exposure. Absence of transgene-linked or nonspecific viral toxicities implies that NG-350A maintains strict tumor-focused oncolysis, replication, and transgene output even when given intravenously. Based on these positive data, the intravenous schedule of NG-350A from this trial will proceed to evaluation alongside pembrolizumab in the FORTIFY trial and alongside chemoradiotherapy in the FORTRESS trial.

Acknowledgments: None

Conflict of Interest: None

Financial Support: None

Ethics Statement: None

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