

Analysis of Clinical Benefit Using DNG64-CAR-V Chimeric Tumor Targeted Amphotropic RNA Vector in *CCNG1* Expressing Cancers

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Abstract

Background/Aim: Metastatic cancer is almost always fatal, with few promising clinical options. DNG64-CAR-V is an off-the-shelf, replication-incompetent Chimeric Amphotropic tumor-targeted RNA Vector encoding a cytotoxic Cyclin G1 (*CCNG1*) inhibitor construct.

Patients and Methods: *CCNG1* expression level in cancer types; Clinical benefit rate or CBR [complete response (CR), partial response (PR), or stable disease (SD)], confirmed by computed tomography or magnetic resonance imaging by RECIST v1.1; Overall response rate (ORR) and incidence and severity of adverse events were assessed. Eligibility criteria included: Previously treated male or female patients ≥ 12 years old with advanced sarcomas and patients ≥ 18 years old with advanced pancreatic ductal adenocarcinoma (PDAC), breast carcinoma or ovarian adenocarcinoma; Patients were treated with DNG64-CAR-V (1.7×10^{10} VC $3 \times$ a week \times 3 weeks/month) plus metronomic low doses of FDA approved drugs (DNG64-CAR-V+); Statistical analysis was performed with Simon 2-stage design with Type I error rate=0.1 and power=0.8. A CBR $\geq 30\%$ warrants a Phase II study using DNG64-CAR-V+ for *CCNG1* expressing tumors.

continued



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Results: Ten subjects with *CCNG1* expressing sarcomas (n=6), PDAC (n=2), breast ductal carcinoma (n=1), and ovarian adenocarcinoma (n=1) were treated with DNG64-CAR-V+. Five of 10 (50%) had PR; 9/10 (90%) had clinical benefit. Median progression-free survival was 6.7 months for sarcoma. No serious treatment-related adverse event is reported. **Conclusion:** A response rate of 50% and a CBR of 90% for all groups meet the Simon 2-stage threshold of CBR \geq 30%, thereby qualifying all groups for a Phase II study using DNG64-CAR-V+ in *CCNG1* expressing advanced cancers.

Keywords: Gene therapy, tumor targeting, RNA vector, cell cycle control, *CCNG1*, human cyclin G, chimeric amphotropic RNA vector (CAR-V), DNG64.

Introduction

Advanced or metastatic cancer is almost always associated with a fatal outcome with few advantageous clinical options. DNG64-CAR-V is an off-the-shelf, replication-incompetent chimeric amphotropic tumor-targeted RNA vector (i) displaying a *Sig* (pan-collagen)-binding domain targeting abnormally exposed collagenous (*i.e.*, *signature or Sig*) proteins within the tumor microenvironment (TME), and (ii) encoding a Cyclin G1/*CCNG1* inhibitor construct (1, 2) (Figure 1). DNG64-CAR-V not only kills cancer cells and tumor-associated neovasculature, but also kills the pro-inflammatory, cytokine secreting, growth-promoting, immune-suppressing, stroma-producing cancer associated fibroblasts (CAFs) (2, 3). Hence, DNG64-CAR-V reduces cancer cells, cytokine-induced inflammation, and extracellular matrix production in the TME, which facilitates the entry of innate immune cells, cytotoxic drugs and/or immunotherapies. Therapeutically, DNG64-CAR-V represents the first, and so far the only, off-the-shelf, intravenously administered, tumor-targeted gene therapy that has been progressively tested in the clinic and shown to be both safe and effective in Phase I and II clinical trials, revealing a dose-dependent relationship between patient survival and DNG64-CAR-V dose (4-8).

The *CCNG1* oncogene encodes the inducible, non-canonical Cyclin G1, a keystone effector of the executive Cyclin G1/CDK/Myc/Mdm2/p53/p18-Hamlet Axis, which governs stem cell survival, thereby linking DNA fidelity and tumor suppression with cellular proliferation and fate. The Cyclin G1 protein, the molecular target of DNG64-

CAR-V, is opposed by the expression of a dominant-negative (mutant) construct, which is cytotoxic to cancer cells derived from all three germ layers. Expression of the Cyclin G1 oncogene is activated by proximal (immediate-early) oncogenic signal transduction events that link the mitogen-activated and the proline-directed (*i.e.*, CDK) protein kinase cascades with tumor suppression and oncogenesis (9). Briefly, the dualistic Cyclin G1/Mdm2/p53/p18-Hamlet Axis of tumor suppression and checkpoint control is subject to viral subversion, mutation, and loss of function, which up-regulates Cyclin G1, while the growth promoting Cyclin G1/CDK/Myc Axis, which programs mitotic competence to cell proliferation, is subject to oncogene addictions. Upon its expression, the unstable Cyclin G1 protein interacts with Protein Phosphatase 2A to activate MDM2, a ubiquitin ligase which mediates the degradation of the p53 tumor suppressor, the *guardian* of DNA fidelity which then allows for proliferation of mutated or damaged cells. Further, Cyclin G1 interacts with cellular cyclin dependent kinases (CDKs) to activate and stabilize the executive Myc oncogene, which governs the state of mitotic competence. Thus, overexpression of Cyclin G1 enhances cancer stem cell renewal, and tumorigenicity, including the troublesome chemoresistance and dormancy of tumor cells (10, 11); its aberrant expression represents a putative biomarker for inhibition by anti-cyclin G1 targeted therapies (12, 13). This article focusses on evaluating the *CCNG1* expression levels in various cancer types and on the first 10 patients with enhanced *CCNG1* expression who were treated with DNG64-CAR-V+.

Patients and Methods

Vector production. Clinical grade DNG64-CAR-V was manufactured under GMP conditions using a transient transfection procedure described previously (1, 4-7).

Clinical protocol. Expanded access for DNG64-CAR-V for an intermediate population of pancreatic cancer, sarcoma and breast cancer was authorized by the United States Food and Drug Administration (FDA-CBER; NCT04091295) and approved by WCG (formerly Western) IRB in 2020. In 2024, FDA CBER and WCG IRB authorized an amendment for the use of DNG64-CAR-V as platform therapy upon which other FDA approved drugs may be added (DNG64-CAR-V+). The authorization was based on reports that *CCNG1* (the oncogene target of DNG64-CAR-V) expression was enhanced in all tumor types tested (13, 14). Written informed consent was obtained from each subject prior to treatment initiation with DNG64-CAR-V+.

Endpoints of the current study. The endpoints of the study included: (i) *CCNG1* expression level in cancer types, (ii) Clinical benefit rate or CBR [complete response (CR), partial response (PR), or stable disease (SD)], confirmed by computed tomography (CT) or magnetic resonance imaging (MRI) by RECIST v1.1; (iii) Overall response rate (ORR); and (iv) Incidence and severity of adverse events.

Eligibility and treatment. The eligibility included: Up to 40 subjects, ≥ 12 years old with advanced sarcomas, ≥ 18 years old with pancreatic cancer or breast cancer will be treated. The patients were treated with DNG64 -CAR-V IV (1.7×10^{10} RV copies 3x a week \times 3 weeks/month) plus FDA approved drugs (DNG64-CAR-V+ regimen).

***CCNG1* RNA sequence analysis.** RNA-seq libraries were sequenced to generate 50 million reads, aligned using Kallisto v0.42.4 to GENCODE v23 transcripts with default parameters. Only protein-coding, IGH/K/L- and TCR-related transcripts were retained for downstream processing, resulting in 20,062 protein-coding genes. *CCNG1* expression

levels were assessed by RNA analysis of archived formalin-fixed paraffin-embedded (FFPE) tumor samples (15). Expression was quantified as transcripts per million (TPM) and log₂-transformed. Expression is presented in relation to the distribution of TPM values in the reference cohort, a diagnosis-stratified patient cohort. Expression levels are categorized as Low (<17%), Medium-Low (17%-49%), Medium-High (50%-83%), and High(>83%).

Statistical analysis. Baseline demographics were statistically analyzed using descriptive statistics. RECIST v1.1 was used to evaluate response and clinical benefit rate (16). A Simon 2-stage design with Type I error rate=0.1 and power=0.8 was used (17) to determine if the study would warrant further clinical development. Accordingly, a CBR $\geq 30\%$ would warrant continuing Phase II studies using DNG64-CAR-V in *CCNG1* expressing tumors. Kaplan Meier graphs were used to determine progression free survival and overall survival (18).

Results

***CCNG1* expression in cancer.** Notably, all 247 tumor samples tested at the Sarcoma Oncology Research Center/ Cancer Center of Southern California showed enhanced *CCNG1* expression. Figure 2 shows a panoramic view of *CCNG1* expression levels in solid tumors. Table I shows the cancer types tested and their corresponding *CCNG1* expression levels. Forty-three (17%) tumors showed high *CCNG1* expression, ninety-five (38%) showed medium-high *CCNG1* expression, ninety-eight (40%) displayed medium-low *CCNG1* expression, and eleven (4%) had low *CCNG1* expression. Ninety-one percent (n=224) of tumors were sarcomas; 2% (n=6) were breast cancer; 1% (n=3) were pancreatic ductal adenocarcinoma (PDAC); and 5.7% (n=14) were other cancer types. Median *CCNG1* level was 57.5% for sarcomas (range=2-100%, n=224), 32.5% for breast cancer (range: 9-57%, n=6), and 55% for PDAC (range=24-75%, n=3). One hundred eighty-four (74%) subjects had metastatic disease, and sixty-three (26%) had locally advanced disease.

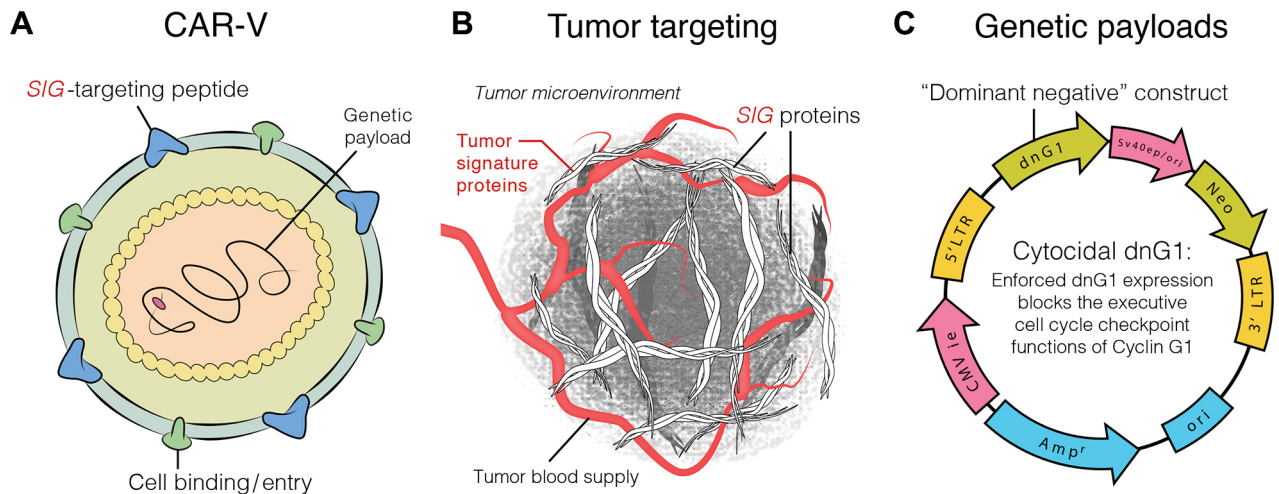


Figure 1. Artistic Illustration of DNG64-CAR-V. The DNG64 chimeric amphotropic RNA vector [CAR-V] displaying a SIG targeting peptide (A-C), for binding to abnormally exposed Signature [SIG] proteins in the tumor microenvironment (TME) (B), and encoding a cytosolic dominant negative human cyclin G1 inhibitor gene (C). Injected intravenously, DNG64-CAR-V nanoparticles find and bind to abnormal SIG proteins exposed in the TME, which augments effective vector concentration in tumors. DNG64-CAR-V kills not only cancer cells and tumor associated vasculature, but also kills cancer associated fibroblasts and reduces stroma production which facilitates entry of immune cells, cancer drugs and immune checkpoint inhibitors into the TME (adapted from Chawla 2019).

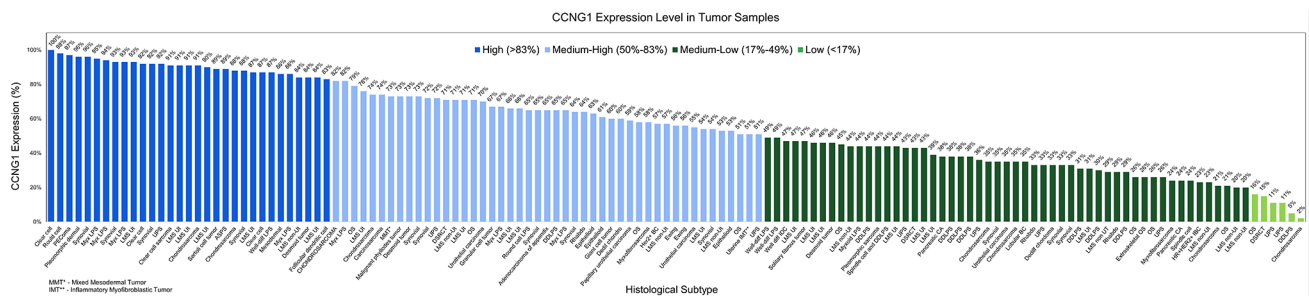


Figure 2. Waterfall illustration of CCNG1 expression levels in solid tumors. CCNG1 expression levels are plotted on the vertical axis in tumor samples tested.

Demographics and baseline characteristics of 10 recipients of the DNG64-CAR-V+ regimen. Ten subjects with advanced sarcomas (n=6), PDAC (n=2), breast ductal carcinoma (n=1) and ovarian adenocarcinoma (n=1) were treated with DNG64-CAR-V plus selected FDA-approved drugs, immune checkpoint inhibitors and/or SNK01, an autologous enhanced natural killer cell therapy (DNG64-CAR-V+). The subjects consisted of 5 men and 5 women, ages ranging from 33 to 85 years. Nine of 10 subjects had previously failed standard chemotherapy and/or targeted

therapies (median number of chemo and/or targeted therapy regimens=3 (range=2-10). Table II shows the prior regimens received by each individual patient. Due to poor disease prognosis, one subject with metastatic ovarian adenocarcinoma was treated with DNG64-CAR-V+ as first line therapy.

Analysis of clinical benefit using DNG64-CAR-V+ in subjects with advanced sarcomas, PDAC, breast ductal carcinoma and ovarian adenocarcinoma. Five of 10 (50%) subjects

Table I. Histological subtypes, CCNG1 expression levels in 247 cancer types.

Histological type	n	% of Samples	CCNG1 level median	CCNG1 level range
All histological types	247	100%	56.0%	2%-100%
Sarcoma	224	90.7%	57.5%	2%-100%
Leiomyosarcoma (All)	47	19.0%	53.0%	18%-93%
Uterine	30	12.1%	60.0%	27%-93%
Non-uterine	17	6.9%	34.0%	18%-93%
Liposarcoma	41	16.6%	45.0%	5%-96%
Synovial sarcoma	19	7.7%	68.0%	33%-96%
Undif. pleomorphic sarcoma	17	6.9%	43.0%	11%-92%
Osteosarcoma	14	5.7%	42.0%	16%-92%
Chondrosarcoma	14	5.7%	68.5%	2%-91%
Ewing	3	1.2%	56.0%	56%-99%
Chordoma	2	0.8%	45.5%	33%-58%
Other	67	27.1%	67.0%	5%-100%
Carcinoma	22	8.9%	51.0%	6%-89%
Breast	6	2.4%	32.5%	9%-57%
Urothelial carcinoma	4	1.6%	57.0%	35%-70%
Pancreatic carcinoma	3	1.2%	55.0%	24%-75%
Adenocarcinoma of the lung	2	0.8%	46.0%	26%-66%
Other	7	2.8%	58.0%	6%-89%
Other	1	0.4%	49.0%	49%
Diffuse large B-cell lymphoma	1	0.4%	49.0%	49%

had PR and 9/10 (90%) subjects had a confirmed PR or SD by RECIST v1.1. By *CCNG1* expression level, high *CCNG1* expressors (n=4) had a 25% (1/4) overall response rate (ORR) and 75% (3/4) clinical benefit rate (CBR); Medium-high expressors (n=3) had a 66% (2/3) ORR and 100% (3/3) CBR; and medium-low expressors (n=3) had a 100% (3/3) CBR. By tumor type, subjects with sarcoma had 33% (2/6) ORR and 83% (5/6) CBR; PDAC, 100% (2/2) CBR; breast cancer 100% (1/1) CBR; and ovarian adenocarcinoma 100% (1/1) CBR. Of note, the two subjects with PDAC and one subject with sarcoma failed the same previously received chemotherapy regimen and regained stable disease or partial response when DNG64-CAR-V was added. Table III presents each individual patient's diagnosis, *CCNG1* expression level, response at month four, and concurrent therapies with DNG64-CAR-V.

Notable reduction in tumor volume with proton therapy + DNG64-CAR-V in a patient with osteosarcoma, a notoriously radioresistant tumor. One subject with recurrent

osteosarcoma of T4-T5 spine and high (89%) *CCNG1* expression had a 65% decrease in tumor volume, when DNG64-CAR-V was administered in conjunction with proton therapy (Figure 3). Mechanistically, intravenously administered DNG64-CAR-V nanoparticles find and accumulate in injured tissues (*i.e.*, irradiated tumors in this case), thereby reducing cancer cell survival, while favoring enhanced efficacy of tumor killing with proton therapy in a subject with advanced unresectable osteosarcoma, a well-known radioresistant type of sarcoma (Table III, Subject #7).

Progression-free survival using DNG64-CAR-V + in subjects with advanced sarcomas. The median progression-free survival in the six subjects treated with DNG64-CAR-V was 6.7 (95%CI=1.875-12.039) months.

Adverse events. Reported DNG64-CAR-V treatment-related adverse reactions included Grade 1 pruritus (n=1) and grade 2 fever (n=1). No treatment-related serious adverse events were reported.

Table II. Prior treatment regimen by individual patient.

Subject #	Diagnosis	All Prior Chemo/Immuno/Targeted Therapy Regimens
1	Follicular dendritic cell sarcoma, metastatic	1. Doxorubicin + Ifosfamide 2. Alternative Therapy Regimen
2	Pancreatic ductal adenocarcinoma, metastatic	3. Trabectedin + Ipilimumab + Nivolumab (SOC 1702) 1. Gemcitabine, 5-Fluorouracil/Leucovorin, Cisplatin, Pembrolizumab, Mitomycin C 2. Compassionate Use + Modified Capecitabine, Irinotecan, Oxaliplatin
3	Leiomyosarcoma, uterine, metastatic	3. Gemcitabine, nab-Paclitaxel, Pembrolizumab, Cisplatin 1. Gemcitabine, Docetaxel 2. Doxorubicin 3. Clinical trial 4. Clinical trial 5. Clinical trial 6. Nivolumab Monotherapy 7. Trabectedin, Nivolumab 8. Clinical trial 9. Denosumab regimen 10. Unesbulin/Dacarbazine
4	Liposarcoma, metastatic	1. Eribulin 2. Trabectedin 3. Clinical trial 4. Gemcitabine, Doxorubicin, Docetaxel 5. Clinical trial 6. Clinical trial 7. Gemcitabine, Docetaxel, Doxorubicin, Nivolumab clinical trial
5	Triple negative breast cancer, Her2 low, metastatic	1. Letrozole + Ribociclib 2. Docetaxel, Carboplatin 3. Enhertu
6	Pancreatic ductal adenocarcinoma, metastatic	1. Capecitabine, Oxaliplatin, Irinotecan, Bevacizumab 2. Gemcitabine, nab-Paclitaxel, Mitomycin C 3. Ipilimumab, Nivolumab
7	Osteosarcoma, locally advanced, unresectable	1. Doxorubicin, Cisplatin 2. Doxorubicin, Ifosfamide/Mesna 3. Compassionate Use of SQ3370
8	Synovial sarcoma, metastatic	1. Doxorubicin, Ifosfamide/Mesna 2. Pazopanib
9	Extraskeletal myxoid chondrosarcoma, metastatic	1. Denosumab, Pazopanib 2. Clinical trial 3. Clinical trial 4. Cabozantinib
10	Ovarian adenocarcinoma, metastatic	None

Discussion

Personalized medicine, metronomic low dose chemotherapy, immunotherapy, targeted therapy, and gene and cell therapy are rapidly changing the practice of cancer medicine in the world. DNG64-CAR-V is uniquely positioned to make a significant impact in the field of cancer gene therapy as the first, and, so far only, off-the-

shelf intravenously administered, tumor targeted, tumor agnostic, gene therapy product that has been shown, in formal clinical trials and in this Expanded Access program, to have a wide margin of safety, undeniable anti-tumor activity, with no reported delayed therapy-related adverse event in long term (up to 17 years) cancer survivors (4-8, 13, 14, 19, 20). Specifically, DNG64-CAR-V's safety and efficacy as monotherapy was evaluated

Table III. *Diagnosis, CCNG1 expression level, response and treatment with DNG64-CAR-V+.*

Patient #	Diagnosis	<i>CCNG1</i> expression level	Response at month 4 RECIST v1.1	DNG64-CAR-V + Drugs/ImmuneRx (metronomic/low dose)
1	Follicular dendritic cell sarcoma, metastatic	83% medium high	Partial response	Trabectedin, nivolumab + autologous enhanced NK cells (SNK01)
2	Pancreatic adenocarcinoma, metastatic	55% medium high	Stable disease	Gemcitabine, cisplatin
3	Leiomyosarcoma, metastatic	87% high	Stable disease	Dacarbazine
4	Liposarcoma, metastatic	29% medium low	Stable disease	Dacarbazine
5	Triple negative breast CA, Her2 low metastatic	35% medium low	Partial response	Trastuzumab, deruxtecan
6	Pancreatic adenocarcinoma, metastatic	79% medium high	Partial response	Gemcitabine, nab-paclitaxel
7	Osteosarcoma, locally advanced, unresectable	89% high	Partial response	Proton therapy + autologous enhanced NK cells (SNK01)
8	Synovial sarcoma, metastatic	96% high	Progressive disease	Trabectedin, nivolumab + autologous enhanced NK cells (SNK01)
9	Extraskeletal myxoid chondrosarcoma	84% high	Stable disease	Dacarbazine
10	Ovarian adenocarcinoma	38% medium low	Partial response	Nab-paclitaxel, carboplatin, bevacizumab

in three phase I and one phase II United States-based clinical trials, which demonstrated a dose-dependent relationship between patient survival and DNG64-CAR-V dose (4-8). The use of DNG64-CAR-V monotherapy was not associated with nausea, vomiting, hair loss, bone marrow or immune suppression, or organ dysfunction; no replication competent retrovirus, vector neutralizing antibodies, or vector integration in non-target organs has been reported at the maximum administered doses. No serious treatment-related adverse events were reported, indicating that DNG64-CAR-V has minimal, if any, systemic toxicity (1, 4-8). Further, no therapy-related leukemias, liver toxicities, or delayed adverse events have been reported to date with the use of DNG64-CAR-V monotherapy unlike other viral vectors, including adenoviral vectors (21, 22) or adeno-associated viral vectors with recently reported life threatening and/or fatal hepatotoxicity (23, 24).

In 2020, FDA CBER authorized expanded access for DNG64-CAR-V for an intermediate size population of pancreatic cancer, sarcoma and breast cancer (named Blessed: NCT04091295). In 2024, FDA CBER authorized

the amended use of DNG64-CAR-V as platform therapy upon which other FDA approved drugs may be added (DNG64-CAR-V+) based on reports that *CCNG1* (the oncogene target of DNG64-CAR-V) expression was enhanced in all tumor types tested (13, 14). Since then, an increasing number of subjects with advanced bone and soft tissue sarcomas, pancreatic ductal adenocarcinoma, breast cancer and ovarian adenocarcinoma have been treated with DNG64-CAR-V+ metronomic low doses of FDA approved chemotherapies and/or immunotherapy with immune checkpoint inhibitors or autologous enhanced natural killer cell therapy with significant tumor shrinkage or stabilization of tumor growth.

In this study, an overall response rate of 50% and a clinical benefit rate (PR or SD, confirmed) of 90% using DNG64-CAR-V+ for advanced sarcomas, PDAC, breast cancer and ovarian adenocarcinoma attest to the predictable efficacy of the DNG64-CAR-V+ regimen in *CCNG1* expressing tumors. The noteworthy observation that patients who previously failed chemotherapy regained disease control when DNG64-CAR-V was added, supports evidence that DNG64-CAR-V not only kills cancer

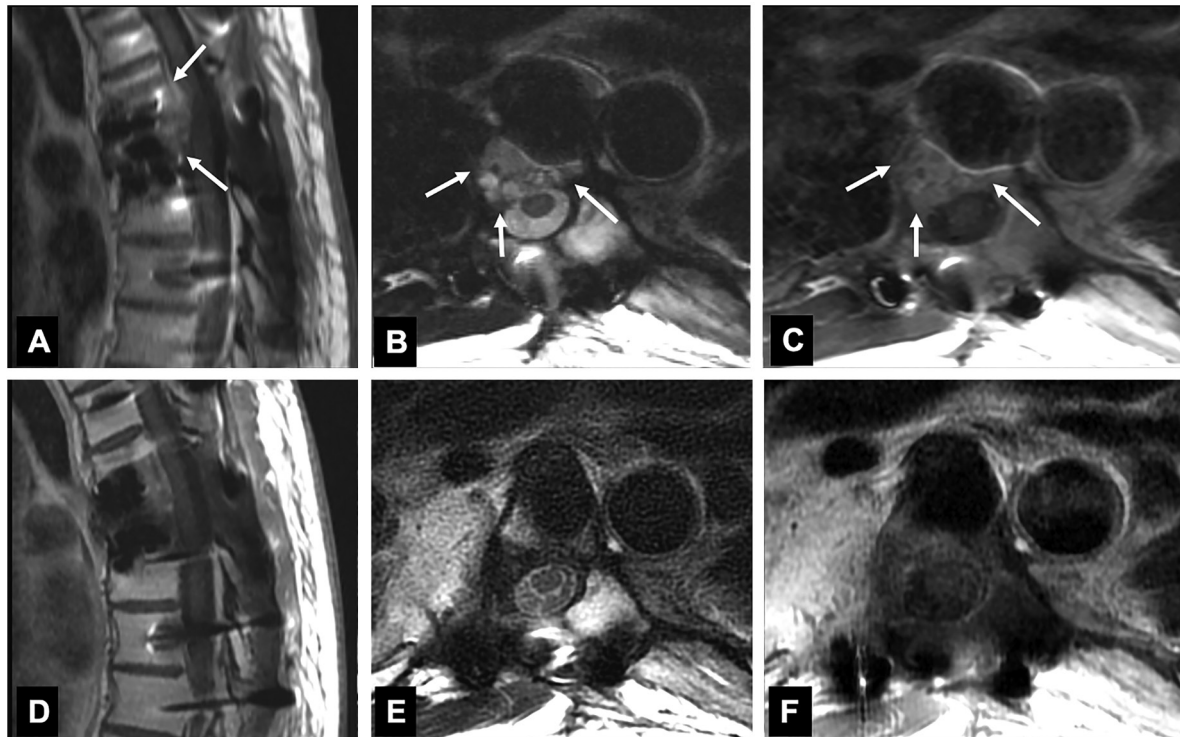


Figure 3. Dramatic reduction in tumor volume with proton therapy and DNG64-CAR-V. Sagittal T1-postcontrast, axial T2 and axial T1 postcontrast magnetic resonance (MR) images of thoracic spine are shown before (A, B, C) and after (D, E, F) treatment with proton therapy and concurrently with DNG64-CAR-V, a tumor targeted gene therapy bearing a cytotoxic *CCNG1* inhibitor gene. There is a 16×14×25 mm mass in the ventral epidural space at T5-T6 level with heterogeneous T2 hyperintense signal (arrows on B) and postcontrast enhancement (arrows on A and C). MR images obtained two months after proton therapy and DNG64-CAR-V show significant decrease in the size (10×14×14 mm) and degree of T2 signal and postcontrast enhancement suggestive of treatment response.

cells but also prepares the tumor microenvironment for improved drug entry by reducing inflammation and stroma production. Further, an ORR of 33%, clinical benefit rate of 83%, and a median progression-free survival of 6.7 months in subjects with advanced *CCNG1* expressing sarcomas provide a reliable prediction of efficacy in a planned phase II randomized study of DNG64-CAR-V + in subjects with *CCNG1* expressing sarcomas.

The chimeric/targeted DNG64-CAR-V vector is designed to seek out and accumulate in injured tissues (e.g., irradiated tissues, cancerous lesions, inflamed TME), wherein abnormally exposed collagenous proteins are present and abundant. This *partitioning effect* favored enhanced efficacy of tumor cell killing in combination with proton therapy in a patient with osteosarcoma, a well-

known radioresistant type of sarcoma. This anecdotal report encourages the development of a Phase II study using DNG64-CAR-V and proton therapy in radioresistant tumors such as osteosarcoma and most sarcomas, gliomas, pancreatic adenocarcinoma, melanoma, renal cell carcinoma, and adenoid cystic carcinoma (25).

In conclusion, our data and analyses indicate that: (i) All tested tumors showed enhanced *CCNG1* oncogene expression, reinforcing the use of intravenous DNG64-CAR-V + therapy for all *CCNG1* expressing cancer types; (ii) An overall response rate of 50% for all groups and a clinical benefit rate of 90% for all groups, when categorized by both tumor *CCNG1* expression level and cancer type, meet the Simon 2-stage design threshold of clinical benefit rate of 30%, thereby qualifying all groups for further studies

of efficacy and safety in planned randomized phase II/III studies using the DNG64-CAR-V + regimen for *CCNG1* expressing tumors.

Conflicts of Interest

SPC, SJ, SSP, RLJ, SVG, TH, JK, NSC, CBS, KN, HB, AG have no conflict of interest. FLH and EMG are co-inventors of the targeted gene delivery system represented by DNG64-CAR-V and are founders and equity members of Delta Next-Gene, LLC. RC is an employee and equity member of BostonGene Corporation; PYS is employee and equity member of NKGen Biotech, Inc.

Authors' Contributions

Investigation, data analysis, review and editing of manuscript, final review and approval of manuscript: SPC, RLJ, SVG, TH, JK, NSC, CBS, KN, HB, AG, EMG; Conception, protocol design, execution, investigation, resources, data analysis, initial writing, review and editing of manuscript, final review and approval of manuscript: FLH, EMG; Resources, data analysis, review and editing of manuscript, final review and approval of manuscript: PYS; Data collection and analysis, review and editing of manuscript, final review and approval of manuscript: SJ, SSP, RC.

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Artificial Intelligence (AI) Disclosure

No artificial intelligence (AI) tools, including large language models or machine learning software, were used in the preparation, analysis, or presentation of this manuscript.

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