Optimization of DNA to siRNA Ratio in SNS01 – an eIF5A-Based Gene Therapy Nanoparticle Designed for the Treatment of Multiple Myeloma

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ABSTRACT

INTRODUCTION: The eukaryotic translation initiation factor 5A (eIF5A) is the only protein to be regulated by post-translational addition of a hypusine residue. Hypusine-eIF5A has been identified as a marker of neoplastic growth and metastasis. However, in its unhypusinated form, eIF5A is pro-apoptotic and thus functionally distinct from hypusine-modified eIF5A. In vitro cell studies and in vivo xenograft studies have demonstrated that simultaneous suppression of eIF5A expression and over-expression of a non-hypusinable mutant of eIF5A (eIF5A^{K50R}) potently induces apoptosis in multiple myeloma cells. SNS01-T is a gene therapy nanoparticle targeted to the treatment of multiple myeloma. SNS01-T is comprised of three components: a B cell-specific plasmid expressing eIF5A^{K50R}; an siRNA that targets the native eIF5A that promotes growth/anti-apoptosis of cancer cells; and polyethylenimine. Previous studies have demonstrated that intravenous (iv) delivery of PEI nanoparticles containing either the eIF5A siRNA or an eIF5A^{K50R} expression plasmid were able to delay tumor growth in a murine model of multiple myeloma. However, co-administration of eIF5A siRNA and eIF5A^{K50R} plasmid delayed tumor growth more effectively than either the DNA or siRNA alone and resulted in tumor regression. SNS01 is a PEI nanoparticle formulation used in the preclinical development of SNS01-T. The effect of altering the N/P ratio or the DNA:siRNA ratio on the anti-tumoral activity of the nanoparticles was examined. Comparisons were made between nanoparticles prepared at N/P ratios of 6 or 8 and at DNA:siRNA ratios of 1:1, 2:1 (ratio used in SNS01-T and SNS01), and 5:1. Anti-tumoral activity of SNS01 nanoparticles following room temperature storage for 0.5, 4, and 6 hours was also investigated.
METHODS: Human KAS-6/1 multiple myeloma cells were transfected with PEI nanoparticles prepared at various DNA to siRNA ratios and apoptosis was quantified by Annexin V/PI labeling and flow cytometry. KAS-6/1 cells were injected subcutaneously into SCID mice. Mice were treated with freshly prepared (30 minutes to 6 hours storage) SNS01 at 1.5 mg/kg by iv injection twice per week for 38 days.
RESULTS: Transfection of KAS-6/1 cells with SNS01 induced greater apoptosis when prepared at a DNA to siRNA of 2 to 1. SNS01 induced apoptosis to a greater degree than PEI nanoparticles containing either plasmid or siRNA alone. In the myeloma model, all treatment groups that were treated with SNS01 exhibited statistically significant inhibition of tumor growth of greater than or equal to 75 % inhibition compared to control animals. Altering the DNA to siRNA ratio from 1:1, 2:1, or 5:1 did not negatively impact tumor growth inhibition, indicating that the eIF5A siRNA and eIF5A^{K50R} expression plasmid are equally important in the anti-tumoral effect observed in response to treatment with SNS01. SNS01 was found to be stable for at least 6 hours after preparation.
CONCLUSION: Multiple myeloma tumors were significantly inhibited following treatment with intravenous administration of SNS01. Reduucing the N/P from 8 to 6 or altering the DNA:siRNA ratio in SNS01 did not impact activity. SNS01 is stable for at least 6 hours at room temperature.

BACKGROUND

eIF5A : the eukaryotic translation initiation factor 5A (eIF5A) is the only known protein to undergo the post-translational modification of a conserved lysine to the unique amino acid hypusine

Hypusine$_{50}$- eIF5A

- is the dominant form of eIF5A in growing cancer cells
- has been identified as a marker of neoplastic growth [1]
- has been correlated to K-*ras* mutations and reduced survival in lung cancer patients [2]
- Deoxyhypusine synthase, the primary enzyme in the hypusine pathway, has also been found to be up-regulated in cancers [3] and has been identified as a marker for metastatic disease [4]
- inhibition of eIF5A expression reduces pro-inflammatory cytokine production and increases survival of LPS-challenged mice [5]

Lysine$_{50}$-eIF5A

- accumulates during apoptosis due to decreased DHS activity
- mutants of eIF5A that cannot be hypusinated (eIF5A^{K50R}) induce apoptosis in numerous cancer cell lines including colon [6], lung [7], and multiple myeloma and improves survival of tumour-bearing mice [7]

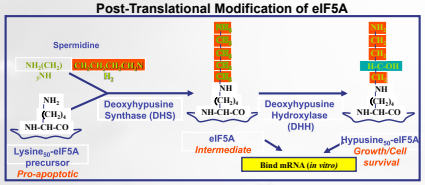
SNS01

- is a polyethylenimine (PEI) nanoparticle designed for treatment of multiple myeloma
- Consists of :
  - an siRNA that targets the native eIF5A that promotes growth/anti-apoptosis of cancer cells
  - a plasmid with a B cell-specific promoter/enhancer expressing a pro-apoptotic mutant of eIF5A (pExp5A plasmid)
  - a synthetic polymer (invivo-jetPEI) that acts as a delivery vehicle

OBJECTIVE

The ability of PEI nanoparticles containing a B cell-specific eIF5A$_{K50R}$ expression plasmid and an eIF5A siRNA (SNS01) to inhibit growth of myeloma cells in vitro and in vivo was examined in this study. The KAS-6/1 subcutaneous tumor model was used to test the room temperature stability of SNS01 and the effect of altering the N/P ratio (the ratio of positively charged amines of PEI to the negatively charged phosphate groups of nucleic acid) or the DNA to siRNA ratio on anti-tumoral activity.

Post-Translational Modification of eIF5A



MATERIALS & METHODS

Cell Culture
The KAS-6/1 human multiple myeloma cell line was obtained from Dr. John Lust (Mayo Clinic, Rochester, Minnesotta). KAS-6/1 cells were maintained in S10 culture media (RPMI-1640 + 10% FBS + 4 ng/mL recombinant human IL-6).
Nanoparticle Preparation
PEI nanoparticles were prepared by diluting 200 µg of pExp5A DNA and 100 µg of eIF5A siRNA in 5% glucose for a total volume of 500 µL. Invivo-jetPEI sufficient for an N/P ratio of 6 (36 µl) or 8 (48 µl) was diluted in 5 % glucose for a total volume of 500 µL in a separate tube and then added to the diluted nucleic acids. Nanoparticles were also prepared using alternate DNA:siRNA ratios while maintaining a nucleic acid concentration of 0.3 mg/mL.
KAS-6/1 Transfection and Apoptosis Assays
KAS-6/1 were diluted to 1x10^6 cells per mL in S10 media and seeded at 0.3 mL per well of a 24-well plate and transfected with 5 µL of PEI nanoparticles. Forty-eight hours later the cells were harvested, washed, and stained with Annexin/PI (BD Transduction Laboratories). The percentage of cells undergoing apoptosis was assessed by flow cytometry
Dynamic Light Scattering
SNS01 was diluted 1:10 in water and loaded into a folded capillary cell. Zeta diameter and polydispersity and zeta potential were measured on a Zetasizer Nano
KAS-6/1 Tumors
Female C.B-17/IcrHsd-Prkdc^{scid} (scid) mice of 4-6 weeks of age were implanted subcutaneously with 1.25 x 10^7 cells each. The mice were treated by intravenous injection twice-weekly with 1.5 mg (nucleic acid)/kg of PEI nanoparticles. Tumors were measured twice weekly with digital calipers and umor volume was calculated using the equation: tumor volume (mm^3) = L * W^2 * 0.5; where length (L) is the longest diameter of the tumor and W (width) is the smaller diameter. All aspects of this study were conducted in accordance with the guidelines set out by the University of Waterloo Animal Care Committee (Waterloo, Ontario, Canada) as established by the Canadian Council on Animal Care and the Province of Ontario Animals for Research Act.

SUMMARY

• SNS01 nanoparticles have an average zeta diameter of 143 nm and a zeta potential of 42.8 mV, while nanoparticles prepared at an N/P ratio of 8 are slighly smaller (122.8 nm).

• SNS01 (N/P = 6) had equivalent anti-tumoral activity to nanoparticles with a higher N/P ratio (N/P = 8) as both were able to inhibit growth of multiple myeloma tumors by >95 % (p<0.05).

• Transfection of human myeloma KAS-6/1 cells with PEI nanoparticles containing both pExp5A plasmid DNA and eIF5A siRNA (SNS01) induced apoptosis to a greater extent than either pExp5A plasmid or eIF5A siRNA individually. Additionally, apoptosis in response to PEI nanoparticles was greatest when the ratio of pExp5A DNA to eIF5A siRNA was 2:1, which is the ratio used in SNS01. Apoptosis in response to SNS01 occurred in the presence of the myeloma survival factor, IL-6.

• Altering the DNA:siRNA (from 2:1 tto 5:1 or 1:1) did not negatively impact anti-tumoral activity, suggesting that the pExp5A plasmid and the eIF5A siRNA activities are equally important in the anti-cancer activity of SNS01.

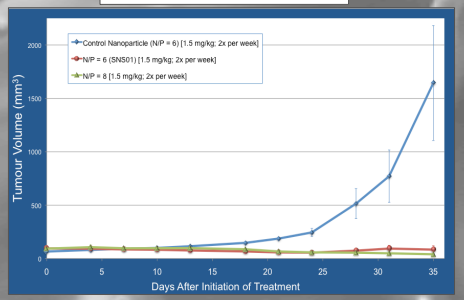
• No loss in anti-tumoral activity was observed when SNS01 was incubated for up to 6 hours at room temperature prior to administration.

In conclusion, it was determined that a 2:1 ratio of pExp5A plasmid DNA to eIF5A siRNA and an N/P ratio of 6 was optimal for activity. Furthermore, SNS01 nanoparticles retain anti-tumoral activity in a myeloma xenograft model following intravenous administration for at least 6 hours after preparation

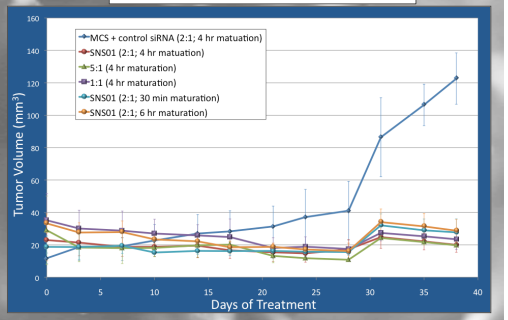
Dynamic Light Scattering

N/P Ratio	Z-Average (nm)	PDI	Zeta Potential (mV)
0 (no PEI)	159.0[1]	0.521	-25.1[1]
2	155.7	0.273	32.1
4	139.0	0.362	35.5
5	135.6	0.342	44.3
6 (SNS01)	143.0	0.344	41.8
7	105.6	0.268	46.4
8	122.8	0.276	45.7
10	123.6	0.303	50.8
12	103.6	0.261	48.7

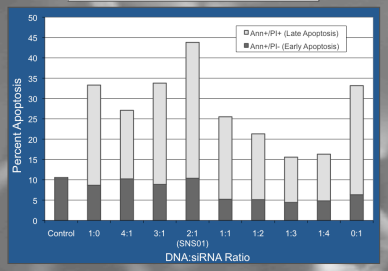
KAS-6/1 Tumor Model – N/P Ratio



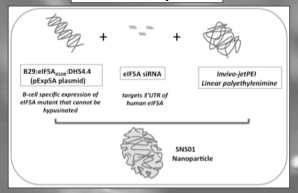
KAS-6/1 Tumor Model – DNA:siRNA Ratio



Apoptosis Assay in KAS-6/1 Cells



SNS01 Nanoparticle



Percent Inhibiton of Tumor Growth

Treatment Group	Maturation Time (hours)	DNA : siRNA Ratio (mg/mL nucleic acid)	Percent Inhibition Compared to Control	p value
MCS + control siRNA	4 hours	2:1 (0.3 mg/mL)	0.0 %	
SNS01	4 hours	2:1 (0.3 mg/mL)	83.7 %	0.00003
5:1 (4 hrs)	4 hours	5:1 (0.3 mg/mL)	83.9 %	0.002
1:1 (4 hrs)	4 hours	1:1 (0.3 mg/mL)	80.9 %	0.002
SNS01	30 minutes	2:1 (0.3 mg/mL)	77.4 %	0.0004
SNS01	6 hours	2:1 (0.3 mg/mL)	76.6 %	0.0004

REFERENCES: **[1]** Cracchiolo BM, Heller DS, Clement DMJ *et al.* (2004) *Gynecol Oncol* 94, 217-222 **[2]** Chen G, Gharib TG, Thomas DG *et al.* (2003) *Proteomics* 3, 496-504 **[3]** Clement PM, Henderson CA, Jenkins ZA *et al.* (2003) *Eur J Biochem* 270, 4254-4263 **[4]** Ramaswamy S, Ross KN, Lander ES *et al* (2003) *Nat Genet* 33, 49-54 **[5]** Moore CC, Martin EN, Lee G, Taylor C *et al.* (2008) *J Infect Dis* 198, 1407-1414 **[6]** Taylor CA, Sun Z, Cliche DO , *et al.* (2007) *Exp Cell Res* 313, 437-449 **[7]** Jin S, Taylor CA, Liu Z *et al.* (2008) *Gene Ther Mol Biol* 12, 207-218