

Preclinical development of SNS01-T: A polyethylenimine nanoparticle with significant anti-tumoral activity in murine models of multiple myeloma

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ABSTRACT

Originally identified as a eukaryotic translation initiation factor, the eukaryotic translation initiation factor 5A (eIF5A) has been implicated in the regulation of cell proliferation, inflammation, differentiation, and more recently apoptosis. eIF5A is the only known protein to be regulated by the post-translational addition of a hypusine residue. Both hypusinated eIF5A and deoxyhypusine synthase, the enzyme that mediates eIF5A hypusination, have been identified as markers of neoplastic growth and metastasis. Recent studies have indicated that, in its unphosphorylated form, eIF5A is pro-apoptotic and thus functionally distinct from hypusine-modified eIF5A.

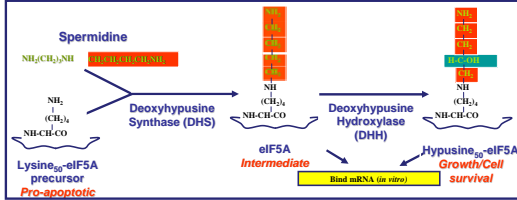
SNS01-T is a cancer gene therapy biologic targeted to the treatment of multiple myeloma. SNS01-T is comprised of three components: a DNA vector containing a B-cell-specific (B29) promoter driving expression of a pro-apoptotic single-point mutant of eIF5A (eIF5A_{K50R}) that cannot be hypusinated; an siRNA that targets the native hypusinated eIF5A that promotes growth of cancer cells; and a synthetic polymer called polyethylenimine (PEI). This approach has been found to effectively induce apoptosis in myeloma cells both in vitro and in vivo. In this study, we examined the physical characteristics and in vitro and in vivo biological activity of SNS01-T.

Dynamic light scattering (DLS) and transmission electron microscopy were used to monitor the size and zeta potential of SNS01-T nanoparticles. An in vitro cell-based assay comprising of SNS01-T transfection followed by RT-qPCR analysis of eIF5A and eIF5A_{K50R} transgene expression has been developed to assess potency of SNS01-T. The effect of SNS01-T on activity of NF-κB, an important mediator of myeloma cell survival, has been assessed using a cell line transfected with an NF-κB-responsive luciferase reporter plasmid. In vivo activity of SNS01-T was assessed following systemic administration in murine subcutaneous human myeloma tumor models.

TEM revealed that SNS01-T is a colloidal mixture of predominantly rod-shaped nanoparticles with a width of 20 to 30 nm and a length of 40 to 60 nm. DLS analysis of SNS01-T gives an average zeta-diameter of 69.10 ± 2.0 nm and a zeta potential of 37.80 ± 5.7 mV. RT-qPCR experiments demonstrated that treatment of the HEK-293 cells with SNS01-T results in greater than 95 % reduction in the expression of eIF5A mRNA (mediated by the siRNA) as well as the accumulation of ~ 100,000 copies of the plasmid-derived eIF5A_{K50R} mRNA per ng of total RNA. Suppression of endogenous eIF5A by the siRNA component of SNS01-T was found to profoundly inhibit the activation of NF-κB in response to stimulation by TNF-α. The anti-tumoral activity of SNS01-T was assessed in SCID mice bearing subcutaneous human multiple myeloma (KAS-6/1) tumors using twice-weekly intra-venous injections at a dose of 0.375 mg/kg. Six weeks of treatment with SNS01-T inhibited tumor growth by 89 % (p = 0.0002) compared to control animals. Furthermore, tumors that re-grew following the cessation of treatment responded to a second cycle of SNS01-T treatment. SNS01-T also showed significant anti-tumoral activity (52 % growth inhibition, p = 0.0001) at 0.375 mg/kg in the human RPMI 8226 myeloma subcutaneous tumor model.

In summary, our preclinical data indicate that systemic administration of SNS01-T nanoparticles is an effective anti-cancer therapy in animal models of multiple myeloma.

Eukaryotic translation initiation factor 5A



* 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

* hypusination status regulates function and cellular localization of eIF5A

Hypusine₅₀-eIF5A

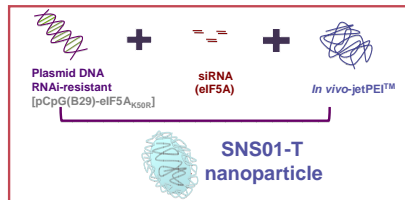
- * translation elongation factor
- * facilitates translation of a subset of mRNAs by acting as nucleo-cytoplasmic shuttle protein
- * is the predominant form in dividing cells and cancer cell lines
- * has been identified as a marker of neoplastic growth [1]
- * DHS, the primary enzyme in the hypusine pathway, has also been found to be up-regulated in cancers [2] and has been identified as a marker for metastatic disease [3]
- * is involved in inflammatory pathways and activation of T cells [4] and dendritic cells [5]
- * inhibition of eIF5A expression reduces pro-inflammatory cytokine production and increases survival of LPS-challenged mice [6]

Lysine₉₀-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 [7], lung [8], and multiple myeloma and improves survival of tumour-bearing mice [7]

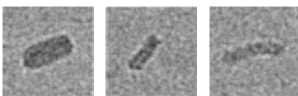
SNS01-T

- * is a nanoparticle designed for treatment of multiple myeloma and 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 non-hypusinated mutant of eIF5A
 - * a synthetic cationic polymer (polyethylenimine) that acts as a delivery vehicle



	[Nucleic Acid] mg/mL	Buffer	Tonicity Agent	pH	Zeta Diameter (nm)	Zeta Potential (mV)	PDI
SNS01	0.30	None	5 % Glucose	2.8 – 3.2	138.02 ± 12.4	39.07 ± 5.7	0.264 ± 0.07
SNS01-T	0.075	Trizma (5 mM)	5 % Glucose	5.8 – 6.5	69.10 ± 2.0	37.80 ± 5.7	0.169 ± 0.001

Visualization of SNS01-T Nanoparticles by Cryogenic Transmission Electron Microscopy



Particles are predominantly oblong in shape with most having length in the range 40nm - 60nm and width in the range 20nm - 30nm.

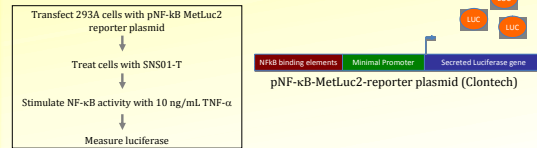
RT-qPCR Potency Assay

Assay	eIF5A			Transgene (eIF5A ^{K50R})		
	Rel. Expression	% of Control	Copy Number per ng total RNA	Rel. Expression	% of Control	Copy Number per ng total RNA
Untreated	1.095 ± 0.085	100	0.006 ± 0.00	0.006 ± 0.00	100	0.000 ± 0.00
2 μl	0.997 ± 0.462	90.99 ± 42.41	0.067 ± 0.03	0.067 ± 0.03	3.70x10 ⁴ ± 1.7x10 ⁴	
5 μl	0.654 ± 0.089	59.68 ± 8.13	0.142 ± 0.03	0.142 ± 0.03	5.73x10 ⁴ ± 5.2x10 ⁴	
10 μl	0.149 ± 0.053	13.60 ± 4.82	0.475 ± 0.14	0.475 ± 0.14	3.04x10 ⁴ ± 9.3x10 ³	
15 μl	0.614 ± 0.009	5.608 ± 0.84	1.259 ± 0.14	1.259 ± 0.14	8.27x10 ⁴ ± 8.7x10 ⁴	
20 μl	0.271 ± 0.006	2.473 ± 0.50	1.474 ± 0.33	1.474 ± 0.33	9.48x10 ⁴ ± 2.1x10 ⁴	

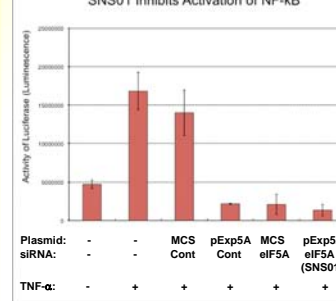
Biological activity of SNS01-T was assessed using RT-qPCR to detect changes in the expression of eIF5A in cultured cells that had been exposed to increasing volumes of SNS01-T. SNS01-T was incubated with HEK-293 cells in the presence of 5 % serum for 4 hours and then removed and replaced with fresh complete media. Twenty-four hours later the cell lysate was harvested and the total RNA was isolated. The RNA was quantified using the RiboGreen Assay (Molecular Probes) and 20 ng of RNA was used in a cDNA synthesis reaction. One microliter of cDNA was used for qPCR using EvaGreen Supermix (Bio-Rad) and quantitative real time PCR was performed using primers specific for either endogenous eIF5A (targeted by the siRNA), the transgene (encoded by the plasmid), or β-actin. Incubation with SNS01-T caused a 9 % (2 μl SNS01-T) to 97 % (20 μl SNS01-T) reduction in the expression of endogenous eIF5A compared to untreated cells. Accumulation of the transgene mRNA ranged from 3700 copies per ng total RNA (2 μl SNS01-T) to 94,800 copies per ng total RNA (20 μl SNS01-T).

NF-κB Assay

Assay Design

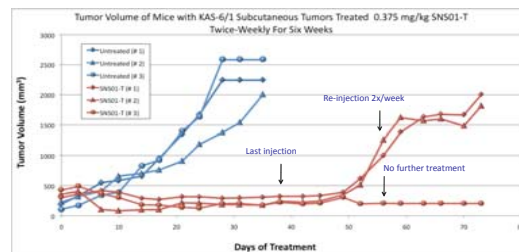


SNS01-T Inhibits Activation of NF-κB



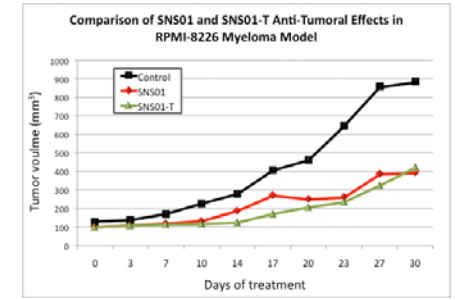
- * NF-κB is an important transcription factor that regulates survival in many cells, including malignant cells.
- * Constitutive activation of NF-κB is a common feature of multiple myeloma.
- * Inhibition of NF-κB is associated with increased apoptosis in multiple myeloma cells.
- * Transfection with both the pExp5A expression plasmid and the eIF5A siRNA (SNS01-T) inhibits NF-κB activity
- * Hypusine₅₀-eIF5A is required for proper response of NF-κB to TNF-α stimulation in 293A cells

KAS-6/1 Myeloma Model



NOD-SCID mice were injected subcutaneously with 1.5 x 10⁷ KAS-6/1 human multiple myeloma cells. Treatment was initiated one tumor volume exceeded 250 mm³. Mice were treated with twice-weekly intravenous injections of SNS01-T @ 0.375 mg/kg for 6 weeks (12 doses). Tumor progression was monitored following the cessation of treatment. Two out of three of the mice experienced tumor progression 10 days following the end of treatment. Treatment with SNS01-T was re-initiated once tumor growth was observed. In one mouse, no further growth of the tumor was observed for the remainder of the experiment (~ 4 weeks after end of treatment). The data shown is the tumor volume for individual mice.

RPMI 8226 Myeloma Model



SNS01 (1.5 mg/kg; n = 4) p < 0.001
 SNS01-T (0.375 mg/kg; n = 4) p < 0.0001

Summary

- * SNS01-T nanoparticles are rod-shaped particles 40 – 60 nm in length and 20 - 30 nm in width.
- * RT-qPCR of HEK-293 cells treated with SNS01-T demonstrated up to a ~ 96 % decrease in siRNA-mediated eIF5A expression as well as the accumulation of up to 94,800 copies of the transgene per ng of total RNA.
- * Reducing levels of hypusine₅₀-eIF5A by transfection of HEK-293 cells with SNS01-T dramatically reduces activation of NF-κB in response to TNF-α. SNS01-T may impair survival of myeloma cells by interfering with NF-κB activity.
- * Treatment of mice bearing either KAS-6/1 or RPMI 8226 subcutaneous tumors with SNS01-T results in significant inhibition of tumor growth and in the case of one mouse, elimination of the tumor.
- * Tumors treated with SNS01-T can respond to a second round of SNS01-T treatment.
- * SNS01-T is an effective anti-cancer therapy in a preclinical models of multiple myeloma and is currently being evaluated for use in a clinical trial with multiple myeloma patients.
- * SNS01-T inhibits NF-κB activity which likely contributes to its anti-tumoral activity.

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