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Preclinical Development of siRNA Therapeutics for AL Amyloidosis

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Abstract

AL (amyloid light chain) amyloidosis is a rare hematologic disorder characterized by the accumulation of a misfolded monoclonal immunoglobulin light chain (LC) as fibrillar protein deposits. Current treatments, including cytotoxic chemotherapy and immunomodulatory therapy, are directed at killing the plasma cells that produce the LCs, but have significant toxicity for other cell types. We have designed small interfering RNAs (siRNAs) targeting the amyloidogenic LC mRNA in order to reduce expression of the amyloid precursor protein. Using nanomolar concentrations of siRNAs, we have inhibited synthesis of LC in transfected cells *in vitro* in a dose-dependent fashion. Furthermore, in an *in vivo* plasmacytoma mouse model of AL amyloidosis, we have demonstrated that these siRNAs can significantly reduce local production and circulating levels of LC. This model system highlights the therapeutic potential of siRNA for AL amyloidosis.

Keywords

siRNA; AL amyloidosis; immunoglobulin light chain; RNAi; electroporation; plasmacytoma

Introduction

AL amyloidosis is a rare hematologic disease characterized by the accumulation of misfolded immunoglobulin light chains (LC) as fibrillar amyloid deposits, usually occurring

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in the setting of a clonal plasma cell dyscrasia in the bone marrow¹. These extracellular deposits cause organ failure and eventually death; in untreated patients, the median survival is 1.5 years following diagnosis². Current treatments are directed at the LC-producing clonal plasma cells and include oral melphalan and dexamethasone, immunomodulatory drugs (IMiDs), proteasome inhibitors, multi-drug regimens, and in selected patients, intravenous high dose melphalan followed by autologous stem cell transplantation (HDM/SCT)². These therapies can reduce or eliminate the plasma cell clone, allowing recovery of organ function and amelioration of disease. However, each of these therapies is potentially toxic in patients compromised by organ dysfunction due to the protein deposition disease. In addition to the myelosuppressive and immunosuppressive effects of chemotherapy, treatment with corticosteroids can exacerbate symptoms of nephrotic syndrome or congestive heart failure often present in patients with AL amyloidosis. Moreover, IMiDs are often poorly tolerated^{3, 4} and HDM/SCT has a much higher rate of complications compared to patients with other hematologic conditions⁵.

In addition to treatment strategies aimed at obliterating the source of the amyloid protein, there have been other approaches which focus on the fibril protein or the circulating precursors. The first effective targeted therapy for a protein deposition disorder was eprodisate. This is a small molecule that binds directly to amyloid fibrils composed of serum amyloid A protein, those that occur in patients with AA (formerly called secondary or reactive) amyloidosis. In a recent multicenter clinical trial, the use of eprodisate was correlated to slower progression of renal failure in patients with AA amyloidosis⁶. In amyloid diseases associated with transthyretin (TTR), agents that stabilize the native form of the protein are being tested as a potential therapeutics in several clinical trials. Diflunisal and other related compounds have been shown to prevent dissociation of tetrameric TTR *in vitro*, thereby blocking misfolding, aggregation, and amyloid fibril formation⁷.

An alternative to these protein-based strategies is a regimen that reduces production of the fibril precursor protein by using siRNA. Components of the endogenous RNA interference (RNAi) pathway, siRNAs are non-coding RNAs that regulate protein expression at the translational level by binding to mRNA. This interaction can block protein synthesis directly by interfering with mRNA translation and/or by promoting mRNA degradation⁸. Synthetic siRNAs have proven to be a powerful tool for investigating cellular gene function *in vitro*, and a Nobel prize was awarded for the development of this technology⁹. At present, there is a great deal of interest in siRNAs as potential therapeutics in a variety of diseases. One example is the use of RNAi to reduce liver-targeted TTR expression either through antisense nucleotides¹⁰ or siRNAs encapsulated with stable nucleic acid lipid particles (SNALP)¹¹; clinical trials are planned using these methodologies. Other studies include the use of siRNAs to prevent the production of amyloid fibril precursor proteins in models of Alzheimer's disease (targeting APP)^{12, 13}, Huntington's disease (targeting Huntington protein)^{14, 15}, Parkinson's disease (targeting alpha-synuclein)¹⁶ and prion diseases (targeting PrP)^{17, 18}. Here, we report the development and preclinical testing of siRNAs that are targeted to amyloidogenic immunoglobulin LC mRNA.

Results

Design of siRNA against amyloidogenic light chains

Among the sequences in AL-Base derived from patients seen in our center, the most frequently expressed LCs are from the $\kappa 1$ family, comprising 21.1% of the sequences in the database¹⁹. Thus, we chose to utilize a $\kappa 1$ LC as a paradigm for these studies. The LC sequence was amplified by reverse transcription PCR and the products were cloned into pcDNA3 for subsequent expression in cell lines.

Two publicly available tools were used to identify siRNA sequences targeted towards the $\kappa 1$ LC sequence. siRNAs targeting either the constant region (CL) of the LC to be “family specific” or the variable (VL) region to be “patient specific” were designed. The locations of the siRNA target sequences are depicted in Figure 1.

In vitro knock down of amyloidogenic light chains

In order to test the LC siRNAs *in vitro*, we created stable NIH-3T3 cell lines expressing the AL-009- $\kappa 1$ LC. The siRNAs were transfected by lipofection into the stably expressing $\kappa 1$ -NIH-3T3 cells at a concentration of 20 nM. By 48 hours, cells with two of the three siRNAs showed decreased amounts of LC mRNA; mean relative mRNA levels with CK1.4 and VK1.2 were significantly different ($p < 0.05$) compared to untreated (Figure 2A). Concentration effects were investigated in $\kappa 1$ -NIH-3T3 cells transfected with siRNAs by lipofection. LC protein amounts, analyzed by immunoblot and normalized to actin, were assessed over a broad concentration range of siRNA from 0.1 to 200 nM. When cells were treated with increasing doses of siRNAs, there was a dose dependent decrease in LC protein expression. This effect is demonstrated both in a representative blot and graphically seen in Figure 2, panels B and C, respectively. The IC₅₀ was between 1 and 10 nM for VK1.2 and CK1.4 and 50 nM for VK1.1, with maximal protein reduction of >70% for two of the siRNAs, VK1.2 and VK1.4. Of note, once the maximal inhibition is reached, increased doses of siRNA have no additional effect; this phenomenon is not abnormal for siRNA efficacy and has been reported by others⁸. Time course studies over a 7 day (168 hour) period were conducted using $\kappa 1$ -NIH-3T3 cells transfected with 20 nM siRNA. As can be seen in Figure 2, panels D and E, maximal inhibition of LC expression occurred between 48 and 96 hours, after which protein levels begin to rise again slightly. Concentrations of LC did not return to baseline by 7 days.

In vivo knock down of amyloidogenic light chains

Having demonstrated that siRNAs can reduce the expression of amyloidogenic LC in cell culture, we investigated whether siRNA delivered to cells *in vivo* could similarly reduce LC expression and secretion. For these studies, a plasmacytoma transplantation model was used. Briefly, in this model, SP2/0 mouse plasmacytoma cells stably expressing human amyloidogenic AL-009- $\kappa 1$ LC were injected subcutaneously into RAG^{-/-} recipient mice. The cells form plasmacytomas that continuously secrete light chain for several weeks. During this time period, LC can be detected in the circulation, as well as, in the kidney where amorphous aggregates and casts are present. Generally, after 25 days, the mice are sacrificed due to tumor burden. While amyloid fibrils are not observed in this model, some

mice eventually become bradycardic, consistent with a toxic effect of prefibrillar aggregates upon the heart²⁰. We used this *in vivo* model to assess the efficacy of siRNA in reducing LC expression. The delivery of siRNA was accomplished with *in vivo* electroporation^{21–23}.

By qRT-PCR, the relative levels of amyloidogenic LC mRNA in the plasmacytomas electroporated with VK1.2 were reduced almost 80% compared with plasmacytomas electroporated with control siRNA (n = 10 per group, p = 0.0016, Figure 3A). Immunoblots of plasmacytoma protein extracts were quantitated and normalized to actin amounts. LC protein levels were decreased in 7 of 10 of the samples electroporated with κ 1 siRNA compared with controls (Figure 3B). For all 10 samples, the mean reduction in κ 1 LC protein was 50%, and in 5 samples, protein was undetectable after treatment with the VK1.2 siRNA. Compared to the control group, the experimental group had a statistically significant reduction in tumor LC protein levels (p = 0.0051, n = 10 per group). As a further assessment of LC protein expression, representative sections from the plasmacytomas were immunohistochemically stained for human κ 1 LC and scored in a blinded fashion based upon the percentage of cells with high, moderate, or low LC expression (Figure 3C). The weighted score in the treated samples was 1.69, compared with a control score of 2.24 (p = 0.033, n = 6 per group).

To determine whether the reduction in plasmacytoma LC mRNA and protein expression led to a reduction in circulating serum levels of LC, equal volumes of pre- and post-treatment sera were compared by immunoblotting. In the controls, the mean serum LC ratio level was 2.71 over the two day experiment, while in the κ 1 siRNA treated mice, LC ratio levels were reduced to 0.23 (p = 0.0003, n = 10 per group, Figure 4).

Discussion

The purpose of the present study was to explore the use of siRNAs as a potential treatment for AL amyloidosis *in vitro* and *in vivo*. It has been shown that a reduction in serum LC levels leads to organ function improvement in patients with AL amyloidosis^{24, 25}. Thus, it is plausible that reducing LC levels through an siRNA-mediated mechanism, alone or in combination with other therapies, could halt AL disease progression and enable patients to recover organ function.

We designed siRNAs that were directed at either the variable (VL) or the constant (CL) regions of the LC in order to reduce amyloidogenic precursor protein expression. The basis of our strategy for targeting the unique VL domain was to develop sequence- and patient-specific siRNAs for treatment directed specifically at the amyloidogenic LC; one aim of this approach was to spare other LCs, i.e. non-amyloidogenic proteins from same LC family that might contribute to immune competence in a patient. Alternatively, by targeting shared sequences in the CL domain, siRNAs might target many populations of LC proteins and could be effective for many patients. All three siRNAs, designed against the amyloidogenic AL-009- κ 1 protein, caused a significant reduction in LC levels compared to controls *in vitro*. The most potent siRNAs had IC₅₀s between 1 and 10 nM, and were effective at reducing LC levels by 75–80% at concentrations of 20 nM. In kinetic studies, LC reduction

in vitro was maximal at 48–96 hours and persisted for at least 7 days, suggesting a weekly dosing regimen might be feasible.

In addition to our *in vitro* studies, we sought to provide proof of concept *in vivo*. For these studies, an innovative electroporation technique was used for delivery of siRNA to plasmacytomas secreting human amyloidogenic LC. In this model, we were able to reduce mRNA and protein levels in the plasmacytomas and also in the circulation with a single dose of siRNA. In these short-term experiments limited by plasmacytoma growth kinetics, we were not able to study the effects of siRNA treatment on amorphous LC deposition in the kidney or on the LC-induced bradycardia. Nonetheless, these results provide a proof-of-concept for this approach of reducing LC message and protein levels. Another recent publication has made use of siRNA against AL LC *in vitro*²⁶, and antisense RNA against LC has also been explored using multiple myeloma cell lines²⁷.

Advantages of the siRNA approach include high specificity and low toxicity. There is minimal sequence homology between immunoglobulin LC and other genes; therefore, there should be few off target effects on other mRNAs and proteins. A consequence of LC knockdown could be a reduction in the humoral immune repertoire. By testing siRNA targeting both the variable and constant domains of the LC, we explored the balance between a sequence-specific, patient-specific therapy and subfamily therapy. VL-targeted sequences would affect only the pathologic LC sequence, but each siRNA would be different and would have to be designed and tested for each patient. Development of CL-directed siRNA sequences would have broader specificity, but still should not significantly impair immune responses, as anti-pathogen antibody responses are not confined to particular subfamilies. This is an advantage over current chemotherapy and non-specific immunomodulatory therapy, which is broadly myelo- and immuno-suppressive.

While our studies indicate that a LC-directed siRNA approach to treatment for AL amyloidosis is feasible, several issues are yet to be addressed in translating this technology to a clinical application. Patients will need to have their amyloidogenic LC isotype, subfamily, and possibly even specific sequence determined to select an appropriate siRNA. In our database of LC sequences, AL-Base, 26% (124/477) of the AL light chain sequences are κ and of those 81% (100/124) are $\kappa 1$ light chain with 40% (47/124) derived from the $\kappa 1$ 018 germline gene donor¹⁹. Thus, a single effective $\kappa 1$ siRNA targeted toward the framework regions of the variable domain could be used for almost half of κ patients. Patients with λ light chain AL amyloidosis have more genetic diversity. Thus, the specific light chain subfamily would have to be targeted, unless an siRNA could be identified that targeted all λ V regions. This seems an almost impossible requirement due to the sequence dissimilarity of the framework regions. In the lambda family, $\lambda 1$, $\lambda 2$, $\lambda 3$, and $\lambda 6$ are found at similar frequencies (19.4%, 15.7%, 19.9%, and 17.6% respectively) in our AL clinic population¹⁹. Though not been synthesized or validated, several siRNAs have been designed *in silico* to target the λ constant domains from $\lambda 1$, $\lambda 2$, $\lambda 3$, and $\lambda 6$, the highest represented AL λ light chain families. These siRNAs have 100% identity in the seed region and minor mismatches in the remainder of the oligonucleotides²⁸, and can be tested in future experiments *in vitro*. Additionally, of the lambda sequences, $\lambda 6$ is of particular interest because it is rarely found in non-AL settings and represents <2% of sequences from patients

with multiple myeloma or from polyclonal B cells. Preliminary results from *in vitro* studies indicate that, like those siRNAs targeting $\kappa 1$ LC, $\lambda 6$ targeting siRNAs are effective in reducing LC mRNA and protein levels (data not shown).

Further preclinical studies aimed at optimizing the pharmacokinetics and improving the delivery of LC-directed siRNA are necessary prior to testing in patients with AL amyloidosis. We have shown *in vitro* that siRNA knockdown can persist for up to 7 days. This suggests that intermittent therapy is possible *in vivo*. Nonetheless, it is likely that siRNA oligonucleotides will need to be altered to extend their duration in the circulation as unmodified oligonucleotides typically have a serum half-life of minutes to hours. By modifying the oligonucleotides, half-life can be extended to days²⁹. Approaches include encapsulating the oligonucleotides in liposomes, nanoparticles, or other substances that are non-toxic and non-immunogenic³⁰.

Another requirement in the development of siRNA as an AL therapeutic is for a delivery system that specifically targets plasma cells in the bone marrow, since amyloidogenic plasmacytomas are rare in patients. Possible technologies to direct siRNA delivery include conjugation with aptamers, lipophilic substances, peptides, or antibodies³¹. For example, antibodies against CD138 could be employed for targeted delivery to plasma cells, although CD138 (syndecan-1), a heparan sulfate-bearing proteoglycan, is also expressed on epithelial cells³². There would presumably be no effect of targeting LC siRNA to epithelial cells; however, these cells would provide a “sink” for the injected siRNA.

In summary, siRNA directed against amyloidogenic light chain can reduce light chain synthesis and secretion in cells in culture and *in vivo*. With the development of improved delivery and targeting techniques, siRNA therapeutics hold promise as an effective and less toxic approach for treatment of AL amyloidosis and possibly as a therapy in other protein misfolding and deposition diseases.

Materials and Methods

Design of siRNA against amyloidogenic light chains

A $\kappa 1$ LC sequence was chosen from those available publicly in AL-Base¹⁹. This database, developed with the approval of the Boston University Medical Campus Institutional Review Board, is comprised of over 400 amyloidogenic and non-amyloidogenic immunoglobulin LC gene sequences, including more than 250 that were cloned and sequenced from patients seen at the Boston University School of Medicine/Boston Medical Center Amyloid Treatment & Research Program. A well-characterized $\kappa 1$ sequence, AL-009- $\kappa 1$, Genbank ID EF589383 (Kappa1 O18 family), was chosen for these studies and will be referred to as AL-009- $\kappa 1$.

Online design algorithms from the Thermo Scientific siDESIGN® Center and the GenScript Corp siRNA Target finder were used to search for sequences targeting either the constant region to be “family specific” or the variable region to be “patient specific.” Synthetic siRNAs were purchased from Thermo Scientific Dharmacon RNA Technologies (Lafayette, CO), brought up in sterile nuclease free water, and stored at -20°C until used. The siRNA

sense sequences are as follows: VK1.1 5' GAUCUACGAUGCUUCCAAU 3'; VK1.2 5'CUGUCAACAAUAUGCUUCU 3'; CK1.4 5' CAAAGCAGACUACGAGAAA 3'; and control siRNA GG1.1 5' GAAUUCACUCUCACAAUCA 3'. All were synthesized as complimentary sequences with 3' UU overhangs.

Generation of stable cell lines and *in vitro* siRNA transfection

The LC sequences were amplified by reverse transcription PCR and the products were cloned into pcDNA3 for subsequent expression in cell lines. The pcDNA3 plasmid containing AL-009- κ 1 LC was linearized and transfected NIH-3T3 cells Lipofectamine™ 2000 (Invitrogen). Stable clones were generated. Clones were tested for LC expression using immunoblot analysis of cell lysates. Those expressing LC were used for siRNA transfection experiments. Similar lines of the transfected and cloned SP2/0 mouse plasmacytoma cells with AL-009- κ 1 have been previously described³³.

siRNA was transfected into 70% confluent AL-009- κ 1 expressing NIH-3T3 cells using Lipofectamine, following manufacturer's instructions. Transfected cells were grown in DMEM media supplemented with 10% FCS, 5% L-glutamine, and 1% penicillin/streptomycin for 24–120 hours at 37 °C, 5% CO₂, depending upon the experiment. Cells were lysed in Laemmli SDS loading buffer (Boston BioProducts).

Plasmacytoma transplantation and *In vivo* electroporation

All mouse experiments were carried out under protocols approved by the Boston University Medical Campus Institutional Animal Care and Use Committee. RAG1^{-/-} mice³⁴ (>8 weeks of age) were anesthetized with isoflurane and injected subcutaneously with 10⁷ SP2/0 cells expressing AL-009- κ 1 light chain. Mice were monitored bi-weekly for the development of subcutaneous plasmacytomas. When plasmacytomas reached 0.75 cm in greatest diameter, the mice were anesthetized with 100 mg/kg ketamine, 16 mg/kg xylazine, and 75–100 μ L blood was collected; at the same time, the fur over the plasmacytoma area was depilated using Nair® (Church & Dwight Co., Inc) or Veet® (Reckitt Benckiser). Fifty μ L of sterile PBS containing 12 μ g siRNA (Thermo Scientific) and 40 units rRNasin (Promega) was injected into the center of the plasmacytoma. Thirty seconds following injection, 70 mm Tweezertrodes (Harvard Apparatus) were applied and the plasmacytoma was pulsed with four square waves of alternating polarity (480 V, pulse duration 5 ms, pulse frequency 1 Hz). The mice were anesthetized and sacrificed by exsanguination/cardiac puncture followed by cervical dislocation 48 hours later. The plasmacytomas were removed and saved for immunohistochemistry (IHC), and protein and RNA analyses. Serum was prepared from the blood samples.

mRNA isolation and qPCR

Cells were harvested and RNA prepared using TRIzol® (Invitrogen) according to the manufacturer's instructions. Plasmacytoma samples were frozen in RNAlater (Ambion) and stored at –80 °C until use. Tissues were homogenized in TRIzol; RNA isolation was performed per manufacturer's instructions. Quantitation of mRNA was performed on a SmartSpec™ Plus spectrophotometer (BioRad) and samples were stored at –80 °C until further analysis. RNA samples were treated following manufacturer's instructions (RQ1

RNase Free DNase (Promega)). DNase-digested samples were run over Purelink RNA mini kit columns (Invitrogen) to remove DNA fragments. Subsequently, cDNA was synthesized using the iScript™ cDNA Synthesis Kit (Bio-Rad) following the manufacturer's instructions.

qPCR was performed on a BioRad C1000 Thermocycler using Sybr green (BioRad) with primers for AL-009-κ1 LC (forward primer: CACCCTGACGCTGAGCAAA, reverse: TGA CTTCGCAGGCGTAGACTT, product size 59 nt), GAPDH, used as a housekeeping gene control (forward primer: CAACGGGAAGCCCATCAC, reverse: GCCTCACCCCATTTGATGTTA, product size 63 nt), and mouse Blimp1, used as a plasma cell specific control (primers: AAAGGACATGGATGGCTTTTCG and GTGCCCGGATAGGATAAACCA, product size 80 nt). LC primers were designed by Primer Express (Applied Biosystems); GAPDH and Blimp1 primers were designed using Primer Blast (NCBI). All primers were synthesized by Invitrogen. Relative amounts of RNA were quantitated using the Ct method^{35, 36}. Ct values were normalized to Blimp1 or GAPDH expression. Statistics were performed using GraphPad Prism v.5.

SDS-PAGE and immunoblotting

10% SDS polyacrylamide gels with 5% stacking gels were used to separate samples. For cell lysate and plasmacytoma homogenate samples, equal protein amounts were loaded; for serum samples, equal volumes (5 μL) were loaded. Gels were run in 1X TGS running buffer. The proteins were transferred to PVDF Immobilon-P transfer membranes (Millipore). After transfer, the membrane was blocked in 5% milk in TBS-T for at least one hour. For detecting K1 LC proteins, polyclonal goat anti-human kappa immunoglobulin (Ig) LC (Strategic Biosolutions) and polyclonal donkey anti-goat IgG (Santa Cruz) antibodies were used. For actin blots, the mouse anti-beta actin (Sigma) and goat anti-mouse IgG (Santa Cruz) antibodies were used. Samples were detected using chemiluminescence (Enhanced Chemiluminescence, Pierce) with film (Kodak) or visualized on the Kodak Image Station 4000MM (Kodak). Plasmacytoma homogenate and cell lysate LC values were normalized to actin. Serum LC values were not normalized, as equal volumes of samples were loaded. Band mean intensities were quantitated using ImageJ software (NIH). Serum membranes were visually inspected for proper loading using Ponceau staining (data not shown).

Immunohistochemistry

Tissues were fixed in 10% formalin, dehydrated, and embedded in paraffin. Five μm sections were cut, baked at 60 °C for 1 hour, deparaffinized, and washed in dH₂O. To block endogenous peroxide reactivity, the sections were placed in 0.3% hydrogen peroxide in methanol for 10 minutes. The antigen was retrieved with Citra plus solution (BioGenex) in a microwave for 3 minutes on high and 8 minutes on medium. Immunostaining was performed on an Autostainer (Dakocytomation, Carpinteria, CA) using polyclonal rabbit anti-human LC antibodies (Dako). Sections were incubated in primary antibody and secondary antibody, each for 30 minutes, and followed by exposure to DAB substrate for 5 minutes. Buffer washings were carried out between incubations; dH₂O washing after DAB substrate (Dako) incubation was performed to stop the reaction. After completion of immunostaining, the sections were counterstained with Harris hematoxylin for 1 minute,

washed with dH₂O until clear, dipped in 0.25% acid alcohol, washed with distilled water, blued in 1% ammonia for 30 seconds, dehydrated, and mounted for microscopy.

Immunohistochemistry Scoring

The immunohistochemical staining was reviewed and scored in a blinded fashion by a hematopathologist (C.O.). The percentage of cells with strong cytoplasmic staining (3+), moderate staining (2+), and weak or no staining (1+) were estimated and a weighted score was assigned as the sum of the percentage x score for each. Weighted scores were compared by Student's t-test and plotted as an average score for each group.

Statistics

All statistical analyses were performed in GraphPad Prism v.5. For immunoblots, quantitation was performed with ImageJ software. Mean band intensities were compared between groups using a one-tailed Student's t-test. Immunohistochemistry weighted scores were compared between groups with a one-tailed Student's t-test. qPCR samples were compared using the Ct method. Ct values were normalized to mouse BLIMP1 expression and compared with a one-tailed Student's t-test.

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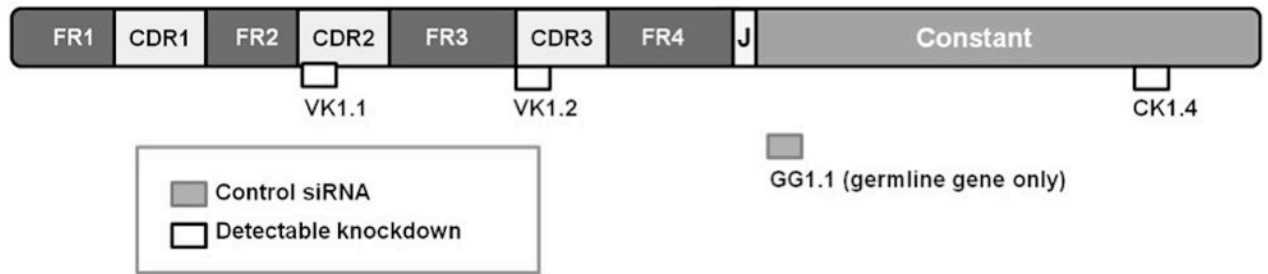


Figure 1. siRNA target sites on a prototype $\kappa 1$ LC

Diagram of siRNA target sites on patient amyloidogenic $\kappa 1$ immunoglobulin LC, AL-009- $\kappa 1$. Two siRNAs were designed to target different regions of the light chain variable (VL) domain and one to target the light chain constant (CL) domain. One siRNA, GG1.1, was designed as a control directed at the $\kappa 1$ germline gene, but not the AL-009- $\kappa 1$ sequence.

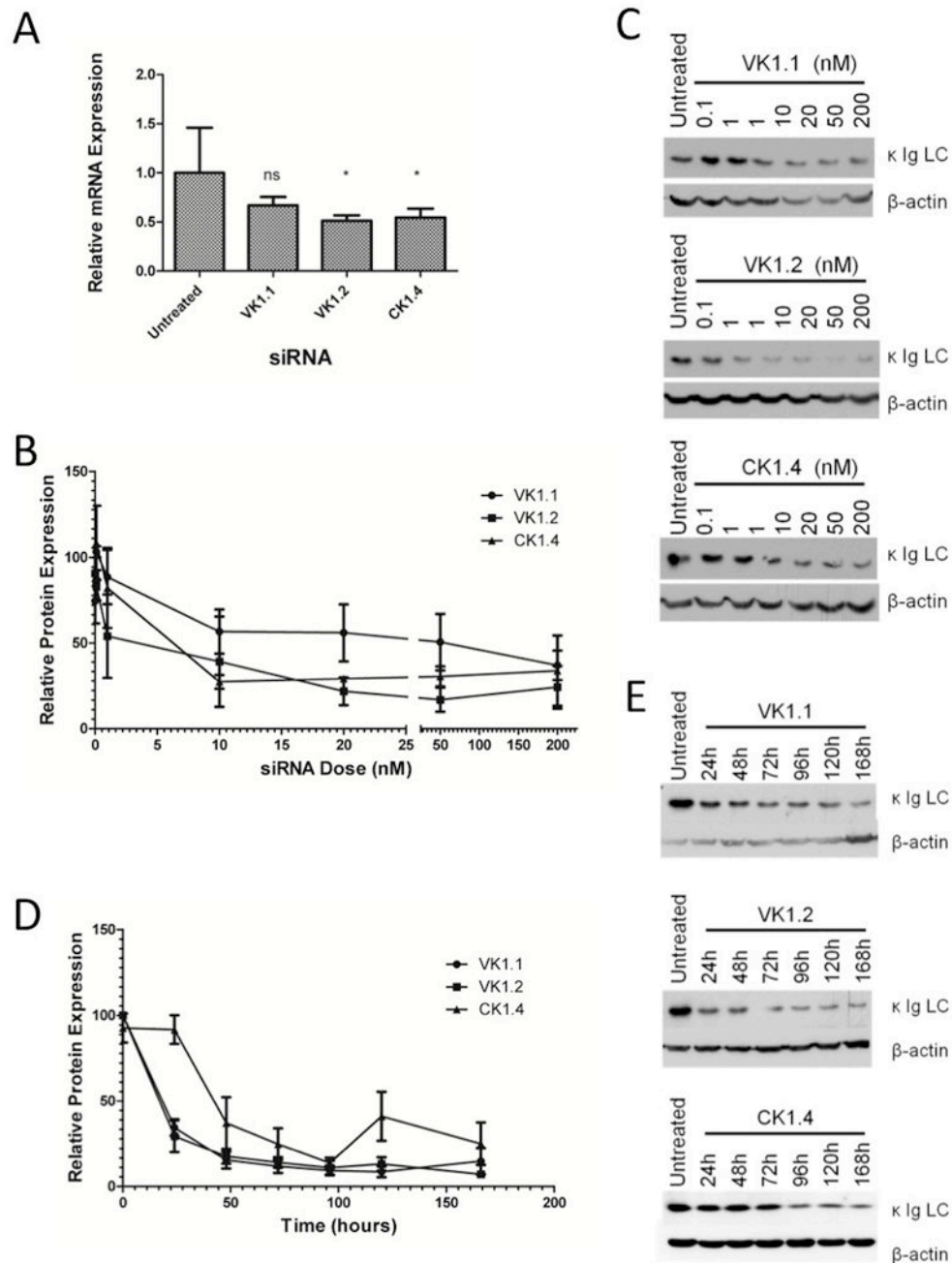


Figure 2. In vitro $\kappa 1$ mRNA and protein expression after siRNA treatment

(A) NIH-3T3 cells stably expressing AL-009- $\kappa 1$ were treated for 48 hours with 20nM of either VK1.1, VK1.2, or CK1.2 siRNAs; mean relative mRNA levels are depicted (* indicates $p < 0.5$). Reduction in cellular LC protein at 48 hours with increasing concentrations of siRNA are plotted in (B); representative immunoblots are shown in (C). Time course of reduction of protein levels cells following treatment with 20 nM of VK1.1, VK1.2 or CK1.2 siRNA are plotted in (D); representative immunoblots are shown (E).

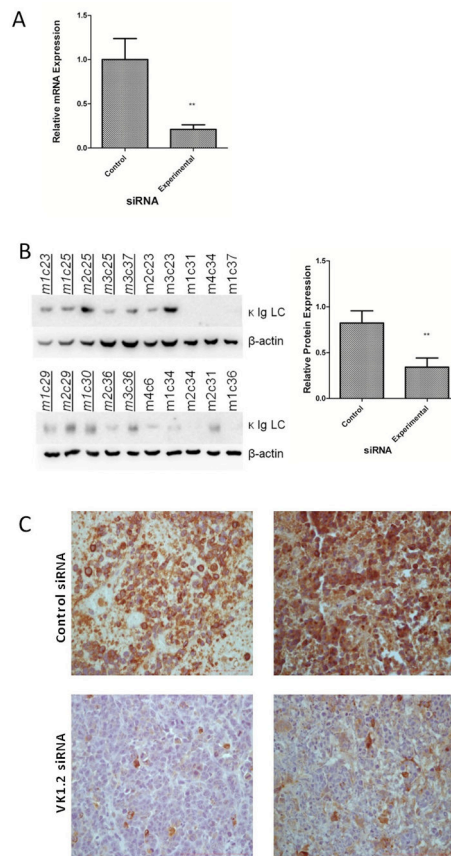


Figure 3. Effect of siRNA, delivered by *in vivo* electroporation, on plasmacytoma LC mRNA and protein levels

Plasmacytomas were formed over 2–3 weeks in mice by subcutaneous injection of SP2/0 cells transfected with human amyloidogenic LC. The plasmacytomas were then injected with 12 μ g of control or experimental (VK1.2) siRNA and *in vivo* electroporation was performed. 48 hours later, the mice were sacrificed for analysis and plasmacytoma tissue was collected. (A) Plasmacytoma κ 1 LC mRNA expression levels relative to Blimp1, a plasma cell specific marker, are depicted (** indicates $p = 0.0016$, $n = 10$ per group). (B) Plasmacytoma lysates immunoblotted for human κ 1 LC; each lane represents an individual plasmacytoma, control treated samples underlined and in italics. Data depicted as the mean for control siRNA vs. VK1.2 siRNA treated plasmacytoma LC protein levels (** indicates $p = 0.0051$, $n = 10$ per group). (C) Representative sections of plasmacytomas treated with control or experimental siRNA, brown staining for human κ 1 LC.

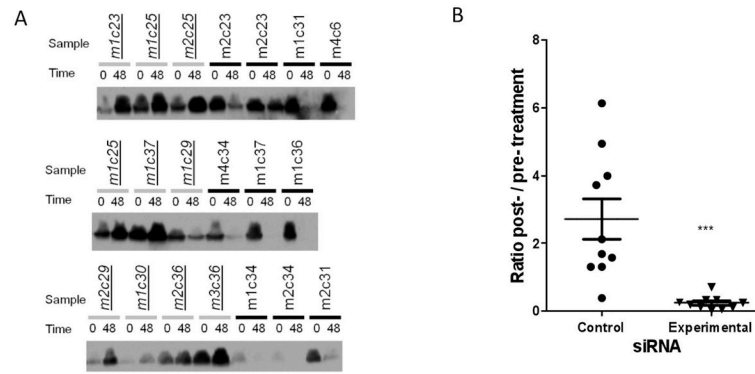


Figure 4. Effect of siRNA, delivered by *in vivo* electroporation, on circulating LC protein levels (A) Comparison by immunoblot of $\kappa 1$ LC levels in sera taken from the same mouse pre- and post-treatment (48 hours). Control-treated samples are underlined and italicized. (B) Graph depicting the ratio of post-treatment to pre-treatment circulating $\kappa 1$ Ig LC levels (post/pre) quantitated from the associated immunoblot for control and experimental siRNA treatment (***) $p = 0.0003$, $n = 10$ per group).