Systemic RNA Interference Therapy for Rheumatoid Arthritis Joints through Novel Nanopieces Delivery

Koichi Okamura[1,2], Brandon Vorrius[1], Yupeng Chen[1], Hongchuan Yu[1], Chathuraka T. Jayasuriya[1], Douglas C. Moore[1], Hirotaka Chikuda[2], Michael G. Ehrlich[1] and Qian Chen[1]

[1]Department of Orthopaedics, Rhode Island Hospital/Brown University, Providence, RI, USA, [2]Department of Orthopaedic Surgery, Gunma University, Maebashi, Gunma, Japan

Disclosures: Koichi Okamura (N), Brandon Vorrius (N), Yupeng Chen (4-Nanode Inc.), Hongchuan Yu (N), Chathuraka T. Jayasuriya (N), Douglas C. Moore (N), Hirotaka Chikuda (N), Michael G. Ehrlich (4-Nanode Inc.) , Qian Chen (4-Nanode Inc.).

INTRODUCTION: Rheumatoid arthritis (RA) is a chronic disease that causes joint inflammation, pain, and destruction. Typically, drugs including antibodies against cytokines such as TNF-α are used as a treatment for RA. Such drugs have been remarkable in improving the life quality of RA patients. However, these drugs are not effective for some patients. Other patients may develop drug resistance by forming antibodies against the protein drugs after long term use. Thus, new therapeutic approaches other than protein drugs against RA are needed. In the present study, our approach was to inhibit the TNF-α gene responsible for the progression of RA by delivering small interference RNA (siRNA) into RA afflicted mice. However, siRNAs are notoriously difficult to infiltrate joint by systemic delivery. This challenge was overcome by a novel Nanopieces (NPs) delivery system consisting of siRNA and non-covalent nanotubes of a small biomimetic molecule named JBaK (Janus Base with amine or lysine (K)).

METHODS: **Approvals**: Collagen induced arthritis (CIA) was generated in 8-week-old DBA/1J mice with approval from Institutional Animal Care and Use Committee (IACUC). **Systemic Delivery of siRNA**: ON-TARGETplus siRNA for mouse TNF-α (siTNF) or mouse non-target siRNA (scrRNA) were encapsulated within JBaK NPs and administered to CIA mice or non-CIA control mice, respectively, via retro-orbital injections twice per week from 21 days to 49 days after the first induction of arthritis. **Clinical evaluation**: Mice were evaluated using total arthritis score, paw thickness, and ankle swelling. Von Frey testing was performed to assess the mechanical nociception. **Gene expression analysis**: TNF-α gene expression was quantified using real-time RT-PCT; 18S and 36B4 ribosomal RNA were used for normalization. **Images and bone volume analysis**: X-ray images and high-resolution (10 μm isometric) 3D volume images using a desktop μCT scanner (MicroCT40, Scanco Medical. Tube Settings: 55 kVp and 145 μA. 300 ms integration time) were generated. Standard trabecular bone indices (e.g. volumetric density (BV/TV), bone mineral density (BMD), trabecular number, trabecular thickness and trabecular separation of the subchondral bone) were calculated from manually-outlined volumes of interest in the distal femur and proximal tibia using the scanner's built-in analysis routines. **Statistics**: Mann-Whitney U test, One-way ANOVA and Turkey's post-hoc analysis were used for statistical analysis. Error bars represent one standard error (SE) of the mean.

RESULTS: After systemic delivery of siTNF in NPs via retro-orbital injections for two times, at the time of week 4, the TNF-α mRNA expression levels were significantly suppressed in peritoneal exudate macrophages (PECs) from abdominal cavity, knee joints, and hind paws in comparison to NP delivery of scrambled siRNA in CIA mice (**Fig. 1 A, B, C**). NP systemic delivery achieved 96% and 90% knockdown of TNF-α mRNA levels in knee and hind paw joints respectively, indicating NP delivery can achieve highly efficient RNAi in joint tissues. Total arthritis score was significantly reduced in siTNF treatment group in comparison to the sham treatment group (scrRNA) after CIA induction for 7 and 8 weeks (**Fig.2**). In addition, siTNF mice had higher mechanical nociception threshold than scrRNA mice. siTNF treatment significantly inhibited bone erosions, and joint destructions and reduction of volumetric density, bone mineral density, and trabecular number and thickness in the CIA mice (**Fig.3**).

DISCUSSION: In the present study, we demonstrate that 1) Systemic delivery of siRNA by NPs can achieve high efficacy (>90%) in knocking down TNF-α gene expression in joint tissues, indicating its superiority in infiltrating peripheral joints for treatment of joint diseases; and 2) Knocking down TNF-α mRNA in the joint tissues reduced the severity of inflammation and joint swelling, increased the threshold for the mechanical pain, and inhibited bone erosion and reduction of joint destruction and BMD in arthritis mice. Since such RNAi therapy utilizes encapsulated RNA rather than protein as a therapeutic, it may circumvent the protein drug resistance problem for some patients. Furthermore, RNAi therapy inhibits the synthesis of cytokines rather than neutralizing them after they are made, it may be used as an alternative approach for RA therapy in the cases protein drug is ineffective.

SIGNIFICANCE: This is the first study to demonstrate that RNAi/NP therapy is highly efficacious in inhibiting cytokine expression in the joint and progression of arthritis in mouse RA model. This systemic siRNA administration technology has great potential to treat RA patients if the results in humans match those in mice.

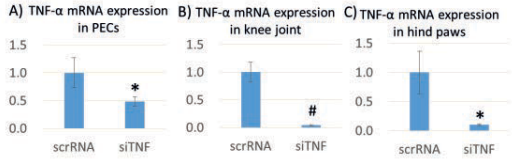

Fig.1 TNF-α mRNA expression of each group in PECs from abdominal cavity (A), knee joints (B) and hind paws (C) after twice injection of siRNA/NPs. In the mice with siTNF, the TNF-α mRNA expression of was significantly knocked down. PECs: Peritoneal exudate cell macrophages, scrRNA: non-target siRNA, siTNF: TNF-α siRNA, CIA: collagen induced arthritis, * $p < 0.05$, # $P < 0.005$

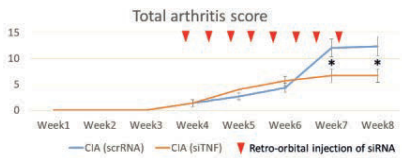

Fig.2 Total arthritis score of CIA mice with each treatment.
scrRNA: non-target siRNA, siTNF: TNF-α siRNA, CIA: collagen induced arthritis, * $p < 0.05$

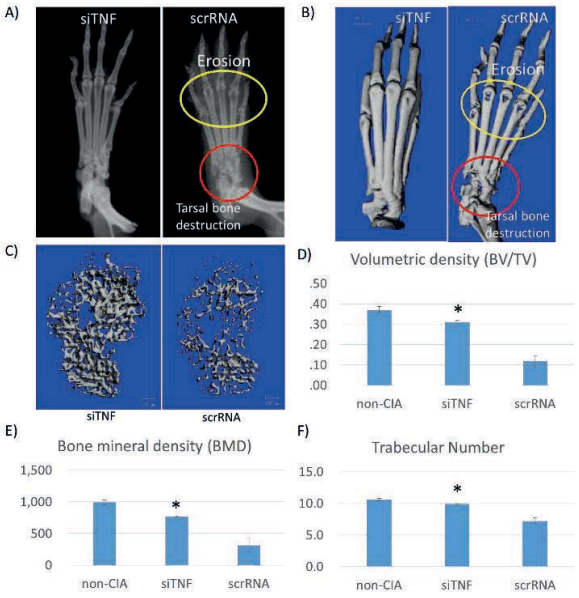

Fig.3 Representative X-ray (A) and μCT (B) images of hind paws with each treatment. Microstructure of subchondral cancellous bone at tibia (knee joint) (C). The volumetric density (D), BMD (E) and trabecular trabecular thickness (F) were significantly higher in siTNF mice compared to scrRNA mice. siTNF: TNF-α siRNA, scrRNA: non-target siRNA, BV: Bone Volume, TV: Total Volume, CIA: collagen induced arthritis, * $P < 0.005$

Systemic RNA Interference Therapy for Rheumatoid Arthritis Joints through Novel Nanopieces Delivery

Koichi Okamura[1,2], Brandon Vorrius[1], Yupeng Chen[1], Hongchuan Yu[1], Chathuraka T. Jayasuriya[1], Douglas C. Moore[1], Hirotaka Chikuda[2], Michael G. Ehrlich[1] and Qian Chen[1]

[1]Department of Orthopaedics, Rhode Island Hospital/Brown University, Providence, RI, USA, [2]Department of Orthopaedic Surgery, Gunma University, Maebashi, Gunma, Japan

Disclosures: Koichi Okamura (N), Brandon Vorrius (N), Yupeng Chen (4-Nanode Inc.), Hongchuan Yu (N), Chathuraka T. Jayasuriya (N), Douglas C. Moore (N), Hirotaka Chikuda (N), Michael G. Ehrlich (4-Nanode Inc.) , Qian Chen (4-Nanode Inc.).

INTRODUCTION: Rheumatoid arthritis (RA) is a chronic disease that causes joint inflammation, pain, and destruction. Typically, drugs including antibodies against cytokines such as TNF-α are used as a treatment for RA. Such drugs have been remarkable in improving the life quality of RA patients. However, these drugs are not effective for some patients. Other patients may develop drug resistance by forming antibodies against the protein drugs after long term use. Thus, new therapeutic approaches other than protein drugs against RA are needed. In the present study, our approach was to inhibit the TNF-α gene responsible for the progression of RA by delivering small interference RNA (siRNA) into RA afflicted mice. However, siRNAs are notoriously difficult to infiltrate joint by systemic delivery. This challenge was overcome by a novel Nanopieces (NPs) delivery system consisting of siRNA and non-covalent nanotubes of a small biomimetic molecule named JBaK (Janus Base with amine or lysine (K)).

METHODS: **Approvals**: Collagen induced arthritis (CIA) was generated in 8-week-old DBA/1J mice with approval from Institutional Animal Care and Use Committee (IACUC). **Systemic Delivery of siRNA**: ON-TARGETplus siRNA for mouse TNF-α (siTNF) or mouse non-target siRNA (scrRNA) were encapsulated within JBaK NPs and administered to CIA mice or non-CIA control mice, respectively, via retro-orbital injections twice per week from 21 days to 49 days after the first induction of arthritis. **Clinical evaluation**: Mice were evaluated using total arthritis score, paw thickness, and ankle swelling. Von Frey testing was performed to assess the mechanical nociception. **Gene expression analysis**: TNF-α gene expression was quantified using real-time RT-PCT; 18S and 36B4 ribosomal RNA were used for normalization. **Images and bone volume analysis**: X-ray images and high-resolution (10 μm isometric) 3D volume images using a desktop μCT scanner (MicroCT40, Scanco Medical. Tube Settings: 55 kVp and 145 μA. 300 ms integration time) were generated. Standard trabecular bone indices (e.g. volumetric density (BV/TV), bone mineral density (BMD), trabecular number, trabecular thickness and trabecular separation of the subchondral bone) were calculated from manually-outlined volumes of interest in the distal femur and proximal tibia using the scanner's built-in analysis routines. **Statistics**: Mann-Whitney U test, One-way ANOVA and Turkey's post-hoc analysis were used for statistical analysis. Error bars represent one standard error (SE) of the mean.

RESULTS: After systemic delivery of siTNF in NPs via retro-orbital injections for two times, at the time of week 4, the TNF-α mRNA expression levels were significantly suppressed in peritoneal exudate macrophages (PECs) from abdominal cavity, knee joints, and hind paws in comparison to NP delivery of scrambled siRNA in CIA mice (**Fig. 1 A, B, C**). NP systemic delivery achieved 96% and 90% knockdown of TNF-α mRNA levels in knee and hind paw joints respectively, indicating NP delivery can achieve highly efficient RNAi in joint tissues. Total arthritis score was significantly reduced in siTNF treatment group in comparison to the sham treatment group (scrRNA) after CIA induction for 7 and 8 weeks (**Fig.2**). In addition, siTNF mice had higher mechanical nociception threshold than scrRNA mice. siTNF treatment significantly inhibited bone erosions, and joint destructions and reduction of volumetric density, bone mineral density, and trabecular number and thickness in the CIA mice (**Fig.3**).

DISCUSSION: In the present study, we demonstrate that 1) Systemic delivery of siRNA by NPs can achieve high efficacy (>90%) in knocking down TNF-α gene expression in joint tissues, indicating its superiority in infiltrating peripheral joints for treatment of joint diseases; and 2) Knocking down TNF-α mRNA in the joint tissues reduced the severity of inflammation and joint swelling, increased the threshold for the mechanical pain, and inhibited bone erosion and reduction of joint destruction and BMD in arthritis mice. Since such RNAi therapy utilizes encapsulated RNA rather than protein as a therapeutic, it may circumvent the protein drug resistance problem for some patients. Furthermore, RNAi therapy inhibits the synthesis of cytokines rather than neutralizing them after they are made, it may be used as an alternative approach for RA therapy in the cases protein drug is ineffective.

SIGNIFICANCE: This is the first study to demonstrate that RNAi/NP therapy is highly efficacious in inhibiting cytokine expression in the joint and progression of arthritis in mouse RA model. This systemic siRNA administration technology has great potential to treat RA patients if the results in humans match those in mice.

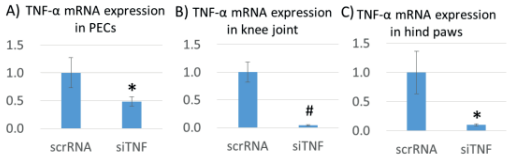

Fig.1 TNF-α mRNA expression of each group in PECs from abdominal cavity (A), knee joints (B) and hind paws (C) after twice injection of siRNA/NPs. In the mice with siTNF, the TNF-α mRNA expression of was significantly knocked down. PECs: Peritoneal exudate cell macrophages, scrRNA: non-target siRNA, siTNF: TNF-α siRNA, CIA: collagen induced arthritis, * $p < 0.05$, # $P < 0.005$

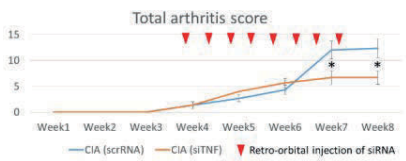

Fig.2 Total arthritis score of CIA mice with each treatment. scrRNA: non-target siRNA, siTNF: TNF-α siRNA, CIA: collagen induced arthritis, * $p < 0.05$

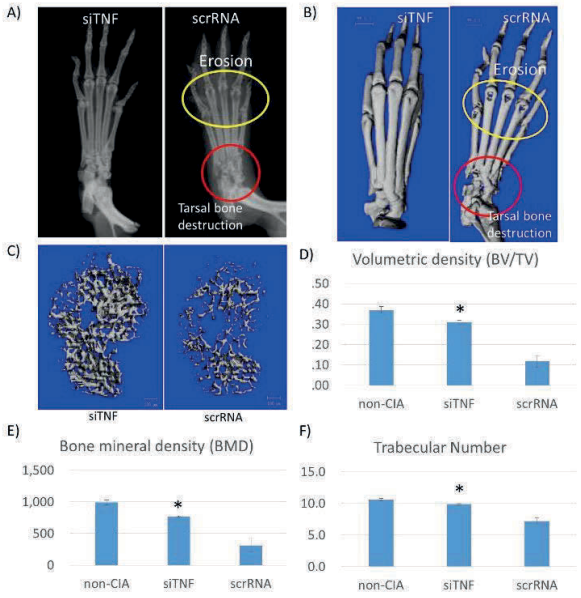

Fig.3 Representative X-ray (A) and μCT (B) images of hind paws with each treatment. Microstructure of subchondral cancellous bone at tibia (knee joint) (C). The volumetric density (D), BMD (E) and trabecular trabecular thickness (F) were significantly higher in siTNF mice compared to scrRNA mice. siTNF: TNF-α siRNA, scrRNA: non-target siRNA, BV: Bone Volume, TV: Total Volume, CIA: collagen induced arthritis, * $P < 0.005$