# Elevated LINE-1 Expression in SLE Keratinocytes Leads to LINE-1 Reverse Transcriptase-dependent Type I Interferon Responses

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## Background/Purpose

Long Interspersed Element-1 (LINE-1) are retrotransposable DNA elements that make up ~17% of the human genome, and their role in health and disease is still being evaluated. Published studies have suggested that LINE-1 may contribute to the development and progression of autoimmune diseases such as lupus by triggering the type I interferon (IFN) production via activation of the innate immune system through nucleic acid sensing pathways. Additionally, studies have shown that lupus patients have higher level of LINE-1 in kidneys and blood compared to healthy controls, but expression of LINE-1 in SLE skin, where type I IFN signatures are dominant, has not been studied. We thus examined LINE-1 RNA and protein expression in lupus patient skin biopsies, and the impact on inflammatory signals upon a LINE-1 reverse transcriptase (RT) inhibitor in cellular and murine models.

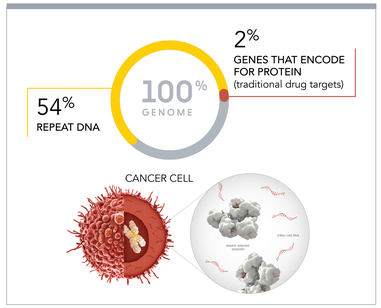
## Methods

All human subjects gave informed, written consent for the study. Formalin-fixed, paraffin embedded skin sections from non-lesional, non-sun exposed and UVB-treated skin were examined for changes in interferon-stimulated gene (ISG) expression via RNA-seq, LINE-1 ORF1p and phospho-STING via immunohistochemistry, and for LINE-1 ORF1 and ORF2 transcripts by RNA-scope. A novel, potent LINE-1 RT inhibitor, RPT-A, was characterized using a LINE-1 RT enzyme assay, a cellular LINE-1 retrotransposition assay, a cellular model of Aicardi-Goutières syndrome, and a UV-irradiated keratinocyte model. We also studied the impact of RPT-A on disease in a TREX1 knockout interferonopathy mouse model.

## Results

- Non-lesional biopsies from SLE skin exhibit increased ORF1p protein and increased ORF2 transcript staining and a concurrent increase in phospho-STING staining, suggestive of activation of nucleic acid sensing pathways. UV stimulation increased expression of LINE-1 transcript and protein in both healthy control and SLE skin.
- Tri-phosphorylated form of RPT-A inhibited enzymatic activity of LINE-1 RT with an $IC_{50}$ of 0.03 μM. RPT-A inhibited cellular LINE-1 retrotransposition, decitabine-induced interferon response in TREX1 deficient THP-1 cells, and UV-induced phospho-TBK1 in human HaCaT keratinocytes.
- LINE-1 knockdown with shRNA and siRNA in the THP1 and HaCaT cells mimicked the inhibitory effect of RPT-A.
- TREX1 knockout mice dosed with RPT-A orally once a day for 28 days exhibited reduced serum anti-dsDNA antibodies, reduced myocardial ISGs and reduced immune infiltrates in the heart and kidney.

## Repeat elements are only active in diseased cells




**REPEATS:**

54% of human genome is comprised of repetitive nucleic acid sequences derived from viruses and retrotransposons that have integrated into our DNA during evolution – part of the genome considered "dark"

**REPEATS ARE "OFF" IN HEALTHY CELLS:**

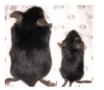
Mostly dormant in healthy cells, repeats are only "activated" and transcribed when cells are injured or diseased

## 2 RPT-A is a LINE-1 reverse transcriptase inhibitor and inhibits the cGAS/TBK1/interferon pathway

| Assay | Experimental system | $IC_{50}$ (μM) |
|---|---|---|
| LINE-1 RT enzyme assay[1] | Recombinant LINE-1 RT | 0.048 |
| LINE-1 retrotransposition assay | HeLa Cis-AI[2] reporter cells | 0.001 |
| Decitabine-induced IFN reporter assay | THP1-TREX1 KO cells (cellular model of Aicardi-Goutières syndrome) | 0.049 |
| UV-induced pTBK1 | Human HaCaT keratinocyte cell line | 0.30 |

[1]Assay performed with RPT-A triphosphate
[2]AI: Antisense Intron

## 5 Murine TREX1 knockout Aicardi-Goutières Syndrome model



wildtype mutant

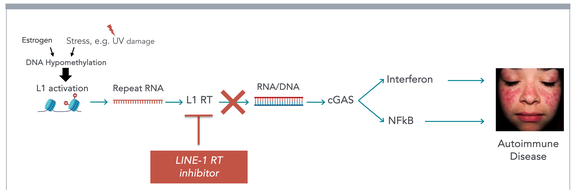
**TREX-1 KO**

- elevated IFN/ISGs
- anti-dsDNA antibodies
- stunted growth
- cardiomyopathy

**AGS patient**

- elevated IFN/ISGs
- anti-dsDNA antibodies
- small head size
- encephalopathy
- skin lesions

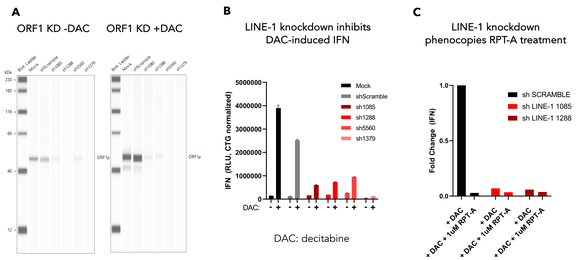
## LINE-1 reverse transcriptase inhibitors (RTIs) for autoimmune disease




**AUTOIMMUNE DISEASE, such as**

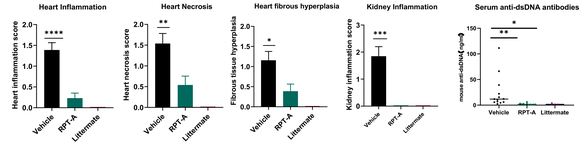
- Type I interferonopathies
- SLE
- Inflammatory Bowel Disease
- Psoriasis
- Myositis
- Systemic sclerosis
- Sjogren syndrome
- Aicardi-Goutières syndrome

## 3 LINE-1 knockdown blocks decitabine-induced IFN in THP1-TREX1 KO cells

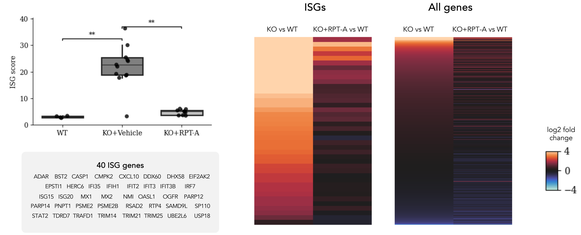



## 6 RPT-A is highly efficacious in the murine TREX1 KO interferonopathy model

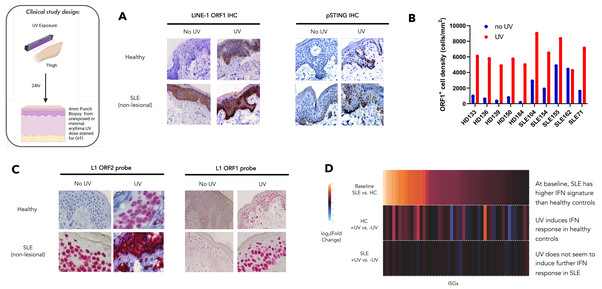
28 days of 100 mg/kg QD dosing initiated when TREX1 KO mice were 4-5 weeks of age




## 7 RPT-A reduces elevated IFN signature genes in the heart of TREX1 KO mice

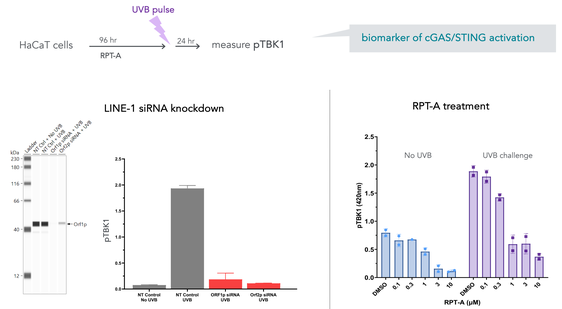



## 1 LINE-1, pSTING and ISGs are elevated in SLE skin and induced by UV in healthy & SLE skin




**(A)** LINE-1 and pSTING are elevated in SLE skin and induced by UV in healthy & SLE skin
**(B)** ORF1+ cell density in epidermis
**(C)** LINE-1 RNA is elevated in SLE skin and induced by UV in healthy and SLE
**(D)** ISGs are elevated in SLE skin & increase with UV in healthy control (HC) skin

## 4 LINE-1 knockdown blocks UVB-induced pTBK1 in human HaCaT keratinocytes




## Conclusions

LINE-1 RNA and protein are increased in SLE skin and is induced by UV exposure. Inhibition of LINE-1 RT activity results in decreased ISG expression and improved disease activity in a murine interferonopathy model. Inhibition of LINE-1 reverse transcriptase holds promise as a novel therapy for diseases in which type I IFNs drive disease.