Novel LINE-1 Reverse Transcriptase Inhibitors Can Suppress Type I Interferon Responses and Are Promising Therapeutics for Lupus

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Background/Purpose

Long Interspersed Element-1 (LINE-1) retrotransposon encodes for two proteins, ORF1p and ORF2p. ORF1p is a chaperone protein while ORF2p contains reverse transcriptase (RT) and endonuclease activities. LINE-1 RT can reverse transcribe LINE-1 and other RNAs into RNA:DNA hybrids and double stranded DNA. These nucleic acid products can trigger the cGAS/STING pathway to induce Type I interferon (IFN) response. LINE-1 is quiescent in healthy tissues but can be induced under pathological conditions and cellular stress. We have previously shown that higher levels of LINE-1 protein and RNA are present in SLE skin, and that a LINE-1 RT inhibitor (RTI) can block cGAS/STING-mediated IFN response. In this study we investigated the ability of LINE-1 RTIs to suppress Type I IFN responses in human skin explants and in a murine interferonopathy model. In addition, we have developed a UV-B skin challenge model in healthy volunteers to use in Phase 1 clinical studies.

Methods

LINE-1 RTIs, RPT-A and RPT-B, were characterized by using a LINE-1 RT polymerase biochemical assay and various cellular assays. We assessed the impact of *ex vivo* treatment with the inhibitors on UV-induced IFN responses in skin explants. We also studied the efficacy of these inhibitors in an interferonopathy mouse model (TREX1 knockout mice). Finally, we conducted a clinical study in which the skin of 10 healthy subjects was irradiated with UV-B on two different study days, two weeks apart, to investigate UV-induced skin inflammation and IFN response. All human subjects gave informed, written consent for the study. The inflammatory reaction was monitored using non-invasive imaging and skin biopsies were collected and examined for interferon-stimulated genes (ISG) expression.

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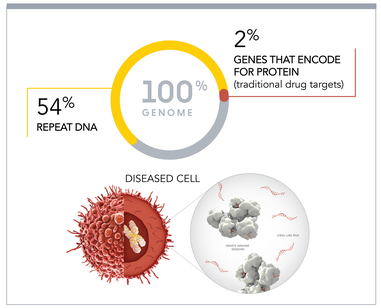
Results

RPT-A and RPT-B potently inhibited the polymerase activity of LINE-1 RT, as well as cellular LINE-1 retrotransposition and UV-induced pTBK1 in human HaCaT keratinocytes. *Ex vivo* treatment of skin explants from healthy subjects with the inhibitors suppressed UV-induced sunburned cells and ISG expression. Five to six-week-old TREX1 knockout mice dosed orally with RPT-A and RPT-B showed reduced serum anti-dsDNA antibodies, heart and kidney immune infiltrates, and myocardial ISGs. In the healthy volunteer UV challenge study, UV-B increased erythema and perfusion as assessed by imaging which reached peak induction by 6 hours post UV and remained elevated 24 hours post UV. The level of induction is consistent between subjects and between the two periods of UV provocation. RNA-seq data of the skin biopsies revealed ISG induction at 24 hours post UV in both periods.

Repeat elements are only active in diseased cells



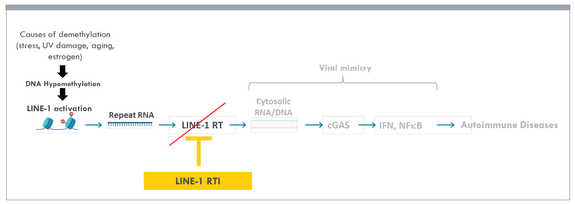
REPEATS:

54% of our genome 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

LINE-1 reverse transcriptase inhibitors (RTIs) represent a novel potentially non-immunosuppressive therapy



AUTOIMMUNE DISEASE, such as

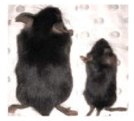
- Type I interferonopathies
- SLE
- Inflammatory Bowel Disease
- Psoriasis
- Myositis
- Systemic sclerosis
- Sjogren syndrome
- Aicardi-Goutières syndrome

1 RPT-A and RPT-B are potent LINE-1 RTIs that suppress the cGAS/TBK1/interferon pathway in cells

Assay	Experimental system	RPT-A IC_{50} (μM)	RPT-B IC_{50} (μM)
LINE-1 RT enzyme assay[1]	Recombinant LINE-1 RT	0.048	0.31
LINE-1 retrotransposition assay	HeLa Cis-AI[2] reporter cells	0.001	0.05
UV-induced pTBK1	Human HaCaT keratinocyte cell line	0.30	2.95

[1]Assay performed with RPT-A and RPT-B triphosphate
[2]AI: Antisense Intron; Xie, Y. *et al.* Cell division promotes efficient retrotransposition in a stable L1 reporter cell line. *Mob. DNA* **4**, 10 (2013)

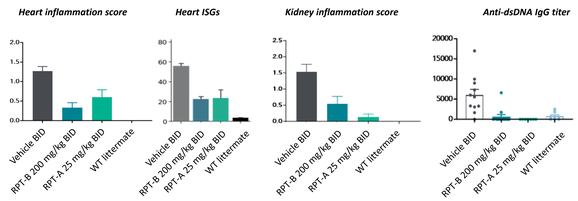
2 Murine TREX1 knockout Aicardi-Goutières Syndrome interferonopathy model



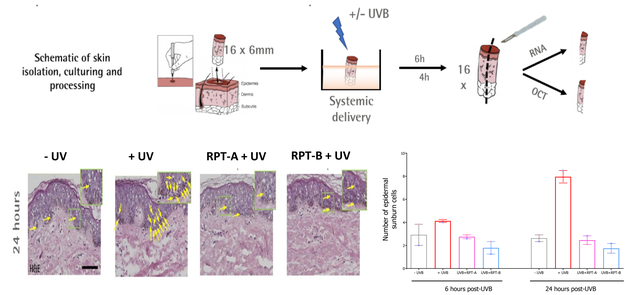
TREX-1 KO	AGS patient
• elevated IFN/ISGs	• elevated IFN/ISGs
• anti-dsDNA antibodies	• anti-dsDNA antibodies
• stunted growth	• small head size
• cardiomyopathy	• encephalopathy
	• skin lesions

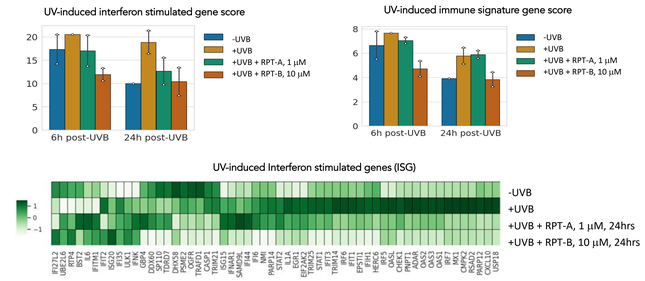
RPT-A and RPT-B are highly efficacious in the murine TREX1 KO interferonopathy model

Dosing initiated when TREX1 KO mice were 4-5 weeks of age

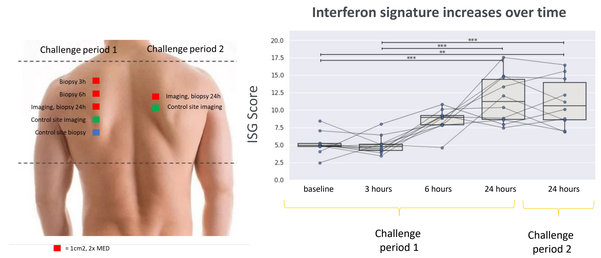


3 LINE-1 RTIs inhibit UV-induced interferon signaling in human skin explant cultures

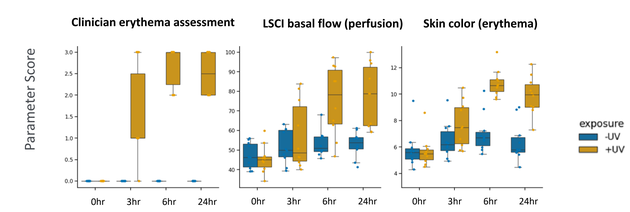




4 A single-center study to characterizing the response of UVB skin response in healthy volunteers demonstrates ISG and inflammation induction as measured by RNA and quantitative, non-invasive imaging markers



* p = [0.01, 0.05]; ** p = [-.001, 0.01]; *** p = [0.0001, 0.001]



Challenge Period 1: Subjects are exposed to UVB light (1 cm2, 2XMED) and biopsies and imaging parameters obtained at 3, 6 and 24hrs post-UVB
Challenge Period 2: Subjects are exposed to UVB light (1 cm2, 2XMED) 2 weeks after period 1 UVB exposure

*Full biopsy analysis of mRNA still pending

Conclusions

- RPT-A and RPT-B are potent LINE-1 RT inhibitors.
- Inhibition of LINE-1 RT activity results in suppression of UV-induced IFN response in skin explants, and decreased disease activity in a murine interferonopathy model.
- Our non-interventional clinical study demonstrated the feasibility of using UV provocation in healthy volunteers to increase skin IFN signaling, enabling a future proof-of-mechanism clinical study for LINE-1 RTIs.
- Together, inhibition of LINE-1 reverse transcriptase holds promise as a novel potentially non-immunosuppressive therapy for Type I interferon driven diseases.