Selective ITK Blockade Modulates T Helper Cell Function and Is Efficacious in Preclinical Models of T-cell Mediated Inflammatory Disease

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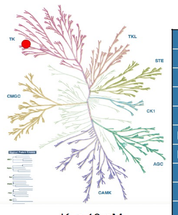
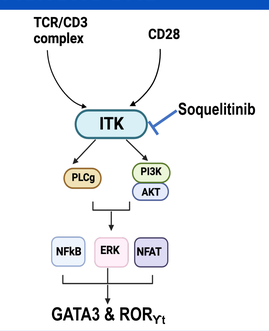
BACKGROUND

Interleukin-2 inducible T cell kinase (ITK) is critical for T cell receptor (TCR) signaling and T helper (Th) cell differentiation.

Aim- In this study, we investigated the activity of a selective, covalent drug, soquelitinib (SQL) in models of immune mediated diseases.



	SQL Kd in nM
ITK	2.5
BLK	4700
BMX	9100
BTK	1200
EGFR	>10000
ERBB2	>10000
ERBB4	>10000
JAK3	2800
MKK7	>10000
TEC	540
RLK	2700



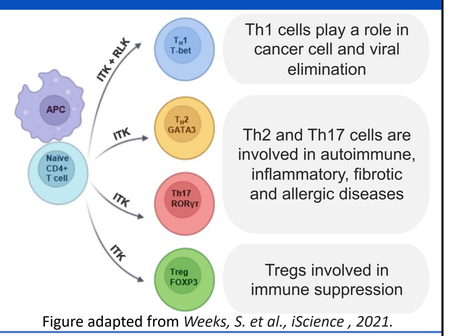


Figure adapted from *Weeks, S. et al., iScience , 2021.*

METHODS

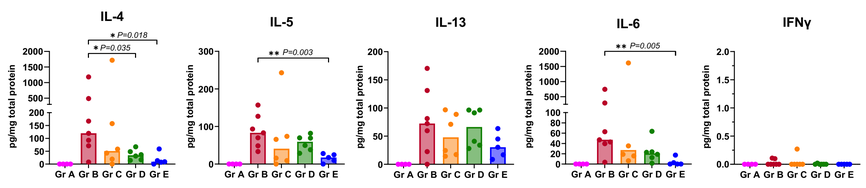
In vivo dosing: In acute asthma and bleomycin-induced fibrosis models, soquelitinib was formulated in a solution and administered to mice at doses of either 10 or 30mg/kg by oral gavage. In chronic asthma and imiquimod-induced psoriasis models, animals were treated with chow diets containing soquelitinib. Three chow formulations of 1.2g SQL/kg chow (low dose); 2.4g SQL/kg chow (intermediate dose) and 7.2g SQL/kg chow (high dose) were evaluated. **Measurement of cytokines:** Cytokines in Bronchoalveolar lavage fluid (BALF) and cell culture supernatants from mouse, human Th differentiated cells were analyzed by MSD Multiplex assay kits. Mouse, and Human CD4 naïve cells (CD44hiCD62L^{low}) & human (CD45RA+CCR7+) were sorted, cultured for Th17 differentiation condition [IL-6 (10 ng/ml), IL-1b (10 ng/ml), IL-23 (10 ng/ml), TGF-b (10 ng/ml), anti-IFNg (10 µg/ml), anti-IL-4 (10 µg/ml)]. **Statistics:** Statistical analysis between two groups were performed using unpaired t test or Mann Whitney test, and between multiple groups using One way ANOVA. * p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001.

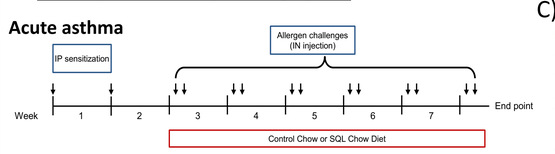
SOQUELITINIB TREATMENT SUPPRESSES TH2 CYTOKINES IN OVALBUMIN INDUCED ASTHMA MODEL

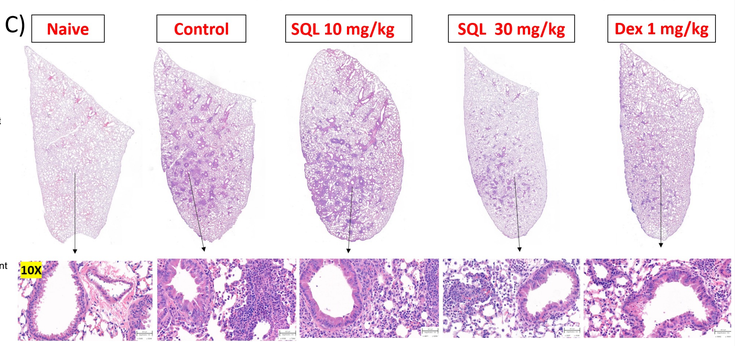
Fig. 1

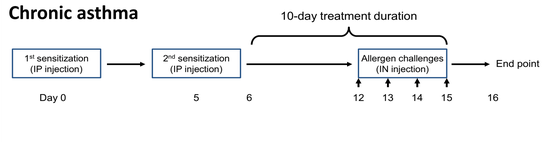
A)

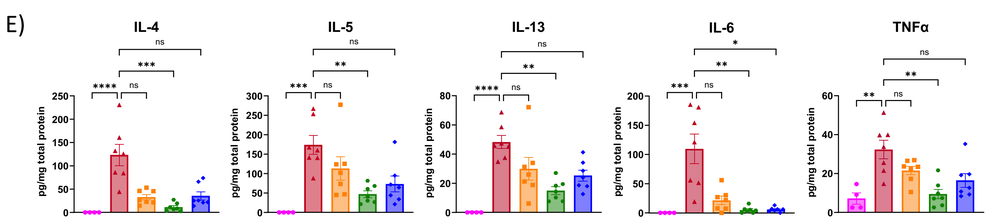
Group	Challenge	Treatment
A	PBS	Naïve
B	Ovalbumin	Control
C	Ovalbumin	SQL, 10 mg/kg
D	Ovalbumin	SQL, 30 mg/kg
E	Ovalbumin	Dexamethasone, 1 mg/kg







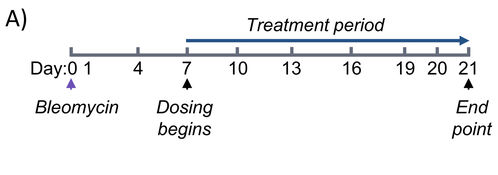




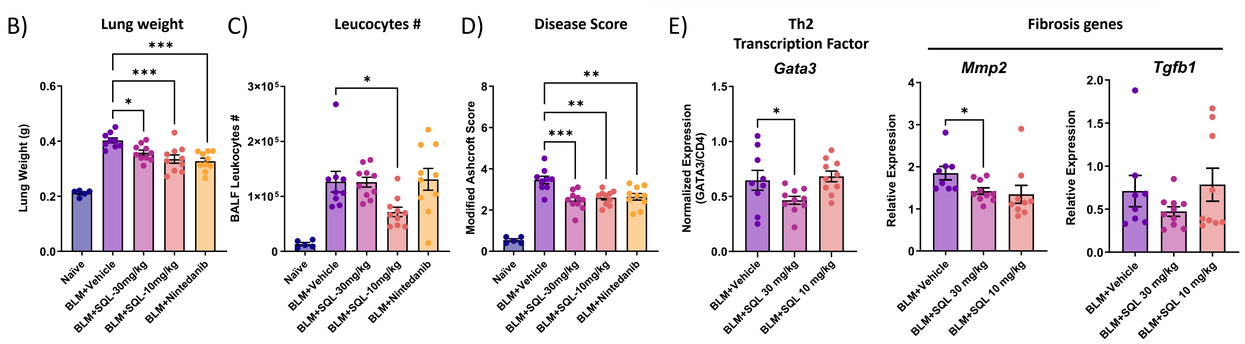
SQL 30 mg/kg dose significantly reduced the levels of Th2 cytokine IL-4 in OVA induced acute asthma model, whereas in the chronic asthma model, SQL intermediate dose was found to suppress Th2 associated and inflammatory cytokines IL-4, IL-5, IL-13, IL-6 and TNF-alpha levels significantly in BALF. Histologically, SQL reduced airway inflammation similar to that of dexamethasone treatment. n=4-7 per group.

SOQUELITINIB TREATMENT REDUCES BLEOMYCIN-INDUCED FIBROSIS

Fig. 2



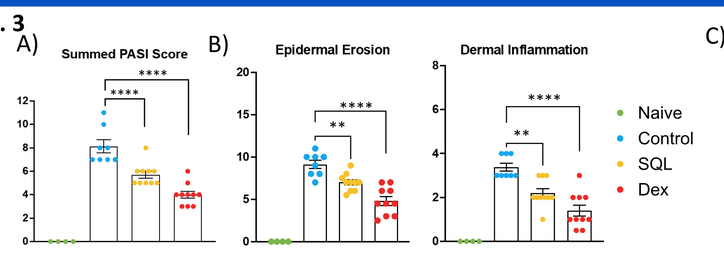
Group	Challenge	Treatment
1	PBS	Naïve
2	Bleomycin 1.5 U/kg oropharyngeal dose	Vehicle
3		SQL 30 mg/kg
4		SQL 10 mg/kg
5		Nintedanib 50 mg/kg

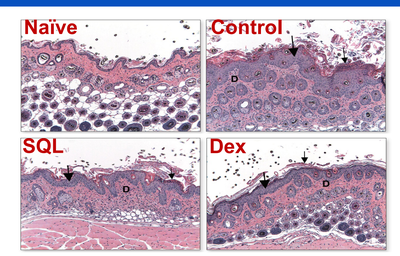


Bleomycin induced fibrosis disease model (Th2 mediated) schematic and Experimental groups (A) are mentioned above. Pharmacological treatment using soquelitinib significantly reduced the absolute lung weight (B) at the doses tested in this model. Intermediate dose of SQL significantly inhibited the number of infiltrating leukocyte collected from BALF samples (C), whereas the severity of fibrosis disease score (D) (Modified Ashcroft scores) was reduced at both doses. Analysis of the mRNA levels of the Th2 transcription factor GATA-3 and fibrosis-associated gene MMP2 in lung tissues (E) were found to be significantly downregulated at 30 mg/kg dose of SQL. Tissue mRNA levels were analyzed by quantitative RT-PCR from 9-10 mice in each group. CD4 was used for normalization of infiltrating CD4 T cell numbers. Fold changes of two fibrosis-associated genes relative to vehicle control are shown. n=4-10 per group.

SOQUELITINIB IS EFFECTIVE IN TREATING TH17 MEDIATED MODEL OF IMIQUIMOD-INDUCED PSORIASIFORM SKIN LESIONS

Fig. 3

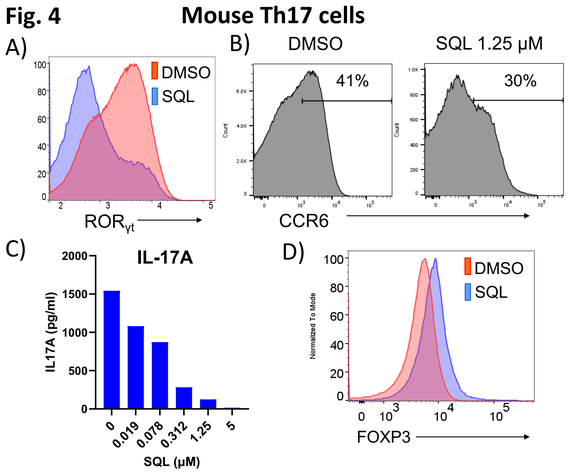




Soquelitinib is found to be efficacious in Th17 mediated disease model of psoriasis. SQL significantly reduced overall disease score [Mice skin Psoriasis Area and Severity Index (PASI) scores] (A) which is a summed score of erythema, scaling, & skin thickening. Soquelitinib reduces epidermal erosion & dermal inflammation (B) which is evident in the representative H&E stained skin sections (C) above, n=4-10 per group.

SOQUELITINIB CAN EFFECTIVELY SUPPRESS THE TH17 PHENOTYPE

Fig. 4 Mouse Th17 cells



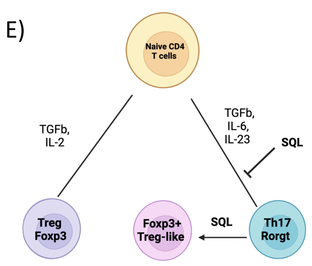
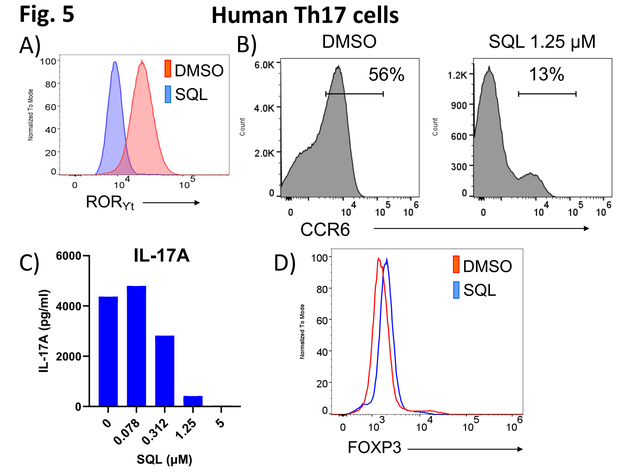


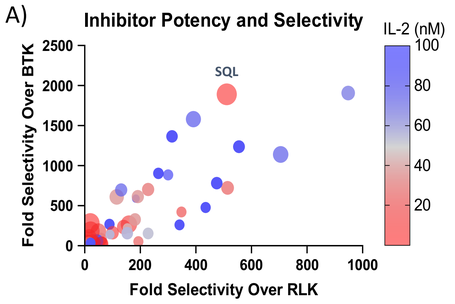
Fig. 5 Human Th17 cells

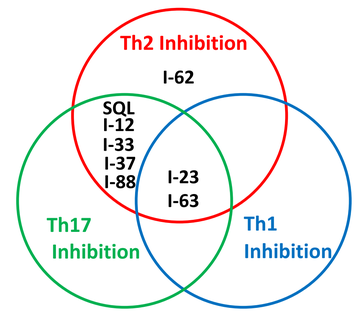


SQL treatment inhibited expression of ROR$_{yt}$, a Th17 defining Transcription factor (A) and CCR6, a chemokine receptor important for Th17 cell migration (B), and IL-17A production (C) in Th17 differentiated cells. Moreover, upregulation of FOXP3 (D) was observed in Th17 cells treated with SQL 1.25 µM concentration. Fig. 4,5. Differentiation of sorted naïve CD4 T cells from Balb/c mouse and healthy human donors at day 6. (E) Working model: Selective ITK inhibition by soquelitinib impaired Th17 cell differentiation and promoted Treg-like phenotype with increased Foxp3 expression. Similar finding was observed in the August's group (See reference 2).

NEXT-GENERATION ITK INHIBITORS WITH DIFFERENT BIOLOGICAL PROPERTIES

Fig. 6





Several next-generation selective ITK inhibitors have been synthesized. (A) Feature plot comparing selectivity over two related Tec kinases (BTK and RLK) and potency of IL-2 inhibition among different ITK inhibitors (larger circle = higher potency; all compounds have IC50<17nM). Each circle represents individual compound. Color of the circle indicates the range of IC50 in IL2 blocking assay. (B) Venn diagram illustrating different biological properties of the next-gen ITK inhibitors in T cell differentiation assays. Results suggest that chemical structures may be refined to perform more specific biologic functions, enabling targeting of various disease types. I-number indicates individual compounds.

CONCLUSIONS

- Soquelitinib (SQL) is a selective ITK inhibitor, which demonstrated efficacy in several models of immune mediated diseases. It recapitulates the published results in ITK-/- mice in Th2/Th17 disease models.
  - ➢ In acute and chronic OVA induced asthma model, Soquelitinib significantly suppressed Th2 cytokines, inflammatory cytokines.
  - ➢ In bleomycin induced lung fibrosis model, SQL treatment significantly decreased the severity of fibrosis (Ashcroft Score), as well as reduced the mRNA levels of the Th2 transcription factor GATA-3 and fibrosis-associated gene MMP2 in lung tissues.
  - ➢ SQL treatment was found to be efficacious in the Th17 mediated model of imiquimod-induced skin inflammation, where it significantly reduced the total disease score (PASI score), epidermal erosion & dermal inflammation.
- Soquelitinib treatment modulated the Th17 differentiation, by inhibition of Th17 transcription factor RORγt, chemokine receptor CCR6 and IL-17A production in both mouse and human Th17 differentiated cells.
- Next-generation ITK inhibitors have been identified and exhibit precise biologic properties.

REFERENCES
1). Weeks, S., Harris, R. Karimi, M. Targeting ITK signaling for T cell-mediated diseases. iScience, August, 2021. **2).** Anannya O., Huang W., August A. ITK signaling regulates a switch between T helper 17 and T regulatory cell lineages via a calcium-mediated pathway. bioRxiv April 3, 2023.