Identification of a neutrophil-specific PIK3R1 mutation facilitates targeted treatment in a patient with Sweet syndrome

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**Graphical abstract**

Neutrophil specific PIK3R1 gain-of-function mutation increases sensitivity towards IL-1. IL-1R inhibitor prevents neutrophil migration towards IL-1 and resolution of Sweet Syndrome.

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Title: Identification of a neutrophil-specific PIK3R1 mutation facilitates targeted treatment in a patient with Sweet Syndrome

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ABSTRACT

Background
Acute febrile neutrophilic dermatosis (Sweet syndrome) is a potentially fatal multiorgan inflammatory disease characterized by fever, leukocytosis, and a rash with a neutrophilic infiltrate. Disease pathophysiology remains elusive, and current dogma suggests Sweet syndrome is a “reactive” process to an unknown antigen. Corticosteroids and steroid-sparing agents remain front-line therapies, but refractory cases pose a clinical challenge.

Methods
A 51-year-old woman with multiorgan Sweet syndrome developed serious corticosteroid-related side effects and was refractory to steroid-sparing agents. Blood counts, liver enzymes, and skin histopathology supported the diagnosis. Whole genome sequencing, transcriptomic profiling, and cellular assays of patient’s skin and neutrophils were performed.

Results
We identified elevated IL-1 signaling in lesional Sweet syndrome skin caused by a PIK3R1 gain-of-function mutation specifically found in neutrophils. This mutation increased neutrophil migration towards IL-1β and neutrophil respiratory burst. Targeted treatment with an IL-1R1 antagonist in the patient resulted in a dramatic therapeutic response and enabled tapering of corticosteroids.

Conclusion
Dysregulated PI3K-AKT signaling is the first signaling pathway linked to Sweet syndrome and suggests Sweet syndrome may be caused by acquired mutations that modulate neutrophil function. Moreover, integration of molecular data across multiple levels identified a distinct subtype within a heterogenous disease that resulted in a rational and successful clinical intervention. Future cases will benefit from efforts to identify potential mutations. The ability to directly interrogate diseased skin allows this method to be generalizable to other inflammatory diseases and demonstrates a potential personalized medicine approach for challenging patients.

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INTRODUCTION
Sweet syndrome (acute febrile neutrophilic dermatosis) presents with fever, leukocytosis, and an eruption of erythematous plaques with a dense dermal neutrophilic infiltrate on histology (1, 2). Systemic multiorgan involvement may occur and carries considerable mortality risk. Sweet syndrome is often classified into classic (associated with infection, inflammatory disorders, vaccination, or pregnancy), malignancy-associated, and drug-induced subtypes (1-3). The molecular pathogenesis of Sweet syndrome remains unknown, but current dogma suggests a cell-extrinsic mechanism of neutrophil hypersensitivity to an unknown trigger leading to cytokine dysregulation during the inflammatory response (4). Systemic corticosteroids are the first-line therapy for Sweet syndrome, and their chronic use results in well-characterized morbidity. Steroid-sparing anti-inflammatory agents such as colchicine, dapsone, and potassium iodide may be utilized for long-term management, but patients refractory to these agents pose a clinical challenge (4).

Here we describe a Sweet syndrome patient with systemic disease who required chronic use of systemic corticosteroids and suffered four hospitalizations over two years for disease flares. Detailed genomic and transcriptomic studies identified the underlying genetic mutation and molecular mechanism responsible for her disease. We started therapy with a targeted treatment, the IL-1R inhibitor anakinra, which resulted in complete disease remission and enabled tapering off of all systemic corticosteroids.

RESULTS
Clinical Presentation
A 51-year-old Caucasian woman with a past medical history of stable pulmonary hyalinizing granuloma was referred to dermatology for diffuse red plaques (Figure 1A, Supplemental Figure 1A). She reported joint pain and waxing and waning skin lesions over the preceding 6 months. Skin biopsies repeatedly demonstrated typical dermal neutrophilic inflammation consistent with Sweet syndrome; histiocytoid morphology was not seen (Figure 1A, Supplemental Figure 1A). The patient developed additional symptoms of fevers, neutrophilia, sterile pyuria, and right upper quadrant abdominal pain with her disease flares, all of which responded to systemic steroid therapy (Figure 1, B and C). A thorough evaluation was conducted to rule out other causes of her presentation and to evaluate for underlying conditions associated with Sweet syndrome. Repeated elevations in transaminases and alkaline phosphatase during disease flares led to a referral to hepatology, and liver imaging demonstrated features of primary sclerosing cholangitis, but serologic workup and rheumatologic evaluation were negative for autoimmunity and infection, including negative anti-neutrophilic cytoplasmic antibodies and negative tissue cultures. Liver biopsy demonstrated periportal fibrosis and mild bile ductular proliferation with predominantly neutrophilic inflammation. A bone marrow examination revealed a normal karyotype, cytogenetics, and morphology. Review of her prior pulmonary hyalinizing granuloma pathology and biopsy of her sinuses ruled out IgG4-mediated disease. Taken together, she was diagnosed with multiorgan Sweet syndrome with cutaneous and hepatic involvement.

For five years, the patient was unable to taper off of prednisone without suffering a flare of her symptoms. Multiple steroid-sparing agents were trialed, including dapsone, potassium iodide, colchicine, hydroxychloroquine, sulfapyridine, and methotrexate. She developed significant steroid-related side effects including ecchymoses, weight gain, severe osteopenia,
spontaneous vertebral fractures. She continued to experience clinical flares and presented to the emergency department frequently, resulting in four hospital admissions within two years. During these flares, her complete blood count consistently demonstrated leukocytosis and neutrophilia, and her transaminases and alkaline phosphatase were markedly elevated (Figure 1, B and C). Repeat skin biopsies remained consistent with Sweet syndrome (Supplemental Figure 1A).

Given the failure to identify an effective and safe long-term therapy and her significant morbidity from corticosteroids, we hypothesized that a molecular characterization of her skin may enable identification of altered pathways that may be readily targeted by currently available FDA-approved therapies. Targeted augmentation of immunological pathways has become a powerful approach to treat human inflammatory diseases. In addition to this personalized treatment approach, understanding the transcriptomic changes in her disease could expand our insight into Sweet syndrome pathophysiology.

**Transcriptomic analysis of lesional skin reveals IL-1β dominant inflammation**

To determine what genes were differentially expressed in our patient’s Sweet syndrome lesions, microarray profiling was performed on dermal RNA extracted from paraffin-embedded skin biopsy samples obtained from the patient (n=3, taken from 3 consecutive years), other Sweet syndrome patients (n=7), and healthy controls (n=13). Principal component analysis demonstrated separation between the three groups, indicating that the patient’s disease was different from the other sampled Sweet syndrome patients (Figure 2A). We identified 1622 differentially expressed genes in the patient’s skin lesions compared with healthy control skin (false discovery rate (FDR) <0.1; fold change (FC), >1.5 or <−1.5; Supplemental Table 1). Gene ontogeny pathways and transcriptome analysis revealed enrichment for neutrophil-specific functions, regulation of interleukin (IL)-1 production, and activation of phosphoinositide 3-kinase (PI3K)–AKT signaling pathway (Figure 2B). Indeed, IL1β gene expression was substantially increased in the refractory patient’s microarray samples (Fig. 2C). We confirmed this result by quantitative PCR on additional dermal RNA samples from the patient and healthy controls (Supplementary Figure 1B). Among the seven other Sweet syndrome patients, three patients exhibited similar increased IL-1β transcript levels in their microarray compared to healthy controls (Figure 2C, yellow box). Finally, transcript levels for neutrophil-marker genes (including myeloperoxidases, matrix metallopeptidases, arginases, defensins) exhibited no significant increase in either the refractory patient or other Sweet Syndrome patients compared to healthy controls, suggesting that the increase in IL-1β is not simply due to more neutrophil recruitment (Supplemental Table 1). Taken together, these data suggested a role for IL1β production in a subset of Sweet Syndrome patients, including our index patient.

**PIK3R1 gain-of-function mutation in neutrophils promotes cell migration towards IL-1β.**

We next performed whole genome sequencing on the patient’s skin infiltrating neutrophils collected by laser capture microscopy to explore whether one or more somatic mutations could be contributing to their disease. Non-lesional skin epidermis was used to establish a reference genome and to rule out naturally occurring single nucleotide polymorphisms (SNPs). 71 missense and nonsense neutrophil-specific mutations were identified (Supplemental Table 2). Four of the mutated genes were among the differentially expressed genes identified in the patient (Figure 3A). Of these, a mutation in the Phosphoinositide-3-Kinase Regulatory Subunit 1 (PIK3R1) gene was of particular interest, because PI3K signaling was among the differentially
PIK3R1 encodes the p85 regulatory protein of the PI3K-AKT signaling pathway, which is important for cellular proliferation, apoptosis, and migration (5, 6). The p.W335C mutation is located within the nSH2 domain, which mediates p85 binding to tyrosine kinase receptors and allows the p110 catalytic binding partner to phosphorylate and activate AKT (Figure 3B) (5, 6). To elucidate the function of this mutation, we used an established in vitro cell culture system, where human HL-60 promyeloblast cells exposed to dimethyl sulfoxide (DMSO) differentiate into a neutrophil-like state (7). We generated stable HL-60 cell lines that overexpress wild-type (WT) and mutant p.W335C (Mut) p85 proteins by lentiviral transduction. Flow cytometry analysis confirmed appropriate differentiation of these cell lines (Supplemental Figure 2, Supplemental Figure 3A). Differentiated cells were used for all subsequent experiments.

mTOR is a well-established target of AKT, and mTOR acts through two functionally distinct complexes, mTOR complex 1 (mTORC1) and 2 (mTORC2) (5, 6). mTORC1 is the rapamycin-sensitive protein complex that regulates protein synthesis, and we observed no difference in mTORC1 activation between WT and Mut p85 cells as assessed by phosphorylation of the classical downstream target S6 (Supplemental Figure 3B). mTORC2 is the rapamycin-insensitive protein complex that regulates cell proliferation, survival, and migration. mTORC2 phosphorylates AKT specifically at serine 473 (S473), and Mut p85 cells exhibited increased phospho-AKT at S473 activation compared to WT p85 cells (Figure 3C). We observed no differences between WT and Mut p85 cells in cell proliferation or survival (Supplemental Figure 3C-D). However, WT and Mut p85 cells demonstrated notable differences in neutrophil respiratory burst and cellular migration.

We assessed neutrophil respiratory activity using the 2,7-dichlorofluorescein diacetate (DCFDA) dye in LPS and nigericin-treated WT and Mut p85 cells. Mut p85 cells exhibited increased neutrophil respiratory burst compared to WT p85 cells (n=8 for each group) (Figure 3D). Two cytokines, IL-1β and IL-8, are powerful inducers of chemotaxis in neutrophils (8-11). Although IL-8 had a similar effect in WT and Mut p85 cells, IL-1β increased cell migration of Mut p85 cells compared to WT cells in a dose-dependent manner (Figure 3E, Supplemental Figure 3F). Consistent with this, Mut p85 cells also exhibited higher transcript levels of cell migration-associated genes compared to WT p85 cells (Supplemental Figure 3E). Prior work demonstrated that AKT, the kinase downstream of PI3K, can induce the expression of IL-1 receptor (IL-1R) (12). Indeed, Mut p85 cells expressed higher levels of IL-1R1 transcript and protein compared to WT p85 cells (Figure 3F-G). Furthermore, treatment with an IL-1R antagonist, anakinra, blocked IL-1β-mediated migration of Mut p85 cells (Figure 3H). Finally, LPS and nigericin-treated Mut p85 cells did not secrete more IL-1β protein compared to WT p85 cells (Supplemental Figure 3G).

Activated PI3Kdelta syndrome (APDS) type 2 results from germline gain-of-function PIK3R1 mutations that activate both mTORC1 and mTORC2. We introduced the canonical p85 deletion mutant (amino acids 434-475) into HL-60 cells (13), and differentiated cells did not exhibit
increased migration towards IL-1β (Supplemental Figure 4A). We also noted that Mut p85 promyeloblasts, the progenitor cell type for neutrophils, do not exhibit increased phospho-AKT S473 activation compared to WT p85 promyeloblasts (Supplemental Figure 4B). These results may explain why our refractory patient did not share a similar phenotype with APDS2 patients and had a normal bone marrow biopsy.

Taken together, we demonstrate that the PIK3R1 p.W335C is a gain-of-function missense mutation that activates the PI3K pathway specifically through mTORC2, enhances neutrophil respiratory burst, and increases cell migration towards IL-1β.

**Clinical remission of refractory Sweet syndrome with anti-IL-1R1 antagonist**

We confirmed that primary neutrophils from the refractory patient also exhibited increased phospho-S473 AKT phosphorylation and IL-1R1 protein (Figure 4A). Neutrophils and macrophages differentiate from the granulocyte-monocyte progenitor cell, and we did not detect the p.W335C mutation in primary macrophages from the refractory patient by Sanger sequencing (Supplemental Figure 5). We conclude that this mutation is strictly within the neutrophil lineage. These results suggested that IL-1R blockade could benefit our refractory Sweet syndrome patient. Remarkably, treatment with anakinra achieved a dramatic therapeutic response with complete resolution of symptoms and allowed tapering of all systemic corticosteroids (Figure 4B-D). Notably, upon trials of stopping anakinra, patient’s disease flared within days with recurrence of fevers, skin lesions, neutrophilia, liver enzyme elevation, and right upper quadrant pain. Re-initiation of therapy again led to complete disease remission (Figure 4D). Notably, we isolated neutrophils post-anakinra treatment and still detected the p.W335C mutation (Figure 3B). Since starting anakinra, the patient has not experienced any further flares, hospitalizations, or additional corticosteroid use over three years. Given our findings, we queried her family history, and no family members have Sweet syndrome.

**DISCUSSION**

We describe the use of transcriptomics and genomic analysis to identify a gain-of-function PIK3R1 mutation in neutrophils from a Sweet syndrome patient. The mutation increased expression of IL-1 receptor, cell migration towards IL-1β, and neutrophil respiratory burst. Sweet syndrome patients typically exhibit pathergy, which is the induction of dermatosis-associated skin lesions at the site of minor skin trauma. Although the molecular mechanism causing pathergy remains undetermined, we speculate that incidental trauma triggers release of additional IL-1 from injured cells and creates a signaling gradient that induces recruitment of IL-1-sensitive PIK3R1 mutant neutrophils to the skin. These insights led to successful treatment with an IL-1β antagonist, which blocks PIK3R1 mutant neutrophils from being recruited to the skin.

Dysregulated PI3K-AKT signaling is the first signaling pathway linked to Sweet syndrome. PIK3R1 mutations are associated with genetic and acquired human diseases (13-17), and this particular mutation has not been previously identified. As mentioned above, germline gain-of-function PIK3R1 mutations may cause APDS2 syndrome, but these mutations are generally exon-skipping, splice site, or deletion variants (13, 18). The canonical APDS2 deletion mutant in our neutrophil-like cell line did not increase IL-1β mediated cell migration. Cell-type specific and biochemical differences may explain the lack of phenotypic overlap between the APDS2 and our patient. Additional work is needed to understand how PIK3R1 specifically activates
mTORC1 and mTORC2 complexes. Finally, FDA-approved mTOR inhibitors, including sirolimus, are used to treat PI3K-mediated processes. However, these inhibitors specifically target mTORC1, and we do not believe they will be effective in this subset of mTORC2-dominant Sweet syndrome patients.

This study counters current dogma that Sweet syndrome is a reactive process, where an unidentified signal activates normal neutrophils and recruits them into the skin. The finding of a somatic mutation that confers sensitivity to IL-1β demonstrates that Sweet syndrome may be caused by a cell-intrinsic process. Sweet syndrome is a member of a larger group of non-infectious neutrophilic dermatoses, including pyoderma gangrenosum, pustular psoriasis, and Behcet’s disease. Patients with Behcet’s disease and pyoderma gangrenosum have been reported to harbor somatic mutations in NFKB1 that modulate IL-1β production (19-21). And patients with germline errors of immunity may also develop neutrophilic dermatoses, including mutations in PSTPIP1, NLRP3, and IL36RN (22). Thus, a common theme emerges from this highly analogous data that neutrophilic dermatoses may result from inherited or acquired mutations affecting signaling pathways that modulate neutrophil function. Some cases of Sweet syndrome are associated with malignancy, most commonly hematologic disease (1-2). The finding of a somatic mutation without a concurrent malignancy is an important distinction in this case. Future Sweet syndrome cases will benefit from a systematic approach to identify these potential mutations.

How does this mutation only affect neutrophils? As mentioned earlier, neutrophils and macrophages share a common progenitor cell, and patient primary macrophages did not harbor the mutation. Myeloblasts are the common progenitor cell type for neutrophils, basophils, and eosinophils and represent the cell-of-origin for myeloproliferative neoplasms. Our patient lacks overt changes to her basophil or eosinophil populations. Neutrophil-specific diseases do exist, including chronic neutrophilic leukemia, a rare but lethal disorder involving increased neutrophil infiltration and proliferation in the blood, marrow, and other organs. A subset of these patients carries activating point mutations in the CSF3R gene (23). Therefore, genetic mutations specifically targeting neutrophils may occur in a cell-specific stem cell population downstream from the myeloblast stage. In our refractory patient, the return of clinical symptoms when pausing anakinra confirmed that her circulating neutrophils exhibit a permanent sensitivity to IL1β. It is curious that mutated promyeloblasts carrying our missense mutation do not exhibit increased AKT activity, and more work is needed to understand how neutrophil differentiation uncovers this change.

Sequencing of the patient’s neutrophils revealed a single T nucleotide peak in PIK3R1 gene at position 335, and sequencing of their macrophages and skin revealed a single G nucleotide peak (Fig. 3B, Supplemental Fig. 5, Supplemental Table 1). The absence of a heterozygous gain-of-function mutation was unusual. There are two possible explanations for this difference. The first, that the patient acquired the same somatic mutation in both alleles of the PIK3R1 gene, is less likely. More likely, the patient developed a mutation in a single allele of the PIK3R1 gene within a neutrophil progenitor cell. This change conferred a selective advantage and resulted in the eventual loss of the second allele. Similar loss of heterozygosity is frequently identified in dysplastic syndromes and cancers (24). This finding suggests additional screening of the
patient’s neutrophil counts over time may be warranted, and more work is needed to understand this mechanism.

Increased IL-1β transcript levels were seen in three out of seven other Sweet syndrome patients, and some patients may exhibit increased circulating IL-1β (25-28). These data suggest that increased IL-1β signaling may play a role in a subset of Sweet syndrome patients. Indeed, IL-1 blockade has been reported anecdotally to be effective in other cases of refractory disease (25-28). Nevertheless, a limitation of this study is that our findings are in a single patient, and thus need to be extended to a larger cohort.

In summary, integration of molecular data across multiple levels helped identify a distinct pathotype within a heterogenous disease that resulted in a successful clinical intervention enabling disease control. This rational and personalized therapy is, to our knowledge, the first example of identifying a targeted treatment for Sweet syndrome. The ability to directly interrogate diseased skin allows this approach to be generalizable to other inflammatory disorders.
METHODS

Transcriptomic analysis
Laser Capture Microdissection (LCM) was performed on formalin-fixed paraffin-embedded (FFPE) blocks to capture the dermis from 10μm sections. RNA was isolated using AllPrep DNA/RNA FFPE kit (Qiagen). 10ng of RNA was provided to the University of Pennsylvania Genomic Analysis Core for downstream processing. Complementary DNA (cDNA) was prepared and biotinylated using the GeneChip WT Pico reagent kit (Thermo Fisher Scientific). Clariom D Human array plates was used for hybridization of labeled cDNA followed by washing, staining and scanned using the GeneChip 3000 7G system (Thermo Fisher Scientific). CEL files were generated after quality control and RMA (robust multi-array average). Further analysis was performed using Transcriptome Analysis Console (TAC) software. The filtering criteria for transcriptional network for the differentially expressed genes is FDR, <0.1; FC, >1.5 or <-1.5. Data is accessible on the GEO repository (Accession# GSE190335).

For quantitative PCR (qRT-PCR), RNA isolation was performed using RNeasy Mini Kit (Qiagen). 1ug of RNA was converted to cDNA by SuperScript IV VILO (Invitrogen). Samples were run on a 7900HT Fast Real-Time PCR System (Applied Biosystems) with Sybr Green Universal PCR Master Mix (Thermo Fisher Scientific). Primers sequences are listed in Supplemental Table 3.

Sequencing
Infiltrating dermal neutrophils and non-lesional epidermis were isolated by LCM from FFPE sections. Genomic DNA was isolated using DNeasy Blood and Tissue kit (Qiagen). Sequencing was performed at Beijing Genome Institute USA (BGI), where sample QC, library construction using TruSeq DNA PCR-Free Library Prep Kit and sequencing using DNBSEQ™ sequencing technology was performed. Sequencing depth was 30X, and bioinformatic analysis was performed by BGI. For Sanger sequencing, peripheral blood neutrophils were isolated using Dextran Sedimentation and Ficoll-Paque density gradients. Genomic DNA was isolated using DNeasy Blood and Tissue kit (Qiagen), and PCR amplification of the PIK3R1 genomic region was performed prior to submission.

Cell Culture
HL60 cells, a human promyeloblast cell line, were obtained from American Type Culture Collection (ATCC). Cells were grown in Iscove’s Modified Dulbecco’s Medium (IMDM, catalog 12440053, Thermo Fisher Scientific) supplemented with 10% FBS and Penicillin/Streptomycin. For differentiation into neutrophil-like state, HL60 cells were treated with 1.3% dimethyl sulfoxide (DMSO) in complete IMDM media for 5 days.

Flow Cytometry
Flow cytometry analysis was performed with CD11b-APC (BioLegend, catalog # 301329) and CD14-FITC (BioLegend, catalog # 325603) to validate HL-60 cell differentiation as previously described (7). Cells were collected at day 5 for western blot and migration assay. Data was analyzed on FlowJo software v10.

Generation of PIK3R1 overexpressed cell lines
We purchased pBluescriptR plasmid containing human full-length PIK3R1 coding sequence (Horizon Discovery, Clone ID: 30528412). PIK3R1 was cloned into a lentiviral FUW plasmid (Addgene, #14882). Q5 site directed mutagenesis kit (NEB) was used to incorporate the G335T mutation into the PIK3R1 coding sequence, and the mutation was confirmed by PCR amplicon sequencing. Lentiviral transduction was performed using standard methods. HL60 cells were infected with lentivirus in presence of 8µg/ml polybrene (Sigma) for 48 hours. Antibiotic selection with Zeocin (200µg/mL for 7 days) was used to select positive clones.

Cellular Functional Assays
Cell survival was assessed by Trypan Blue dye exclusion assay on days 3 to 6 after DMSO addition to HL60 cells. A Countess Automated Cell counter (Thermo Fisher Scientific) quantified live cells. Cell proliferation was assessed by CyQuant direct cell proliferation assay (C35011, Thermo Fischer Scientific). Transwell migration assay was performed on differentiated HL60 cells at day 5. The top chamber of the Transwell contained cells in IMDM media with DMSO, and the bottom chamber contained IMDM supplemented with IL1b or IL8. To inhibit migration, IL1RA (IL1 receptor antagonist, Sigma Aldrich, SRP3084) was added to the top chamber. The number of live cells in the bottom chamber was quantified at 16-18 hours by Trypan Blue dye exclusion assay. Cellular respiratory burst was detected using the cell permeable redox sensitive fluorescent probe 2,7-dichlorofluorescein diacetate (DCFDA) per manufacturers protocol (Cayman Industries). Briefly, 20 x 10³ cells were seeded into 96 well plate. Cells were treated with 100ng/ml LPS for 4 hours followed by 10 µM Nigericin for 1 hour. 100 µM H2O2 for 1 hour served as the positive control. Cells were washed with PBS and incubated with 20 µM DCFDA for an hour. Cells were washed with PBS to remove unassimilated DCFDA and the fluorescence read on a plate reader, Excitation: 485/20, Emission: 528/20 (BioTekHT). IL-1β secretion was detected by ELISA (R&D Systems). Cells were treated with 100ng/mL LPS for 4 hours followed by 10 µM Nigericin for 1 hour.

Western Analysis
Whole cell lysates were prepared using RIPA buffer with PMSF and protease and phosphatase inhibitors (Invitrogen). 30µg of total cell lysate was used for protein quantification by Pierce BCA protein assay (Thermo Fisher Scientific, #23225). The cell lysate was resolved using 4-12% NuPage gels (Thermo Fisher Scientific) and transferred to nitrocellulose membrane using a wet transfer system (Bio-rad). After blocking in 5% milk, membranes were incubated with primary antibody overnight at 4°C. The following primary antibodies were used: anti-AKT (1:1000, #9272, Cell Signaling Technologies), anti-phospho AKT (1:1000, #9271, Cell Signaling Technologies), anti-S6 kinase (1:1000, #9202, Cell Signaling Technologies), anti-phospho S6 kinase (1:1000, #9204, Cell Signaling Technologies) and anti-β-actin (1:5000, #4970, Cell Signaling Technologies), anti-PI3K p85a (1:1000, #60225, Proteintech) and anti-IL1R1 (1:2000, #PA5-97866, Thermo Fisher Scientific). Membranes were washed and incubated with anti-rabbit or mouse HRP-linked secondary antibody (anti-rabbit, #7074S, Cell Signaling Technologies; anti-mouse, #sc-2005, Santa Cruz Biotechnologies) and were visualized by Chemiluminescence using ECL prime kit (Sigma Aldrich, #RPN2232) and ChemiDoc imaging system (Bio-Rad Laboratories). Densitometry analysis was performed using ImageLab software (Bio-Rad).

Statistics
Presented data combines all experiments, and unless noted, all experiments were repeated 3-5 times independently. Experiments were not randomized, and investigators were not blinded to allocation during experiments and outcome assessment, unless noted in the text. 2-tailed Student’s t test was used to determine significance, with p-values of less than 0.05 considered significant. Higher levels of significance are indicated by the following: ** p < 0.01, *** p < 0.001 in the text. When appropriate, specific p values are provided in figure legends.

**Study approval**
The study protocol was approved by the Institutional Review Board of the University of Pennsylvania School of Medicine. All patients provided written informed consent prior to participation. The funding sources did not participate in any part of the trial, from conception through manuscript preparation. The diagnosis of Sweet syndrome was made by trained dermatologists and dermatopathologists using published criteria based on clinical and histologic findings. Within the informed consent, we explicitly explain that our protocol is for discovery work, and that patients should have no expectation of a specific medical intervention, treatment, or altered approach to modify their health outcome. Blood and skin biopsies were obtained. Healthy control skin samples were deidentified discarded tissue obtained from the Skin Biology and Disease Resource Center at the University of Pennsylvania. Written informed consent was provided for clinical photographs appearing in the manuscript.

**AUTHOR CONTRIBUTIONS:**
Writing of the manuscript: S.B., T.H.L.
Reviewing and editing the manuscript: S.B., S.Basu, C.A.N., J.S.B., K.S., R.G.M., W.D.J., M.R.

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**Data and materials availability:** All data are available in the main text or the supplementary materials. Microarray data is submitted to GEO (GEO Accession Number - GSE190335).
REFERENCES


Figure 1. Refractory Sweet Syndrome patient required multiple hospitalizations for disease control. (A) Clinical photographs of patient’s skin lesions. The middle and right panels show hematoxylin and eosin staining of patient’s skin biopsy, which demonstrates a diffuse dermal neutrophilic infiltration. Scale bar 200μM. (B and C) Time course of her peripheral neutrophilia (B) and liver enzyme fluctuations (C) during her multiple clinical flares. Her four hospital admissions for disease flares are denoted by the green boxes.
Figure 2. Refractory Sweet syndrome lesions reveal an IL-1β dominant inflammation. (A) Principal component analysis of gene expression generated from skin dermis from the refractory patient \( n=3 \), purple, other Sweet syndrome patients \( n=7 \), blue, and healthy controls \( n=13 \), red. (B) Gene ontology categories that are most highly enriched in the transcriptome of the refractory patient compared to healthy controls. (C) Increased IL1β transcript detected in the refractory patient dermis (purple) compared to healthy control dermis (blue). Individual Sweet syndrome patient dermis demonstrated varied levels of IL1β transcript levels. 3 patients had levels greater than 2.5 standard deviations above healthy control average (yellow box). Data represents mean ± SEM. 2-tailed Student’s t test. ***P<0.001.
**Figure 3.** PIK3R1 gain-of-function mutation increases neutrophil migration towards IL-1β.

(A) Venn diagram which displays the intersection between differentially expressed genes and neutrophil-specific gene mutations in the refractory patient. Gene names are listed in the box. (B) Schematic depicting the PIK3R1 gene. The mutation location is identified by the black arrow. Adjacent to schematic is the DNA sequence chromatogram that confirms the mutation. (C) Western panel demonstrates increased phosphorylation of AKT on serine 473 in overexpressed p.W335C p85 (MT) cells compared to overexpressed p85 (WT) cells. Experiment repeated independently 3 times. (D) Cellular respiratory burst detected by 2,7-dichlorofluorescein diacetate (DCFDA) in LPS-treated WT and MT p85 cells (n=8 for each group). (E) Transwell migration of WT and MT p85 cells in response to IL-1β (n=6 for each group). (F) qRT-PCR (n=3 for each group) and (G) Western panel demonstrates increased IL1 receptor 1 (IL1-R1) in MT p85 cells compared to WT p85 cells. Experiment repeated independently 3 times. (H) Pharmacologic treatment with an IL1 receptor antagonist (IL1RA) block IL-1β-mediated Transwell migration (n=3 for each group). Data represents mean ± SEM. 2-tailed Student’s t test. * P<0.05; **P<0.01.
Figure 4. Clinical remission of refractory Sweet syndrome with IL-1 receptor antagonist
(A) Patient neutrophils exhibit increased AKT activation and IL-1R1 expression. Experiment repeated 2 times. (B) Photograph demonstrating disease clearance post-anakinra treatment. (C) Hematoxylin and eosin staining of post-treatment skin biopsy with the absence of a neutrophilic infiltration. (C) Neutrophil and liver enzyme counts of the refractory patient after initiation of anakinra. When anakinra was paused, her clinical symptoms reflected in laboratory values returned over several days.