Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Vanderver A, Adang L, Gavazzi F, et al. Janus kinase inhibition in the Aicardi–Goutières syndrome. N Engl J Med 2020;383:986-9. DOI: 10.1056/NEJMc2001362

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1 FUNDING SUPPORT

Eli Lilly and Company was the sponsor of the expanded access program for baricitinib. Baricitinib was provided by Eli Lilly and Company (NCT01724580). Outcomes were studied under grants from CURE (Commonwealth Universal Research Enhancement program), NICHD (U01HD082806) and AGSAA.

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2 TRIAL REGISTRATION

ClinicalTrials.gov NCT01724580 and NCT03047369

3 SUPPLEMENTAL METHODS

3.1.1 Study approval and consent

This program was approved by the IRB of Children's Hospital of Philadelphia. Informed consent for both a natural history study (IRB#14-011236; NCT01724580) and the expanded access (IRB#16-013205; NCT03047369) program were obtained. The Declaration of Helsinki and Good Clinical Practice guidelines were followed. Individuals were referred by local providers or were self-referred.

3.1.2 Study design and participants

Thirty-five subjects with AGS were treated (Figure S1, Table 1). This study is part of a larger, ongoing compassionate use protocol (NCT01724580) (see Supplemental protocol). Three additional patients without genetically confirmed AGS were enrolled, but excluded from analyses. Four enrolled patients did not complete the initiation period because of infectious risk and disease-related death. This report represents the analysis of a minimum length of treatment of 12 months for all subjects (collected March 2016 through October 2019) (Figure S1B). The medication was provided to families without charge, and no incentives were provided.

3.1.3 Intervention

Individuals were dosed with baricitinib using the dosing tables in the Supplemental Protocol. Dose was increased to control symptoms, according to weight and renal function. In individuals with normal renal function, and a weight of less than 20 kg, a dosing goal of approximately 0.3 mg/kg/day was used, with highest final doses of 0.5 mg/kg/day in some individuals. In individuals with weight between 20-40 kg, a goal of 0.15-0.3 mg/kg/day was used up to 8 mg per day. In individual with a weight of >40 kg, a maximum of 12 mg per day was used. In individuals with altered eGFR, lower dosing was used. In some cases, dosing adjustments were made for hematologic parameters. Participants continued to receive standard of care, including medication and surgical interventions. Children were advised to avoid all live vaccinations while on baricitinib.

3.1.4 Safety assessments

Safety was evaluated by the incidence of adverse events, changes in clinical laboratory findings, and physical examination. Because of the chronic medical issues experienced by individuals with AGS, serious adverse events (SAEs) were defined as those that result in death, are life-threatening, require hospitalization, and all serious adverse drug-related events as judged by appropriate medical professionals, as attributable to the intervention. Elective admissions and known complications of AGS were not considered SAEs.[1,2] Laboratory abnormalities were graded using Common Terminology Criteria for Adverse Events (CTCAE v5.0), supplemented with grading definitions for thrombocytosis.[3,4]

Based on the FDA-approved prescribing information, participants were monitored for serious infections, thrombosis, and key laboratory measurements of leukocytes, neutrophils, hemoglobin,

liver enzymes, creatinine kinase, alkaline phosphatase, creatinine, estimate glomerular filtration rate (eGFR), and lipids. Statistical analysis was performed on the full set of data.

3.1.5 Clinical measures

Patient-proxies (parents) completed a daily symptom diary during the run-in and study period. This diary allowed parents to evaluate neurologic disability, crying, sleep, irritability, seizures, fevers, and skin inflammation of the body and extremities (Table S5). Clinical scores were generated for the intervals before study drug initiation, and then for each visit interval by averaging scores between visits.

All available medical records pre-treatment and on study were retrospectively evaluated neurologic function using quantitation of ten key developmental milestones: mobility (1: head control, 2: sitting, 3: rolling/crawling, 4: supported ambulation, 5: independent ambulation), fine motor control (6: pincer grasp or self-feeding) and social communication (7: smiling, 8: babbling, 9: single words, 10: sentences) (Table S6). AGS developmental information were available from disease onset or a minimum of 12 months prior to treatment initiation and on study.

Baseline neurologic skills were obtained from all 35 subjects. Baseline was collected medical record review from disease onset to study enrollment. Less than 12 months of data was collected from patients with new onset disease. We collected pre-treatment data of a median of 16.5 months (25th to 75th percentile of 7.4 to 41.5) and mean of 36.9 months (standard deviation 53.2; 95% confidence interval 18.7 to 55.2).

3.1.6 Interferon Signaling Gene Expression Scores (AGS Biomarker)

Levels of mRNA for 6 IFN-inducible genes: IFI27, IFI44L, IFIT1, ISG15, RSAD2, and SIGLEC1, and 4 housekeeping genes: ALAS1, HPRT1, TBP, and TUBB, were assayed as previously described.[5,6] In brief, patient blood samples were collected in PAXgene blood RNA tubes (PreAnalytiX), and RNA was purified using PAXgene blood RNA kits (Qiagen). Copy number of mRNA transcripts was quantified using Nanostring nCounter™ Digital Analyzer using 200ng of sample RNA. The raw copy number of mRNA transcripts of each type I IFN-inducible gene was standardized (stdGene) to the housekeeping genes for each individual. The 6-gene IFN signature was calculated using the sum of the median Z scores of these genes. The IFN signature was positive (IFN high) if ≥1.96 (>98centile) by one tail analysis. The baseline measurement was calculated as the average of all pre-treatment ISG values for each subject (minimum of 3 per subject). On study samples were collected with safety laboratory testing. The average number of ISG scores per patient was 8.8 (minimum = 6, maximum = 12).

3.1.7 Overall Statistical Analyses

Analyses were performed with Stata 16.0. Prism8 was used to construct graphs. Preliminary analyses were descriptive and included frequency tables of categorical variables and summaries (mean, median, 25th to 75th percentile, range) of outcomes, and changes from baseline in each outcome. Graphical displays of the data included histograms and quantile-quantile plots to assess normality; Lowess smoother plots (locally weighted regression of outcome versus day on treatment) to evaluate linearity of change over time; box plots to assess the distribution of outcomes and of change in outcomes versus visit; and individual level profile plots to examine

outcomes within individuals over time. McNemar's test for paired dichotomous measurements was used in some cases to assess pre and post treatment change with 95% confidence intervals (CI). In addition, heat maps were used to visually represent gradation in change.

For each outcome, we calculated the intra-subject changes from baseline: immediate (evaluated at the first visit post baseline), at one year, and overall (final measurement) (Table S4). We also obtained the median (25th, 75th percentile) and mean with 95% confidence-intervals (CI).

For each outcome, we also fit longitudinal models. These longitudinal models were fitted using quasi-least squares (QLS) regression, a computational approach for estimation of intra-subject correlations in the framework of generalized estimating equations (GEE)[7]. We modelled the intra-subject correlation of measurements using AR(1), Markov, and exchangeable structures, with robust sandwich estimators of covariance. To evaluate temporal changes in outcomes, we included time as a continuous variable as well as an indicator variable for each visit, with baseline as the reference category. Goodness of fit criteria for GEE and QLS[8,9] were used to choose between models. The adjusted QLS model included age at symptom onset, age at drug initiation, microcephaly, male gender, and indicator variables for the more severe TREX1 and RNASEH2B genotypes. We then used the lincom procedure in Stata 16 to estimate the average change on therapy based on the estimated regression coefficient.

We fitted models that included all the pre-treatment development skill counts for each subject. When we included the pre-treatment data, we fitted models with indicator variables for each visit (with pre-treatment as the reference category); in addition, we fitted models that allowed us to decompose the longitudinal effects of treatment versus the cross-sectional effects due to participants starting treatment at different ages[10]. The results for the QLS analysis for AGS are based on the final model for the data set that includes all pre-treatment values for AGS.

Adjustment for multiplicity: No adjustment for multiplicity was made to the confidence intervals.

Classification of evidence. This interventional study provides Class IV evidence that baricitinib is safe in children with AGS. This study provides Class IV evidence that baricitinib results in a significant improvement compared with baseline neurologic function.

Data availability. Individual level data will not be shared due to (1) the expanded access program does not meet requirements for data sharing as outlined by the ICMJE, and (2) we are unable to adequately anonymize the data for data sharing given the rarity of the disorder.

4 SUPPLEMENTAL RESULTS

4.1.1 Demographics

Forty-two patients were screened, and 35 patients with molecularly confirmed AGS have received medication (Table S1, Figure S1). The median age of symptom onset was six months and the median age at drug initiation was 2.9 years (range of 0.2 - 21.8 years).

Analyses included data collected from study initiation to a minimum of 12 months of data collection (October 31, 2019). All patients (n=35) had visits at baseline and at months 1, 3, 6, 9 and 12. In addition, 31 patients had a visit between baseline and within the first ten days on treatment. The median time on treatment was 570 days (range of 360 to 1332 days) (Figure S1B).

4.1.2 Safety

Overall, baricitinib was well tolerated by the AGS population. Over the duration of collected data, there were 49 hospitalizations in 22 subjects, all attributable to AGS and complications related to severe neurologic disease. This included urinary tract infections, seizures, admission for viral syndromes with respiratory distress or dehydration, pneumonia-including aspiration pneumonia, erythema multiforme, fracture, acute neurologic change, and planned interventions). One patient died during the enrollment phase, prior to drug initiation from AGS-related complications. On study, one individual died secondary to pulmonary hypertension related to AGS while on study at day 428.[11] A second individual died after the data lock, while on study at day 1357. This death occurred in the context of a multisystem illness resulting in worsening pretreatment chronic liver failure with ascites and hypogammaglobulinemia, worsening of pretreatment pulmonary hypertension, leukopenia, anemia, worsening pretreatment thrombocytopenia requiring chronic steroids, and renal insufficiency. Autopsy revealed a fungal pneumonia, which had not been detected on pre-mortem cultures, and was possibly attributable to the study medication.

Throughout the trial, dose adjustments were based on changes in weight, pharmacokinetic data, baseline eGFR and re-emergence of symptoms, according to the study protocol.7,11 In most children, dose was stable or increased during the study. However, the medication was additionally decreased in several cases due to changes in laboratory parameters. In 4 children, drug dosage was decreased during the study period for BK viremia (n=1), thrombocytosis (n=2), anemia (n=1).

Many individuals began the study with abnormal laboratory values (Table S4A). We used odds-ratios to compare the odds of having specific laboratory abnormalities at baseline and on study (Figure S2). After starting study medication, markers of liver dysfunction were transiently abnormal in a subset of subjects (ALT abnormal in n=10/35; AST n=20/35; GGT n=30/35), but overall improved on therapy (Table S4B). ALT decreased overall in treated subjects [estimated odds-ratio = .3 (95% CI = 0.2 to 0.5). After treatment, a decreased number of subjects met grade 3-4 severity criteria for GGT (n=4), ALT (n=1) and AST (n=1) abnormalities (Figure S2, Table s4B).

Nineteen individuals had a grade 1-2 or greater anemia [estimated odds-ratio = 2 (95% CI 0.9-4.4)]] (Figure S2). One individual had a history of iron-infusion dependent anemia and required this therapy for grade 3 anemia (hemoglobin <8.0 g/dL) in the second year of treatment, at which point the dose was decreased. This was the same individual who later died from infection.

The majority (n=20/35) subjects were found to have platelet abnormalities on study. The majority of these abnormalities represented an increase in platelet numbers, all grade 1-2 [estimated odds-ratio = 2.5 (95% CI = 1 to 6.1)] which resulted in decreased dosing in two individuals (Figure S2). Four individuals had Absolute Neutrophil Counts (ANC) that were transiently between 500-1000 cells/ul [estimated odds-ratio = 5.8 (95% CI = 0.7 to 46.6)] (Figure S2). No individuals had

decreases of the Absolute Lymphocyte Counts (ALC) <500 during the reporting period (estimated odds-ratio = 0.8 (95% CI 0.1-4.1)] (Figure S2).

Two individuals had reversible elevations of alkaline phosphatase after treatment, which were investigated and found to be consistent with transient hyperphosphatasemia of childhood, a common finding in this age group with no clinical significance. Two individuals had transiently elevated creatinine kinase. In general, creatinine and estimated glomerular filtration rates (eGFR) were stable during the study period and did not require dose adjustments after starting intervention.

Five children received systemic steroids at prior to the study initiation for symptom management (n=3 for skin, n=1 for neurologic dysfunction, n=1 for thrombocytopenia), and these were decreased (n=2) or discontinued (n=3) by their local teams while on study.

4.1.3 Biomarker evaluations

IFN signaling gene (ISG) expression scores were calculated for each patient at baseline and every three months on study (Main Figure, Table S4). ISG scores were elevated at baseline, except for one individual with RNASEH2B-dependent AGS [median score 24.39 (1.89-65.49)]. There was improvement in ISG scores from baseline to the measurements within the first week on study, to the 1-year values, and to the last value available. Although overall scores were decreased, each individual demonstrated continued variability in the face of triggers, such as infection. Three individuals demonstrated an immediate increase in ISG scores, but over time, these scores had also decreased. The QLS approach was used to model longitudinal change of the ISG score on study, which demonstrated in an immediate change of -14.3 (95% CI = -19.7 to -8.8), -7.4 after one year on study (95% CI = -13.3 to -3.5); and -8.0 at 24 months (95% CI = -13.6 to -2.5).

4.2 Efficacy evaluations

4.2.1 Clinical Scores

A disease-specific symptom diary (“clinical score”) was designed to be a patient-centric measure of the impact of disease (Table S5). Clinical scores improved (decreased) on study (Main Figure, Table S4). There was improvement in ISG scores from baseline to the measurements within the first month on study, to the 1-year values, and to the last value available. In the QLS model, the expected changes were -0.1 at 1 month (95% CI = -0.1 to 0.0), -0.7 at 12 months (95% CI = -1.0 to -0.4), and -1.1 at 24 months (95% CI = -1.6 to -0.6), demonstrating a small, but persistent improvement in the burden of disease as measured by the families. Although skin involvement varied in individuals during the study due to seasonal fluctuation, overall skin involvement as assessed by domains in the AGS clinical diary improved (mean final minus pre change = -1.4; 95% CI = -2.2, to -0.6).

4.2.2 Functional assessments

The impact of baricitinib on neurologic function was analyzed in post-hoc analysis (Figure S3). Because of the severe limitations in neurologic function in children with AGS, traditional outcomes measures would be unable to sensitively measure change in our population and demonstrate significant floor effects. As such, we collected developmental skill information

through retrospective chart review to assessment neurologic performance, focusing on the presence of key skills in the AGS population, both prior to study drug and on study drug. The skills were selected based on their presence across the AGS population: mobility (1: head control, 2: sitting, 3: rolling/crawling, 4: supported ambulation, 5: independent ambulation), fine motor control (6: pincer grasp or self-feeding) and social communication (7: smiling, 8: babbling, 9: single words, 10: sentences). Head circumference, which did not change, was also included to bring the maximum score to 11.[12]

Underscoring the severity of AGS, at baseline, each individual only had a median of 3 developmental skills (range of 0-11 points; mean 4.5 points + 3.67 points). Prior to treatment, neurologic skills were stable (n=18/35), lost (n=8/35), or improved (one skill: n=8/35; two skills: n=1/35). On study, twelve individuals (34.3 %) demonstrated a gain of two or more skills between baseline and final assessment. Overall, 20 individuals demonstrated a gain of one or more skills (57.1%). In one subject, the drug was briefly decreased from 6 mg/day to 4 mg/day in the context of hospitalization for encephalopathy, and number of developmental skills decreased from 10 to 7, which was not recovered.

There were clinically meaningful improvements in AGS developmental skills at three months, one year, and at the final assessment. In the best-fitting QLS model, there was an improvement of 0.6 developmental skills by 3 months (95% CI = 0.2 to 1.0), 0.7 skills by 1 year (95% CI = 0.2 to 1.3), and 1.2 new developmental skills at 24 months (95% CI = 0.6 to 1.9) (Table S4). The improvement in the developmental skills correlated with reaching target dosing at the final evaluation across all weight brackets (Figure S4).

5 SUPPLEMENTAL DISCUSSION

Since the identification of the genetic etiology of AGS, there has been intense interest in the application of immunomodulation as disease modifying therapy.[13] Initial efforts focused on use of steroids, intravenous immunoglobulin, cyclophosphamide, and tocilizumab. More recently, with the development of JAK inhibitors, case reports treating AGS with ruxolitinib and baricitinib have emerged, addressing the impact of this class of molecules on cerebral vasculopathy, skin manifestations, and ISGs.[14-18] JAK inhibitors have the potential to more directly protect affected individuals from the end-organ damage associated with uncontrolled IFN production, though much remains to be understood about the penetration of these agents to key organs, including the blood brain barrier.

This is the first study to assess safety and efficacy of a JAK inhibitor in AGS. While Baricitinib was generally well tolerated in this cohort, there were several notable safety events while on study, primarily related to thrombosis and infection. One individual died during treatment due to disease related pulmonary hypertension[19]. AGS is known to affect platelets as well as the vascular system.[20-24] The complex interplay between JAK inhibition and AGS on systemic vasculopathy is under-characterized.

A second individual, on chronic steroids, died of a multisystemic illness that was thought to be related to worsening of his underlying disease, but a fungal pneumonia with dissemination was

identified on autopsy. While the 95% CI did not suggest significant changes within our cohort across the population, the impact of JAK inhibition on the absolute leukocyte and neutrophil count should be closely followed. No other treatment-associated severe adverse events were identified, although the presence of increased upper respiratory infections often associated with this class of medications would be difficult to identify in this population. Additionally, several individuals had pneumonia and urinary tract infections, but this complication is common in children with leukodystrophies, and differences in frequency are difficult to quantify in this small cohort.[25]

Children recovered without drug interruption from infections, including skin infections, a baclofen pump infection, influenza, urinary tract infections or pneumonia. No other serious infections were identified. Of note, one patient experienced a severe and persistent loss of neurologic function in the context of a dose decrease.

Several target organs appeared to have therapeutic improvement with baricitinib administration, particularly thrombocytopenia, skin inflammation, and hepatitis. Chronic thrombocytopenia improved in one individual, and in two individuals, liver enzyme abnormalities of more than 15-fold upper limit of normal improved on study. Severe skin manifestations by subjective description and symptom diaries also improved, consistent with a prior report.[14,17] Several subjects were on chronic steroids at enrollment, all of which were decreased while on study.

We found that while ISG scores declined immediately (<10 days) with baricitinib initiation and were overall decreased on study, there was continued variability in ISG scores over time. This may be representative of insufficient pathway inhibition, variability of disease activity, or exogenous stressors such as intercurrent illnesses. Environmental factors that may influence ISG levels at the time of sample collection, such as intercurrent infection or recent vaccination, were not available for analysis.

We designed the AGS symptom diary to allow families to report the daily burden of disease. This score improved while on therapy, and this improvement was sustained. We hypothesized that while injury to the brain was likely to be irreversible in AGS, ongoing inflammation may contribute to the poor developmental trajectory through encephalopathy and muscle inflammation. Neurologic improvement or stabilization is challenging to measure in AGS. Some individuals in this study had more than 10 years of disease progression before treatment initiation. In addition, the severe neurologic manifestations made objective measurement of neurologic improvement difficult due to floor effects using traditional outcome measures. To address the limitations of existing outcome measures, we utilized the simple approach of quantitating clinically meaningful developmental skills. We found that baricitinib resulted in an improvement in a subset of individuals. These data suggest that individuals with longstanding disease have a potential for neurologic improvement. While limited by the retrospective nature, our cohort was stable in the period prior to treatment and responsive to therapy initiation.

Further research will be necessary to define whether earlier treatment may prevent neurologic decline in pre-symptomatic individuals. However, these findings suggest that baricitinib may be an effective disease modifying therapy in AGS. Caution should be used in treating individuals who are also treated with other immunosuppressive regimens, including corticosteroids.

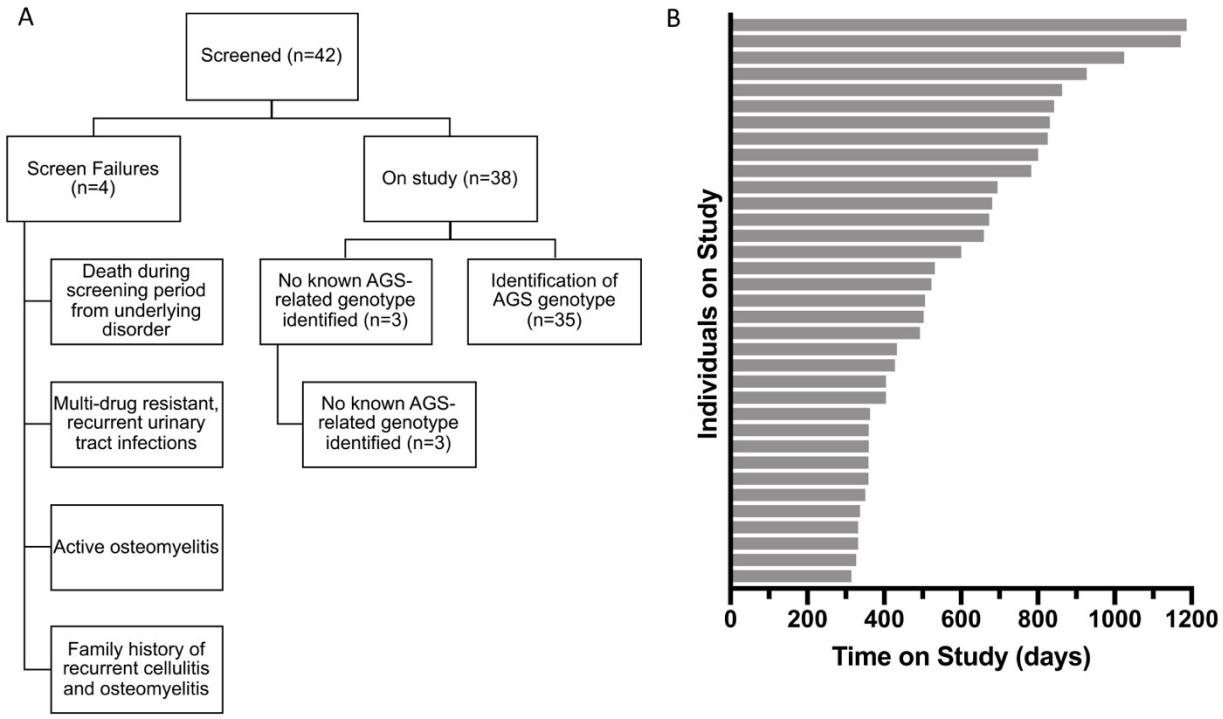

FIGURE S1. TRIAL PROFILE. (A) Subject disposition. (B) Swim lane plot of time on study.

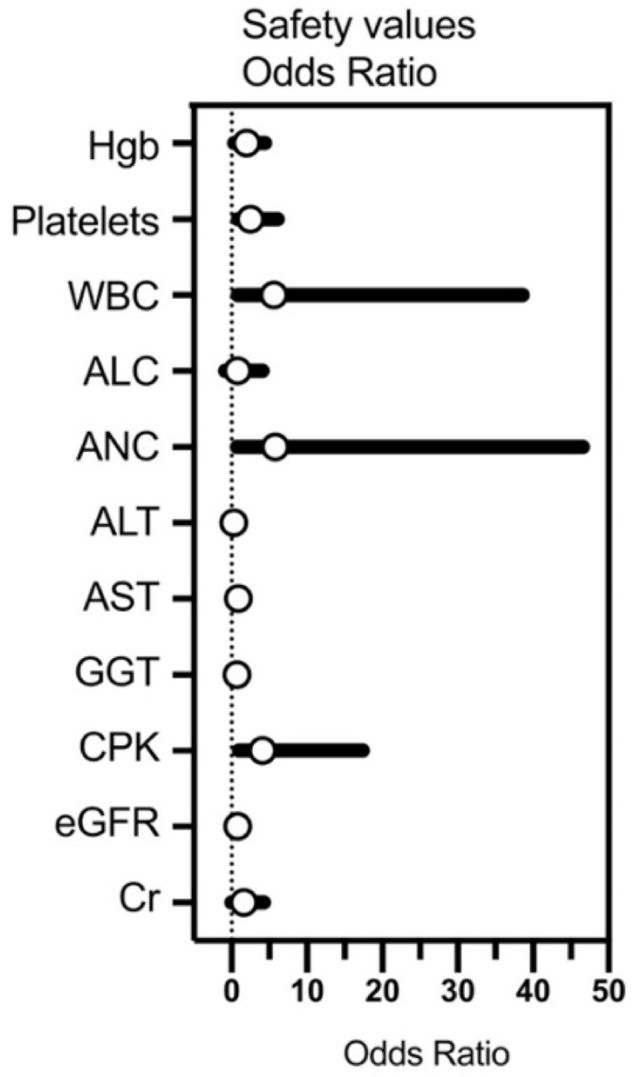

FIGURE S2. ODDS-RATIO CALCULATIONS FOR SAFETY PARAMETERS, COMPARING SAFETY GRADES BEFORE AND AFTER STUDY. Presented as Odds-Ratio with 95th percentile confidence interval. For eGFR and Creatinine, we performed logistic regression because these were coded as normal versus abnormal.

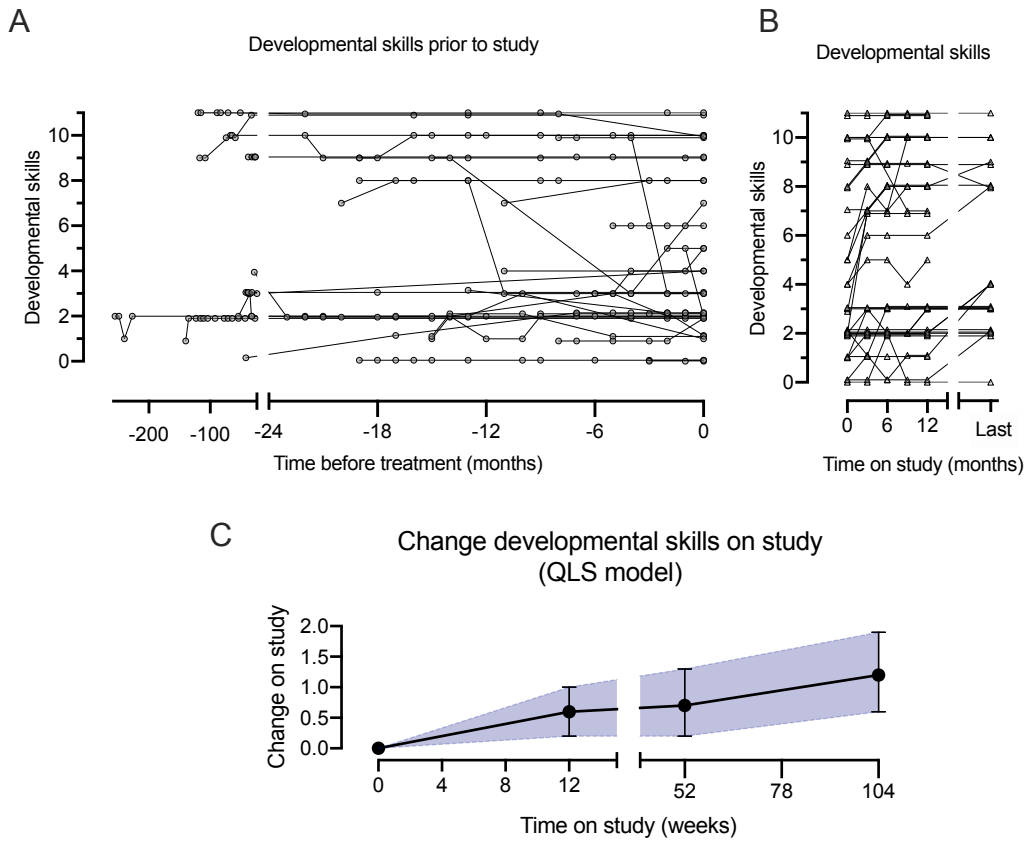

FIGURE S3. CHANGE IN NEUROLOGIC FUNCTION ON STUDY. Developmental skills (including head circumference) were retrospectively collected from medical records pre-treatment (A) and on study (B). The QLS model was used to model longitudinal change on therapy (C).

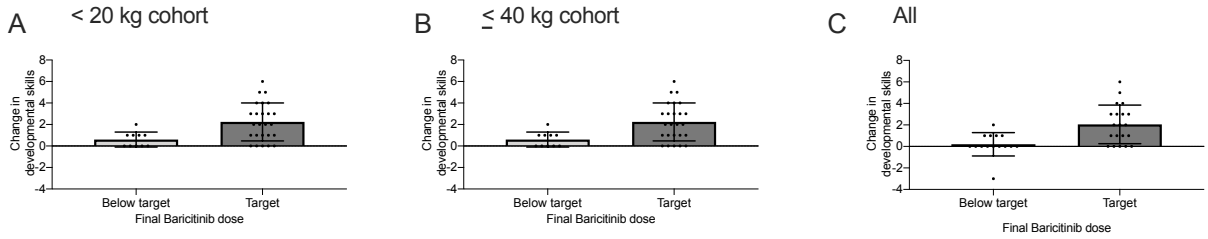

FIGURE S4. CHANGE IN NEUROLOGIC FUNCTION ON STUDY CORRELATES WITH FINAL DOSAGE. Individuals within weight categories were divided into 'Below target' and 'Target'. Target represents > 0.3 mg/kg/day for individuals less than or equal to 40 kg or 12 mg for individuals over 40 kg. Change in developmental scores are presented as box plot of mean with standard deviation whiskers.

TABLE S1. Baseline demographics and clinical characteristics.

Baseline demographics		
Sex [Female n (%)]	13 (37.1)	
Genotype [n (%)]		
TREX1	5 (14.3)	
RNASEH2B	8 (22.9)	
RNASEH2A	1 (2.9)	
RNASEH2C	1 (2.9)	
SAMHD1	5 (14.3)	
ADAR1	7 (20.0)	
IFIH1	8 (22.9)	
Baseline clinical characteristics		
	mean (SD)	median (25%, 75%)
Age symptoms onset (years)	0.8 (0.9)	0.5 (0.1, 1.2)
Age at enrollment (years)	5.9 (6.1)	2.9 (1.7, 7.8)
IFN stimulated gene expression score	25.5 (14.4)	24.4 (17.8, 32.1)

TABLE S2. Classification of AGS Causative Variants in the treated population

Genotype	Nucleotide change 1	Amino acid change 1	Inheritance	ACMG	SIFT	Polyphen	Nucleotide change 2	Amino acid change 2	Inheritance	ACMG	SIFT	Polyphen	Neuro	Skin	Liver	OCM	ISG
TREX1	c.341G>A	p.Arg114His	U	PA	D	PRD	c.667G>A	p.Ala223Thr	U	LP	D	PRD	1	1	0		1
TREX1	c.341G>A	p.Arg114His	U	PA	D	PRD	c.416delC	p.Ala139ValfsX21	U	PA	N/A	N/A	1	0	0	G	1
TREX1	c.341G>A	p.Arg114His	M	PA	D	PRD	c.667G>A	p.Ala223Thr	P	LP	D	PRD	1	1	1	DI	1
TREX1	c.341G>A	p.Arg114His	U	PA	D	PRD	c.907A>C	p.Thr303Pro	U	LP	D	POD	1	1	1	DM1	1
TREX1	c.341G>A	p.Arg114His	M	PA	D	PRD	c.341G>A	p.Arg114His	P	PA	D	PRD	1	0	1	TH	1
TREX1	c.52G>C	p.Asp18His	U	PA	D	PRD							1	1	0		1
RNASEH2B	c.128C>A	p.Pro43His	P	LP	D	PRD	c.529G>A	p.Ala177Thr	M	PA	D	B	1	0	0	TH	0
RNASEH2B	c.428_434delAGGAAAA	p.Glu144Valfster5	M or P	PA	N/A	N/A	c.529G>A	p.Ala177Thr	M or P	PA	D	B	1	0	1		1
RNASEH2B	c.529G>A	p.Ala177Thr	M	PA	D	B	c.529G>A	p.Ala177Thr	P	PA	D	B	1	1	0		1
RNASEH2B	c.3G>A	p.M1?	P	PA	N/A	N/A	c.412C>T	p.Leu138Phe	M	LP	D	POD	1	0	1		1
RNASEH2B	c.529G>A	p.Ala177Thr	U	PA	D	B	c.529G>A	p.Ala177Thr	U	PA	D	B	1	1	0		1
RNASEH2B	c.510+1G>A	–	P	PA	N/A	N/A	c.529G>A	p.Ala177Thr	M	PA	D	B	1	1	1		1
RNASEH2B	c.529G>A	p.Ala177Thr	M	PA	D	B	c.529G>A	p.Ala177Thr	P	PA	D	B	1	1	0		1
RNASEH2B	c.529G>A	p.Ala177Thr	M	PA	D	B	c.510+1G>A	–	P	PA	N/A	N/A	1	1	0		1
RNASEH2A	c.557G>A	p.Arg186Gln	M	LP	D	PRD	c.557G>A	p.Arg186Gln	P	LP	D	PRD	1	0	0		1
RNASEH2C	c.472C>G	p.His158Asp	M	VUS	D	PRD	c.155T>G	p.Ile52Ser	P	VUS	D	PRD	1	1	1		1
SAMHD1	Exon 1 deletion	–	U	PA	N/A	N/A	Exon 1 deletion	–	U	PA	N/A	N/A	1	1	1		1
SAMHD1	chr20:35,578,204-35,583,998 (deletion)	–	U	PA	N/A	N/A	chr20:35,578,204-35,583,998 (deletion)	–	U	PA	N/A	N/A	1	1	1	G	1
SAMHD1	c.602T>A	p.Ile201Asn	M	LP	D	PRD	c.1293A>T	p.Leu431Phe	U	VUS	D	PRD	1	1	0		1
SAMHD1	chr20:35,578,204-35,583,998 (deletion)	–	U	PA	N/A	N/A	chr20:35,578,204-35,583,998 (deletion)	–	U	PA	N/A	N/A	1	1	0		1
SAMHD1	Exon 1 deletion	–	U	PA	N/A	N/A	Exon 1 deletion	–	U	PA	N/A	N/A	1	1	1	G	1

ADAR1	c.3019G>A	p.Gly1007Arg	DN	PA	D	PRD							1	1	0		1
ADAR1	c.3577G>A	p.Glu1193Lys	M	LP	D	PRD	c.1493_1494delAG	p.Glu498ValfsX18	P	PA	N/A	N/A	1	1	1		1
ADAR1	c.577 C>G	p.Pro193Ala	M	PA	D	PRD	c.3020-3C>G	IVS11-3C>G	P	LP	N/A	N/A	1	1	0		1
ADAR1	c.3019G>A	p.Gly1007Arg	M	PA	D	PRD	c.2653G>A	p.Val885Ile	P	LP	T	POD	1	1	0		1
ADAR1	c.3019G>A	p.Gly1007Arg	M	PA	D	PRD	c.2653G>A	p.Val885Ile	P	LP	T	POD	1	0	1		1
ADAR1	c.982C>T	p.Arg328X	U	PA	N/A	N/A	c.577C>G	p.Pro193Ala	P	PA	D	PRD	1	0	1		1
IFIH1	c.2342G>A	p.Gly781Glu	NA	VUS	D	PRD							1	1	1		1
IFIH1	c.2159G>A	p.Arg720Gln	DN	PA	D	PRD							1	0	1	TH, PH	1
IFIH1	c.1009A>G	p.Arg337Gly	DN	PA	T	PRD							1	1	0	PH	1
IFIH1	c.2336G>A	p.Arg779His	DN	PA	T	POD							1	0	1		1
IFIH1	c.2336G>T	pArg779Leu	DN	LP	D	PRD							1	0	1		1
IFIH1	c.2336G>A	p.Arg779His	P	PA	T	POD							1	0	0		1
IFIH1	c.2336G>A	p.Arg779His	P	PA	T	POD							1	0	0		1
IFIH1	c.2407A>T	p.Ile803Phe	DN	VUS	T	B							1	0	1		1

Abbreviations:
ACGM: ACGM classification; B: benign; D: deleterious; DI: diabetes insipidus; DM1: diabetes mellitus type 1; DN: de novo; G: glaucoma; LP: likely pathogenic; M: maternal; OCM: other clinical manifestation; P: Paternal; PA: pathogenic; PH: pulmonary hypertension; POD: possibly damaging; PRD: probably damaging; T: tolerated; TH: thrombocytopenia: U: unknown; VUS: variant of uncertain significance
Scoring notation:
Neurologic (neuro): 0 if normal neurologic exam at screening evaluation, 1 if abnormal neurologic exam at screening evaluation
Skin: 0 if no pre-treatment skin involvement recorded in clinical notes or if skin scores in pre-treatment symptom diary scores <2 (single day) or <1 (average score). 1 if pre-treatment skin involvement recorded in clinical notes or if skin scores in pre-treatment symptom diary scores ≥2 (single day) or ≥1 (average score).
Liver: 0 if AST, ALT, GGT normal or increased <2 folds from normal range value upper limit , 1 if AST, ALT, GGT normal or increased ≥2 folds from normal range value upper limit
ISG: 0 if non-elevated ISG, 1 if elevated ISG

TABLE S3. Safety Measures

Maximum laboratory abnormalities prior to study

Grade	0	1	2	3	4	Total
Laboratory parameter	N (%)	N (%)	N (%)	N (%)	N (%)	N
ABSOLUTE LYMPHOCYTES	32 (94.1)	2 (5.9)	0 (0)	0 (0)	0 (0)	34
ABSOLUTE NEUTROPHILS	31(96.9)	1 (3.1)	0 (0)	0 (0)	0 (0)	32
LEUKOCYTOSIS	32 (100)	0 (0)	0 (0)	0 (0)	0 (0)	32
LEUKOPENIA	32 (91.4)	3 (8.6)	0 (0)	0 (0)	0 (0)	35
THROMBOCYTOSIS	30 (96.8)	1 (3.2)	0 (0)	0 (0)	0 (0)	31
THROMBOCYTOPENIA	30 (88.2)	3 (8.8)	1 (2.9)	0 (0)	0 (0)	34
ALANINE AMINOTRANSAMINASE (ALT/SGPT)	18 (51.4)	15 (42.9)	1 (2.9)	1 (2.9)	0 (0)	35
ALKALINE PHOSPHATASE (AP)	34 (100)	0 (0)	0 (0)	0 (0)	0 (0)	34
ASPARTATE AMINOTRANSAMINASE (AST/SGOT)	14 (40)	20 (57.1)	1 (2.9)	0 (0)	0 (0)	35
CHOLESTEROL	31 (88.6)	3 (8.6)	1 (2.9)	0 (0)	0 (0)	35
CPK	32 (94.1)	3 (5.9)	0 (0)	0 (0)	0 (0)	34
CREATININE	24 (68.6)	11 (31.4)	0 (0)	0 (0)	0 (0)	35
GAMMA GLUTAMYL TRANSFERASE	14 (40)	12 (34.3)	6 (17.1)	3 (8.6)	0 (0)	35
HEMOGLOBIN	29 (82.9)	6 (17.1)	0 (0)	0 (0)	0 (0)	35
RETICULOCYTE COUNT	16 (47.1)	18 (52.9)	0 (0)	0 (0)	0 (0)	34
TRIGLYCERIDES	25 (71.4)	9 (25.7)	1 (2.9)	0 (0)	0 (0)	35
eGFR	29 (82.9)	6 (17.1)	0 (0)	0 (0)	0 (0)	35
Total	391 (75.5)	112 (21.6)	11 (2.1)	4 (0.8)	0 (0)	518

Maximum laboratory abnormalities on study

Grade	0	1	2	3	4	Total
Laboratory parameter	N (%)	N (%)	N (%)	N (%)	N (%)	N
ABSOLUTE LYMPHOCYTES	27 (77.1)	8 (22.9)	0 (0)	0 (0)	0 (0)	35
ABSOLUTE NEUTROPHILS	14 (40.0)	16 (45.7)	5 (14.3)	0 (0)	0 (0)	35
LEUKOCYTOSIS	35 (100)	0 (0)	0 (0)	0 (0)	0 (0)	35
LEUKOPENIA	20 (57.1)	11 (31.4)	4 (11.4)	0 (0)	0 (0)	35
THROMBOCYTOSIS	15 (44.1)	15 (44.1)	3 (8.8)	1 (2.9)	0 (0)	34
THROMBOCYTOPENIA	30 (88.2)	3 (8.8)	1 (2.9)	0 (0)	0 (0)	34
ALANINE AMINOTRANSAMINASE (ALT/SGPT)	13 (37.1)	21 (60)	0 (0)	0 (0)	1 (2.9)	35

ALKALINE PHOSPHATASE (AP)	27 (77.1)	6 (17.1)	0 (0)	2 (5.7)	0 (0)	35
ASPARTATE AMINOTRANSAMINASE (AST/SGOT)	0	30 (85.7)	4 (11.4)	1 (2.9)	0 (0)	35
CHOLESTEROL	21 (60)	13 (37.1)	0 (0)	0 (0)	1 (2.9)	35
CPK	18 (51.4)	12 (34.3)	4 (11.4)	1 (2.9)	0 (0)	35
CREATININE	13 (37.1)	22 (62.9)	0 (0)	0 (0)	0 (0)	35
GAMMA GLUTAMYL TRANSFERASE	5 (14.3)	20 (57.1)	6 (17.1)	4 (11.4)	0 (0)	35
HEMOGLOBIN	15 (42.9)	13 (37.1)	6 (17.1)	1 (2.9)	0 (0)	35
RETICULOCYTE COUNT	3 (8.6)	32 (91.4)	0 (0)	0 (0)	0 (0)	35
TRIGLYCERIDES	17 (48.6)	13 (37.1)	5 (14.3)	0 (0)	0 (0)	35
eGFR	19 (54.3)	16 (45.7)	0 (0)	0 (0)	0 (0)	35
Total	226 (43)	249 (47.4)	38 (7.2)	10 (1.9)	2 (0.4)	525

TABLE S4. Statistical analysis of outcome measures on study

	Immediate Change	Change at 12 months	Change at final measurement [median months on study 18 (25th,75th %ile 12-30)
Change in ISG Score	(within first week) [N = 31]	[N = 35]	[N = 35]
Median (25th, 75th percentile)	-13.8 (-19.7, -6.9)	-8.6 (-10.9, -4.6)	-5.6 (-12.3, -2.8)
Mean (95%CI)	-14.7 (-20.7, -8.6)	-8.3 (-12.8, -3.8)	-7.2 (-12.6, -1.9)
QLS model estimated change (95% CI)	-14.3 (95% CI = -19.7 to -8.8)	-7.4 (95% CI = -13.3 to -3.5)	-8.0 (95% CI = -13.6 to -2.5) at 24 months
Adjusted QLS model estimated change (95% CI)	-14.0 (95% CI = -19.5 to -8.6)	-7.3 (95% CI = -11.2 to -3.4)	-7.2 (95% CI = -12.6 to -1.9) at 24 months
Change in clinical score	(within first month) [N = 35]	[N = 35]	[N = 35]
Median (25th, 75th percentile)	-0.4 (-0.9, 0.2)	-1.1 (-2.4, 0.0)	-1.1 (-2.2,-0.2)
Mean (95%CI)	-0.7 (-1.2, -0.2)	-1.2 (-2.0,-0.40)	-1.4 (-2.2,-0.6)
QLS model estimated change (95% CI), p-value	-0.1 (95% CI = -0.1 to 0.0)	-0.7 (95% CI = -1.0 to -0.4)	-1.1 (95% CI = -1.6 to -0.6)
Adjusted QLS model estimated change (95% CI),	-0.10 (95% CI = -0.1 to 0.0)	-0.6 (95% CI = -1.0 to -0.3)	-1.5 (95% CI = -2.1 to -0.9)
Change in developmental skills	(within first 3 months) [N = 35]	[N = 35]	[N = 35]
Median (25th, 75th percentile)	0.0 (0.0, 1.0)	1.0 (0.0, 1.0)	1.0 (0.0, 2.0)
Mean (95%CI)	0.5 (0.2,0.9)	0.9 (0.4, 1.5)	1.3 (0.6, 1.9)
QLS model estimated change (95% CI)	0.6 (95% CI = 0.2 to 1.0)	0.7 (95% CI = 0.2 to 1.3)	1.2 (95% CI = 0.6 to 1.9)
Adjusted QLS model estimated change (95% CI)	0.6 (95% CI = 0.2 to 0.9)	0.7 (95% CI = 0.3 to 1.2)	1.2 (95% CI = 0.6 to 1.8)

TABLE S5. Clinical scores

Category	Signs and Symptoms	Score
Crying	Cries, but easily consolable	0
	Excessive or high-pitched cry for > 2 min OR intermittently for <10 min	1
	Excessive or high-pitched cry for > 2 min AND intermittently for <10 min	2
	Excessive or high-pitched cry, not consolable (cries >10 mins)	3
Sleep	Sleeps for > 3 hours continuously during night	0
	Sleeps 2-3 hours continuously during the night	1
	Sleeps 1-2 hours continuously during the night	2
	Sleeps < 1 hour continuously during the night	3
Irritability	No irritability	0
	Consoling calms individual in < 6 min	1
	Consoling calms individual in 6-15 minutes	2
	Consoling calms individual in >15 minutes or not at all	3
Seizure	No convulsions or seizures	0
	Experiences convulsions or seizures	8
Fever	No fever (temperature < 98.9 F)	0
	Temperature greater than 99.1 F	1
Skin findings: body	No skin problems	0
	Red patches which fade when pressed with fingers	1
	Red patched not fading when pressed with fingers	2
	Chronic discoloration	3
Skin findings: hands, face, ears	No skin problems	0
	Red patches which fade when pressed with fingers	1
	Red patched not fading when pressed with fingers	2
	Chronic discoloration	3

This form was developed in collaboration between Eli Lilly and Children's Hospital of Philadelphia

TABLE S6. AGS Developmental skills[12]

Item	Points	
	0 Absent	1 Present
Normal head circumference		
Social smile		
Vocalizations (cooing or babbling)		
Single, meaningful words		
Minimum of three-word phrases		
Head control (> 60 seconds)		
Pincer grasp or self-feeding		
Independent sitting (> 2 minutes)		
Rolling or crawling to goal		
Ambulation with assistance (devices or two-hand assist)		
Independent ambulation (no assistive devices)		

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