Treatment of Severe Persistent Asthma with IL-6 Receptor blockade

Brittany Esty, MD, MPH[a*], Hani Harb, PhD [a*], Lisa M. Bartnikas, MD [a], Louis M. Charbonnier, PhD [a], Amir H. Massoud, PhD [a], Carmen Leon-Astudillo, MD[b] , Gary Visner, DO[b] , Meera Subramaniam, MD[b] , Wanda Phipatanakul, MD, MS[a] , Talal A. Chatila, MD, MSc[a]

[a] Division of Immunology, Boston Children's Hospital, Department of Pediatrics, Harvard Medical School, Boston, MA.[b] Division of Respiratory Diseases, Boston Children's Hospital, Department of Pediatrics, Harvard Medical School, Boston, MA.

* Authors contributed equally to the study

Corresponding Author: Talal A. Chatila, MD, MSc, Division of Immunology, Department of Pediatrics, Boston Children's Hospital, Harvard Medical School, Boston, MA 02115. E-mail: talal.chatila@childrens.harvard.edu.

SUPPLEMETARY METHODS

Study approval. Studies on patient peripheral blood samples were all performed at the Boston Children's Hospital and were approved by the Institutional Review Board.

Antibodies: Flow cytometry and intracellular staining. Single-cell suspensions were stained with the indicated antibodies (Ab) and analyzed on LSRIIFortessa cytometer (Becton Dickinson). Cytokine expression in CD4+ T cells was determined by stimulating cells with PMA (20 ng/ml) plus ionomycin (1 μg/ml) for 4 hours in the presence of Golgi-plug (BD Biosciences) followed by intracellular staining for the respective cytokine using the eBioscience Fixation/Permbealization buffer following the manufacturer's instructions. Fluorescence-conjugated mAbs used were obtained from BD Biosciences, Biolegend and eBioscience. Anti-CD3-APC-Cy7, (H1T3a), anti-CD4-PerCP-Cy5.5 and PE (PRA-T4), anti-CD25-PE (CD25-4E3), anti-CD127-PE-Cy7 (A019D5), anti-CRTH2-FITC (BM16), anti-CXCR3-APC (G025H7), anti-CCR4-BV605 (L29H14), anti-CCR6-Amcyan (G034E3). For intracellular staining, the following mAbs were used from BD Biosciences, Biolegend and eBioscience, anti-IFNG-PE-Cy7 (45.B3) anti-IL-13-PerCP-Cy5.5 (JE510-SA2), Anti-IL-4-BV605 (MP4-25D2), anti-IL-17-APC (BL168), anti-FOXP3-Pacific Blue (PCH101).

Cell preparation: Blood was obtained from the patients after a written consent. PBMCs were isolated using Ficoll (GE-Healthsciences). Shortly, 4 mL of Ficoll were layered in a 15 mL tube. Afterwards, the blood will be layered on top of ficoll very slowly to build two separate phases. The ficoll/blood mixture were centrifuged on 300 g for 20 min without breaks to have the PBMCs caught in the middle layer. The cells were aspirated into a new 15 mL tube and washed

twice with 10 mL PBS. The cell pellet was then used for the PMA/Ionomycin/ Golgi-plug stimulation and FACs staining.

Data analysis: The flowcytometric analysis of the data was done using Flowjo software (FlowJo, LLC). The graphs and statistical analysis were done using GraphPad Prism version 7.00 for Windows, GraphPad Software, La Jolla California USA (www.graphpad.com). The time course of cytokine expression post-therapy was analyzed by two-way ANOVA with Tukey post-test analysis. Within each cell type and for each cytokine, the values obtained post therapy were compared to the pre-therapy baseline value. A p value <0.05 was considered statistically significant.

Statistical process control (SPC) charts were created to demonstrate days between admissions for patient 1 and 2. SPC charts demonstrate data over time illustrating common cause and special cause variation. A t-chart is a measure of time between disease incidences for a specified period of time [1]. Common cause variation demonstrates randomly distributed variation around the center line appearing between upper and lower control limits [2.] Common cause variation represents variation inherent in the process. Control limits are positioned 3 standard deviations from the center line (3 sigma limits) [1]. Special cause variation demonstrates non-random variation. Identification of non-random variation is defined by rules of statistical process control. The Western Electric rules for special cause variation include: 1) One or more points beyond a 3-sigma limit, 2) Two out of three successive points beyond a 2-sigma limit, 3) Four out of five successive points beyond a 1-sigma limit, and 4) Eight consecutive points above or below the center line [3]. SPC t-charts were created for the days between admissions in QI Macros, SPC Software for Excel (https://www.qimacros.com) to analyze measures.

References

1. Provost L, Murray S. The Health Care Data Guide: Learning from Data for Improvement. Hoboken, NJ: John Wiley & Sons, 2011:229e30.
2. Montgomery DC. Introduction to statistical quality control. 6. USA: Wiley; 2009. P 182-190.
3. Western Electric Company. Statistical quality control handbook. 2nd Easton: Mack Printing Company; 1958. Available from: http://www.westernelectric.com/support-statistical-quality-control-handbook.html. Accessed 14 Feb 2019.

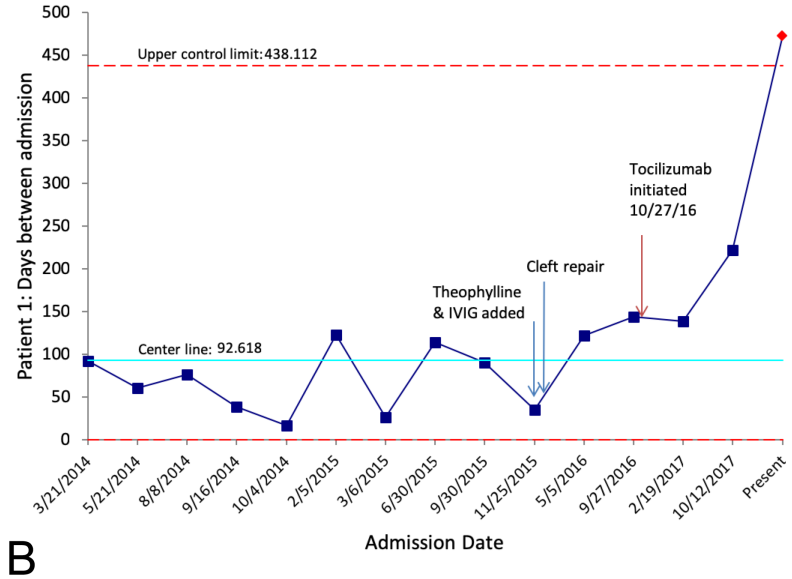
Supplemental Figure Legend

Fig. E1. Statistical process control (SPC) t chart illustrating days between inpatient hospital admissions in patient 1 (Fig E1, *A*) and patient 2 (Fig E1, *B*). Note. Red points indicate special cause variation.

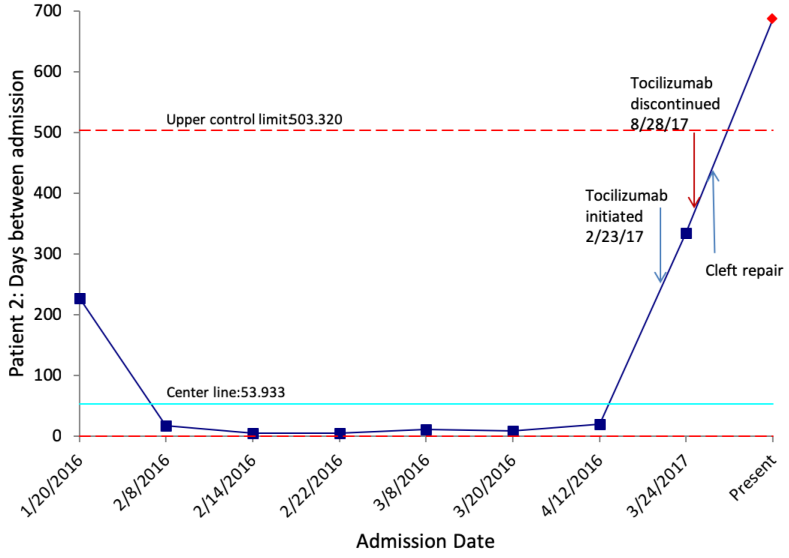
Fig. E2. Analysis of cytokine production by T effector (Teff) cells in patient 1 and 2. **A and B**, Flow cytometric analysis of IL-4 and IL-17 expression in Teff cells at baseline and at 4, 8 and 10 months after tocilizumab treatment in patient 1 (Fig E2, *A***)** patient 2 (Fig E2, *B*).

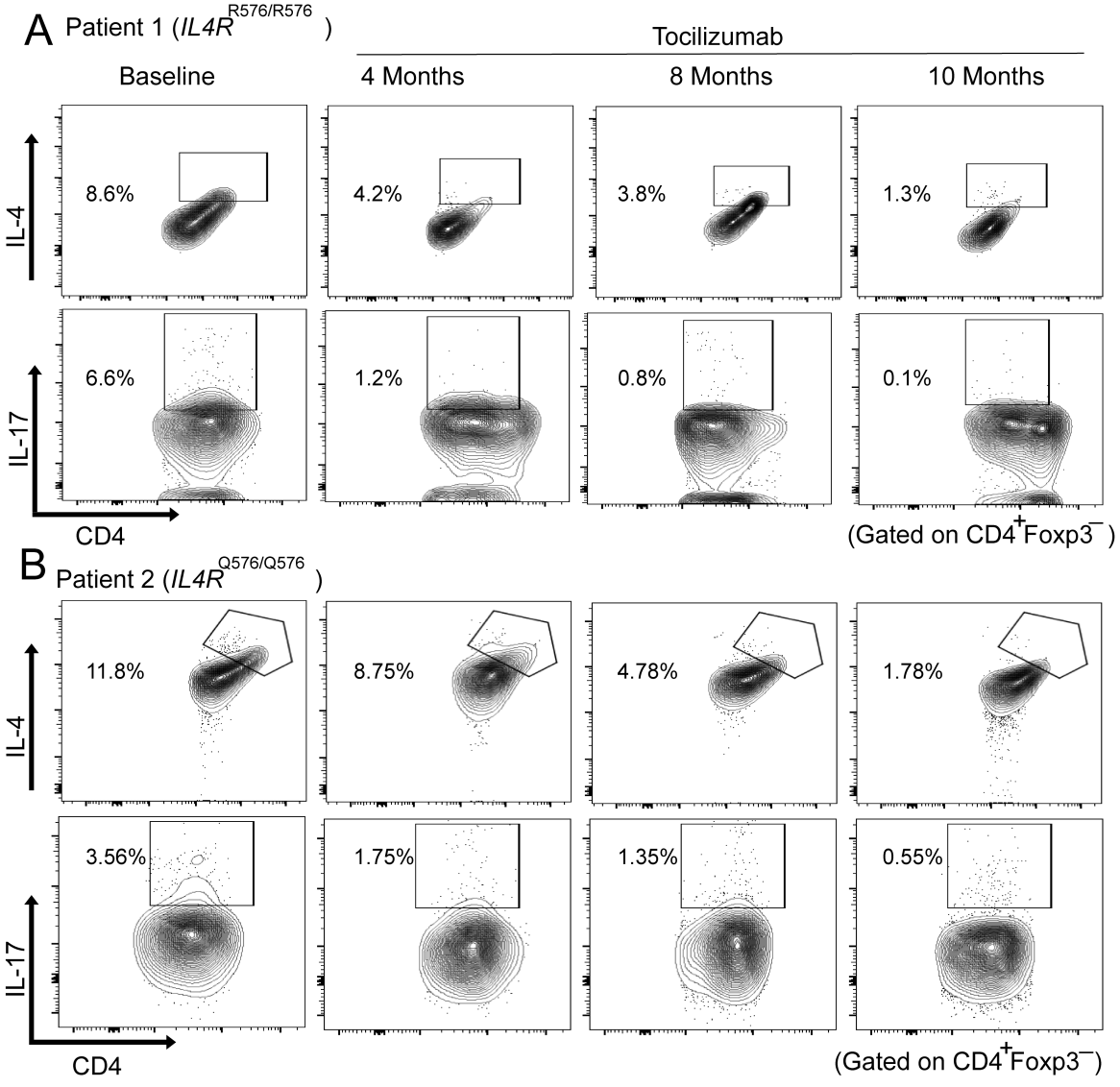
Fig. E3. Peripheral blood eosinophil count at baseline and with each tocilizumab infusion in patient 1 (Fig E3, *A*) patient 2 (Fig E3, *B*).

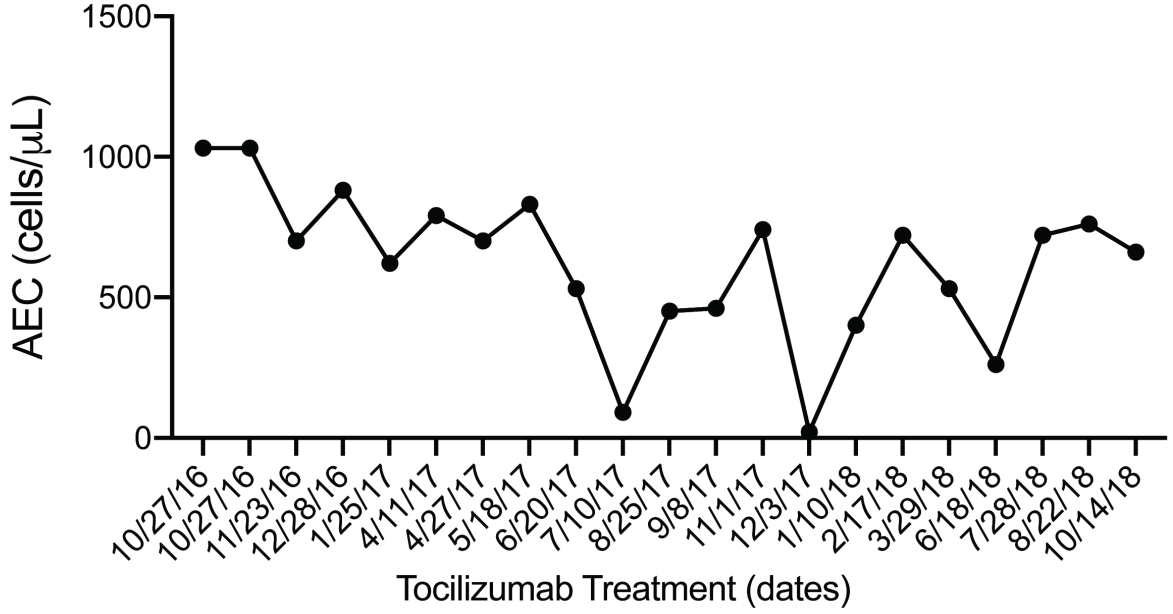
A

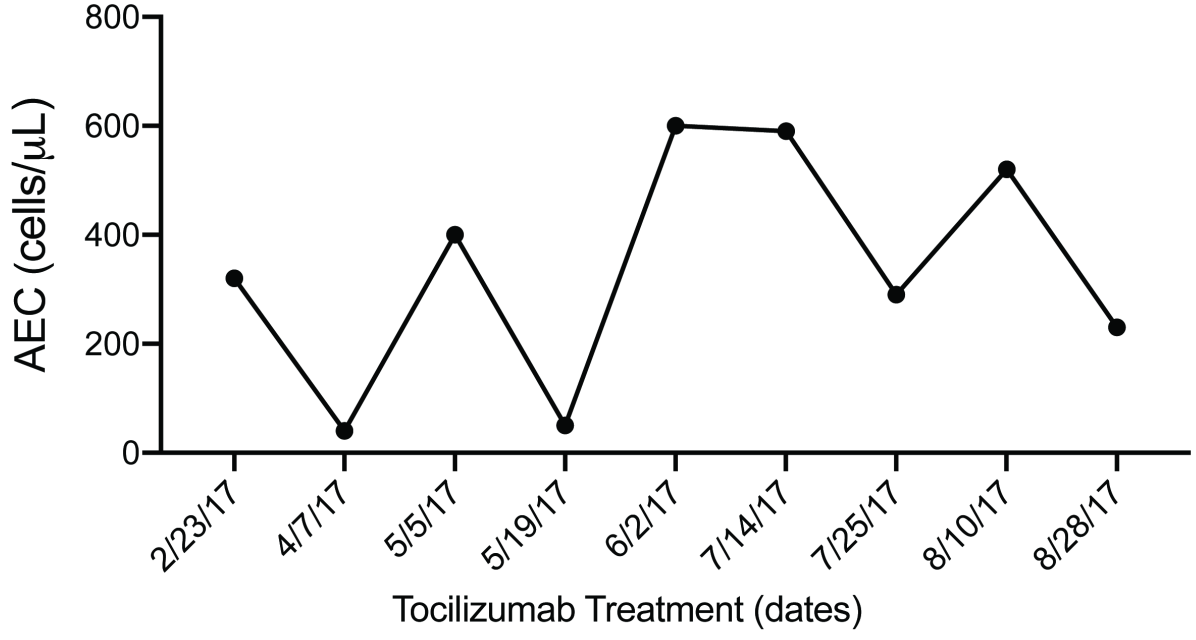

Upper control limit: 438.112
Center line: 92.618
Tocilizumab initiated 10/27/16
Cleft repair
Theophylline & IVIG added
Patient 1: Days between admission
Admission Date
500
450
400
350
300
250
200
150
100
50
0
3/21/2014
5/21/2014
8/8/2014
9/16/2014
10/4/2014
2/5/2015
3/6/2015
6/30/2015
9/30/2015
11/25/2015
5/5/2016
9/27/2016
2/19/2017
10/12/2017
Present

B


Upper control limit503.320
Center line:53.933
Tocilizumab discontinued 8/28/17
Tocilizumab initiated 2/23/17
Cleft repair
Patient 2: Days between admission
Admission Date
700
600
500
400
300
200
100
0
1/20/2016
2/8/2016
2/14/2016
2/22/2016
3/8/2016
3/20/2016
4/12/2016
3/24/2017
Present


A
Patient 1 (IL4R R576/R576)
Tocilizumab
Baseline
4 Months
8 Months
10 Months
IL-4
8.6%
4.2%
3.8%
1.3%
IL-17
6.6%
1.2%
0.8%
0.1%
CD4
(Gated on CD4+Foxp3−)
B
Patient 2 (IL4R Q576/Q576)
IL-4
11.8%
8.75%
4.78%
1.78%
IL-17
3.56%
1.75%
1.35%
0.55%
CD4
(Gated on CD4+Foxp3−)


1500
1000
500
0
AEC (cells/μL)
10/27/16
10/27/16
11/23/16
12/28/16
1/25/17
4/11/17
4/27/17
5/18/17
6/20/17
7/10/17
8/25/17
9/8/17
11/1/17
12/3/17
1/10/18
2/17/18
3/29/18
6/18/18
7/28/18
8/22/18
10/14/18
Tocilizumab Treatment (dates)


800
600
400
200
0
AEC (cells/μL)
2/23/17
4/7/17
5/5/17
5/19/17
6/2/17
7/14/17
7/25/17
8/10/17
8/28/17
Tocilizumab Treatment (dates)