Title: Phase 2 Study of Danicopan in Paroxysmal Nocturnal Hemoglobinuria Patients with an Inadequate Response to Eculizumab

Short Title: Danicopan in PNH Eculizumab Inadequate Responders

Authors: Austin G. Kulasekararaj[1], Antonio M. Risitano[2], Jaroslaw P. Maciejewski[3], Rosario Notaro[4,5], Peter Browett[6], Jong Wook Lee[7], Mingjun Huang[8], Michael Geffner[9], Robert A. Brodsky[10]

[1]King's College Hospital-NHS Foundation Trust, NIHR/Wellcome King's Clinical Research Facility, London, UK & King's College London, London, UK;
[2]AORN Moscati, Avellino, Italy;
[3]Department of Translational Hematology and Oncology Research, Taussig Cancer Institute, Cleveland Clinic, Cleveland, Ohio, USA;
[4]Azienda Ospedaliera Universitaria Careggi, Firenze, Italy;
[5]Instituto per lo Studio, la Prevenzione e la Rete Oncologica, Firenze, Italy;
[6]University of Auckland, Auckland, New Zealand;
[7]Seoul St. Mary's Hospital, The Catholic University of Korea, Seoul, Korea, Republic Of Korea;
[8]Achillion, Inc., a wholly owned subsidiary of Alexion Pharmaceuticals, Inc., New Haven, CT, USA
[9]Achillion, Inc., a wholly owned subsidiary of Alexion Pharmaceuticals, Inc., Blue Bell, PA, USA;
[10]Johns Hopkins University School of Medicine, Baltimore, MD, USA

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Correspondence to:
Robert A. Brodsky, MD
Ross Research Building
720 Rutland Ave, Baltimore MD, 21205
brodsro@jhmi.edu

Supplementary Appendix

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

Supplement to: Kulasekararaj, AG et al. Phase 2 Study of Danicopan in Paroxysmal Nocturnal Hemoglobinuria Patients with an Inadequate Response to Eculizumab

Supplementary Assay Methods

Serum complement C3 concentration and serum classical pathway (CP) activity were measured with the Cobas (Roche) and Complement Activation EIA (Diasorin) kits, using pre-existing methods established in clinical laboratories. Serum alternative pathway (AP) activity was measured with AP Wieslab assay (Euro Diagnostica); Bb concentration was measured with MicroVue Complement Fragment Bb EIA (Quidel); serum FD concentration was measured with Quantikine® ELISA (R&D Systems, Inc.) by a single central laboratory after validating the assays according to the manufacturers' instructions. In normal individuals, the mean ± SD value of plasma Bb is 0.84±0.84 µg/mL, with a range of 0.553–1.357 µg/mL based on the data collected from three phase 1 studies in healthy volunteers (N=100; trial ID: ACTRN12617001521314, ACTRN12618001989235, ACTRN12618000896279) by the same single centralized lab with the same commercial kit.

C3 fragment deposition on erythrocytes was measured by flow cytometry after validating the following protocol: after centrifugation of whole blood collected from patients, the pellet containing erythrocytes was transferred, washed three times with phosphate buffer saline [PBS], and resuspended in GVB^0 buffer (Complement Technology) at a density of erythrocytes about $1\text{-}2\times10^9$/mL. Before the test, an aliquot (5×10^6) of erythrocytes was transferred to a fresh tube, washed once with flow cytometry (FC) wash buffer (PBS + 15 mM EDA + 1% BSA), and incubated with 100 µl FC wash buffer containing FITC conjugated human C3d antibody (Assay Pro, Cat# 11294-05041), and PE conjugated human CD59 antibody (NOVUS, Cat # MEM-43) in the dark at room temperature for 1 hour. An additional aliquot of erythrocytes was processed in the same way, replacing the antibodies in FC wash buffer with isotopic controls. At the end of incubation, erythrocytes were washed three times with FC wash buffer, resuspended in 200 µl FC buffer wash, and finally submitted to flow-cytometry analysis. Intact erythrocytes were gated based on physical parameters on forward and side scatter; gating for the fluorescence dye-conjugated antibodies was established with the isotypic controls. The following values were reported:

$$\frac{\text{erythrocytes stained negative for anti-CD59 antibody \& positive for anti-C3d antibody}}{\text{total erythrocytes}} \times 100\%$$

Supplementary Table 1. Dose of danicopan in mg, TID, at each scheduled visit for each enrolled patient

Visit*	Patient A 41 y/o, F	Patient B 51 y/o, F	Patient C 67 y/o, M	Patient D 29 y/o, F	Patient E 22 y/o, F	Patient F 44 y/o, F	Patient G 35 y/o, F	Patient H 53 y/o, F	Patient I 51 y/o, F	Patient J 19 y/o, M	Patient K 57 y/o, F
Baseline	100	100	150	150	100	100	100	100	100	100	100
Week 1	100	100	150	150	100	100	100	100	150	100	100
Week 2	100	100	150	150	100	100	100	100	150	100	100
Week 4	100	100	150	150	100	100	100	150	150	100	100
Week 8	100	100	150	150	150	100	100	150	150	100	100
Week 12	150	100	150	150	100	100	100	200	150	100	100
Week 16	150	100	150	150	150	100	100	200	200	150	150
Week 20	150	100	150	150	150	150	100	200	200	200	150
Week 24	150	100	150	150	150	150	100	200	200	200	150

F, female; M, male; TID, three times daily.