**Baseline clinical characteristics and complement biomarkers of patients with C3G enrolled in two Phase II studies investigating the factor D inhibitor danicopan**

Steven D. Podosa, Howard Trachtmanb\*, Gerald Appelc, Andrew S. Bombackc, Bradley P. Dixond, Jack Wetzelse, H. Terence Cookf, Samir V. Parikhg, Matthew C. Pickeringf, James Tumlinh\*\*, Craig Langmani, Liz Lightstonef, C. John Speratij, Erica Dainak, Koenraad Peter Boumanl, Kara Ricem, Jane A Thanassia, Mingjun Huanga, Carla Nestern and Giuseppe Remuzzik

# Supplementary tables

**Supplementary table 1.** Key inclusion and exclusion criteria for studies NCT03369236 (Study 204) and NCT03459443 (Study 205).

|  |  |
| --- | --- |
| **NCT03369236 (Study 204)** | **NCT03459443 (Study 205)** |
| **Key inclusion criteria**   * Must have biopsy-confirmed primary C3G * Must be 17 years of age or older and capable of swallowing tablets * Significant proteinuria ≥500 mg/day of protein in a 24-hour urine or equivalent on spot urine * Stable dose of corticosteroids, anti-hypertensive medications, anti-proteinuric medications for at least 2 weeksa   **Key exclusion criteria**   * A pre-treatment renal biopsy, or a recent historical biopsy available for review, which shows more than 50% global fibrosis or more than 50% of glomeruli with cellular crescents * Estimated GFR <30 mL/min/1.73 m2 at the time of screening or at any time over the preceding four weeks | **Key inclusion criteria**   * Must have completed the ACH471-201 Proof of Mechanism (POM) study, followed by a washout period of at least 30 days,   OR   * Must meet all the following criteria: * Must have biopsy-confirmed primary C3G or IC-MPGN * Significant proteinuria ≥500 mg/day of protein in a 24-hour urine * If a pre-treatment biopsy is obtained, or if a historical biopsy is available for review, it must have no more than 50% global fibrosis and no more than 50% of glomeruli with cellular crescents * Must be 12 years of age or older and capable of swallowing tablets * Stable dose of corticosteroids, anti-hypertensive medications, anti-proteinuric medications for at least 2 weeksa   **Key exclusion criteria**   * Estimated GFR <30 mL/min/1.73 m2 at the time of screening or at any time over the preceding four weeks |

**a**For example, angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARBs), or mycophenolate mofetil (MMF). Prior treatment exclusion criteria included eculizumab within 50 days prior to investigational drug dosing and tacrolimus or cyclosporine within 2 weeks of initial investigational drug dose.

**Supplementary Table 2.** Baseline biomarker concentrations and limits of normality.

|  |  |  |
| --- | --- | --- |
| Baseline biomarker | Central pathologist confirmed C3G (n = 29) | Normal rangea |
| **Plasma/serum biomarkers** | | |
| **C3 (µmol/L)b**  Median (IQR)  <5.0 µmol/L (n, %)  <2.5 µmol/L (n, %) | 2.33 (3.72)  23 (79.3)  15 (51.7) | LLN = 4.33 – 5.00 µmol/L  ULN = 9.05 – 11.16 µmol/L |
| **C4 (µmol/L)b**  Median (IQR)  <0.5 µmol/L (n, %) | 1.28 (0.75)  2 (6.9) | LLN = 0.50–0.70 µmol/L  ULN = 2.00–2.60 µmol/L |
| **C5 (µg/mL)c**  Median (IQR)  <50 µg/mL (n, %) | 40.0 (37.6)  17 (58.6) | LLN = 50 µg/mL  ULN = 115 µg/mL |
| **FD (µg/L)**  Median (IQR)  >3966 µg/L (n, %) | 3692 (2852)  13 (45) | LLN = 1437 µg/L  ULN = 3966 µg/L |
| **Ba (ng/mL)**  Median (IQR)  >1164 ng/mL (n, %) | 871 (556)  11 (37.9) | LLN = 338 ng/mL  ULN = 1164 ng/mL |
| **Bb (mg/L)**  Median (IQR)  >1.42 mg/L (n, %) | 1.79 (1.02)  21 (72.4) | LLN = 0.49 mg/L  ULN = 1.42 mg/L |
| **sC5b-9 (µg/L)**  Median (IQR)  >467 µg/L (n, %) | 597 (1194)  19 (65.5) | LLN = 95 µg/L  ULN = 467 µg/L |
| **AP activity (%)d**  Median (IQR)  <30 % (n, %) | 14.0 (57.0)  20 (69.0) | LLN = 30 %  ULN = 113 % |
| **CP activity (IU/L)**  Median (IQR)  <60 IU/L (n, %) | 47 (64)  18 (62.1) | LLN = 60 IU/L  ULN = 144 IU/L |
| **Urinary biomarkers** | | |
| **Urine Ba (ng/mL)e**  Median, (IQR) | 133 (370) | NA |
| **Ba/creatinine (µg/mmol)e**  Median, (IQR) | 10.7 (32.4) | NA |
| **sC5b-9 (ng/mL)e**  Median (IQR) | 126 (317) | NA |
| **sC5b-9/creatinine (µg/mmol)e**  Median (IQR) | 18.4 (31.6) | NA |

AP, alternative pathway of complement; CP, classic pathway of complement; FD, factor D; LLN, lower limit of normality; NA, not applicable; ULN, upper limit of normality.

aThe lower and upper limits of normality (LLN and ULN) were determined by central clinical laboratories except as follows: limits for serum C3 and C4 and for urine protein and creatinine were determined independently by each local laboratory; limits for serum AP activity were utilized as determined by the manufacturer (defined as ±2 SD from mean based on data from 120 donor samples; AP Wieslab, SVAR, Sweden); limits for FD were calculated internally from results of a Phase I trial by Alexion of FD inhibitors (calculated internally as ±2 SD from mean based on data from three human volunteer studies conducted in 178 participants; data on file) and limits for C5 were utilized as determined by the manufacturer (Abcam, UK).  
bC3 and C4 values normal ranges differ by local laboratory; cnon-GLP analysis (normal range for ELISA manufacturer); dAP Wieslab activity values are provided on a scale such that 100% of activity is normal activity as per assay control. Full inhibition of AP corresponds to a value of 0*.* eUrine concentrations are presented as absolute concentration and normalized to creatinine from same Day 1 spot urine collection; sC5b-9 values in 5 C3G patients were below the lower limit of quantitation of 8.8 ng/mL and were set to 8.8 ng/mL for normalization and analysis (concentrations of proteins are low or undetectable in the urine of healthy volunteers).

**Supplementary Table 3.** Baseline biopsy scoresa

|  |  |
| --- | --- |
|  | **Central pathologist confirmed C3G**  **N = 27/29b** |
| **Composite biopsy score** |  |
| Median (IQR) | 11.0 (5.0) |
| **Biopsy activity score** |  |
| Median (IQR) | 6.0 (5.0) |
| **Glomerular C3c staining** |  |
| Median (IQR) | 3.0 (0.0) |
| **Glomerular macrophage infiltration** |  |
| Median (IQR) | 1.0 (1.0) |
| **Chronicity score** |  |
| Median (IQR) | 5.0 (5.0) |

aSee supplementary information S1 for further details of the biopsy scoring system. bn = 27 for ‘central pathologist confirmed’ C3G population (biopsy scoring was not available for two patients at baseline, however a diagnosis was confirmed).

**Supplementary Table 4.** Historical medication usea

|  |  |
| --- | --- |
|  | **Central pathologist confirmed C3G**  **N = 29** |
| **Prior use of ACE/ARB inhibitors - n (%)** |  |
| Yes | 27 (93.1) |
| <3 months | 1 (3.4) |
| ≥3 months | 26 (89.7) |
| No | 2 (6.9) |
| **Prior use of immunosuppressants - n (%)** |  |
| Yes | 21 (72.4) |
| Prednisolone | 8 (27.6) |
| Methylprednisolone | 4 (13.8) |
| Mycophenolate mofetil | 16 (55.2) |
| Tacrolimus | 2 (6.9) |
| <3 months | 1 (3.4) |
| 3–6 months | 1 (3.4) |
| >6 months | 19 (65.5) |
| No | 8 (27.6) |

ACE, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blockers.

aPrior medications taken within 90 days of screening were recorded (four patients [13.8%] previously received eculizumab).

**Supplementary Table 5.** Overview of rare genetic variants in patients with central pathologist confirmed C3G

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Gene** | **Genetic variant** | | | **Computational prediction** | | | | **Reported sourceb** | **ACMG**  **classificationb**  **b** |
| **Coding change** | **Protein change** | **Consequence** | **CADDa** | **PolyPhen2a** | **SIFTa** | **Overall**  **predictiona** |
| C3 | c.2203C>T | p.Arg735Trp | Missense | 25.9 | Probably damaging  (0.993) | Deleterious (0.02) | Predicted  Pathogenic | [1; 2] | Benign |
| C3 | c.4369G>C | p.Asp1457His | Missense | 19.2 | Probably damaging  (0.929) | Deleterious (0.02) | Predicted  Pathogenic | Novel | VUS |
| C3c | c.4855A>C | p.Ser1619Arg | Missense | 15.2 | Possibly damaging  (0.761) | Tolerated (0.07) | Indeterminate | [1; 3] | Benign |
| CD46d | c.38C>T | p.Ser13Phe | Missense | 3.1 | Benign (0.082) | Tolerated (0.32) | Predicted  Benign | [1] | Benign |
| CD46d | c.38C>T | p.Ser13Phe | Missense | 3.1 | Benign (0.082) | Tolerated (0.32) | Predicted  Benign | [1] | Benign |
| CD46c | c.1148C>T | p.The383Ile | Missense | 3.9 | Benign (0.002) | Deleterious (0.01) | Indeterminate | [4; 2; 5] | VUS |
| CFB | c.1352A>G | p.His451Arg | Missense | 25.3 | Probably damaging  (1) | Deleterious (0) | Predicted  Pathogenic | [6; 7; 8] | VUS |
| CFHe | c.2509G>A | p.Val837Ile | Missense | 0.0 | Benign (0.019) | Tolerated (1) | Predicted  Benign | [1] | Benign |
| CFH | c.3133+4C>G | -- | Splice region | 2.0 | -- | -- | Predicted  Benign | [6; 7; 4] | VUS |
| CFHR5f | c.832G>A | p.Gly278Ser | Missense | 24.7 | Probably damaging  (0.971) | Deleterious (0) | Predicted  Pathogenic | [1] | Benign |
| THBD | c.562G>A | p.Ala188Thr | Missense | 9.8 | Benign (0.002) | Tolerated (0.31) | Predicted  Benign | Novel | VUS |
| THBDf | c.844G>C | p.Ala282Pro | Missense | 5.9 | Benign (0.001) | Tolerated (0.27) | Predicted  Benign | Novel | VUS |

Genetic analysis limited to the 27 of 29 C3G patients who provided consent.

aEach variant was automatically annotated with scores from CADD 1.6 [9], PolyPhen2 [10] and SIFT [11] to obtain a preliminary assessment of pathogenicity for novel variants and Variants of Uncertain Significance (VUS). A Deleteriousness threshold of 15 was used for CADD scores [9] with default scoring thresholds used for PolyPhen2 and SIFT. Variants were classed as “Predicted Pathogenic” or “Predicted Benign” if the predictions from all 3 algorithms were aligned, or “Indeterminate” if variant scoring wasn’t concordant among the algorithms. For non-exonic variants (e.g., splicing), only the CADD score was used for a computational prediction. bNo variants were officially classified as pathogenic or likely pathogenic in our analysis, despite computational predictions of pathogenicity, based on reported studies on these variants, which in some cases indicate that a variant should be classified as benign even if computational prediction is pathogenic, from disease versus healthy allele frequencies or from functional studies.

cVariants Benign C3 p.Ser1619Arg and VUS CD46 p.The383Ile were observed in the same patient; dVariant was observed in two patients; once alone and once in the same patient as variant Benign CFH p.Val837Ile; eVariant observed in the same patient as one of the two patients carrying the Benign CD46 p.Ser13Phe variant; fVariants Benign CFHR5 p.Gly278Ser and VUS THBD p.Ala1282Pro were observed in the same patient.

References

1. Osborne A, *et al*. J Immunol 2018;200:2464–2478  
2. Bu F, *et al*. J Am Soc Nephrol 2016;27:1245–1253  
3. Feng S, *et al*. Blood 2013;122:1487–1493  
4. Landrum MJ. Nucl Ac Res 2018;46:1062–1067  
5. Provaznikova D, *et al*. Pediatr Nephrol 2012;27:73–81   
6. Goodship THJ, *et al*. Kidney Int 2017;91:539–551  
7. Richards S, *et al*. Genet Med 2015;17:405–424  
8. Iatropoulos P, *et al*. Mol Immunol 2016;71:131–142  
9. Kircher M, *et al*. Nat Genet 2014;46:310–315  
10. Adzhubei I, *et al*. Curr Protoc Hum Genet 2013;76:7.20.1– 7.20.41   
11. Sim NL, *et al*. Nucleic Acids Res 2012;40:W452–W4