Pharmacological inhibition of PI3Kδ reduces autoantibody formation and is efficacious in a preclinical model of autoimmune hemolytic anemia

M. Scuron[1], B. Fay[1], R. Collins[1], L. Hall[1], A. Connell[1], N. Shin[1], M. Soloviev[1], D. Shuey[1], and P. Smith[1]

[1]Incyte Research Institute, 1801 Augustine Cut-off, Wilmington, Delaware, USA

INTRODUCTION

- Autoimmune hemolytic anemia (AIHA) is a rare acquired disorder in which autoantibodies directed against red blood cell (RBC) membrane antigens lead to accelerated RBC destruction[1].
- AIHA is classified by thermal range of autoantibodies that react with RBCs at temperatures ≥37°C (warm antibody hemolytic anemia, immunoglobulin [Ig] G-driven) or <37°C (cold agglutinin disease, IgM-driven)[2].
- AIHA has an estimated prevalence of 17:100,000 and mortality rate of 11%, with no regulatory approved treatments[2].
- Phosphatidylinositol 3-kinases (PI3Ks) are divided into three classes (Class I, II, and III) according to their structure, regulation, and substrate specificity.
  - The Class I PI3K delta isoform (PI3Kδ) has been implicated in autoimmune diseases associated with aberrant B-cell and antibody responses **(Figure 1)**[3].
- Parsaclisib (INCB050465) is a potent and selective oral, small-molecule PI3Kδ inhibitor being clinically investigated for the treatment of AIHA (see EHA 2021 **EP685** for details)[4,5].

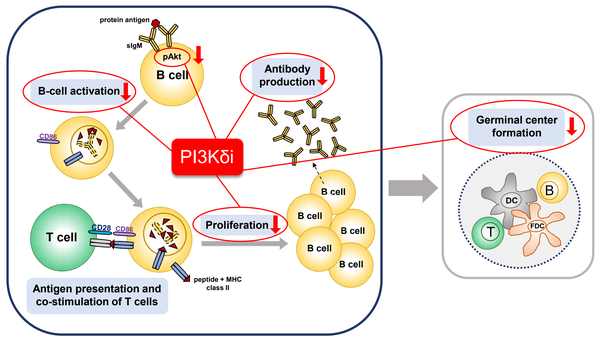

Figure 1. *PI3Kδ is a critical node in B-cell biology.*

OBJECTIVE

The goal of the study was to characterize the effect of PI3Kδ inhibition on autoimmune disease with aberrant B-cell and antibody responses.

METHODS

- Intravenous injection of 2,4,6-trinitrophenyl lipopolysaccharide (TNP-LPS) triggers a rapid antigen-specific B-cell–mediated IgM response. C57BL/6 mice were injected with TNP-LPS on day 0 and dosed with parsaclisib for 6 days. Blood was collected at study termination for *de novo* antibody quantification.
- Intraperitoneal injection of 2,4-dinitrophenyl-keyhole limpet hemocyanin (DNP-KLH) drives an antigen-specific, T-cell–dependent, B-cell–mediated IgG immunity.
- In the acute DNP-KLH model, C57BL/6 mice were injected with DNP-KLH on day 0 and dosed with parsaclisib for 8 days. Blood was collected at study termination for *de novo* antibody quantification.
- In the chronic DNP-KLH model, C57BL/6 mice were immunized on days 0 and 7 and dosed with parsaclisib for 10 weeks. Blood was sampled throughout the study for *de novo* antibody quantification. Spleens and mesenteric lymph nodes were collected at study termination for flow cytometry analysis.
- In the interleukin-2 knockout model, mice spontaneously develop a systemic autoimmune disease, dying from complications of AIHA mediated by autoantibodies. Mice were dosed with parsaclisib from weaning (day 0) until day 17. The pharmacological effect of PI3Kδ inhibition was quantified using complete blood count, ELISA, and flow cytometry.

RESULTS

Table 1. Enzyme inhibitory activity

Enzyme	Human IC_{50} ± SD (nM)	Fold Selectivity for PI3Kδ
PI3Kδ	**1.1 ± 0.5**	–
PI3Kα	>20,000	>20,000
PI3Kβ	>20,000	>20,000
PI3Kγ	19,000 ± 1700	19,000

Table 2. Antiproliferative effects of parsaclisib in primary B cells

Stimulant	Species	IC_{50} ± SD (nM)
Anti-human IgM Ab	Human	0.21 ± 0.12
Anti-human CD40 Ab	Human	0.42 ± 0.21
hCD40 ligand	Human	0.72 ± 0.22
hIL-4	Human	0.47 ± 0.14
LPS + hIL-4	Human	0.73 ± 0.59
hIL-6	Human	0.59 ± 0.42
hBAFF	Human	0.53 ± 0.18
LPS + mIL-4 or mBAFF	Mouse	0.37 ± 0.31

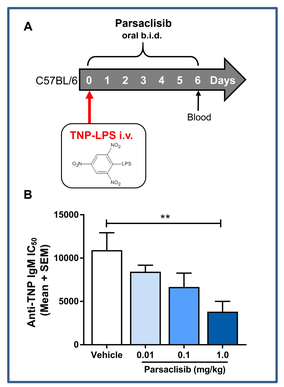

Figure 2. *Oral twice-daily parsaclisib administered to TNP-LPS immunized mice* ***(A)*** *dose-dependently reduced antigen-specific IgM titer with significant inhibition observed in the 1-mg/kg group* ***(B)****; **p<0.01.*

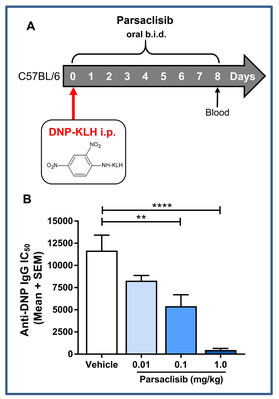

Figure 3. *DNP-KLH immunization in the acute model* ***(A)*** *was associated with a high IgG titer induction on day 8 in the vehicle-treated mice. Prophylactic twice-daily parsaclisib treatment dose-dependently reduced antigen-specific IgG titer with significant inhibition observed in the 0.1- and 1-mg/kg groups* ***(B)****; **p<0.01, ****p<0.0001.*

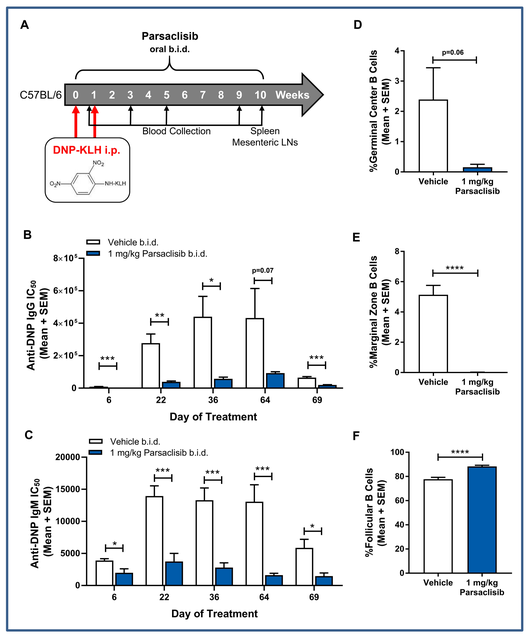

Figure 4. *In the chronic DNP-KLH model* ***(A)****, IgG* ***(B)*** *and IgM* ***(C)*** *titers were significantly and consistently lower in the parsaclisib-treated mice compared with vehicle group. The durability of the response was confirmed by flow cytometry analysis. Prophylactic twice-daily parsaclisib treatment reduced the proportion of germinal center B cells* ***(D)*** *and splenic marginal zone B cells* ***(E)*** *following DNP-KLH B-cell activation. These findings were consistent with the increase in the proportion of follicular B cells* ***(F)*** *in the parsaclisib-treated group; *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.*

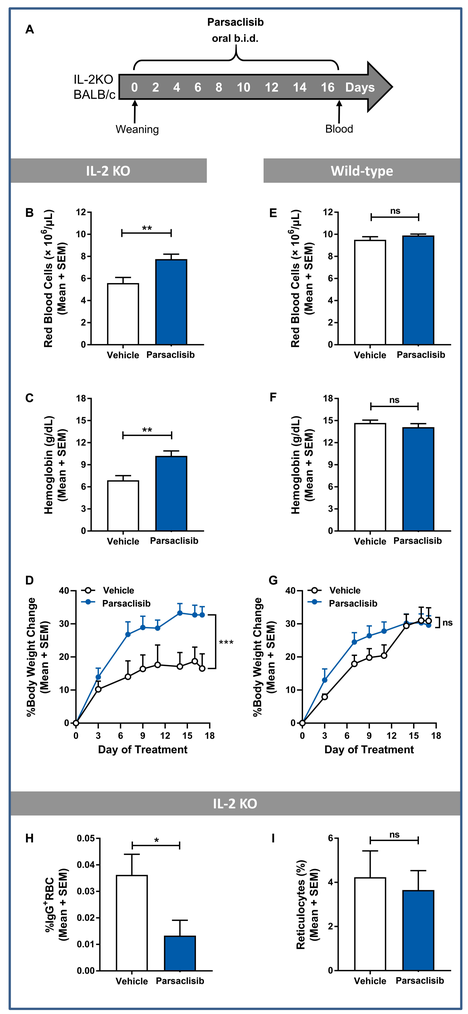

Figure 5. *Interleukin-2 knockout (IL-2 KO) mice that develop a systemic autoimmune disease and healthy, age-matched, wild-type mice were treated twice daily with PI3Kδ inhibitor or vehicle* ***(A)****. Selective pharmacologic inhibition of the PI3Kδ isoform in IL-2 KO animals resulted in significantly increased red blood cell (RBC) counts* ***(B)****, significantly higher hemoglobin levels* ***(C)****, and marked amelioration of mouse body weights* ***(D)*** *as compared to the vehicle group. RBC counts* ***(E)****, hemoglobin levels* ***(F)****, and body weights* ***(G)*** *were unaffected by PI3Kδ inhibition in healthy, age-matched, wild-type mice. Improvement of RBC and hemoglobin levels in IL-2 KO mice was associated with a reduction of IgG binding to RBCs in circulating blood* ***(H)****. Percentage of reticulocytes was unaffected by PI3Kδ inhibition* ***(I)****; *p<0.05, **p<0.01, ***p<0.001, ns=not significant.*

CONCLUSIONS

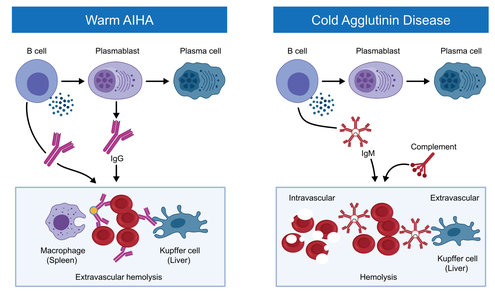

Figure 6. *In warm antibody hemolytic anemia, hemolysis triggered by pan-reactive IgG autoantibodies against red blood cell antigens is often severe and can be fatal. In cold agglutinin disease, the hemolysis occurs largely in the extravascular mononuclear phagocyte system of the liver and spleen. The anemia is usually mild (hemoglobin >7.5 g/dL); autoantibodies in cold AIHA are predominantly IgM.*

- Parsaclisib dose-dependently inhibited *de novo* IgM and IgG humoral responses.
- PI3Kδ inhibition in the spontaneous mouse model of AIHA resulted in:
  - -Modulation of AIHA progression;
  - -Increased RBC counts;
  - -Increased hemoglobin;
  - -Reduction of IgG binding to RBCs in circulating blood.
- Primary findings in toxicology studies in rats and dogs were lymphoid depletion, most notably B-cell regions, of multiple lymphoid organs including lymph nodes, spleen, thymus, and gut-associated lymphoid tissue, similar to results of pharmacology studies reported here and consistent with PI3Kδ inhibition. Secondary effects of immunosuppression occurred only at exposures several fold the anticipated therapeutic exposure in AIHA.
- The preclinical data support the scientific rationale for clinically evaluating parsaclisib in AIHA (NCT03538041) (EHA 2021 EP685).

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DISCLOSURES

All authors: Employment and stock ownership – *Incyte Corporation.*

REFERENCES

1 **Hill A et al.** *Hematology Am Soc Hematol Educ Program* 2018;1:382-389.
2 **Michalak S et al.** *Immun Ageing* 2020;17:38.
3 **Stark A-K et al.** *Curr Opin Pharmacol* 2015;23:82-91.
4 **Yue E et al.** *ACS Med Chem Lett* 2019;10:1554-1560.
5 **Shin N et al.** *J Pharmacol Exp Ther* 2020;374:211-222.

CONTACT INFORMATION

Paul A. Smith, PhD
Sr. Director, Inflammation and Autoimmunity
Incyte Research Institute
+1 302 498 5690
psmith@incyte.com