# Expression of PI3K signalling associated with T cells in psoriasis is inhibited by seletalisib, a PI3Kδ inhibitor, and is required for functional activity

Nicole Yager[1], Ciara Haddadeen[1,2], Mark Powell[3], Andrew Payne[3], Rodger Allen[3] and Eugene Healy (0000-0001-5591-6970)[1,2]

[1]Dermatopharmacology, Sir Henry Wellcome Laboratories, Clinical & Experimental Sciences, Faculty of Medicine, University of Southampton, Southampton, Hampshire, UK

[2]Dermatology, University Hospital Southampton NHS Foundation Trust, Southampton, Hampshire, UK.

[3]UCB Pharma, Slough, Berkshire, UK

Correspondence: Prof Eugene Healy, Dermatopharmacology, Clinical and Experimental Sciences, Faculty of Medicine, University of Southampton, Southampton General Hospital, Southampton, SO16 6YD, Hampshire, UK. E-mail: ehealy@soton.ac.uk

Short title: PI3Kδ inhibition of psoriatic T cell function

Abbreviations: Akt, protein kinase B; mTOR, mammalian target of rapamycin; PI3K, phosphoinositide 3-kinase; pS6, phosphorylated ribosomal protein S6.

**TO THE EDITOR**

The phosphoinositide 3-kinase (PI3K) pathway plays a key role in many cellular processes, including cell proliferation, survival and protein synthesis, with the PI3K isoform, PI3Kδ, involved in normal T cell development and function (Jarmin et al., 2008; Lucas et al., 2016; Vanhaesebroeck et al., 2012). Evidence to suggest that PI3K signalling might play a role in psoriasis comes from reports of increased expression of phosphorylated Akt (pAkt), mTOR and ribosomal protein S6 (pS6), which are downstream in the PI3K signalling pathway, in lesional psoriatic skin compared to non-lesional skin and healthy controls (Buerger et al., 2013; Madonna et al., 2012; Rosenberger et al., 2007). In addition, PI3Kδ knock-in mice, expressing a catalytically inactive form of PI3Kδ ($p110\delta^{D910A/D910A}$), and PI3Kγ knockout mice are greatly protected from imiquimod-induced psoriasis-like skin inflammation compared to wildtype mice (Roller et al., 2012).

As psoriasis is a T cell mediated skin disease, the recent development of the selective PI3Kδ inhibitor, seletalisib (Allen et al., 2017), led us to investigate the effects of PI3Kδ inhibition in T cells of psoriatic subjects. All investigations were conducted under ethics committee approval, with written, informed patient consent. Immunofluorescence staining and confocal microscopy, used to characterise the expression of the PI3K pathway in psoriatic skin and its colocalisation with infiltrating T lymphocytes (see supplementary Materials and Methods online), identified pS6-positive T cells present in the dermis of lesional psoriatic skin (Figure 1a and Supplementary Figures S1 and S2). Western blotting demonstrated enhanced phosphorylation of S6 protein in $CD4^+$ T cells following stimulation with anti-CD3, and separately anti-CD3/CD28 (Figure 1b). Therefore, the effect of seletalisib on PI3K-related generation of pS6 in T cells from individuals with psoriasis was investigated. Using a

phosflow assay, stimulation with anti-CD3/CD28 of T lymphocytes isolated from lesional skin biopsies and PBMCs of subjects with psoriasis led to an increase in pS6, which was dose-dependently inhibited by seletalisib (Figures 1c-g). Inhibition of PI3Kδ by seletalisib at 10 μM reduced the median percentage of pS6-positive dermal lesional T cells and peripheral blood T cells by 74% and 49% respectively ($p=0.0081$ and $p=0.017$ respectively, Kruskal-Wallis test with Dunn's post-test, Figure 1d,e). Comparison of the proportion of lesional $CD3^+$T cells positive for pS6 in the phosflow assay (Figure 1d) with immunofluorescence microscopy of lesional psoriasis biopsies (Supplementary Figures S1, S2) suggests that immunofluorescence microscopy gives an underrepresentation of the total number of psoriatic $CD3^+$T cells with PI3Kδ activity. Although pAkt proved more difficult to measure, a reduction in pAkt in $CD3^+$T cells from PBMCs of psoriatic subjects was seen following inhibition of PI3Kδ in each of three cases where the phosflow assay was conducted (Supplemental Figure S3).

Flow cytometry of T cells from lesional psoriatic dermis showed that they were largely effector/memory ($CD45RO^+$) $CD3^+CD4^+$ cells whilst those from PBMCs were a mixture of both naïve ($CD45RO^-$) and effector/memory $CD3^+CD4^+$ and $CD3^+CD4^-$ cells (Supplementary Figure S4). Analysis of pS6-positive psoriatic dermal memory T cells indicated that 10 μM seletalisib reduced the median percentage of pS6 in $CD4^+CD45RO^+$ cells by 68% and $CD8^+CD45RO^+$ cells by 82% ($p=0.0062$ and $p=0.014$ respectively, Kruskal-Wallis test with Dunn's post-test, Figure 1f,g).

We next wished to determine whether inhibition of PI3K signalling in activated psoriatic T lymphocytes by seletalisib was sufficient to elicit a functional effect. Using a CFSE assay,

PBMCs from subjects with psoriasis were stimulated with anti-CD3 and incubated either in vehicle or seletalisib for 72 h. As the dose of seletalisib increased, there was a corresponding decrease in the number of cell divisions, and inhibition of the percentage of $CD4^+$ and $CD8^+$ cells that had divided, as demonstrated by a reduction in the peaks of $CD4^+CFSE^{lo}$ and $CD8^+CFSE^{lo}$ populations (Figure 2a). Following addition of 1 μM and 10 μM seletalisib, the mean percentage of lymphocytes which had proliferated (i.e. % divided) was reduced in $CD4^+$ cells by 54% and 93% ($p=0.0072$ and $p=0.0002$, 1-way ANOVA with Dunnett's post-test) and in $CD8^+$ cells by 54% and 91% ($p=0.0002$ and $p<0.0001$, 1-way ANOVA with Dunnett's post-test) respectively (Figure 2b,c). Furthermore, at 10 μM seletalisib, in 5 psoriatic subjects <1% of $CD4^+$ cells had proliferated and in 4 psoriatic subjects <3% of $CD8^+$ cells had divided.

Next, we evaluated the effect of seletalisib on IFNγ, TNFα and IL-17 release. PBMCs from psoriatic subjects were stimulated with anti-CD3 and incubated either in vehicle or seletalisib for 48 h, and supernatants then removed and IFNγ, TNFα and IL-17 measured by ELISA. A significant decrease in IFNγ and TNFα release was seen following exposure to seletalisib, with doses of 1 μM and 10 μM seletalisib causing reductions of 79% and 98% for IFNγ ($p=0.0254$ and $p=0.0126$, 1-way ANOVA with Dunnett's post-test) and 59% and 93% for TNFα ($p=0.0017$ and $p=0.0005$, 1-way ANOVA with Dunnett's post-test) respectively (Figure 2d,e). Mean IL-17 release was reduced by 68% and 79% at doses of 1 μM and 10 μM seletalisib, respectively; this was not statistically significant, probably as a result of the undetectable levels of IL-17 in three of the subjects' T cells when stimulated in vehicle (Figure 2f).

This study suggests that PI3K signalling is altered in lesional psoriatic T cells, as seen by the presence of pS6 in psoriatic skin on immunofluorescence staining, and that the use of the PI3Kδ-selective inhibitor, seletalisib, to block PI3Kδ signalling in human psoriatic T cells significantly inhibits S6 phosphorylation, lymphocyte proliferation and cytokine release. Although a previous study reported that another PI3Kδ inhibitor, IC87114, reduced IFNγ and IL-17 production by PBMCs from donors with psoriasis (and provided representative data from two of five individuals) (Roller et al., 2012), a recent first-in-human study of oral seletalisib demonstrated acceptable safety and pharmacological profiles, as well as preliminary evidence of target engagement in psoriatic skin disease (Helmer et al., 2017). Whereas recent psoriatic drug development has mainly centred on injected biological therapies, there is a great need for effective systemic treatments which can be administered orally and the results of this current study suggests that the PI3Kδ pathway would make an excellent target for the treatment of psoriasis and that further studies are warranted on seletalisib as an oral drug in psoriatic subjects.

## CONFLICT OF INTEREST

MP, AP and RA are employees of UCB Pharma. This work was a collaboration between the University of Southampton and UCB Pharma and was funded by UCB Pharma, who also provided the PI3Kδ inhibitor, seletalisib.

## ACKNOWLEDGEMENTS

We are grateful to (i) the subjects who participated in the study, (ii) the NIHR Wellcome Trust Clinical Research Facility in University Hospital Southampton NHS Foundation Trust

for providing support during skin biopsy and phlebotomy procedures, (iii) Richard Jewell and Carolann McGuire in Dermatopharmacology, University of Southampton for assistance with flow cytometry, (iv) Susan Wilson and members of the Immunohistochemistry Laboratory in the Faculty of Medicine, University of Southampton for advice regarding cryostat sectioning of skin samples, and (v) Kenneth Johnson, UCB Pharma, for assistance with Western blotting.

## REFERENCES


Allen RA, Brookings DC, Powell MJ, Delgado J, Shuttleworth LK, Merriman M, et al. Seletalisib; characterisation of a novel, potent, and selective inhibitor of PI3Kδ. J Pharmacol Exp Ther 2017;361:429-40.

Buerger C, Malisiewicz B, Eiser A, Hardt K, Boehncke WH. Mammalian target of rapamycin and its downstream signalling components are activated in psoriatic skin. Br J Dermatol 2013;169:156-9.

Helmer E, Watling M, Jones E, Tytgat D, Jones M, Allen R, et al. First-in-human studies of seletalisib, an orally bioavailable small-molecule PI3Kδ inhibitor for the treatment of immune and inflammatory diseases. Eur J Clin Pharmacol 2017;73:581-91.

Jarmin SJ, David R, Ma L, Chai JG, Dewchand H, Takesono A, et al. T cell receptor-induced phosphoinositide-3-kinase p110delta activity is required for T cell localization to antigenic tissue in mice. J Clin Invest 2008;118:1154-64.

Lucas CL, Chandra A, Nejentsev S, Condliffe AM, Okkenhaug K. PI3Kδ and primary immunodeficiencies. Nat Rev Immunol. 2016 Nov;16(11):702-714.

Madonna S, Scarponi C, Pallotta S, Cavani A, Albanesi C. Anti-apoptotic effects of suppressor of cytokine signaling 3 and 1 in psoriasis. Cell Death Dis 2012;3:e334.

Roller A, Perino A, Dapavo P, Soro E, Okkenhaug K, Hirsch E, et al. Blockade of phosphatidylinositol 3-kinase PI3Kdelta or PI3Kgamma reduces IL-17 and ameliorates imiquimod-induced psoriasis-like dermatitis. J Immunol 2012;189:4612-20.

Rosenberger C, Solovan C, Rosenberger AD, Jinping L, Treudler R, Frei U, et al. Upregulation of hypoxia-inducible factors in normal and psoriatic skin. J Invest Dermatol 2007;127:2445-52.

Vanhaesebroeck B, Stephens L, Hawkins P. PI3K signalling: the path to discovery and understanding. Nat Rev Mol Cell Biol 2012;13:195-203.

## FIGURE LEGENDS

**Figure 1. PI3Kδ inhibition of pS6 in psoriatic T cells.** (a) Identification of $pS6^+$ T cells in psoriatic lesional skin determined by immunofluorescence confocal analysis. Arrows point to $CD3^+pS6^+$ cells. Scale bar, 20 μm. (b) Western blot showing phosphorylation of the ribosomal protein S6 in human $CD4^+$ T cells following stimulation with anti-CD3 and with anti-CD3/anti-CD28. (c) Representative histogram of pS6 during a phosflow assay, gated on $CD3^+$ cells in a lymphocyte population determined by FSC and SSC. (d - g) Combined psoriatic subject data shown as % of (d) $CD3^+pS6^+$ cells from psoriatic lesional dermis, (e) $CD3^+pS6^+$ cells from psoriatic PBMCs, (f) $CD4^+CD45RO^+pS6^+$ cells from psoriatic lesional dermis and (g) $CD8^+CD45RO^+pS6^+$ cells from psoriatic lesional dermis. In d – g, each colour represents a single psoriatic subject. *$p<0.05$, **$p<0.01$.

**Figure 2. PI3Kδ inhibition of psoriatic T cells reduces proliferation and cytokine release.** (a) Representative FACS plots of CFSE proliferation data when cells were activated in the presence or absence of seletalisib; gated on $CD4^+$ or $CD8^+$ cells. (b – f) Combined psoriatic subject data shown as (b,c) CFSE proliferation data based on % divided (b) CD4 and (c) CD8 cells and (d – f) cytokine release of (d) IFNγ, (e) TNFα and (f) IL-17. n=10 subjects for b,c; n=9 subjects for d-f. *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$.