Discovery of DWP212525, a potent JAK3 and TEC family kinase Inhibitor for the Treatment of Autoimmune Diseases

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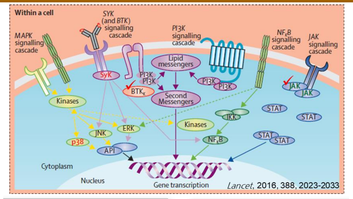
SCAN ME

BACKGROUND



- Janus Kinase (JAK) and TEC family kinase such as BTK, RLK, ITK and BMX play critical roles in activation and function of T cells and B cells. Dysregulation of this process has been known to cause various immune-related diseases.
- Tofacitinib, a JAK inhibitor, was originally developed as a JAK3 inhibitor but it showed limited selectivity against JAK1 and JAK2. Although Tofacitinib has proved therapeutic effects in RA, serious side effects such as anemia, neutropenia and CV risk have been frequently reported.
- We first confirmed BTK which can inhibit B cell in the Tec family kinase. Because, existing BTK inhibitors have insufficient clinical efficacy, which creates higher demands for treatment with improved efficacy.
- DWP212525 is a JAK3 and TEC family kinase inhibitor which may have synergistic effects for the treatment of rheumatoid arthritis and other inflammatory diseases such as pemphigus vulgaris (PV).

METHODS

- Inhibition of JAK3 and TEC family kinase enzyme activity and selectivity against Cys-family kinase group were evaluated by a series of biochemical assays.
- Cellular activity for target phosphorylation in human T and B cells were measured by Phospho-STAT5 or phospho-BTK cellular kit. BCR dependent CD69 expressions were determined in hPBMC.
- To measure BTK occupancy, mouse spleen was extracted at several time points up to 24 hours after receiving an oral administration of DWP212525.
- Occupancy was quantified by the number of unbound BTK in ELISA-based assay using biotinylated-DWP212525, which binds to free active site of target after oral administration of DWP212525.
- Furthermore, the efficacy of DWP212525 was investigated in the mouse Pemphigus vulgaris(PV) model and mouse collagen-induced arthritis (CIA) model.

RESULTS

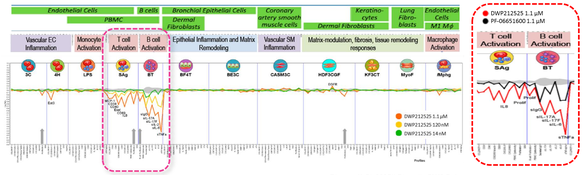
DWP212525 is highly potent irreversible JAK3/TEC family kinase inhibitor

		DWP212525 JAK3/TEC family kinase inhibitor	PF-06651600 JAK3/TEC family kinase inhibitor
Kinase assay		Km ATP$_{(1mM ATP)}$	Km ATP$_{(1mM ATP)}$
IC_{50}, nM	JAK3	0.2$_{(50)}$	0.2$_{(33.1)}$
	TEC	2.3$_{(11.6)}$	*(>833)
	BTK	1.5$_{(81.1)}$	13.9$_{(624)}$
	RLK	*(37.4)	*(155)
	BMX	0.6$_{(18.5)}$	*(666)
	ITK	1.2$_{(191)}$	15.6$_{(935)}$
	EGFR	65.3$_{(10,000)}$	*(>10,000)
Cell-based assay		IC_{50}, nM	IC_{50}, nM
JAK1/JAK3 pairing p-STAT5		101	84
p-BTK		11	68.5
p-EGFR		> 5,000	> 5,000
T_{H1} ($CD4^+$,IFNγ^+)		< 100	> 500
T_{H2} Polarization ($CD4^+$,IL-5$^+$)		< 50	< 100
T_{H17} ($CD4^+$,IL-17$^+$)		< 50	> 100

- DWP212525 showed high potency against JAK3/TEC family kinase including BTK
- DWP212525 has no inhibitory effect on EGFR phosphorylation in squamous carcinoma cell line (A431, IC_{50} > 5000 nM)
- In hPBMC, DWP212525 more suppressed helper-T cell polarization to Th1, Th2 and Th17 than PF-06651600

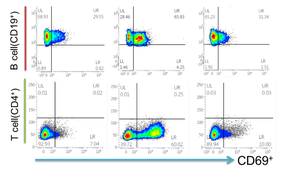
DWP212525 effectively inhibited T and B cell function in cell based assay

Primary human co-culture system



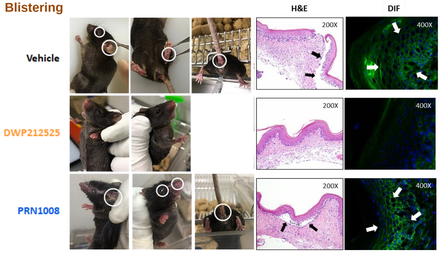
Biological and Disease Relevance Category	Decreased activity
Inflammation-related activities	MCP-1, Esel, IL8, sTNFa & Eot3
Immunomodulatory activities	CD38, CD40, CD69, sigG, sIL-2, sIL-6, sIL-17A, sIL-17F

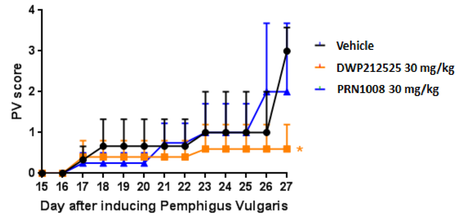
human PBMC T/B cell activation assay



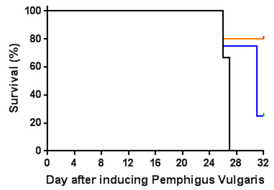
- DWP212525 inhibited T cell activation in human PBMCs with a mean IC_{50} of 146 nM
- DWP212525 inhibited B cell activation in human PBMCs with a mean IC_{50} of 287 nM

DWP212525 is efficacious in a mouse PV model

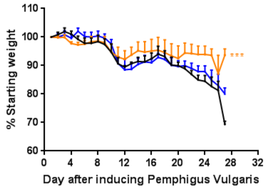




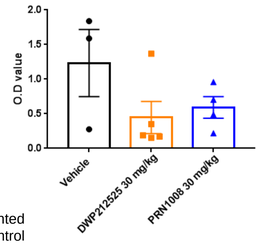
Survival



Body weight



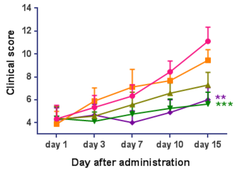
anti-DSG3 antibody



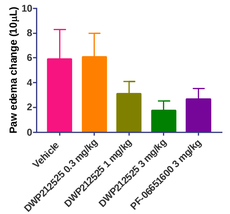
- In mouse PV model, DWP212525 alleviated the severity of disease index score prevented body weight loss and confirmed that the survival rate was higher than the positive control group treated with a BTK inhibitor and the vehicle group.

DWP212525 is efficacious in a mouse CIA model

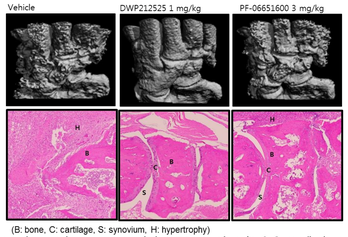
Clinical score



Paw edema



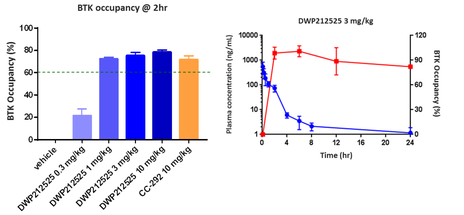
Micro-CT & Histology



(B: bone, C: cartilage, S: synovium, H: hypertrophy)

- In mouse CIA model, treatment of DWP212525 improved arthritis in a dose-dependent manner(The ED_{50} value is 0.8 mg/kg), and significantly improved histological damage

BTK occupancy by DWP212525 on mouse splenocytes



- Results are expressed as the mean±SD. The time course of plasma drug concentration(Blue) was investigated after single administration of 3 mg/kg in ICR mouse (Plotted on the left axis). BTK occupancy(Red) was confirmed on the last day of DWP212525 3 mg/kg administration for 3 weeks in CIA mice (Plotted on the right axis).

CONCLUSIONS

- **DWP212525** is a potent small molecular inhibitor of **JAK3 and TEC family kinase** such as BTK.
- In summary, DWP212525 has potent in vitro and in vivo pharmacological activities compared to existing JAK3/TEC family kinase inhibitor(PF-06651600) and selective BTK inhibitor(PRN1008).
- **DWP212525 can be more effective** due to the addition of JAK3 inhibition than selective BTK inhibitor for the treatment of various **autoimmune diseases, including RA and PV.**
- Therefore, DWP212525 may serve as a next generation therapeutics for autoimmune diseases and now work for IND-enabling study.

REFERENCES

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