PLX-4107, a selective IKZF2 degrader, reprograms suppressive regulatory T cells and demonstrates anti-tumor activity

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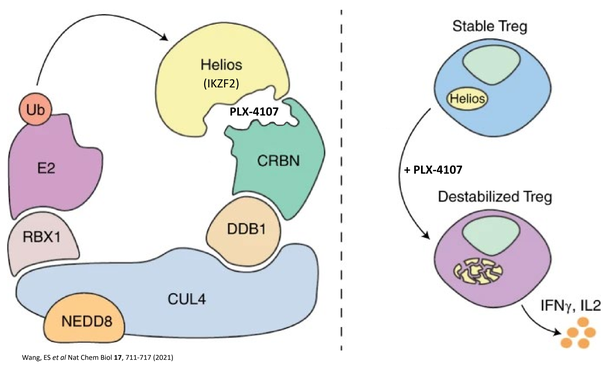
Abstract

Immune checkpoint therapy has demonstrated durable clinical responses in multiple solid tumor types. Reduced clinical response to checkpoint therapy has been linked to the presence of potent immunosuppressive regulatory T cells (Tregs) within the tumor microenvironment that contribute to tumor immune evasion. The transcription factor Helios (IKZF2) is a marker of highly suppressive Tregs and is required to maintain a stable, suppressive Treg cell phenotype in the inflammatory tumor microenvironment. Depletion of IKZF2 in Tregs results in both loss of suppressive activity and conversion of Tregs into effector-like T cells, leading to anti-tumor immunity. Targeted protein degradation using the endogenous Ubiquitin Proteasome System (UPS) has enabled targeting undruggable proteins, such as IKZF2, that have no known small molecule binding pocket. We have designed small molecules that promote a novel interaction between IKZF2 and the E3 ubiquitin ligase substrate receptor, cereblon, leading to proximity induced protein degradation.

PLX-4107 is a novel molecular glue that is a highly selective, potent, and rapid degrader of IKZF2 via the redirection of the E3 substrate receptor, cereblon. Degradation of IKZF2 by PLX-4107 is blocked in the presence of proteasome and neddylation inhibitors as well as a cereblon knock-out cell line, confirming that degradation is mediated by the UPS and specifically through the involvement of cereblon. Proteome-wide analysis demonstrated that PLX-4107 selectively depletes IKZF2 protein levels without degrading other known cereblon neo-substrates. *In vitro*, PLX-4107 mediated degradation of IKZF2 resulted in conversion of suppressive Tregs into CD4+ effector-like T cells, coupled with an increased production of the effector cytokines IL2 and IFN$_\gamma$. Oral administration of PLX-4107 to cynomolgus monkeys demonstrated sustained pharmacodynamic response, persistent depletion of IKZF2, and reprogramming of Tregs, consistent with the catalytic mechanism of protein degradation. *In vivo*, T cell expansion studies showed that administration of PLX-4107 decreased both Treg CD25 expression and proliferation, along with increased activation of CD8+ T effector cells. PLX-4107 was evaluated in *in vivo* xenograft efficacy studies and demonstrated dose dependent single agent anti-tumor activity that was dependent on the presence of T cells. In addition, co-administration of PLX-4107 and anti-PD-1 antibody pembrolizumab resulted in tumor growth inhibition and significant combination benefit.

PLX-4107 is a novel molecular glue that selectively degrades the undruggable transcription factor, IKZF2. PLX-4107 mediated IKZF2 degradation results in conversion of Tregs to an effector-like T cell phenotype, single agent antitumor activity and the ability to enhance the efficacy of immune checkpoint therapy *in vivo*.

Introduction



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- Immune resistance is a hallmark of cancer. Regulatory T cells (Tregs) are a key contributor of tumor immune evasion and compromise antitumor immune responses
- The zinc-finger transcription factor Helios (IKZF2) is a marker of highly suppressive Tregs
- Plexium has discovered PLX-4107 that is derived from a novel chemical series of small molecules that bind to the E3 ligase substrate receptor cereblon (CRBN) and selectively recruit the neosubstrate IKZF2, promoting its ubiquitination and degradation
- PLX-4107 degradation of IKZF2 destabilizes Tregs, increases tumor infiltration of activated T-effector cells, and results in anti-tumor activity
- Combination of PLX-4107 with PD1 antibody demonstrates combination benefit and suggests the potential to improve clinical responses to immune checkpoint therapy

PLX-4107 is a Selective IKZF2 Degrader

A

Compound	CRBN EC_{50}, nM	IKZF2 DC_{50}, nM	IKZF1 DC_{50}, nM	IKZF3 DC_{50}, nM	GSPT1 DC_{50}, nM
PLX-4107	6.3	5.7	>30,000	>30,000	>10,000
DKY709[1]	11.6	74	>30,000	>30,000	>10,000
PLX-5858 (non-degrader)	17.2	>30,000	NA	>30,000	NA

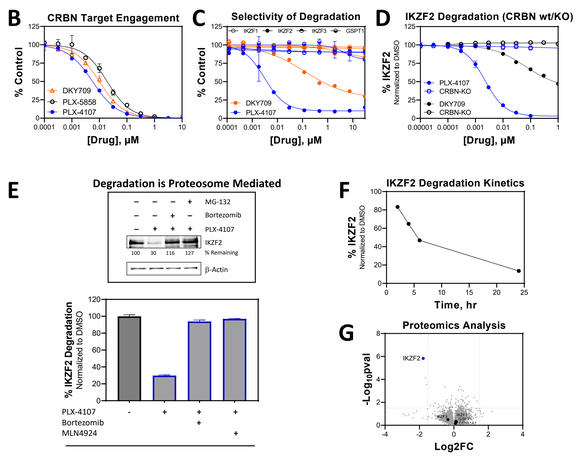

Figure 1: A. Small molecules that bind the E3 ligase substrate receptor, cereblon (CRBN). PLX-4107 and DKY709 recruit the neosubstrate IKZF2 for degradation. **B.** NanoLuc-CRBN target engagement binding curve of PLX-4107, PLX-5858, and DKY709. Bars represent standard deviation (SD). **C.** Detection of IKZF transcription factors or GSPT1 levels with increasing compound concentration using mNeonGreen-IKZF(1, 2, or 3) or HiBiT tagged GSPT1 HEK293 cells. **D.** IKZF2 protein levels after treating with increasing concentrations of PLX-4107 and DKY709 for 24h in CRBN wt and KO Jurkat cells. Intracellular IKZF2 protein was detected using a Miltenyi MACSQuant 16 Flow Cytometer and analyzed using FlowLogic software. **E.** Degradation is mediated by the proteasome. Jurkat cells were incubated +/- proteasome inhibitor (100nM bortezomib or 1μM MG-132) or neddylation inhibitor (1μM MLN4924) for 2h, followed by a 6h incubation with 100nM PLX-4107. IKZF2 protein levels were detected by western blot with β-actin as a loading control (top panel) or by flow cytometry (bottom panel). **F.** Degradation kinetics of IKZF2 in Jurkat cell line incubated with PLX-4107 at its DC_{90} for varying timepoints. **G.** Quantitative proteomic profile of Jurkat cell line treated for 24h with 20nM of PLX-4107. Volcano plot represents the relationship between the log_2 fold-change and the $-log_{10}$(*P value*).

IKZF2 Degradation Derepresses IL2 Expression

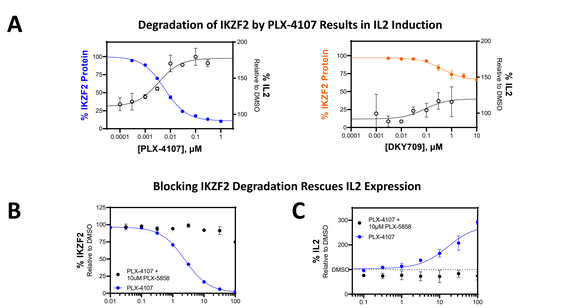

Figure 2: A. Degradation of IKZF2 in Jurkat cell line after exposure to PLX-4107 and DKY709. Compound treatment derepresses IL2 expression. Engineered Jurkat cells expressing a luciferase reporter (TCR/CD3 effector cells) driven by an IL2 promoter were treated with various compound concentrations for 48h. Drug treated cells were stimulated by addition of CD3 antibody leading to an upregulation of IL2 production detected by a luciferase assay. The magnitude (D_{max}) of IKZF2 degradation corresponds with IL2 induction. **B.** Co-treatment of Jurkat cells with various concentrations of PLX-4107 (IKZF2 degrader) ± a fixed concentration of PLX-5858 (CRBN binder, IKZF2 non-degrader). PLX-5858 inhibits PLX-4107 mediated IKZF2 degradation and **C.** IL2 induction. Intracellular IKZF2 levels were detected by flow cytometry.

PLX-4107 Converts Tregs into Effector-like T Cells

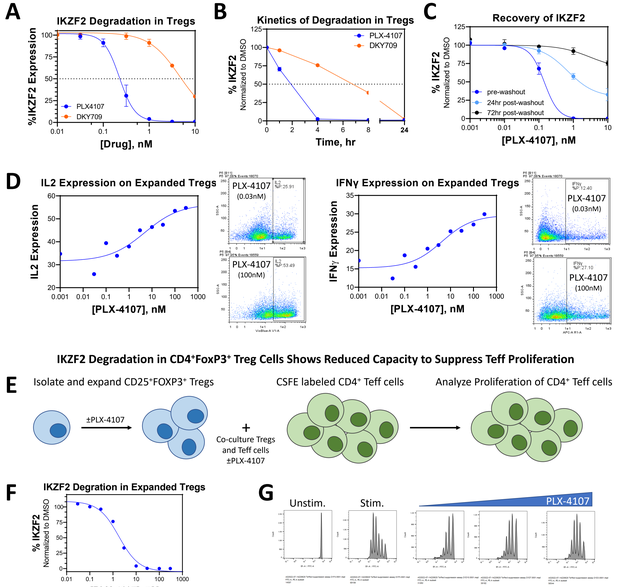

Figure 3: Degradation of IKZF2 converts human CD4$^+$Foxp3$^+$ Treg cells into a T effector-like phenotype and reduces suppression of T effector proliferation. **A**. Dose dependent degradation of IKZF2 in human Treg cells treated with PLX-4107 (DC_{90} = 0.56 ±0.58nM) or DKY709 (DC_{90} = 3.9 ±1.0nM) for 24h. **B.** Treatment of human Treg cells with PLX-4107 at the DC_{90} results in rapid and complete degradation of IKZF2 within 4h. Full degradation of IKZF2 by DKY709 takes 24h. **C.** Human Treg cells treated with PLX-4107 for 24h results in sustained suppression of IKZF2. Recovery of IKZF2 protein levels takes more than 72h after removal of PLX-4107. **D.** Dose dependent induction of IL2 (EC_{50} = 3.0 ±2.5nM) and IFN$_\gamma$ (EC_{50} = 3.9 ±0.5nM) in CD4$^+$Foxp3$^+$ Treg cells with PLX-4107 treatment (representative donor). Treg cells were expanded in the presence of IL2, CD3/CD28 Dynabeads and PLX-4107 for 5-7 days. Representative fluorescence activated cell sorting (FACS) plots and quantification of percent IL2$^+$ or IFNγ^+ CD4$^+$Foxp3$^+$ Treg cells after PMA/Ionomycin stimulation and treatment with Brefeldin A to block cytokine secretion. Representative data from multiple donors. **E.** Schematic of Treg Suppression Assay. **F.** Degradation profile of PLX-4107 in expanded Tregs (DC_{50} = 1.8nM). **G.** Expanded human CD4$^+$Foxp3$^+$ Treg cells were co-cultured with CFSE labeled CD4$^+$ Teff cells (5:1 Teff:Treg) and compound or control for an additional 5 days. Cells were surface stained, fixed and permeabilized and then intracellular stained. Protein levels were detected by flow cytometer and analyzed using FlowLogic software.

PLX-4107 Destabilizes Tregs and Increases Effector T Cells *In Vivo*

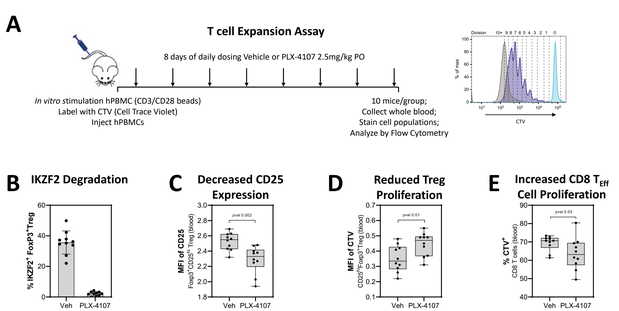

Figure 4: IKZF2 degradation in human CD4$^+$Foxp3$^+$ Treg cells with PLX-4107 destabilizes Tregs. **A.** Schematic of T cell expansion assay. hPMBCs were stimulated *in vitro* with anti-CD3/anti-CD28 overnight and then labeled with Cell Trace Violet (CTV). CTV-labeled hPBMCs were injected into immune incompetent NCG mice and dosed with PLX-4107 (2.5mg/kg, PO, QD) for 8 days. Whole blood was collected, cell populations were stained, fixed and permeabilized for intracellular staining, and analyzed by flow cytometry. Treatment with PLX-4107 results in **B.** complete degradation of IKZF2, **C.** decreased CD25 expression on FoxP3$^+$ CD25^{hi} Treg cells, **D.** reduced Treg proliferation and **E.** increased effector CD8 T cell proliferation.

IKZF2 is Rapidly Degraded by PLX-4107 *in vivo*

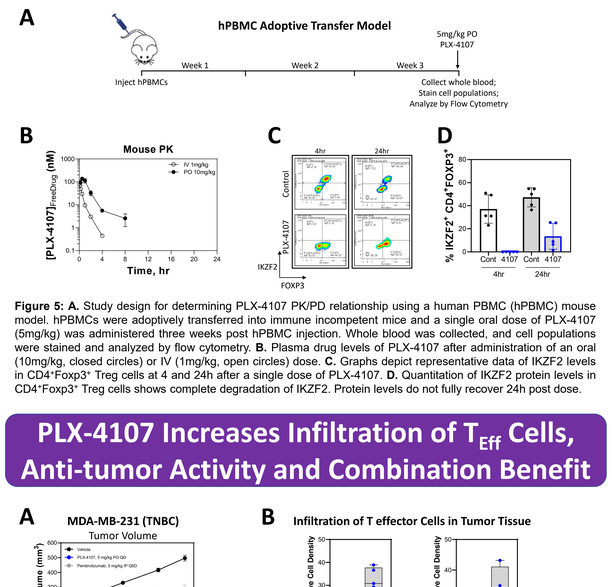

Figure 5: A. Study design for determining PLX-4107 PK/PD relationship using a human PBMC (hPBMC) mouse model. hPBMCs were adoptively transferred into immune incompetent mice and a single oral dose of PLX-4107 (5mg/kg) was administered three weeks post hPBMC injection. Whole blood was collected, and cell populations were stained and analyzed by flow cytometry. **B.** Plasma drug levels of PLX-4107 after administration of an oral (10mg/kg, closed circles) or IV (1mg/kg, open circles) dose. **C.** Graphs depict representative data of IKZF2 levels in CD4$^+$Foxp3$^+$ Treg cells at 4 and 24h after a single dose of PLX-4107. **D.** Quantitation of IKZF2 protein levels in CD4$^+$Foxp3$^+$ Treg cells shows complete degradation of IKZF2. Protein levels do not fully recover 24h post dose.

PLX-4107 Increases Infiltration of T_{Eff} Cells, Anti-tumor Activity and Combination Benefit

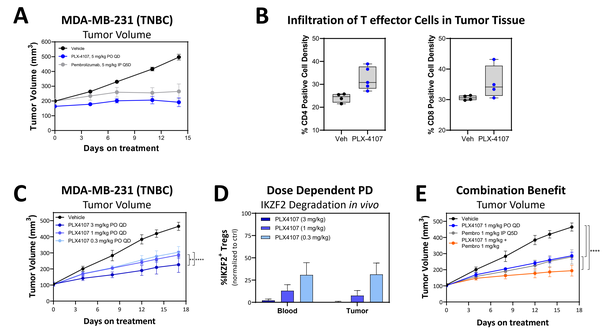

Figure 6: PLX-4107 demonstrates single agent anti-tumor activity and increases infiltration of T effector cells. **A.** MDA-MB-231 xenograft-bearing animals were injected with hPBMCs and treated with PLX-4107 (PO, QD) or pembrolizumab (IP, Q5D) for 14 days. Treatment results in significant tumor growth inhibition. **B.** Tumor tissue was collected 4h post 14 days of PLX-4107 treatment. Tumor tissue was fixed, stained for CD4 and CD8 T cells, and analyzed by immunohistochemistry (IHC). PLX-4107 treatment results in significant increase in effector T cell infiltration. **C.** Dose dependent single agent anti-tumor activity with PLX-4107 treatment at various doses for 17 days. **D.** Dose dependent pharmacodynamic response in blood and tumor tissue. **E.** Combination of PLX-4107 and pembrolizumab, agents with orthogonal MOA, result in significant combination benefit.

Summary

- PLX-4107 is a novel molecular glue that binds cereblon and selectively recruits the undruggable transcription factor, IKZF2, for degradation. PLX-4107 potently degrades IKZF2 resulting in enhanced derepression of IL2
- PLX-4107 destabilizes human CD4$^+$Foxp3$^+$ Treg cells as seen by an induction of IL2 and IFN$_\gamma$, and reduces the capacity of Tregs to suppress Teff proliferation
- Treatment of PLX-4107 decreases CD25 expression in CD4$^+$FOXP3$^+$ Tregs, reduces Treg proliferation, and increases effector T cell activity *in vivo*
- Oral administration of PLX-4107 results in rapid degradation of IKZF2 in CD4$^+$FOXP3$^+$ Tregs *in vivo*, dose dependent single agent anti-tumor activity, and increased infiltration of effector T cells
- Combination of PLX-4107 with PD1 antibody demonstrates combination benefit and suggests the potential to improve clinical responses to immune checkpoint therapy

References:
[1]*Bonazzi et al. WO2020128972A1*

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