# Identification of selective IKZF2 degraders that reprogram suppressive regulatory T cells in solid tumors

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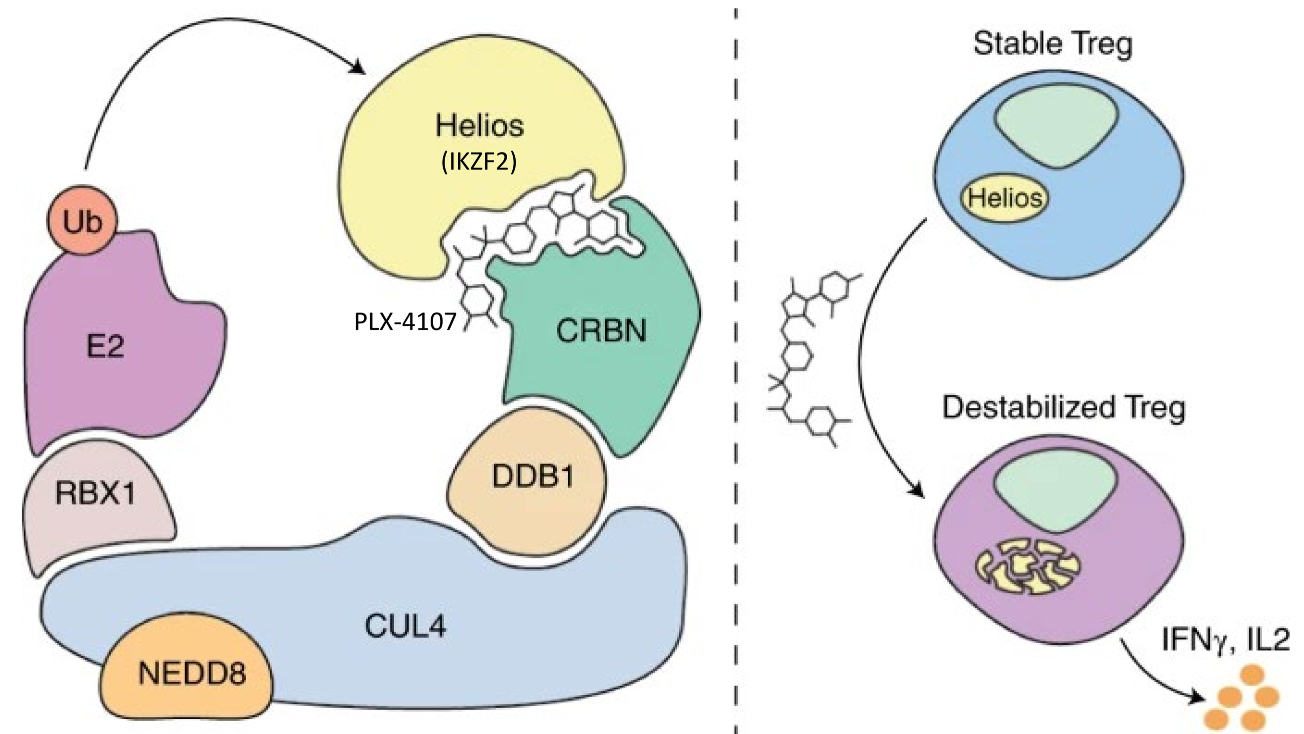
## Abstract

Targeted protein degradation (TPD) using the endogenous Ubiquitin Proteasome System (UPS) is a rapidly growing drug discovery strategy to eliminate pathogenic proteins. Molecular glues are small molecules that promote a novel interaction between a protein of interest with an E3 ubiquitin ligase leading to proximity induced protein degradation. This has enabled targeting undruggable proteins, such as the zinc-finger transcription factor Helios (IKZF2), that have no known small molecule binding pocket.

Despite recent clinical breakthroughs in checkpoint blockade in treating solid tumors, suppression of the antitumor immune response in the tumor microenvironment (TME) is a major obstacle to tumor regression. Regulatory T cells (Tregs) in the TME are potent immunosuppressive cells that promote progression of cancer. IKZF2 has been shown to be a marker of highly suppressive Treg cells and is critical for maintaining the anergic and suppressive phenotype in the highly inflammatory tumor microenvironment. Genetic depletion of IKZF2 in Treg cells results in both loss of suppressive activity and conversion of Tregs into T effector cells, leading to enhanced anti-tumor immunity. Collectively, these findings support that an IKZF2-specific degrader could be beneficial in enhancing the efficacy of current immunotherapies.

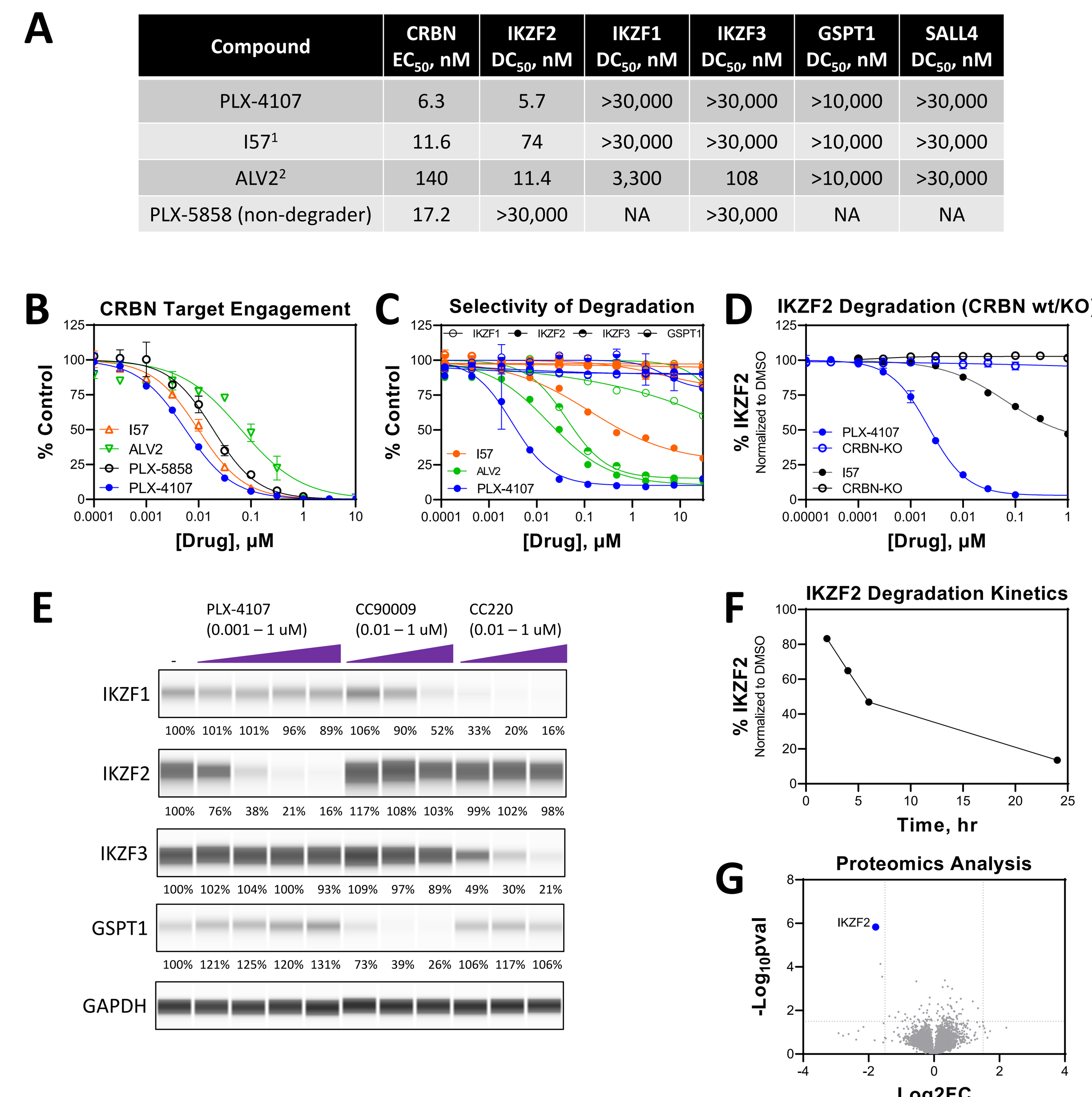
Here we report our efforts leading to the development of a series of potent and selective IKZF2 degraders for the treatment of cancer. Our lead compound, PLX-4107, is a novel molecular glue that was optimized to be a highly selective, deep, and rapid IKZF2 degrader via the redirection of the E3 substrate receptor, cereblon. Proteome-wide analysis demonstrated that PLX-4107 depletes IKZF2 protein levels without degrading other known cereblon neo-substrates. PLX-4107 mediated degradation of IKZF2 resulted in conversion of highly suppressive Tregs into T effector cells, coupled with an increased expression of the effector cytokines IL2 and IFNγ. Oral administration of PLX-4107 to cynomolgus monkeys caused rapid, complete, and prolonged degradation of IKZF2 in Tregs, indicative of the catalytic nature of degraders where sustained pharmacodynamic response is observed well beyond plasma drug exposure levels. These preliminary data provide a strong rationale for developing small molecule therapeutics that target the undruggable IKZF2 transcription factor with the potential to enhance the efficacy of immune checkpoint therapy.

## Introduction




- $CD4^+$ regulatory T cells (Tregs), a specialized subset of T cells, compromise antitumor immune responses
- The zinc-finger transcription factor IKZF2 (Helios) 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
- Depletion of IKZF2 results in converting Tregs into a T-effector like phenotype and increases secretion of cytokines (IL2 and IFNγ)
- IKZF2 degradation has the potential to improve clinical responses to immune checkpoint inhibitor therapy across multiple tumor types

## PLX-4107 is a Selective IKZF2 Degrader



| Compound | CRBN $EC_{50}$, nM | IKZF2 $DC_{50}$, nM | IKZF1 $DC_{50}$, nM | IKZF3 $DC_{50}$, nM | GSPT1 $DC_{50}$, nM | SALL4 $DC_{50}$, nM |
|---|---|---|---|---|---|---|
| PLX-4107 | 6.3 | 5.7 | >30,000 | >30,000 | >10,000 | >30,000 |
| I57[1] | 11.6 | 74 | >30,000 | >30,000 | >10,000 | >30,000 |
| ALV2[2] | 140 | 11.4 | 3,300 | 108 | >10,000 | >30,000 |
| PLX-5858 (non-degrader) | 17.2 | >30,000 | NA | >30,000 | NA | NA |



**Figure 1: A.** Small molecules that bind the E3 ligase substrate, cereblon (CRBN). PLX-4107, I57[1] and ALV2[2] recruit neosubstrates for degradation. **B.** NanoLuc-CRBN target engagement binding curve of PLX-4107, PLX-5858, I57 and ALV2. 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 I57 for 24h in CRBN wt and KO Jurkat cells. Protein was detected by flow cytometry. **E.** Western blot analysis for select CRBN molecular glues. CC90009 (GSPT1 degrader) and CC220 (IKZF1/3 degrader) were used as controls. Proteins were detected using a Jess Simple Western (ProteinSimple) with GAPDH as a loading control. **F.** Degradation kinetics of IKZF2 in Jurkat cell line incubated with PLX-4107 for varying timepoints at its $DC_{90}$. Intracellular IKZF2 protein was detected using a Miltenyi MACSQuant 16 Flow Cytometer and analyzed using FlowLogic software. **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

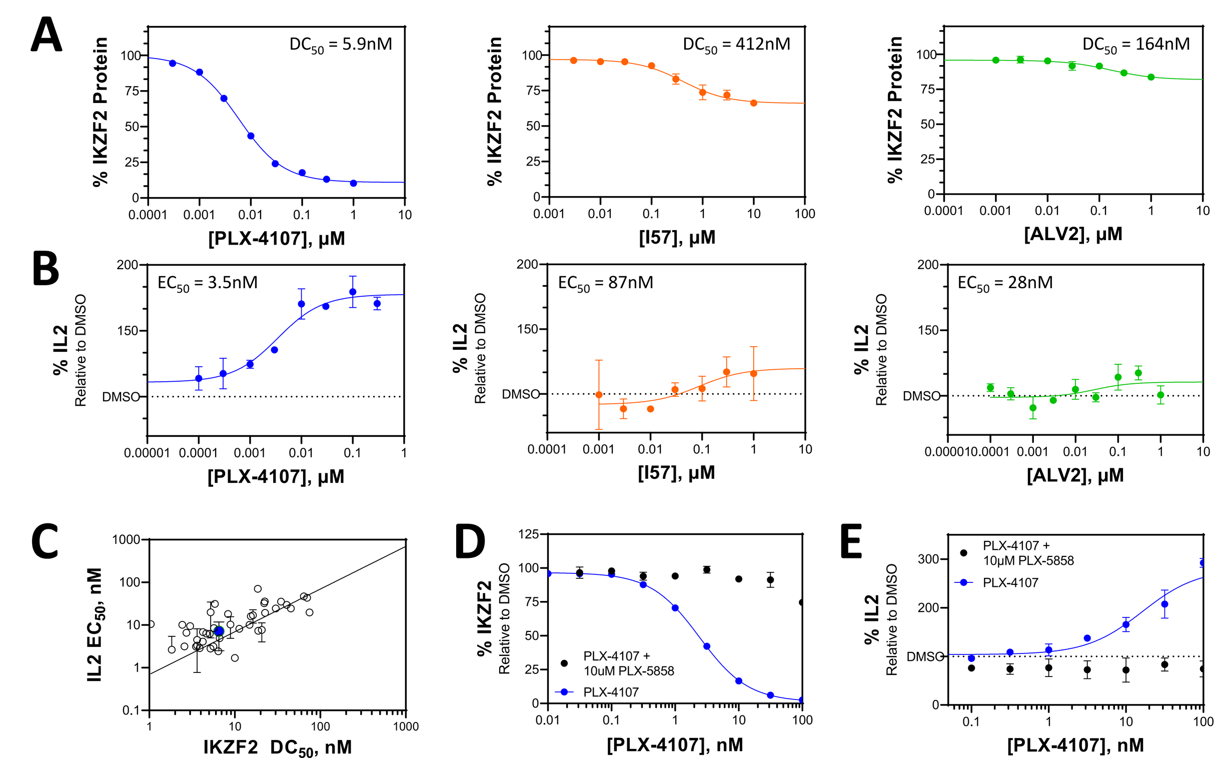



**Figure 2: A.** Degradation of IKZF2 in Jurkat cell line after exposure to PLX-4107, I57[1] and ALV2[2]. **B.** Compound treatment derepresses IL2 expression. Engineered Jurkat cells expressing a luciferase reporter (TCR/CD3 effector cells) driven by an IL2 promoter were treated with various compound concentrations for 48hr. 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. **C.** PLX-4107 (highlighted in blue) is part of a chemical series that selectively degrade IKZF2 and induce IL2. $DC_{50}$ of IKZF2 degradation correlates with IL2 $EC_{50}$ induction. **D.** 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 **E.** IL2 induction. Intracellular IKZF2 levels were detected by flow cytometry.

## PLX-4107 Destabilizes Human Treg Cells

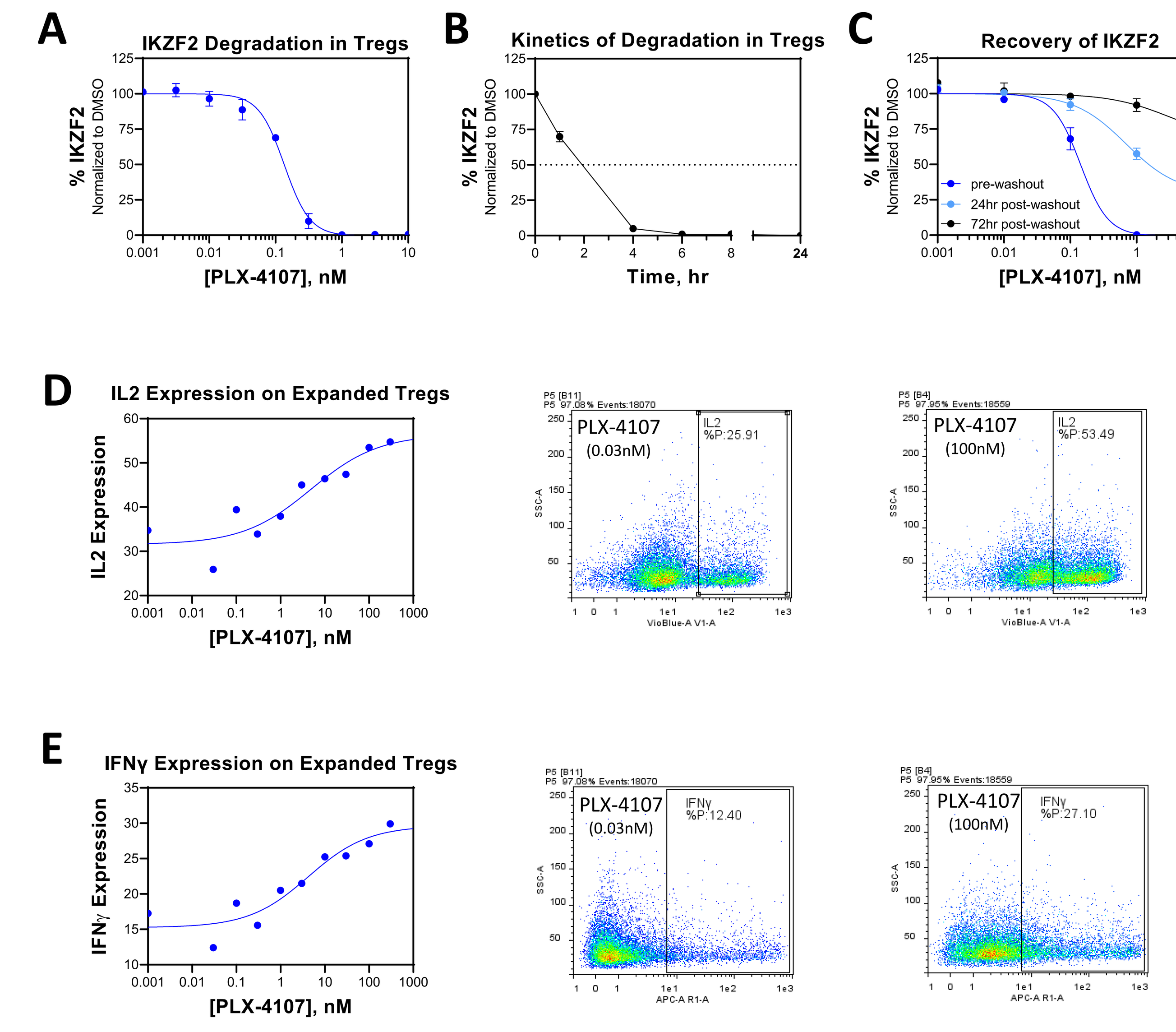



**Figure 3:** Degradation of IKZF2 destabilizes human $CD4^+Foxp3^+$ Treg cells resulting in the induction of cytokine production. **A.** Dose dependent degradation of IKZF2 in human Treg cells treated with PLX-4107 for 24h ($DC_{50}$ = 0.56 ±0.58nM). **B.** Treatment of human Treg cells with PLX-4107 at the $DC_{90}$ results in rapid and complete degradation of IKZF2 within 4h. **C.** Human Treg cells treated with PLX-4107 results in sustained suppression of IKZF2. Recovery of IKZF2 protein levels was observed to take more than 72h after removal of PLX-4107. **D.** Dose dependent induction of IL2 ($EC_{50}$ = 3.0 ±2.5) and **E.** IFNγ ($EC_{50}$ = 3.9 ±0.5) in $CD4^+Foxp3^+$ Treg cells with PLX-4107 treatment from 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 $IFN\gamma^+$ $CD4^+Foxp3^+$ Treg cells after PMA/Ionomycin stimulation and treatment with Brefeldin A to block cytokine secretion. Cells were surface stained, fixed and permeabilized and then intracellular stained. Protein levels were detected using a Miltenyi MACSQuant 16 flow cytometer and analyzed using FlowLogic software. Representative data from multiple donors.

## PLX-4107 Reduces Treg Suppression of Teff Cells

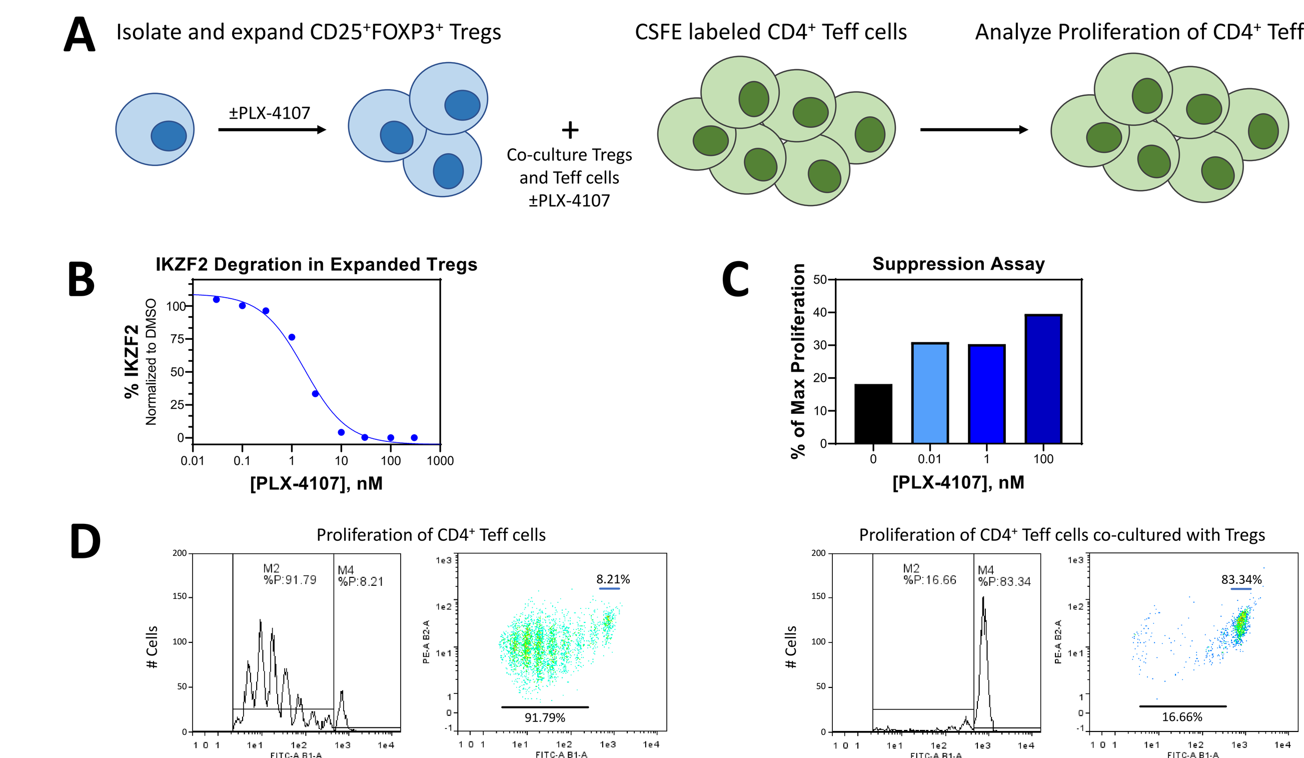



**Figure 4:** IKZF2 degradation in $CD4^+Foxp3^+$ Treg cells with PLX-4107 shows reduced capacity to suppress Teff proliferation. **A.** Schematic of Treg Suppression Assay. **B.** Degradation profile of PLX-4107 in expanded Tregs ($DC_{50}$ = 1.8nM). **C.** Quantitation of Teff cell proliferation co-cultured with Tregs and incubated with PLX-4107 or control. **D.** Representative cell division profiles and quantitation of proliferative $CD4^+$ Teff cells co-cultured with Treg cells. Human $CD4^+Foxp3^+$ Treg cells were expanded in the presence of IL2, CD3/CD28 Dynabeads and various concentrations of PLX-4107 or control for 5-7 days and then co-cultured with CFSE labeled $CD4^+$ Teff cells (1:0.1 Teff:Treg) and compound or control for an additional 5 days. Cells were surface stained, fixed and permeabilized for intracellular staining. Proteins levels were analyzed by flow cytometry.

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

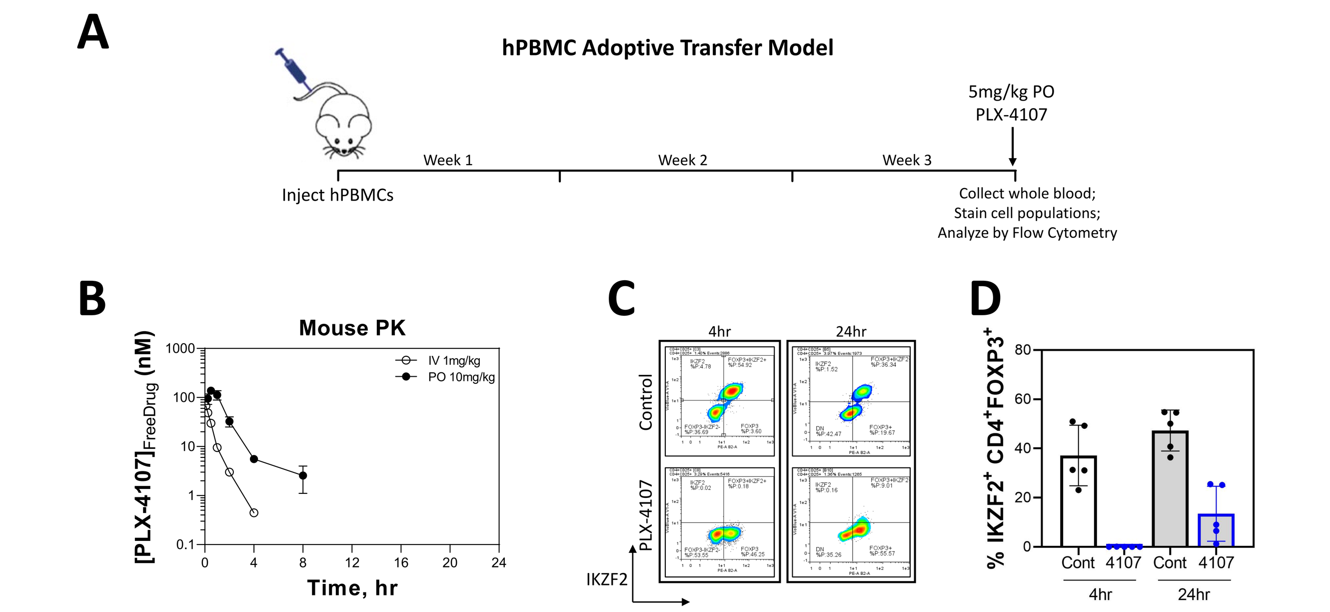



**Figure 5: A.** Study design for determining PLX-4107 PK/PD relationship using a human PBMC (hPBMC) mouse model. hPBMCs were adoptively transferred into immune incompetent mice and a single oral dose of PLX-4107 (5mg/kg) was administered three weeks post hPBMC injection. Whole blood was collected, and 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 and protein levels are not fully recovered 24h post dose.

## Sustained Degradation of IKZF2 Induces IL2/IFNγ Levels *in vivo*

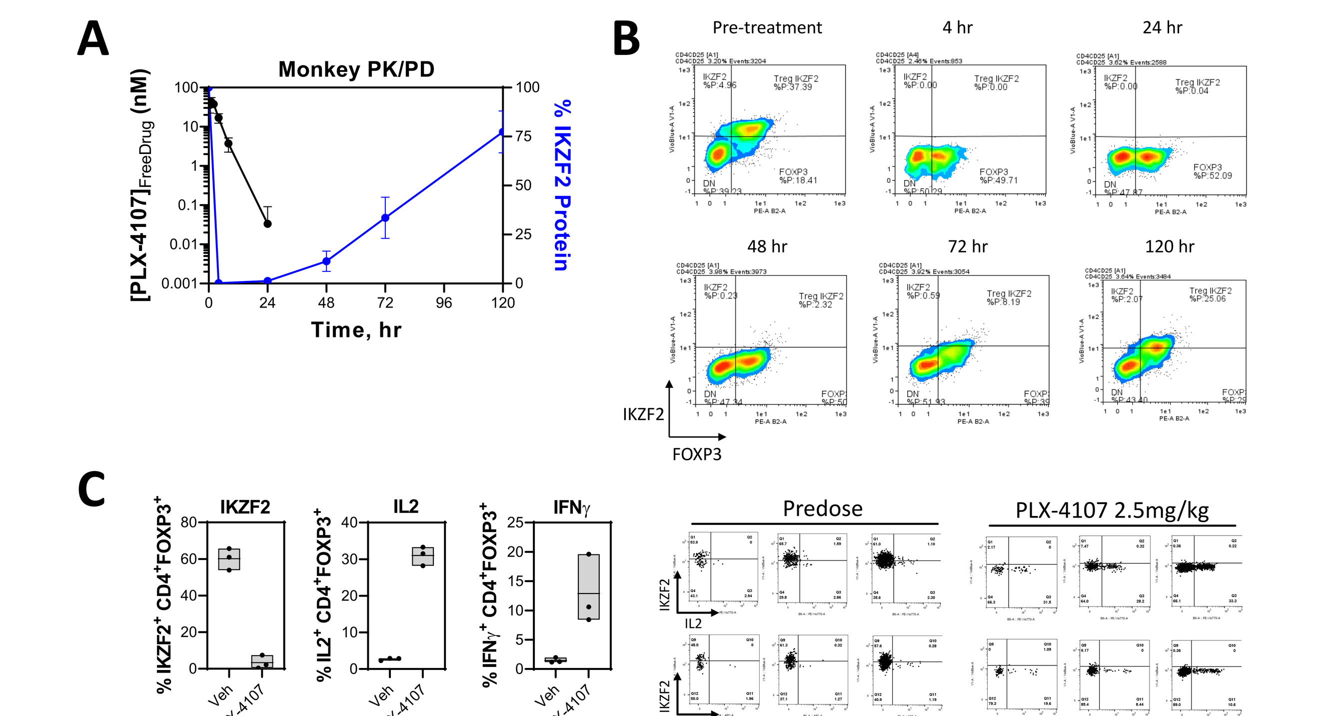



**Figure 6: A.** Plasma drug concentration levels of PLX-4107 in the cynomolgus monkey (black circles) and IKZF2 protein expression (green circles) as determined by flow cytometry in $CD4^+Foxp3^+$ Treg cells after an oral dose of PLX-4107 (2.5mg/kg). IKZF2 is rapidly degraded after administration of PLX-4107 and requires over 5 days for protein levels to fully recover. **B.** Representative flow cytometry graphs for the analysis of IKZF2 in cynomolgus monkey $CD4^+Foxp3^+$ Treg cells as a function of time after administration of PLX-4107. **C.** A single dose of PLX-4107 (2.5mg/kg) in monkeys degrades IKZF2 and induces IL2 and IFNγ cytokine levels in $CD4^+Foxp3^+$ Treg cells. Cells were stimulated with PMA/Ionomycin and treated with Brefeldin A to block cytokine secretion. Protein levels were analyzed by flow cytometry.

## Summary

- PLX-4107 is a novel molecular glue that binds cereblon and selectively recruits the IKZF2 transcription factor for degradation. PLX-4107 more potently degrades IKZF2 resulting in enhanced induction of IL2 compared with competitor compounds
- Degradation of IKZF2 by PLX-4107 derepresses IL2 expression. The magnitude ($D_{max}$) of IKZF2 degradation corresponds with IL2 induction
- PLX-4107 destabilizes human $CD4^+Foxp3^+$ Treg cells as seen by an induction of IL2 and IFNγ and reduces the capacity of Tregs to suppress Teff proliferation
- Oral administration of PLX-4107 in mouse and NHP PK/PD models rapidly and completely degrades IKZF2 and induces cytokine levels in $CD4^+FOXP3^+$ Tregs *in vivo*

***References:***
*[1]Bonazzi et al. WO20201289721A1*
*[2]Wang et al. Nat Chem Biol 2021, 17, 711-717*

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