Discovery of PLX-4107, a selective IKZF2 molecular glue degrader, that modulates suppressive regulatory T cells and demonstrates anti-tumor activity

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Abstract

Background: Immune checkpoint inhibitors (CPI) have significantly advanced cancer treatment; nevertheless, responses are limited to patient subsets, thereby necessitating additional treatment strategies. Regulatory T-cells (Tregs) are a specialized population of CD4+ T-cells that maintain normal immune tolerance and homeostasis; however, in the tumor microenvironment (TME), Tregs are potent immunosuppressive cells that promote tumor immune evasion and reduced clinical response to CPI. The transcription factor IKZF2 is a marker of highly suppressive Tregs and is required to maintain a stable, suppressive Treg cell phenotype in the inflammatory TME. Depletion of IKZF2 reprograms suppressive Tregs into effector-like T-cells leading to anti-tumor immunity, but targeting transcription factors has been challenging due to the lack of defined structures and binding pockets. Protein degradation using the endogenous Ubiquitin Proteasome System (UPS) has enabled accessing undruggable proteins, such as IKZF2, through chemically induced proximity that promotes degradation.

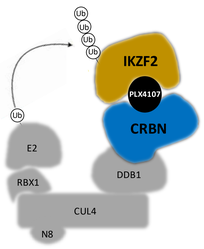
Methods: Through computational modeling, compound screening and optimizing for enhanced reprogramming of Treg function, highly selective IKZF2 degraders were identified.

Results: PLX-4107 is a molecular glue that was designed to promote a novel interaction between IKZF2 and the E3 ubiquitin ligase substrate receptor, cereblon, leading to potent and selective degradation of IKZF2. Degradation of IKZF2 by PLX-4107 is blocked by co-treating with proteasome and neddylation inhibitors or a cereblon knock-out cell line, confirming that degradation is UPS mediated and dependent on cereblon. Global cellular proteomics demonstrated that PLX-4107 selectively depleted IKZF2 protein levels without degrading other cereblon neo-substrates. *In vitro*, PLX-4107 mediated degradation of IKZF2 caused human Tregs to lose their suppressive function and produce effector cytokines (IL2, IFNγ) resulting in increased proliferation of effector T-cells and tumor cell killing. Oral administration of PLX-4107 to primates or humanized mice demonstrated sustained depletion of IKZF2 and reprogramming of Tregs. *In vivo* xenograft efficacy studies showed that administration of PLX-4107 resulted in dose dependent single agent anti-tumor activity. PLX-4107 treatment resulted in destabilization of Tregs by decreasing CD25 expression and increasing Treg expression of effector-like cytokines leading to increased infiltration of activated CD4/8+ effector T-cells into tumor tissue. Co-administration of PLX-4107 and pembrolizumab in xenograft studies resulted in tumor growth inhibition and significant combination benefit.

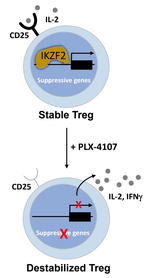
Conclusion: PLX-4107 is a novel molecular glue that selectively degrades IKZF2, a transcription factor of suppressive Tregs. PLX-4107 mediated IKZF2 degradation reverses tumor immune evasion by converting Tregs to an effector-like T-cell phenotype, resulting in single agent antitumor activity and the ability to enhance CPI efficacy.

Background

Targeting Undruggable Transcription Factor (IKZF2) using a Molecular Glue Strategy



Loss of IKZF2 Destabilizes Suppressive Tregs



- Escaping immune destruction is a key step in cancer progression. 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

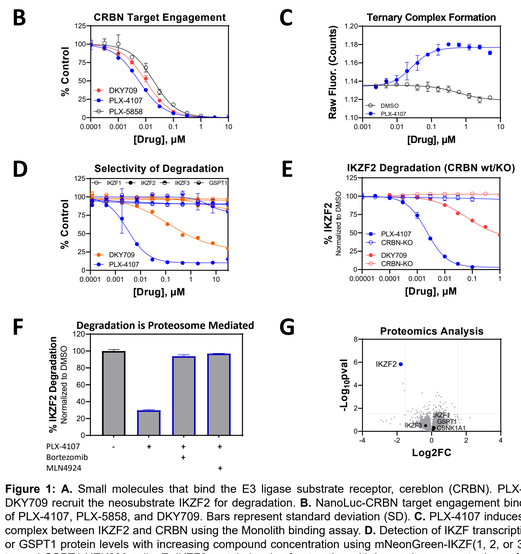


Figure 1: A. Small molecules that bind the E3 ligase substrate receptor, cereblon (CRBN). PLX-4107 and DKY709 recruit the neosubstrate IKZF2 for degradation. **B.** NanoLuc-CRBN target engagement binding curve of PLX-4107, PLX-5858, and DKY709. Bars represent standard deviation (SD). **C.** PLX-4107 induces a ternary complex between IKZF2 and CRBN using the Monolith binding assay. **D.** Detection of IKZF transcription factors or GSPT1 protein levels with increasing compound concentration using mNeonGreen-IKZF(1, 2, or 3) or HiBiT tagged GSPT1 HEK293 cells. **E.** 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. **F.** Degradation is mediated by the proteasome. Jurkat cells were incubated +/- proteasome inhibitor (100nM bortezomib) or neddylation inhibitor (1µM MLN4924) for 2h, followed by a 6h incubation with 100nM PLX-4107. IKZF2 protein levels were detected by flow cytometry. **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 Increases IL-2 Expression

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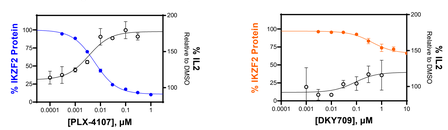

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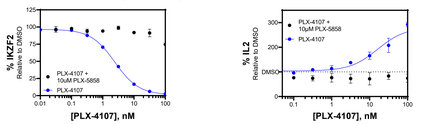


Figure 2: A. Degradation of IKZF2 in Jurkat cell line after exposure to PLX-4107 and DKY709 derepresses IL-2 expression. Engineered Jurkat cells expressing a luciferase reporter (TCR/CD3 effector cells) driven by an IL-2 promoter were treated with various compound concentrations for 48h. Cells were stimulated by addition of CD3 antibody leading to upregulation of IL-2 production detected by a luciferase assay. The magnitude (D_{max}) of IKZF2 degradation corresponds with IL-2 production. **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 IL-2 production.

PLX-4107 Converts Tregs into Effector-like T Cells

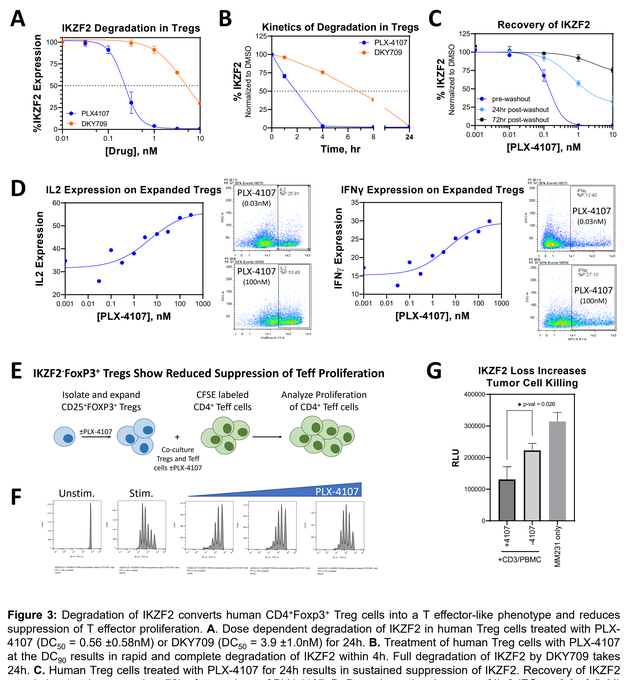


Figure 3: Degradation of IKZF2 converts human CD4+Foxp3+ Treg cells into a T effector-like phenotype and reduces suppression of T effector proliferation. **A**. Dose dependent degradation of IKZF2 in human Treg cells treated with PLX-4107 (DC_{50} = 0.56 ±0.58nM) or DKY709 (DC_{50} = 3.9 ±1.0nM) for 24h. **B.** Treatment of human Treg cells with PLX-4107 at the DC_{50} 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 washout of PLX-4107. **D.** Dose dependent increase of IL-2 (EC_{50} = 3.0 ±2.5nM) and IFNγ (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 IL-2, CD3/CD28 Dynabeads and PLX-4107 for 5-7 days. Representative fluorescence activated cell sorting (FACS) plots and quantification of percent IL-2+ 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.** 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. **G.** MDA-MB-231-luciferase cells were co-cultured with PBMCs stimulated with anti-CD3 antibody ±PLX-4107 for 96h. Target cells were quantitated by monitoring luminescence.

PLX-4107 Reverses Tumor Immune Evasion Resulting in Anti-Tumor Activity *In Vivo*

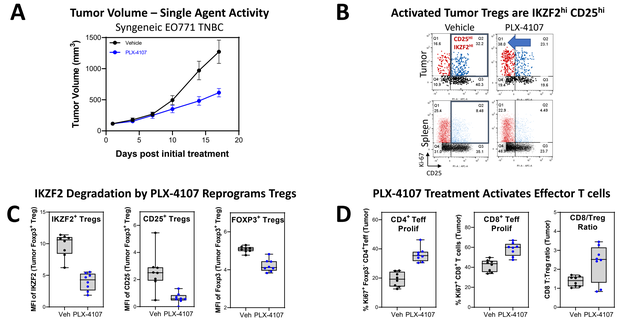


Figure 4: IKZF2 degradation results in single agent anti-tumor activity. **A.** EO771 TNBC syngeneic tumor bearing humanized CRBN knock in mice were treated with vehicle or PLX-4107 PO for 17 days. PLX-4107 treatment results in significant tumor growth inhibition. Tumor or spleen tissue was collected 6h post last dose, immune cells were isolated, stained and analyzed by flow cytometry. **B.** PLX-4107 treatment shifts activated tumor FoxP3+ Tregs from high to low expression of IKZF2 and CD25. **C.** IKZF2 degradation in tumor FoxP3+ Tregs with PLX-4107 administration decreases CD25 and FOXP3 expression resulting in **D.** increased proliferation of CD4 and CD8 effector T cells and CD8:Treg ratio.

PLX-4107 Results in Single Agent Activity and Enhances CPI Efficacy *In Vivo*

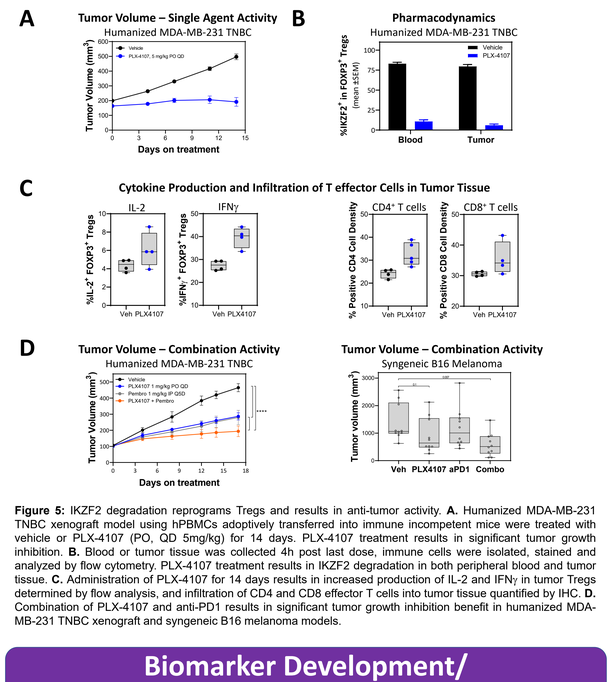
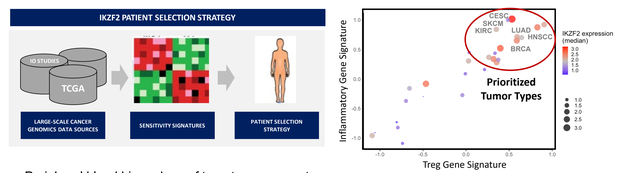


Figure 5: IKZF2 degradation reprograms Tregs and results in anti-tumor activity. **A.** Humanized MDA-MB-231 TNBC xenograft model using hPBMCs adoptively transferred into immune incompetent mice were treated with vehicle or PLX-4107 (PO, QD 5mg/kg) for 14 days. PLX-4107 treatment results in significant tumor growth inhibition. **B.** Blood or tumor tissue was collected 4h post last dose, immune cells were isolated, stained and analyzed by flow cytometry. PLX-4107 treatment results in IKZF2 degradation in both peripheral blood and tumor tissue. **C.** Administration of PLX-4107 for 14 days results in increased production of IL-2 and IFNγ in tumor Tregs determined by flow analysis, and infiltration of CD4 and CD8 effector T cells into tumor tissue quantified by IHC. **D.** Combination of PLX-4107 and anti-PD1 results in significant tumor growth inhibition benefit in humanized MDA-MB-231 TNBC xenograft and syngeneic B16 melanoma models.

Biomarker Development/ Patient Selection Strategy



- Peripheral blood biomarkers of target engagement
- Mechanism based biomarker for enrichment and selection of patients
  - -Supported by bioinformatics analysis of public databases
  - -Comparative analysis of IKZF2 expression with inflammatory and Treg gene signatures
- Prioritized tumor types along with potential predictive gene signatures

Summary

- PLX-4107 is a novel molecular glue that binds cereblon and selectively recruits the undruggable transcription factor, IKZF2, for degradation
- PLX-4107 selectively degrades IKZF2 converting suppressive Tregs to effector-like T cells
  - -Increases Treg production of IL-2 and IFNγ
  - -Reduces capacity of Tregs to suppress Teff proliferation
  - -Decreases CD25+FoxP3+ Tregs
  - -Increases proliferation and infiltration of CD4 and CD8 Teff cells into tumor tissue
  - -Increases tumor cell killing
- PLX-4107 reverses tumor immune evasion resulting in anti-tumor activity *in vivo*
  - -*In vivo* dose dependent PK/PD in immune cells and dose dependent single-agent activity
  - -Shifts Teff:Tref balance in favor of anti-tumor activity
  - -Combination of PLX-4107 with anti-PD1 therapy results in significant TGI benefit
- Initiating clinical trials Q4 2023

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