First-in-class PDE4D Bifunctional Degraders for Inflammatory Skin Diseases

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Abstract

Atopic dermatitis (AD) is a chronic inflammatory skin disease characterized by erythema and pruritis, affecting over 15 million people in the US. Th2/Th17 cytokines play a key role in AD pathogenesis, resulting in an increased inflammatory response, disrupted skin barrier, increased susceptibility to infections and allergen sensitization. Phosphodiesterase 4 (PDE4) is a cyclic 3′,5′-adenosine monophosphate (cAMP)-specific phosphodiesterase and its expression is elevated in AD patients sustaining a highly inflammatory environment. PDE4 inhibition has been shown to effectively suppress proinflammatory cytokines in clinically validated approaches for several chronic inflammatory conditions. The current standard of care therapies including PDE4 inhibitors are linked to broad adverse effects due to their off-target activities. Utilizing our proprietary PRODEGY discovery platform, we have designed potent and selective PDE4D bifunctional degraders. PDE4D degradation dramatically decreases the pro-inflammatory cytokines released by activated T cells *in vitro*. Additionally, PDE4D is degraded efficiently in mice spleens treated with the lead PDE4D degrader in a dose-dependent manner, with no adverse effects. Importantly, our PDE4D degraders do not induce significant degradation of known neo-substrates, suggesting high specificity. Taken together, we propose the introduction of a more potent, specific, and safe PDE4D degrader as novel therapy for inflammatory skin diseases, such as AD.

PDE4 Regulates Multiple Nodes Inflammatory and Immune response

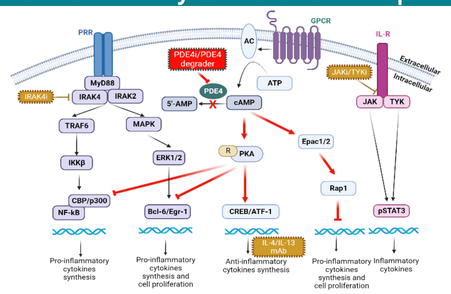


Figure 1: PDE4 Broadly Regulates Inflammation and Immune response.

PDE4 Inhibitors have Limitations Which can be Addressed by a Degrader Modality

Limitations of conventional inhibitors of PDE4

1. Limited efficacy vs. newer agents and biologics
2. Limited success in broader inflammatory indications
3. Blood-brain-barrier (BBB) penetration and pan-isoform inhibition may result in observed safety issues (e.g., GI tox, CNS effects)

Advantages of degrader-based approach for PDE4

1. Far **superior activity** vs. newer agents based on degrader modality
2. Potential for **broader indication opportunity**
3. Isoform **selectivity and likely lack of BBB penetration** enables potential for favorable safety profile

Figure 2: Advantages of degrading PDE4D when compared to approved PDE4 inhibitors

pM Potency Driven by Rapid and Deep Target Degradation

A

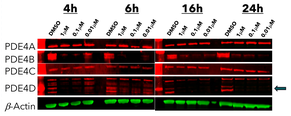
PDE4 bifunctional degraders are selective and pan-isoform degraders of PDE4D in A549 cells

Compounds tested in A549 cells at 24 hrs	PDE4D DC_{50} (Short isoforms) nM	PDE4D DC_{50} (Long isoforms) nM
Apremilast	N/A	N/A
BTX-AP01 (Short isoform degrader)	1.7	>1000
BTX-AP02 (Pan isoform degrader)	0.44	11
BTX-AP03 (Pan isoform degrader)	0.02	1
BTX-AP04 (Pan isoform degrader)	<10	9
BTX-AP05 (Pan isoform degrader)	0.6	4

Bold font denotes orally bioavailable molecules
Note: DC_{50} is the concentration to achieve 50% degradation

B

PDE4 bifunctional degraders selectively degrade PDE4B and PDE4D in human PBMCs



BTX-AP04	D_{max} (max degradation)
Compounds tested in A549 cells at 24 hrs	97%
Compounds tested in PBMCs at 24 hrs	86%

Figure 3: (A) Lead PDE4D degraders were tested in A549 cells which express 2 long isoforms and 2 short isoforms of PDE4D. IC_{50} values for all the lead degraders indicate that the BTX compounds can degrade both the isoforms at low nM levels , while the lead (BTX-AP05) can degrade PDE4D short isoforms at pM levels.

(B) Degradation of various PDE4 isoforms was assessed in human PBMCs with lead oral degrader. Western blots for all the 4 isoforms are shown. Results show that BTX-AP04 can degrade PDE4D rapidly (4h) and deeply (86%).

PDE4 Bifunctional Degraders Display Superior Activity on Mechanistic Targets

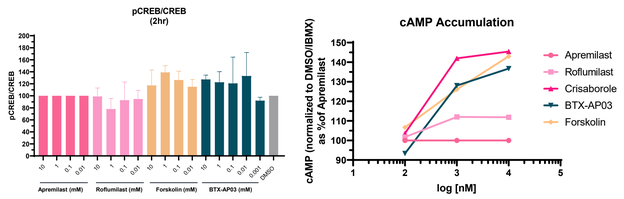


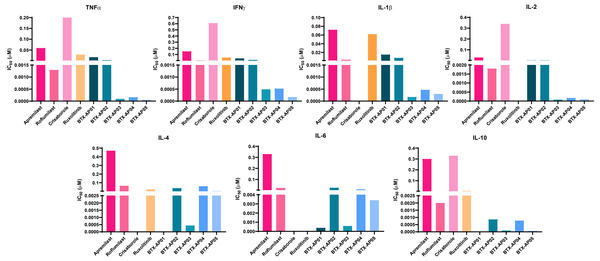
Figure 4: (A) A549 cells were treated with PDE4 inhibitors (Apremilast and Roflumilast) as well as lead PDE4D degrader, BTX-AP03 for 2 hours. Forskolin was included as a positive control. pCREB and total CREB levels were measured in the cell lysates using an MSD based assay. Ratio of pCREB/tCREB is plotted and normalized to Apremilast. Results indicate that PDE4D degrader was not only able to phosphorylate CREB higher than the PDE4 inhibitors, but also equivalent to Forskolin which produces maximum increases in the levels of pCREB.

(B) PDE4 inhibition leads accumulation of cAMP in the cells (explained in Figure 1). We tested PDE4 inhibitors as well as PDE4D degraders for accumulation of cAMP in A549 cells after 2 hours of treatment. We included Forskolin as a positive control. Here too, we observe that PDE4D degrader (BTX-AP03) led to higher accumulation of cAMP than PDE4 inhibitors.

Results of both pCREB and cAMP assays indicate that our PDE4D degraders perform better than approved PDE4 inhibitors.

PDE4 Degraders Display Potent Inhibition of Inflammatory Cytokines

Up to 4,000-fold improvement in IC_{50} vs. PDE4i and up to 500-fold improvement vs. JAK1/JAK2i

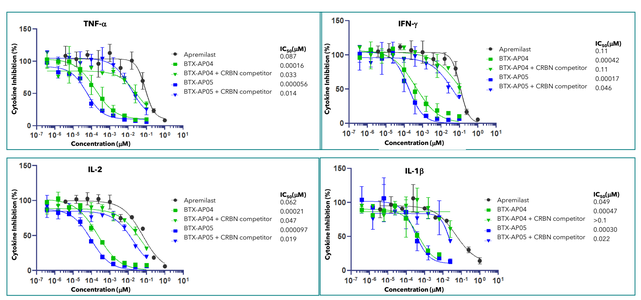


α-CD3/α-CD28 stimulated human PBMCs treated with compounds for 24 hours

Figure 5: PDE4D degradation suppresses expression of multiple inflammatory cytokines. PBMCs were stimulated using αCD3 and αCD28 antibodies. 1h post stimulation, control compounds and BTX degraders were added to the stimulation media, followed by incubation at 37°C for 24h. Conditioned media was collected, and cytokines were assayed for cytokine expression using MSD kits. PBMCs were used in a CTG-assay to assess cell viability. When compared to all the PDE4 inhibitors (Apremilast, Roflumilast and Crisaborole) as well as Jak1/2 inhibitor (Ruxolitinib) tested, PDE4D degraders are 4000-fold more potent than PDE4 inhibitors and 500-fold more potent than Jak1/2 inhibitor, as measured by the IC_{50} of cytokine inhibition by these compounds.

Functional Activity of PDE4 Bifunctional Degraders is CRBN Dependent

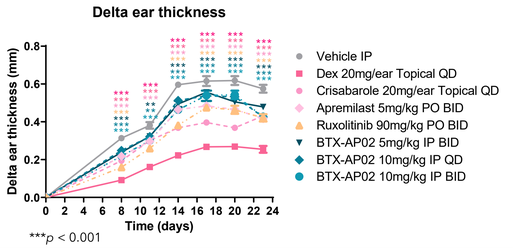
Expression of inflammatory cytokines by PDE4 bifunctional degraders is significantly reduced in the presence of competitor CRBN binder



α-CD3/α-CD28 stimulated human PBMCs treated with compounds for 24 hours

Figure 6: PBMCs stimulated with αCD3 and αCD28 antibodies were incubated with BTX compounds with or without CRBN competitor to determine that the anti-inflammatory effect due to PDE4D degradation is CRBN dependent using an MSD assay. Expression of inflammatory cytokines by PDE4 bifunctional degraders is significantly reduced in the presence of competitor CRBN competitor. Moreover, upon inhibition of the CRBN binding moiety, expression of cytokines is at the level of the inhibitor indicating that the bi-functional degrader is still bound to PDE4D.

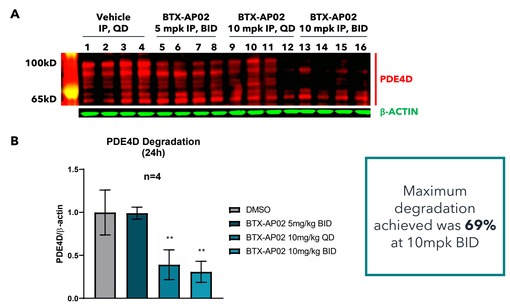
Efficacy Observed in Mouse Model of Atopic Dermatitis with PoC Degrader, BTX-AP02 is Equivalent to SoC



Current leads demonstrate 10x more potent inflammatory cytokine inhibition *in vitro* than PoC early degraders

Figure 7: Efficacy of our proof-of-concept PDE4D degrader (BTX-AP02) was tested in the mouse model of oxazolone induced atopic dermatitis. Ear thickness was measured to determine the efficacy of BTX compound and compared with lead PDE4D inhibitors (Crisabarole and Apremilast) as well as Jak1/2 inhibitor (Ruxolitinib). The results indicate that the PoC BTX compound displays equivalent efficacy to all the commercially available anti-inflammatory agents.

PoC Degrader, BTX-AP02 Demonstrates Deep Degradation of PDE4D Isoforms in Mouse Tissues



Maximum degradation achieved was **69%** at 10mpk BID

Figure 8: (A) Spleens from the mice treated with BTX-AP02 at various dose levels from the study above were evaluated for PDE4D degradation 2, 6 and 24h post last dose. We observed dose dependent degradation of PDE4D at all time points. Maximum degradation of PDE4D was observed at 24h, but rapid PDE4D degradation was observed at 2h and 8h (not shown).

(B) Quantification of PDE4D degradation in spleens at 24h is shown. Maximum degradation of PDE4D achieved was 69% at 24h time point.

PoC degrader BTX-AP02, Downregulates Expression of Inflammatory Cytokines with SoC

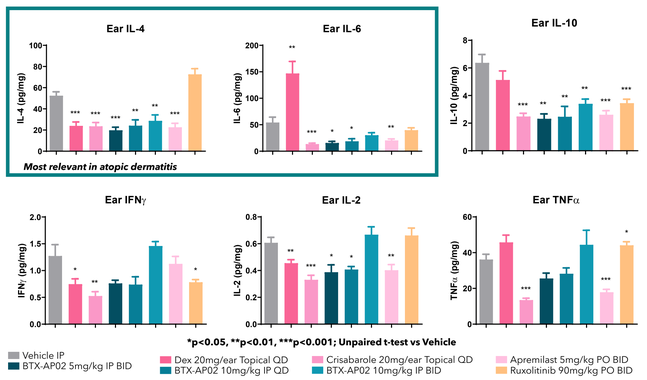
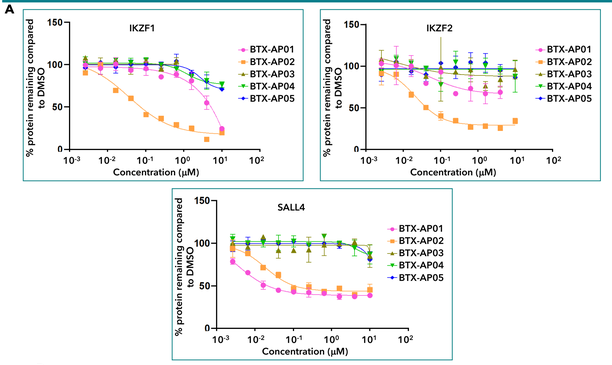


Figure 9: Analysis of inflammatory cytokines was performed on the ear lysates of all the mice enrolled in the efficacy study. Ear lysates were analysed for expression of various cytokines by an MSD assay. Results indicate that the expression of Th2 cytokines (IL-4 and IL-6) was significantly reduced in response to BTX-AP02, which was comparable to the effect shown by approved PDE4D inhibitors and the positive control. Effect on Th1 cytokines (IFNγ, IL-2, TNFα and IL-10) also displayed a similar trend.

Our PDE4 Degraders are Designed to Avoid Degradation of Known Neosubstrates

A



B

Test	Result
AMES	Not Mutagenic
hERG	>30 mM
CYP	>50 mM for CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A-M
Non-cancer epithelial cells	>10 mM
Cardiotoxicity	Assessment ongoing (incl. vs. JAK1/JAK2i)

Figure 10: (A) Lead PDE4D degraders were counter screened for the degradation of known neosubstrates of CRBN in HiBit lines. While earlier leads degraded IKZF1/3 as well as Sall4, the current lead compounds optimized for oral dosing are highly selective for PDE4D degradation and do not degrade any known CRBN neosubstrates.

(B) Oral lead (BTX-AP04) were also tested for *in vitro* toxicological parameters. These compounds were negative for all the parameters, indicating an excellent *in vitro* toxicological profile.

Conclusions….

- Improved **activity** profile versus other oral dermatological agents
  1. Picomolar potency, driven by rapid (4 hours) and deep (90%) target degradation of PDE4
  2. Head-to-head *in vitro* experiments show potent inhibition of a broad panel of inflammatory cytokines with up to 4,000-fold improvement vs. PDE4i and JAK1/JAK2i
  3. Early PoC degrader showed effective degradation and cytokine inhibition in *in vivo* AD model, matching SoC efficacy. Latest lead degraders are 10x more potent.
- Favorable **safety profile**
  4. Potential to overcome PDE4 inhibitor safety limitations due to likely lack of blood-brain-barrier penetration and PDE4D isoform selectivity
  5. Selective degradation of PDE4 versus known neosubstrates
  6. hERG, CYP and AMES negative and good safety in PBMCs and non-cancer epithelial cells
- Convenient **dosing** options
  7. Favorable PK profile with oral bioavailability
  8. Topical formulation feasibility and potential identified

Next Steps….

Key assessments

- **Efficacy**
  1. Head-to-head GvHD *in vivo* efficacy vs. PDE4i, JAK1/2i and IL-4/IL-13 mAb
  2. Additional I&I disease assessments (e.g., RA, IBD, neuroinflammation)
- **Safety**
  3. Blood-brain-barrier penetration assessment ongoing
  4. *in vivo* safety assessment
- **ADMET**
  5. PK assessment across species for oral bioavailability
  6. Complete topical feasibility assessments and formulation development of both oral and topical DC

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