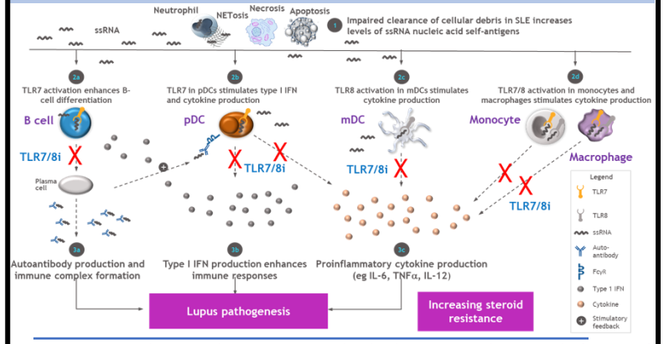
Discovery of BMS-986256, a Small Molecule Dual Antagonist of the Toll-Like Receptors 7 and 8 (TLR7/8) Advanced to Clinical Trials for the Treatment of Lupus

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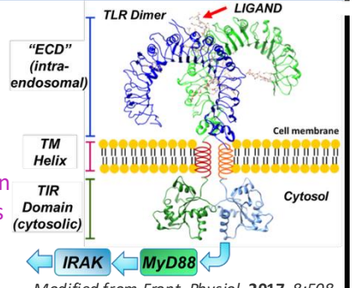
TLR7 & TLR8: Important Mediators of Lupus Pathophysiology



- TLR7, 8: Endosomal receptors which detect nucleic acids (ssRNA)
  - B cell activation, IgG production, cytokine & Type I Interferon
- Potential treatment for Lupus, as patients show:
  - Defects in apoptotic clearance, elevated levels of anti-nuclear autoantibodies, IFNs and cytokines, and B-cell activation

The Toll-Like Receptors: Structure/Signaling

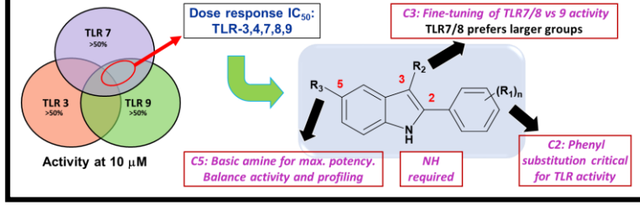
- Type I Transmembrane Proteins with 3 regions
  - ECD (intra-endosomal)
  - Transmembrane helix
  - TIR Domain (cytosolic)
- Signaling requires dimerization
- Intracellular cascades involves MyD88, IRAKs among others



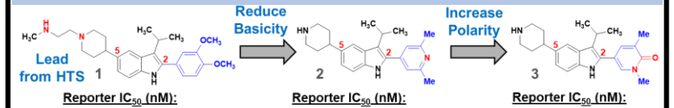
Modified from Front. Physiol. **2017**, *8:508*

Screening Identified Indole-based Inhibitors of TLRs

- HEK cellular reporter based HTS of internal compound collection
  - TLR7/9 primary screen, with TLR3/4 as counterscreen
- 2-Arylindole series of TLR inhibitors was identified

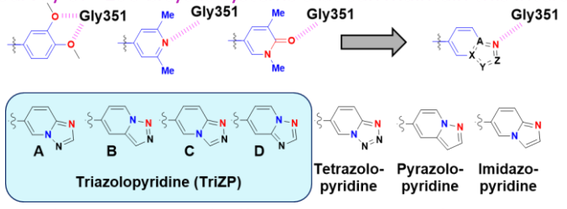


Structure-Guided Optimization of Indole TLR Antagonists



	Reporter IC_{50} (nM): TLR7/8/9	
1	10 / 17 / 530	Very High Vss, CNS Exposure. Ion Channel Activity
2	0.7 / 3 / 300	Very High Vss, CNS Exposure, Improved Ion Channel Profile
3	1.6 / 5.1 / 4,400	Reduced Vss & CNS Exposures Further Improved Ion Channel Introduced Cytotoxicity

- Initial exploration of Right-Side Aryls highlighted a key interaction with Glycine-351 in TLR8 co-crystal structures
- Survey of >100 bicyclic systems that can maintain this interaction



- TriZP (Triazolopyridine) offered best balance of potency, PK properties, and liability profile

Optimization of Triazolopyridine (TriZP) TLR7/8 Antagonists

C5 Piperidine Modifications
Attenuate the Basicity
Evaluate Replacements

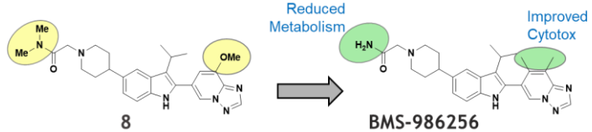
C2 Heteroaryl Modifications
Varied polarity and basicity
Peripheral Substitution
→Polar/Steric effects

Further Refine Potency, PK, Cytotoxicity and Ion Channel Profile



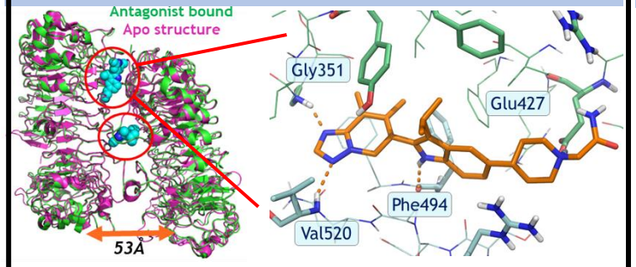
	4	5	6	7	8
TLR7/8 IC_{50} (nM)	1.0 / 9.1	2.5 / 14	1.6 / 6.9	0.6 / 3.9	0.4 / 2.7
hWB TLR7/8 IC_{50} (nM)	6.4 / 6.2	18 / --	4.8 / 16	4.4 / 3.4	2.1 / 2.1
hERG Patch Clamp %inh @ 3 μM	NA	NA (binding 16 uM)	17%	32%	10%
Notes	Poor Permeability	hERG, Cytotox	Poor Permeability & Oral Exposure	CNS exposure	

- Isomer A of TriZP series provided a superior overall profile.



- Fine-tuning of SAR around **8** led to the discovery of **BMS-986256**

BMS-986256 Stabilizes Inactive Form of hTLR8



- BMS-256 binds 2:2 with hTLR8, locking the Apo conformation
  - TriZP heteroaryl H-bonds with Gly351 and Val520
  - Indole NH bonds to backbone carbonyl of Phe494
  - Protonated piperidine forms salt bridge with Glu427
  - C3 isopropyl fills a small hydrophobic pocket

In Vitro and Pharmacokinetic Profile of BMT-986256

In Vitro Potency and Selectivity

HEK Reporter Assay	IC50 (nM)
TLR7	0.65 ± 0.24
TLR8	0.86 ± 0.53
TLR9	6400 ± 2470
TLR3, 4	>50,000

Human PBMC	IC50 (nM)
GDQ-induced IL-6	0.5 ± 0.05
ssRNA40-induced IL-6	0.4 ± 0.04

Human Whole Blood	IC50 (nM)
TLR7 GDQ-induced IL-6	4.5 ± 0.4
TLR8 TL8-506-induced IL-6	1.5 ± 0.1

Mouse Whole Blood	IC50 (nM)
TLR7 GDQ-induced IL-6	2.4 ± 1.3

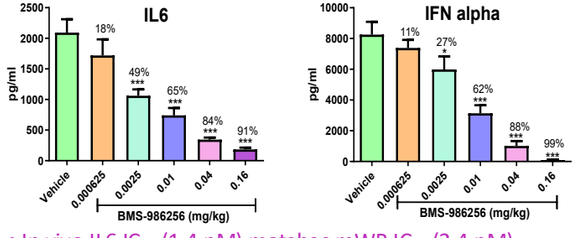
Pharmacokinetic Parameters

Species	CL ml/min/kg	Vss L/kg	%F	t1/2 h	MRT h
Mouse	5.6	4	102	8	11
Rat	4.2	4	65	11	15
Cyno	2.7	6.5	76	30	40
Dog	4.2	10	64	39	40

Select Liability Assays

Cellular Cytotox Panel	>9 μM
hERG Patch Clamp IC50	4 μM
Na, Ca Patch Clamp	<50% (10 μM)
Rabbit Electrophysiology	No sig effects >500×
Genetic tox: Ames:	Negative
In Vitro Micronucleus:	Negative

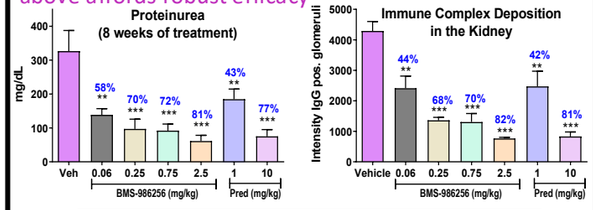
Robust Inhibition of TLR7-induced Cytokines in Mice



- In vivo IL6 IC_{50} (1.4 nM) matches mWB IC_{50} (2.4 nM)
- C57BL/6 mice challenged with GDQ 30 min after dosing with **BMS-986256**. Cytokine readout 2h post-challenge.

High Efficacy in Male MRL/lpr Mouse Lupus Model

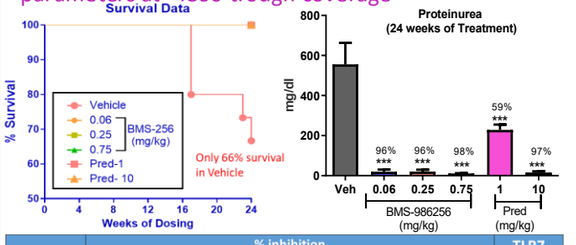
2-3 Month Model: Coverage of TLR7 IC90 at trough and above affords robust efficacy



Treatment	% inhibition: Protein-uria	uNGAL	uTIMP1	Anti- smRNP Ab	Anti-dsDNA Ab	TLR7 Coverage at trough
BMS-986256 (0.25 mg/kg)	70	59	42	45	21	IC92

Striking Efficacy in NZB/W Mouse Lupus Model

6-Month Model: Excellent impact on many disease parameters at <IC90 trough coverage



Treatment	% inhibition: Protein-uria	uNGAL	uTIMP1	Anti-smRNP Ab	Anti-dsDNA Ab	IL12p40	IL10	TLR7 Coverage at trough
BMS-986256 (0.25mg/kg)	96	84	73	48	34	78	93	IC91

Summary

- TLRs play key roles in the pathology of lupus
- Small-molecule TLR7/8 antagonists were identified via high throughput screening
- Structure-guided optimization of an indole series led to the identification of **BMS-986256**
  - Highly potent TLR7/8 antagonist with impressive oral activity in mouse PD (TLR7) and mouse models for lupus
  - Excellent pre-clinical PK and safety profile
- **BMS-986256** advanced to clinical evaluation in patients with CLE (NCT04493541) and SLE (NCT04895696)