Small Molecule Drug Discovery – Immunology Chemistry

Discovery of afimetoran, a small molecule dual antagonist of the toll-like receptors 7 and 8 (TLR7/8) advanced to clinical evaluation in patients with lupus

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Bristol Myers Squibb™

Toll-Like Receptors 7 and 8 (TLR7/8) contribute to the development of lupus and steroid resistance

TLR7 and TLR8 detect ssRNA from pathogens or self-apoptotic sources

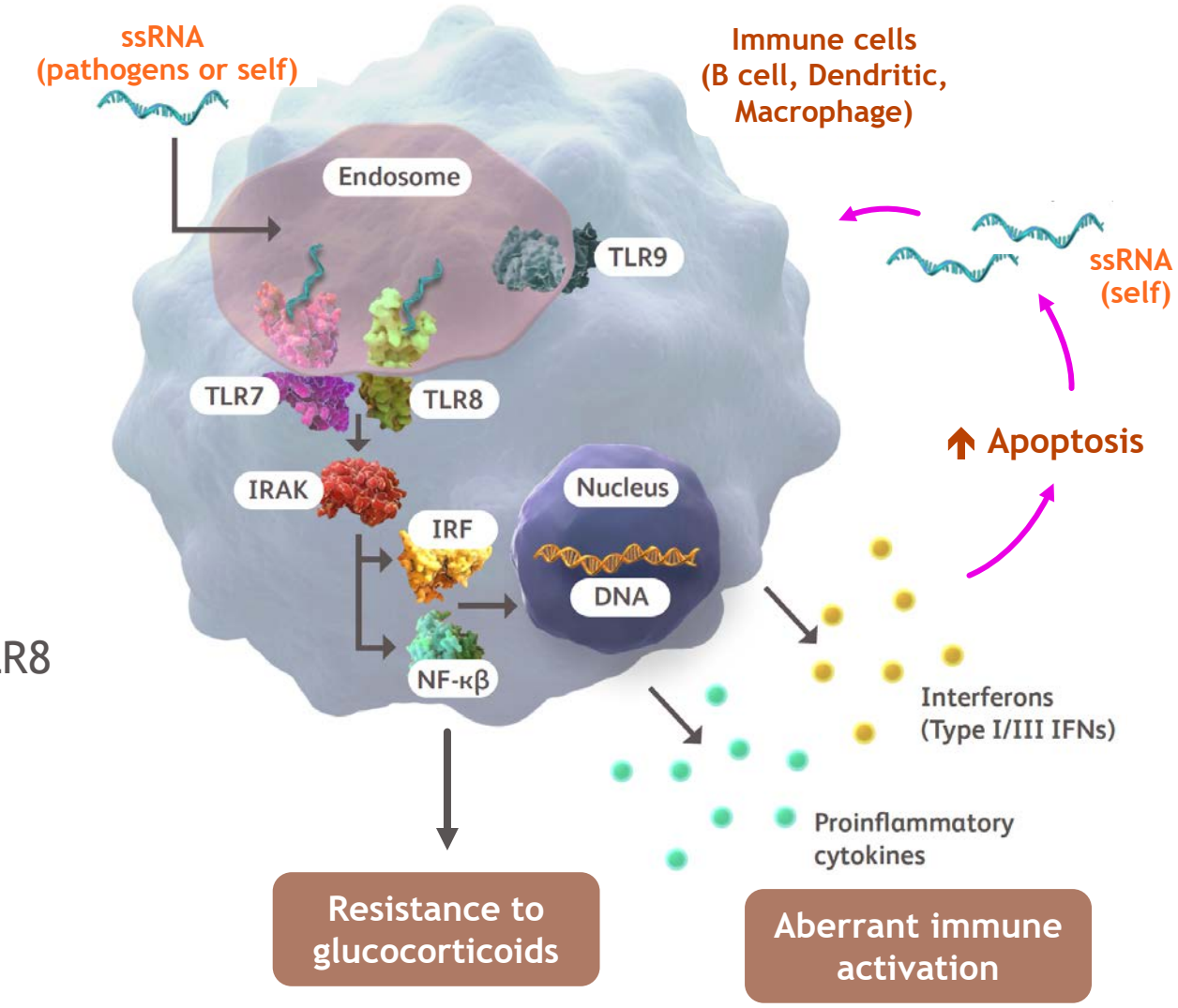
Activation of TLR7 and TLR8 within multiple immune cell types leads to:

- Elevated pro-inflammatory cytokines
- Production of type I/III interferons
- Enhanced autoantibody production (B cells)

TLR7 and TLR8 may play an important role in the initiation and progression of disease in lupus

- Lupus is associated with defects in clearance of apoptotic debris, which can activate TLR7 and TLR8

Increased NF-κβ activation by TLR7 and TLR8 is associated with steroid resistance



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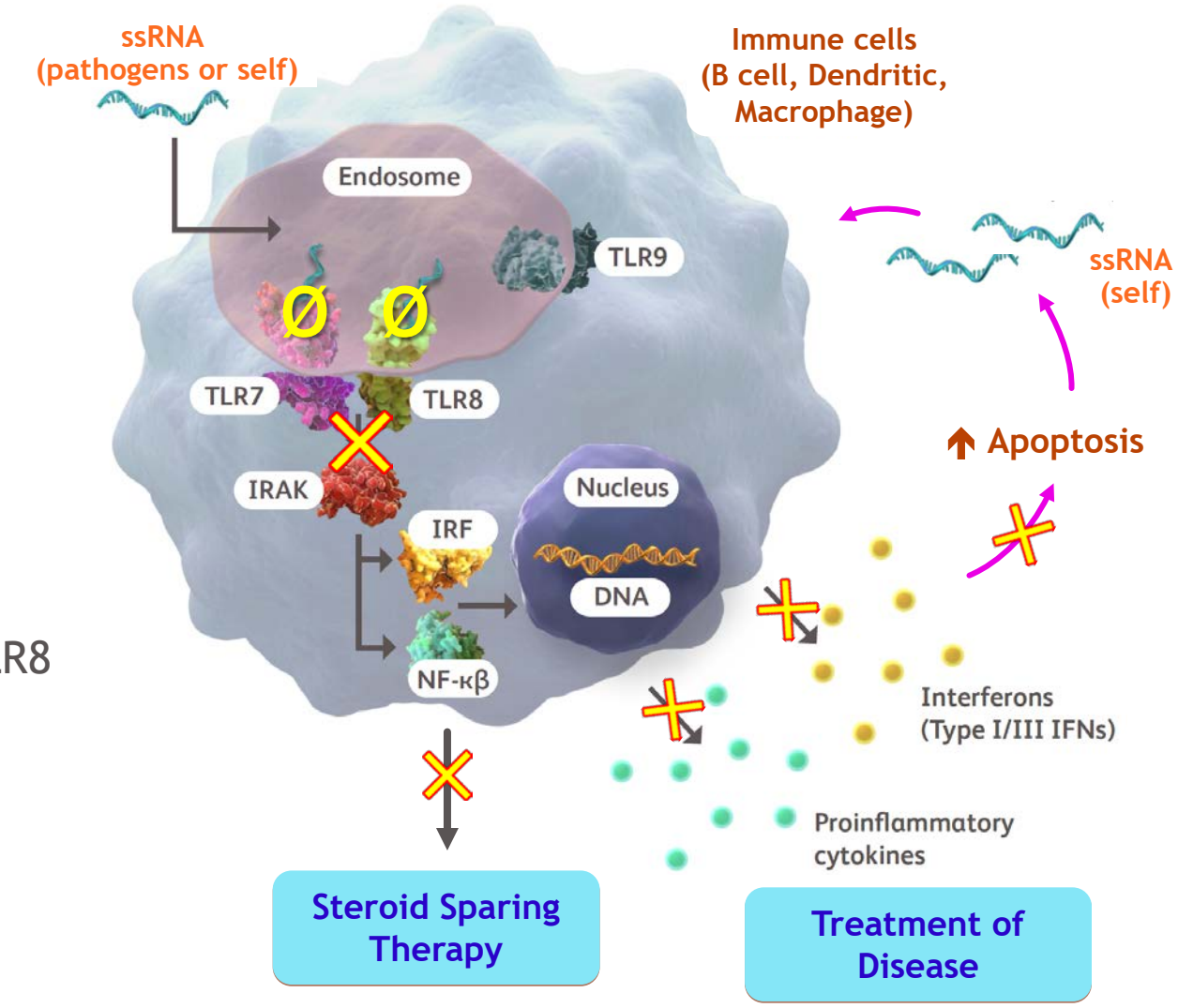
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TLR7 and TLR8 may play an important role in the initiation and progression of disease in lupus

- Lupus is associated with defects in clearance of apoptotic debris, which can activate TLR7 and TLR8

Increased NF-κβ activation by TLR7 and TLR8 is associated with steroid resistance

Goal: Identify small molecule blockers of TLR7/8 signaling capable of disrupting the vicious cycle

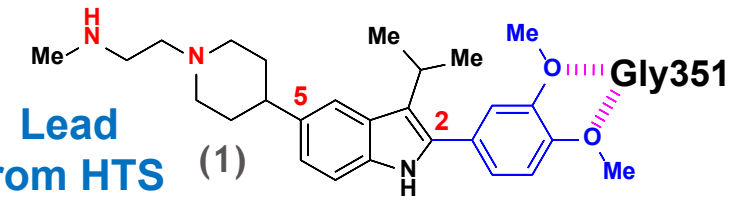


TLR7/8 Antagonist: Screening hit to lead optimization

Cellular reporter assay used for screen of internal compound collection (~1.2M cmpds)

- TLR7 and 9 as primary screen (TLR8 activity tracked with TLR7); TLR3/4 as key counter screens
- 2-Aryl indole series of TLR7/8 inhibitors was identified

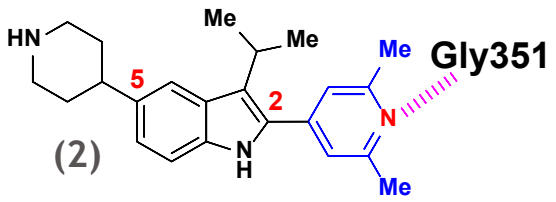
hTLR8 X-ray guided SAR: Key interaction of C2 aryl groups observed with Gly351



TLR7 *reporter IC_{50} (nM): 10
TLR8 *reporter IC_{50} (nM): 17

Very High Vss, CNS Exposure.
Ion Channel Activity

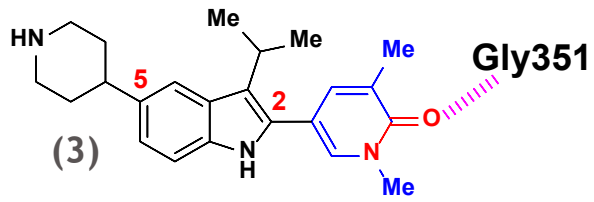
Reduce Basicity



TLR7: 0.7
TLR8: 3.0

Very High Vss, CNS Exposure,
Improved Ion Channel Profile

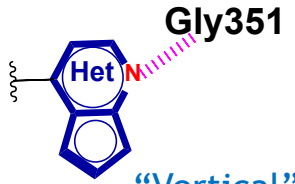
Increase Polarity



TLR7: 1.6
TLR8: 5.1

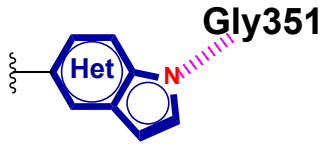
Reduced Vss & CNS Exposures
Further Improved Ion Channel
Unexpected Cytotoxicity

Maintain key interaction within bicyclic ring systems



"Vertical"

OR



"Angular"

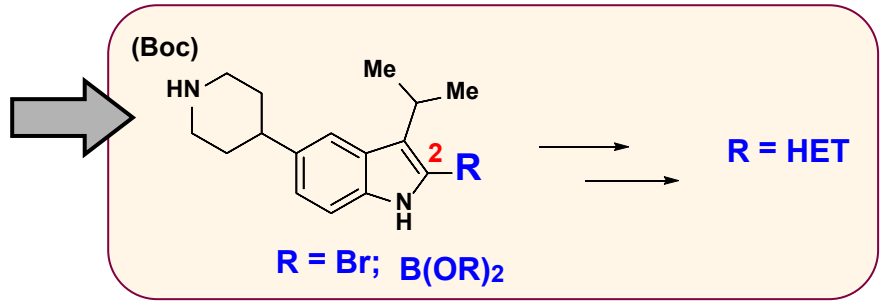
*HEK293 SEAP Reporter Assay

ACS Med. Chem. Lett. **2020**, *11*, 1751–1758; *ACS Med. Chem. Lett.* **2022**, *12*, 812-818.

C2 SAR via late-stage diversification

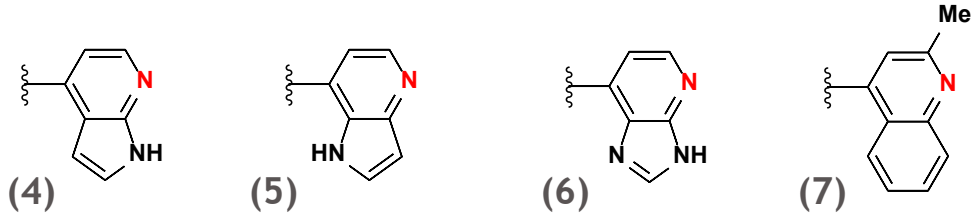
Readily available C2 Bromo/Boronate intermediates enabled rapid SAR

- >100 bicyclic systems surveyed, with a bias on those that could engage Gly351
- Modulate molecule basicity and polarity through selection of heterocyclic replacements



Vertical Bicycles:

R =

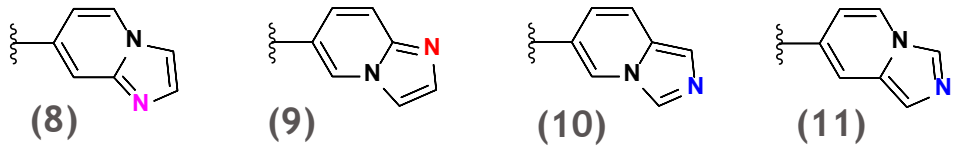


	(4)	(5)	(6)	(7)
TLR7 IC_{50} (nM):	4.5	6.1	6.5	3.6
TLR8 IC_{50} (nM):	3.0	84	12	26

Issues: Generally poor permeability, low PO exposures in mice

Angular Bicycles:

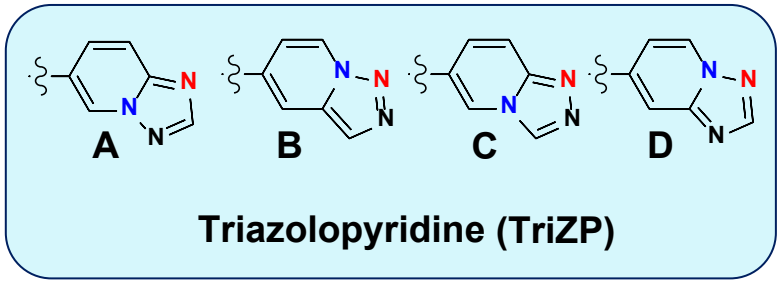
R =



	(8)	(9)	(10)	(11)
TLR7 IC_{50} (nM):	1800	3.5	660	10
TLR8 IC_{50} (nM):	6200	150	130	36

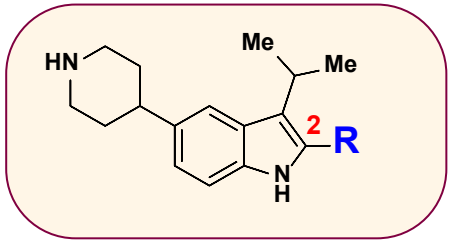
TLR7 particularly sensitive to N position

Except when it's not



Triazolopyridine (TriZP) isomers identified as particularly effective C2 aryl groups

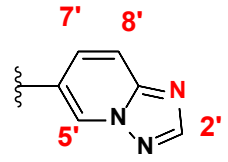
Triazolopyridine A (TriZP A) optimizations

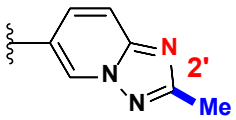


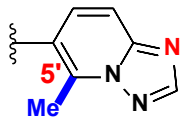
"TriZP A" provided an excellent balance of TLR7/8 potency and overall profiling

- Permeability, metabolic stability, ion channel activity, cytotox. profile
- Fine-tuning of properties via peripheral substitution was explored

R =

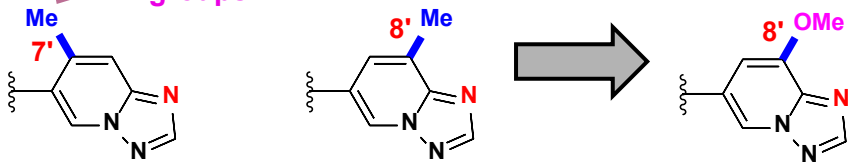




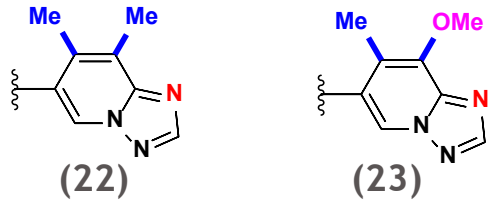


small groups

broader tolerance



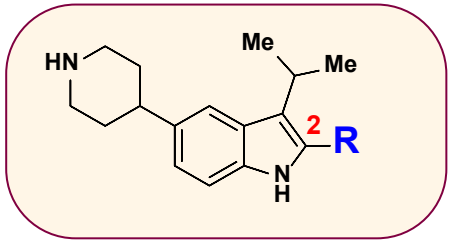
	(12)	(13)	(14)	(15)	(16)	(17)
TLR7 IC_{50} (nM):	32	9.6	29	3.9	1.4	0.4
TLR8 IC_{50} (nM):	140	90	32	2.2	3.2	2.4



	(22)	(23)
TLR7 IC_{50} (nM):	0.5	0.7
TLR8 IC_{50} (nM):	1.0	7.2

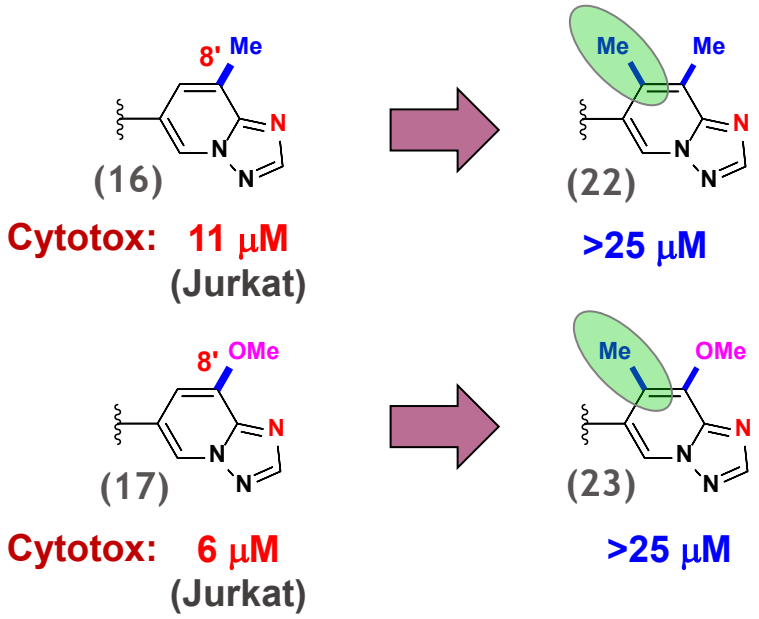
	8' R'	TLR7 / 8
(18)	R' =Cl	0.8 / 3.3
(19)	R'= Et	0.3 / 5.7
(20)	R'= cPr	0.3 / 5.0
(21)	R'= NHEt	2.0 / 7.8

Triazolopyridine A (TriZP A) optimizations



"TriZP A" provided an excellent balance of TLR7/8 potency and overall profiling

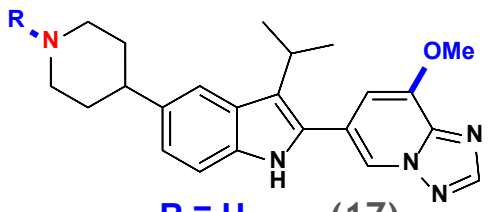
- Permeability, metabolic stability, ion channel activity, cytotox. profile
- Fine-tuning of properties via peripheral substitution was explored



C7' Methyl substitution consistently reduced activity in assays of off-target cellular proliferation.

Piperidine modifications leading to afimetoran

Capping groups varying the degree and location of basicity modulated potency as well as a range of potential in vitro and in vivo liability flags



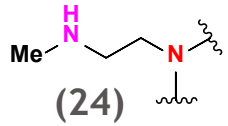
R = H (17)

TLR7 / 8 IC_{50} (nM): 0.4 / 2.4

High Vss, Ion Channel, Cytotox

Monobasic

R =

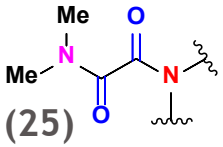


(24)

TLR7 / 8: 0.4 / 0.7

High Vss, Ion Channel, Cytotox, Permeability

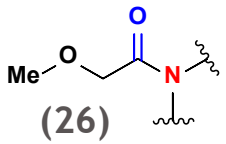
Dibasic



(25)

2.5 / 83

TLR8 Potency

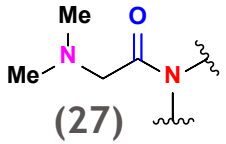


(26)

1.8 / 46

TLR8 Potency

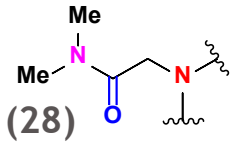
Nonbasic



(27)

1.1 / 9.2

Permeability, Met. Stab.

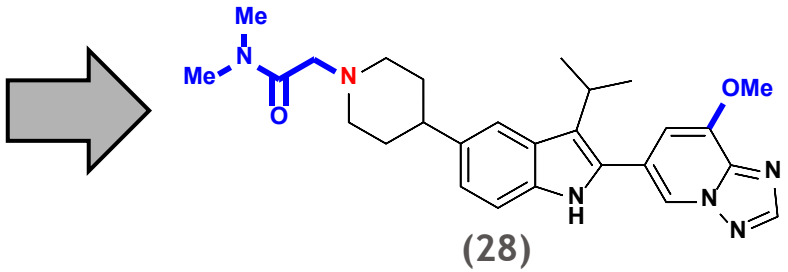


(28)

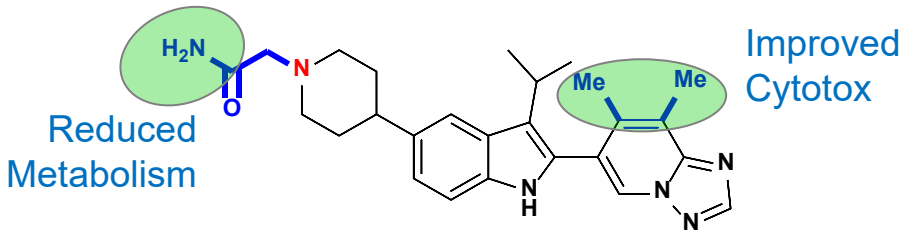
0.4 / 2.7

Favorable Profile

Attenuated Monobasic



(28)



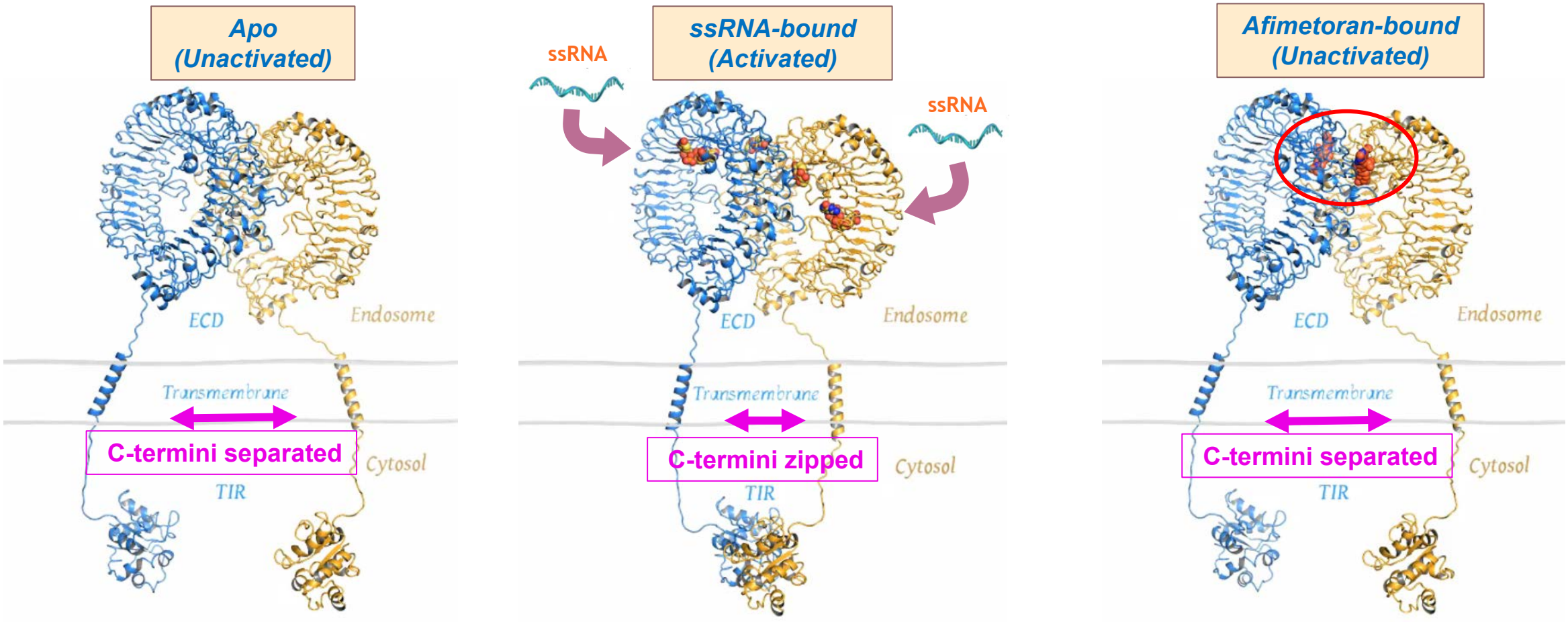
(29): Afimetoran

Fully progressible profile. Advanced evaluations conducted

Opportunities for improvement: Cross-species metabolism
Residual cytotoxicity in some cell lines
In Vitro Micronucleus (positive with S9 fraction)

Consistent cross-species metabolism
No cytotox across cell line panel (>25 μM)
No flags with In Vitro Micronucleus

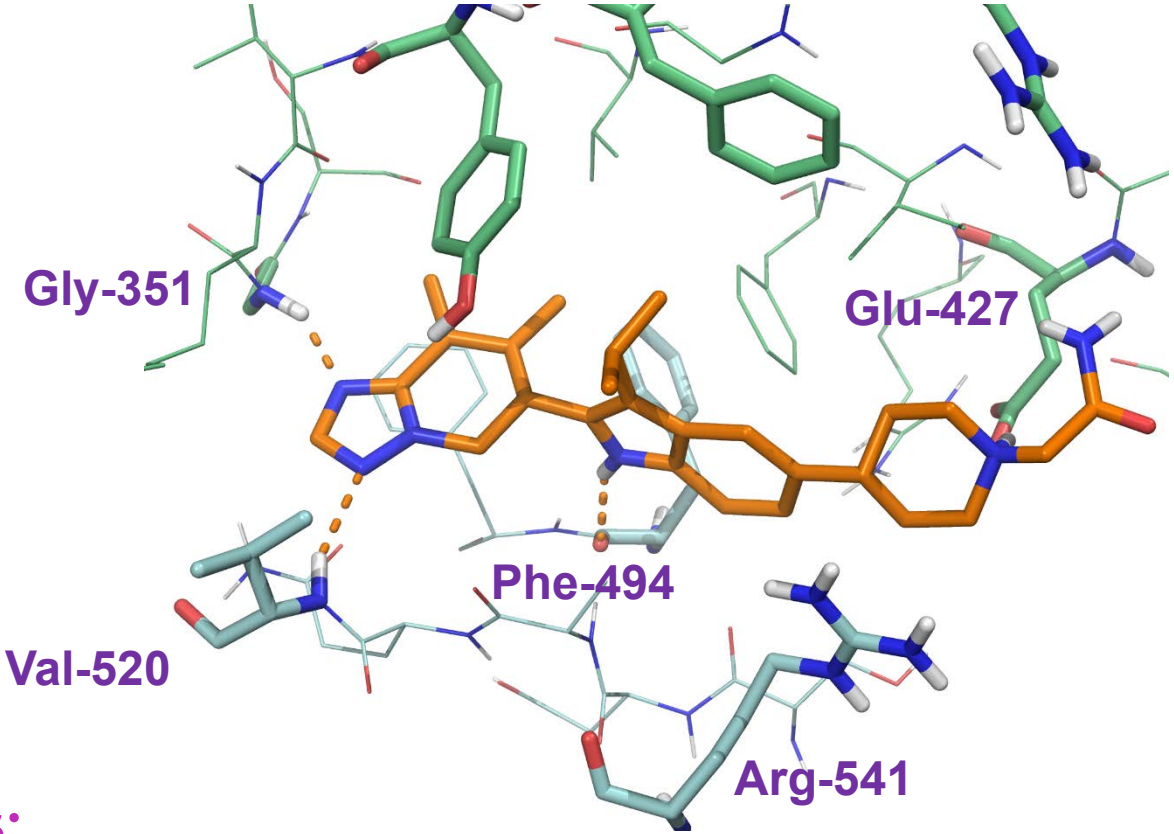
TLR8 structures elucidate afimetoran's mode of inhibition

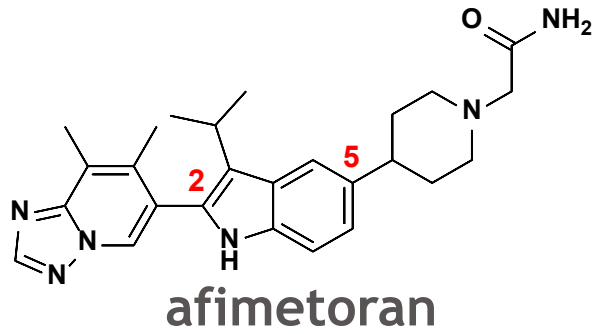


Models of full length TLR8 were based on various X-ray structures with TLR8 ECD

- Activation of TLR8 requires C-terminal closure induced by ssRNA binding
- Afimetoran binds in the ECD and locks TLR8 into an apo conformation (C-termini separated)

Co-crystal structure of afimetoran and hTLR8





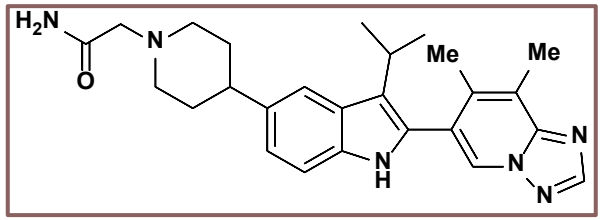
afimetoran

Key Interactions:

- Hydrogen bond between amide N-H of Gly351 and N1 of the TriZP A
- TriZP A group forms a second Hydrogen bond, to Val-520 via N3
- Hydrogen bond between the amide oxygen of Phe494 with indole N-H
- Basic piperidine nitrogen off of C-5 makes a salt bridge with Glu427

Afimetoran: In vitro and in vivo profile

Highly potent and selective, excellent non-clinical pharmacokinetic profile and favorable predicted liability profile



afimetoran
(BMS-986256)

In Vitro Potency and Selectivity

SAR Driving Assays

HEK Reporter Assay	IC50 (nM)
TLR7	0.7 ± 0.2
TLR8	0.9 ± 0.5
TLR9	6400 ± 2500
TLR3, 4	>50,000

Primary Cell Potency

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

Blood Potency

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

Cross-Species Potency

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

In Vivo Potency

Mouse PD Assay	IC50 (nM)
TLR7 GDQ-induced IL-6	1.4

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

Afimetoran improved survival and reversed disease in multiple mouse models of lupus

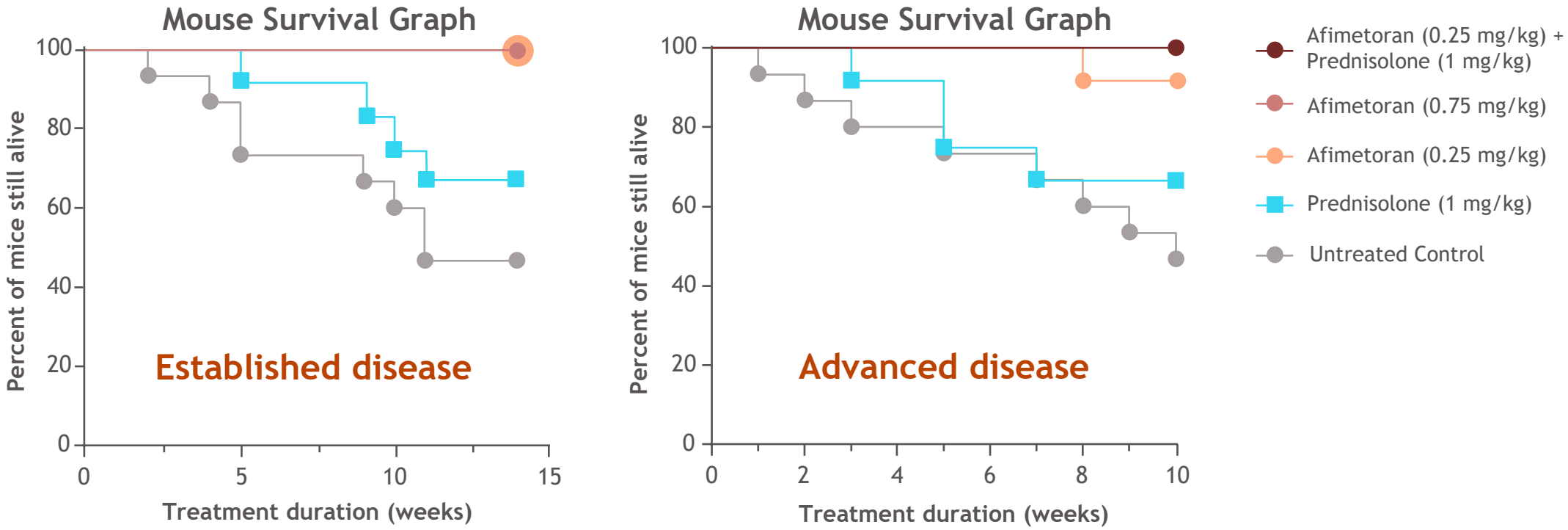
- Afimetoran demonstrated high efficacy in *preventative* regimen in:
  - **MRL/lpr** models (Interferon-independent) of lupus nephritis and cutaneous lupus
  - **NZB/W** model (Interferon-dependent)
    - Significantly decreased proteinuria, autoantibody titer, and cytokines[1,2]
- Those notable results prompted us to examine the *therapeutic* efficacy of afimetoran in the NZB/W model[3]
  1. **Established disease** corresponds to moderate form of disease at the time of treatment initiation with proteinuria 60–100 mg/dL[a]
  2. **Advanced disease** corresponds to severe form of disease at the time of treatment initiation with proteinuria > 100 mg/dL[a]

[a]The proteinuria in normal NZB/W mice without any disease is < 30 mg/dL.

IMRL/lpr, mice homozygous for the lymphoproliferation spontaneous mutation (*Fas*^{lpr}); NZB/W, New Zealand black mouse crossed with a New Zealand white mouse.

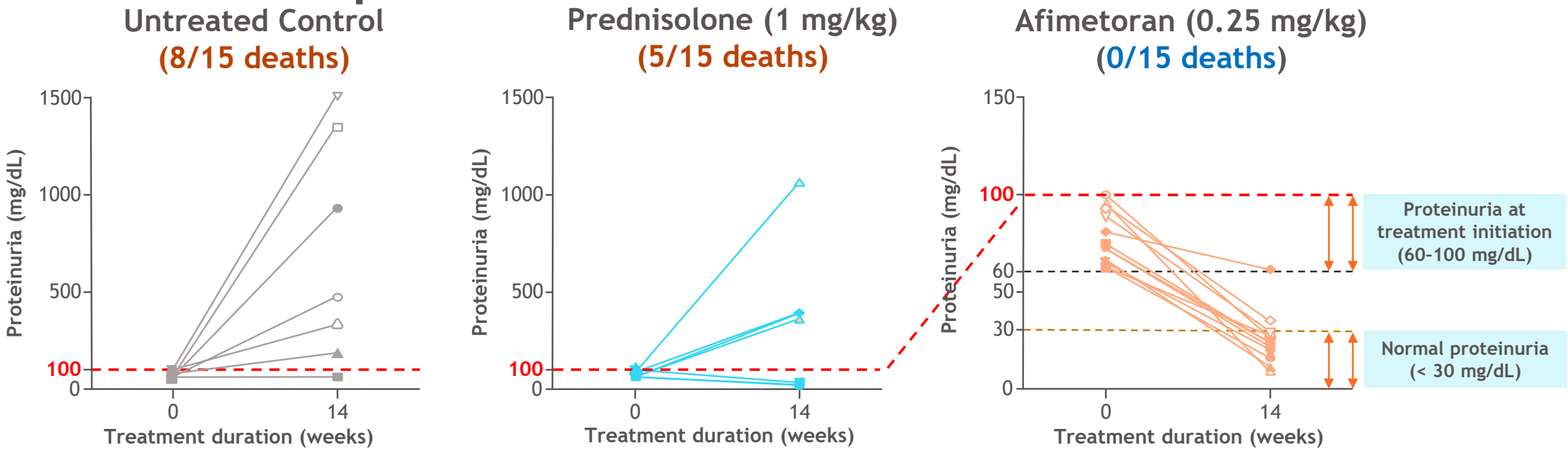
1. Dudhgaonkar S, *et al*. LUPUS/CORA; Oct. 6-9, 2021 Abs# 238; 2. Dyckman AJ, *et al*. LUPUS/ CORA; Oct. 6-9, 2021 Abs# 257; 3. Harrison, M, *et al*. ACR Convergence 2021; Nov 5-9, 2021 Abs#1771.

Afimetoran improved survival in mouse models of established and advanced lupus



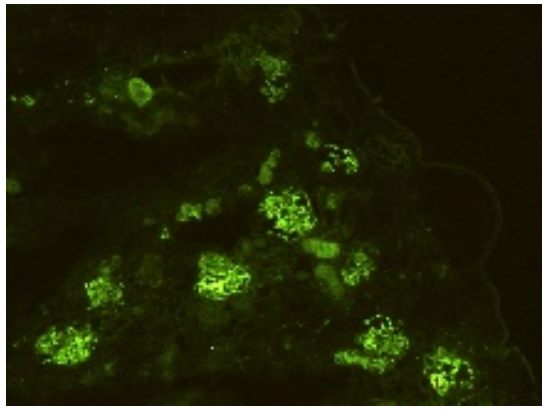
- NZB/W mice develop disease symptoms that mirror human systemic lupus
- In Established and Advanced modes, treatment initiation was delayed until the animals showed moderate or severe signs of disease, close to the point of succumbing to the disease
- Oral, once-daily afimetoran treatment resulted in complete or near-complete survival as monotherapy, superior to the survival benefit afforded by the low-dose steroid positive control

Afimetoran *reversed* proteinuria in a mouse model of established lupus

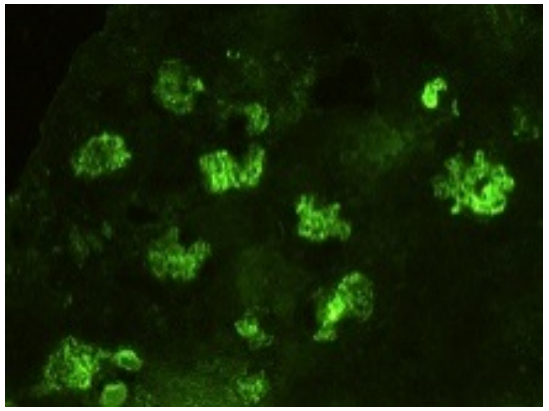


- Proteinuria, or increased protein in urine due to kidney damage, is a common symptom of lupus and is also observed in the NZB/W lupus-like mice
- Proteinuria was controlled, but not reversed, in the group treated with low-dose prednisolone
- Afimetoran treatment ***reversed*** proteinuria levels to pre-disease levels in almost all animals

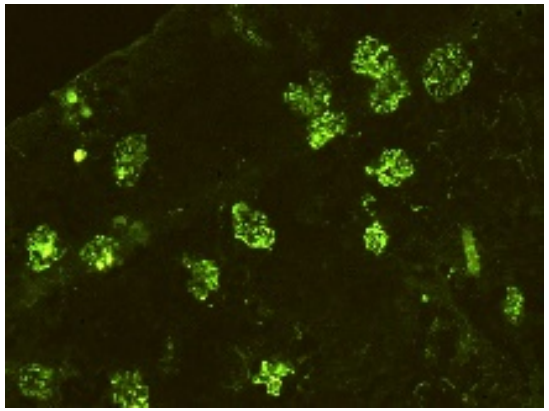
Afimetoran *reversed* IgG deposition in kidneys in the NZB/W murine model of established lupus nephritis



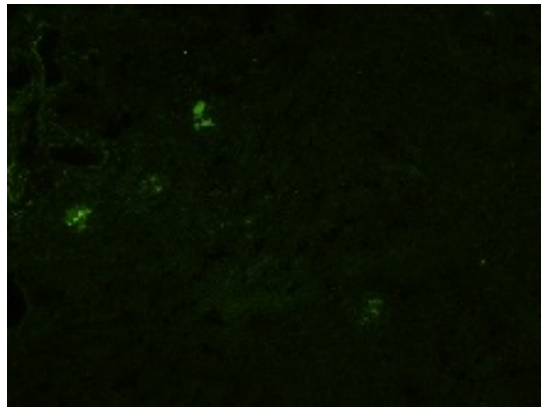
Satellite
(at treatment initiation)



Vehicle
(end of study)



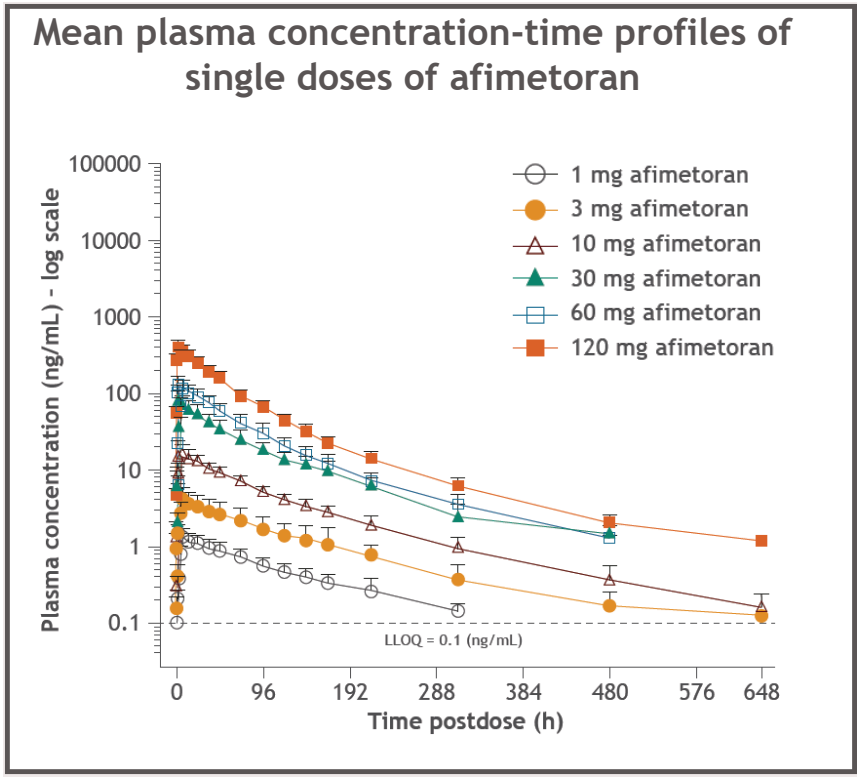
Prednisolone
(1 mg/kg)

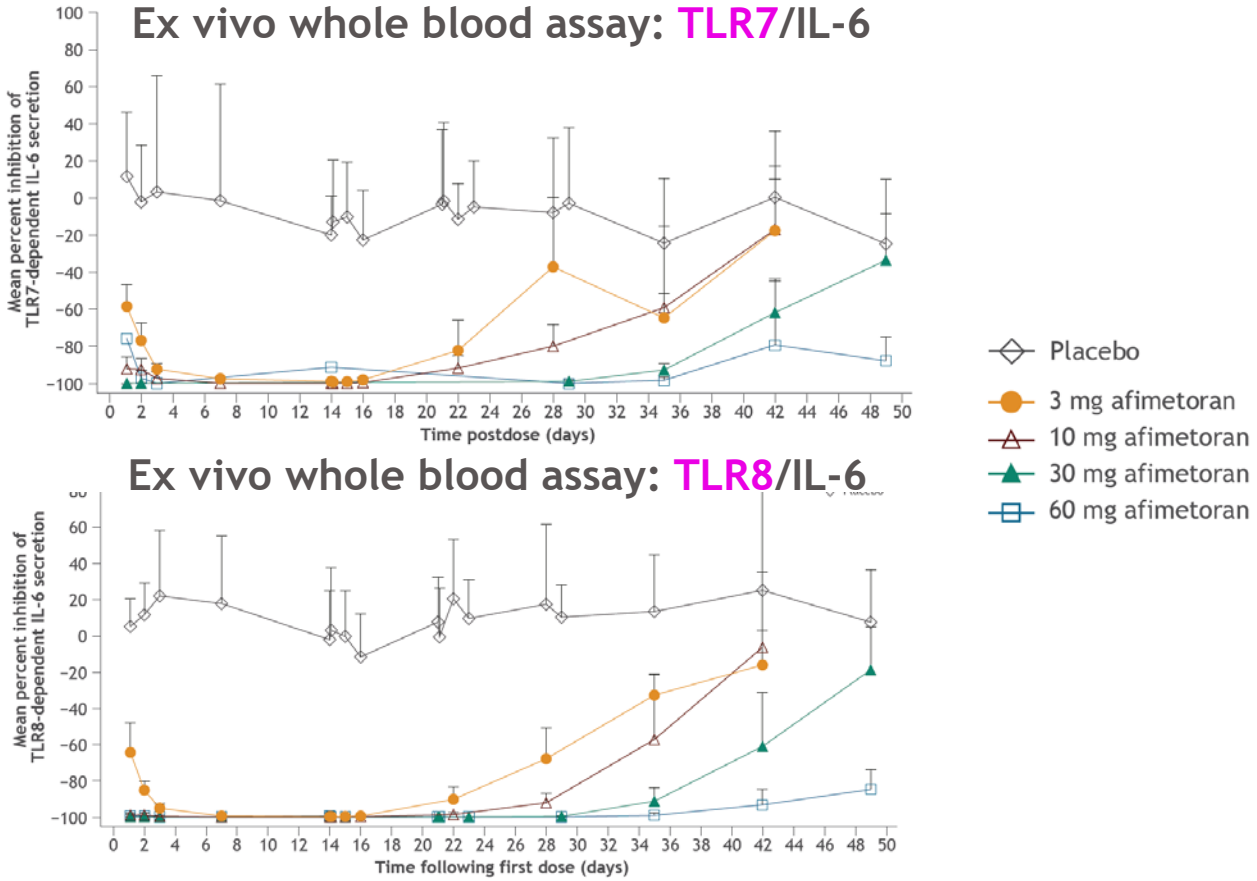


Afimetoran
(0.25 mg/kg)

- Satellite mice showed IgG deposition in kidney before the initiation of treatment
- Afimetoran reversed both the number of positive glomeruli and the amount of IgG deposition, as detected by immunofluorescence
- The combination of afimetoran with prednisolone also reversed both the number of IgG-positive glomeruli and the amount of IgG deposition in glomeruli
- A similar observation was also reported in NZB/W mice with advanced disease

Afimetoran clinical PK profile and PD responses





- In the SAD cohort, afimetoran displayed linear PK with dose-dependent increase in exposures and a half-life (~ 80 h) consistent with its predicted low clearance
- Ex vivo biomarker evaluation demonstrated dose-dependent PD inhibition consistent with a high degree of target engagement from low doses

Harrison, M, *et al*. ACR Convergence 2021; Nov 5-9, 2021. Abstract 1771.

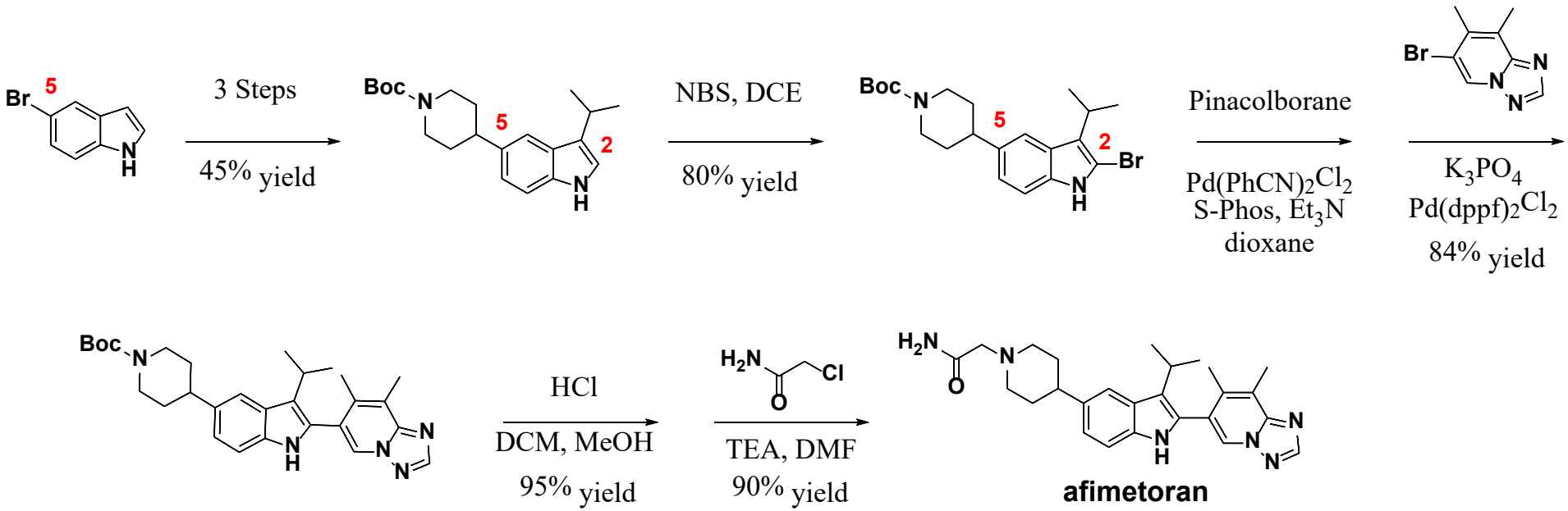
Summary

- TLR7 and TLR8 are believed to play key roles in the pathology of lupus
- Small-molecule antagonists of TLR7/8 were identified via high throughput screening
- Structure-guided optimization of an indole series led to the identification of afimetoran
  - Highly potent TLR7/8 antagonist with impressive oral activity in mouse models for lupus.
  - Excellent non-clinical PK and safety profile supported the evaluation of afimetoran in Phase 1 trials
  - Clinical PK properties were consistent and favorable, with a half-life supporting once daily dosing
  - Response of biomarkers was robust and dose-dependent, with significant PD inhibition starting from a 10-mg single dose of afimetoran
- These findings support further clinical development of afimetoran, with Phase 1b and Phase 2 clinical trials in patients with *cutaneous* (CLE) and *systemic* lupus erythematosus (SLE), respectively, currently underway.[a]

[a] CLE Trial: NCT04493541; SLE Trial: NCT04895696

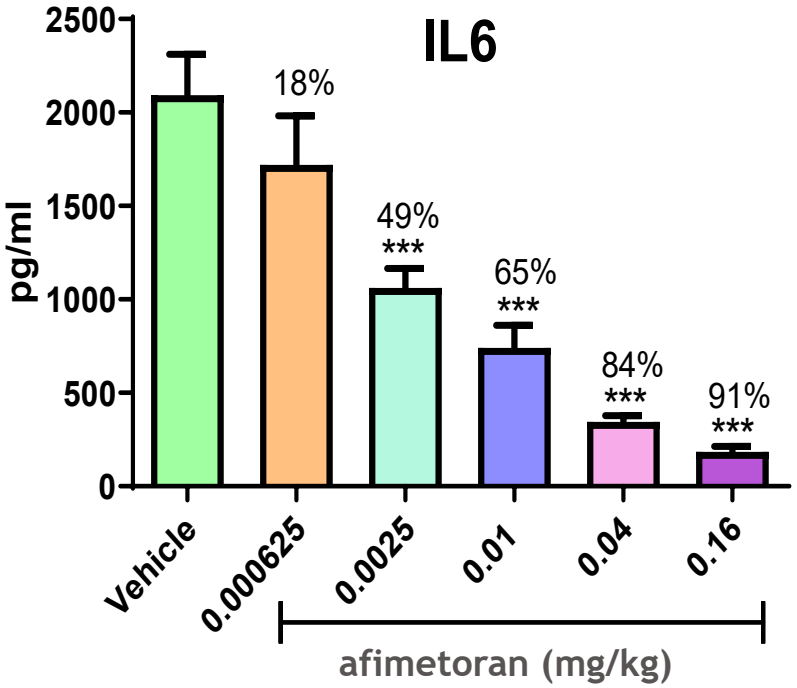
BACKUP SLIDES

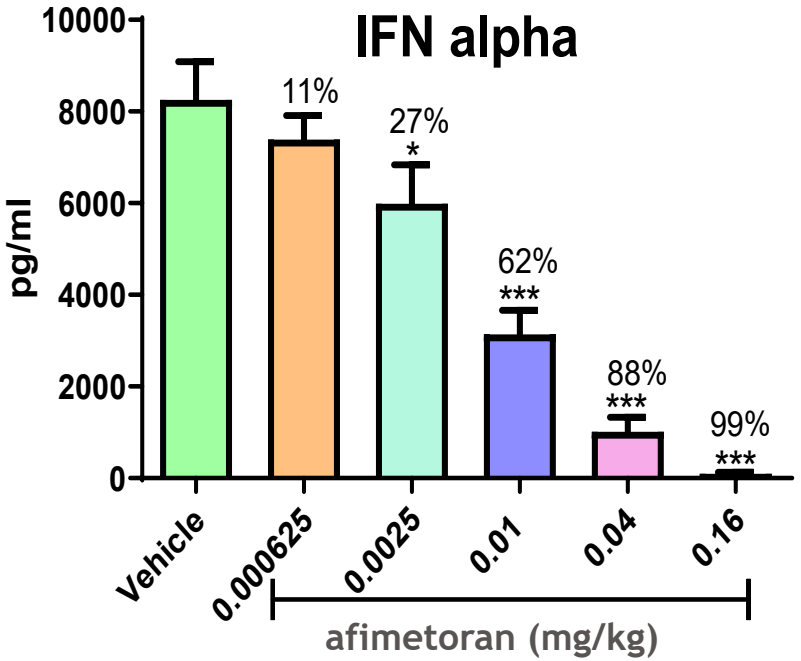
Synthesis of afimetoran



Synthetic route: 7 linear steps. No chiral centers. 10-25% overall yield.

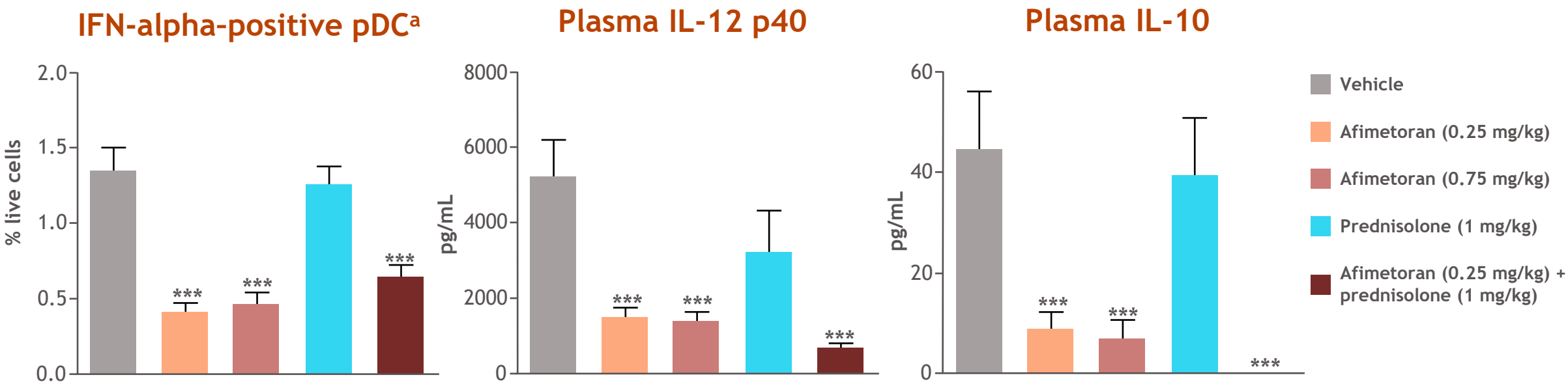
Robust inhibition of TLR7-induced cytokines in mice





- Mice are not responsive to TLR8 agonists, so only TLR7 PD responses were examined
- C57BL/6 mice challenged with gardiquimod 30 minutes after dosing with afimetoran
- Cytokine readout 2h after gardiquimod challenge
- In vivo IL6 IC50 (1.4 nM) matches Mouse Whole Blood IC50 (2.4 nM)

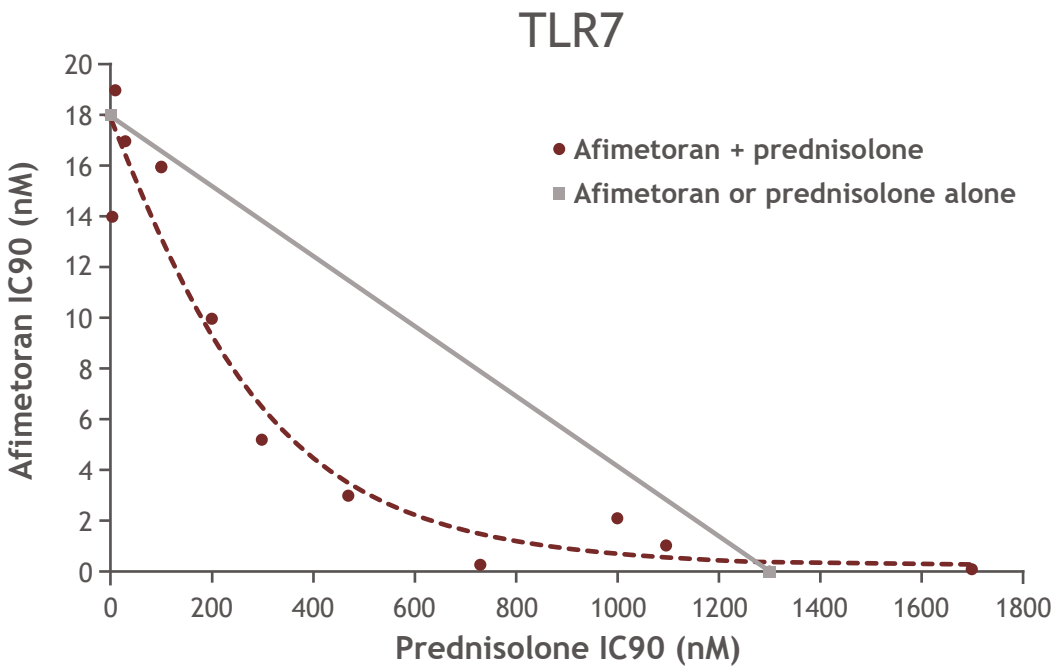
Afimetoran reduced cytokine-secreting cells and circulating cytokines in the NZB/W murine model of established lupus nephritis

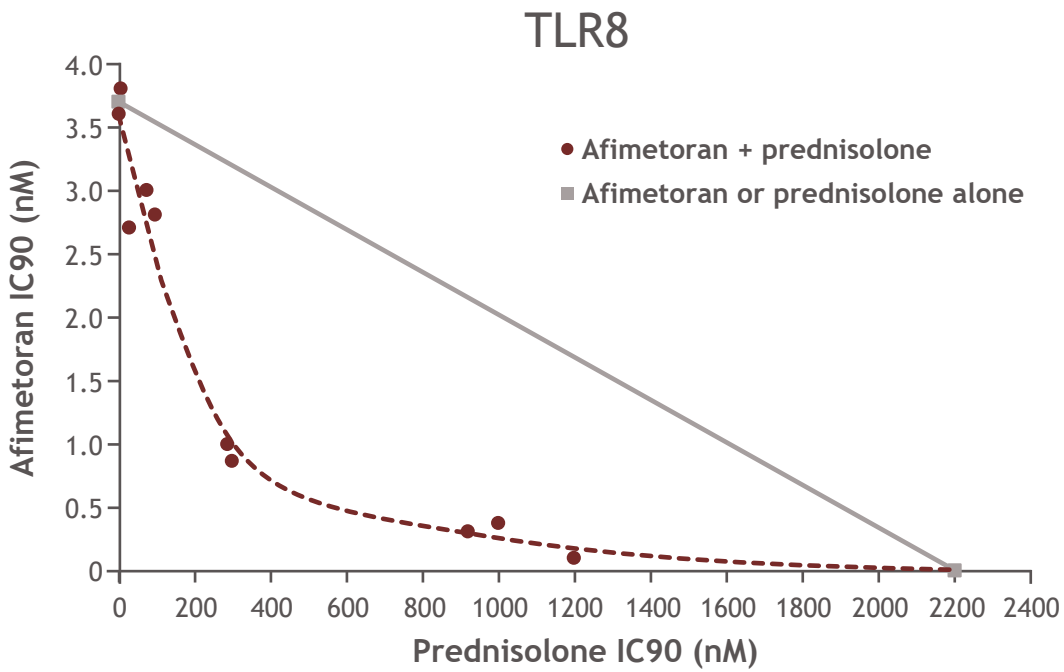


- Afimetoran led to the reduction of cytokine-secreting cells such as pDCs and secreted cytokines
- The combination of afimetoran + prednisolone also reduced cytokine-secreting cells and cytokines
- A similar observation was also reported in NZB/W mice with advanced disease

[a]Cells were considered to be pDC if they were positive for CD317. ****P* < 0.001. IFN-alpha, interferon-alpha; IL, interleukin; pDC, plasmacytoid dendritic cell; NZB/W, New Zealand black mouse crossed with a New Zealand white mouse.

Afimetoran acted synergistically with prednisolone to inhibit pro-inflammatory cytokine secretion in human whole blood



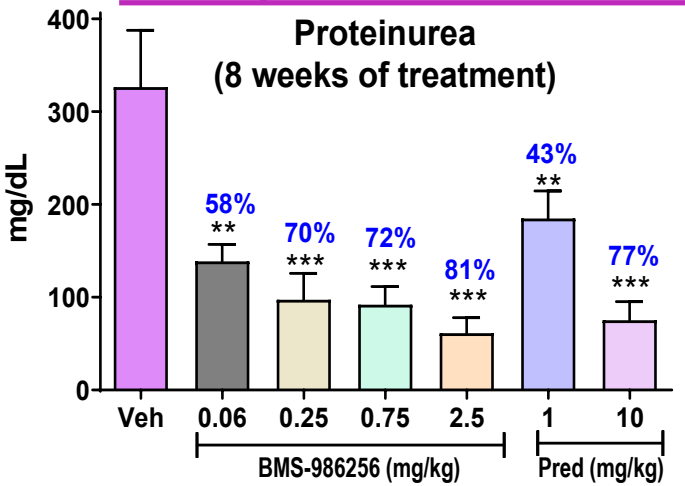


- The IC90 values for the combination afimetoran + prednisolone are lower than the line of additivity
- Afimetoran acted synergistically with prednisolone to inhibit IL-6 secretion induced by either a TLR7 agonist (gardiquimod) or a TLR8 agonist (TL8-506) in human whole blood

IC90, concentration producing 90% inhibition; IL, interleukin; TLR, toll-like receptor.

BMS-986256 is Highly Efficacious in Multiple Pre-clinical Models of Autoimmune Diseases

MRL/lpr Male Mouse Lupus Model

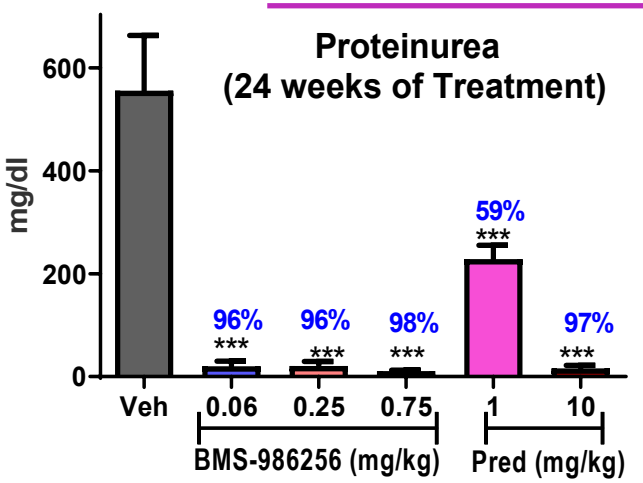


2-3 Month Duration:

- Coverage of TLR7 IC90 at trough and above affords robust efficacy across multiple disease parameters

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

NZB/W Mouse Lupus Model



~6-Month Duration:

- Complete survival in treated groups vs 66% survival in vehicle group
- Excellent impact on many disease parameters even at <IC90 trough coverage

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

Summary

- TLR7 and TLR8 play key roles in the pathology of lupus
- Small-molecule antagonists of TLR7/8 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 and in mouse models for lupus.
  - For additional in vivo characterization of BMS-986256 in a mouse model of CLE, see Abstract #238
  - Excellent non-clinical PK and safety profile supported the successful evaluation of BMS-986256 in Phase 1 trials
- BMS-986256 was advanced to clinical evaluation in patients with CLE (NCT04493541) and SLE (NCT04895696)