Objective and Rationale

Program objective: ***To develop an orally bioavailable small molecule TLR7/8-dual antagonist for the treatment of lupus and other autoimmune diseases***

Select TLRs (3, 7, 8 & 9) are expressed endosomally and detect nucleic acids

TLR 7 and 8 recognize ssRNA fragments

- ssRNA from pathogens or tissue damage
- Signal via MyD88/IRAK4 pathways

TLR 7 acts in pDCs and B cells

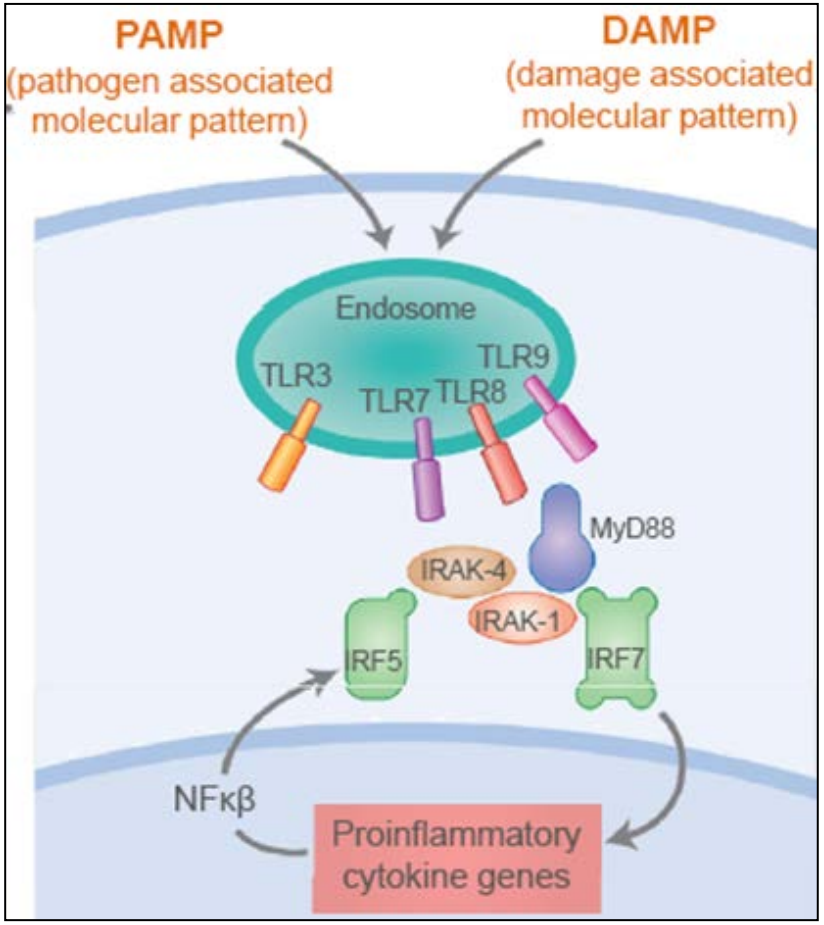
- Strong inducer of type-I IFN production from pDC
- Activate B cells to drive anti-nuclear Ab production
- Induce other pro-inflammatory cytokines

TLR 8 acts in monocytes, macrophages, mDCs

- Induces IL-6, IL-10, TNFa and other pro-inflammatory cytokines

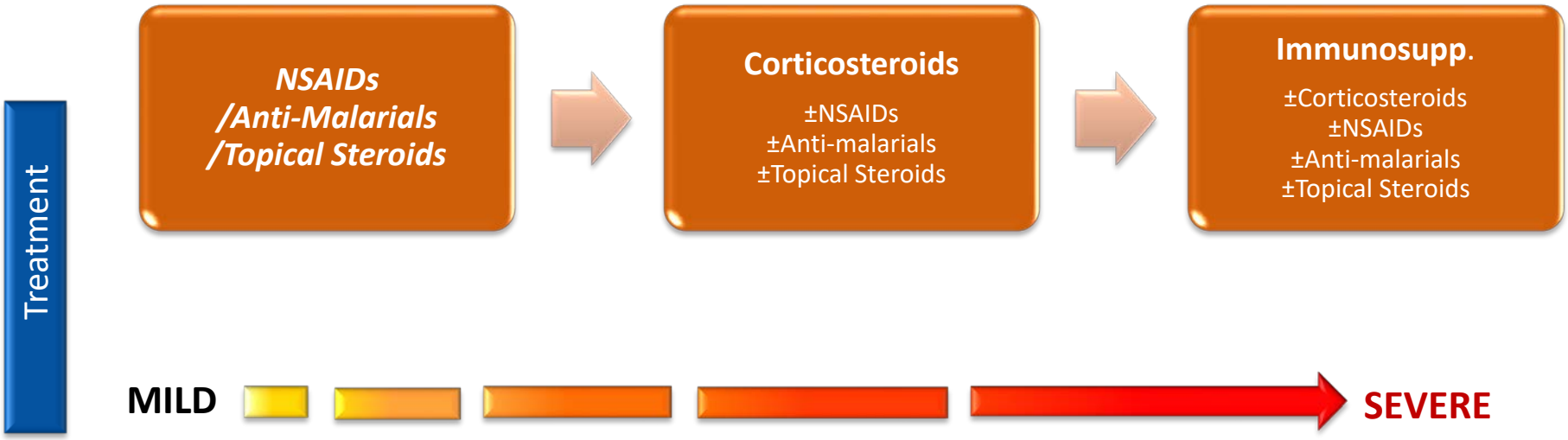
Potential treatment for lupus, as patients show:

- Defects in apoptotic clearance (increased ligands)
- Elevated levels of:
  - Anti-nuclear autoantibodies
  - IFNs and Pro-inflammatory cytokines
  - B-cell activation



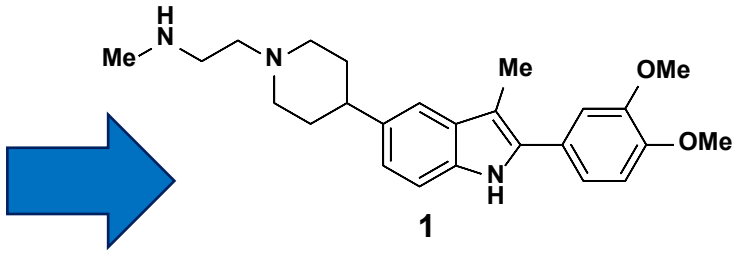
Systemic Lupus Erythematosus (SLE)

- Chronic autoimmune disease that can affect multiple tissues and organs including the joints, skin, kidneys, blood cells, brain, heart and lungs
- Prevalence of ~168,000 cases US; ~5 million cases worldwide
- Combination therapies play a key role in treatment as the severity of the disease increases
- There is a need for safer and more effective treatment options



HTS Hit to Lead Optimization

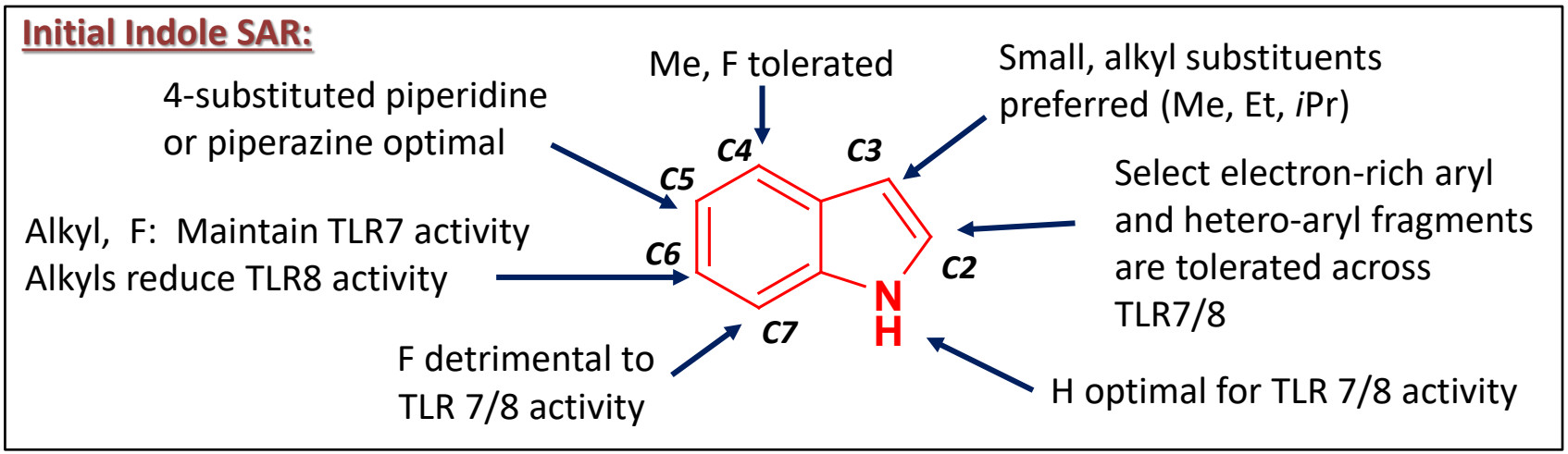
- 1.25 Million compounds screened
- Evaluated for TLR 7/9 inhibition in cellular assays
- Counter-screened against TLR3 and TLR4
- Indoles as potential lead series--active against TLR 7/8/9
  → selected for further exploration
- Program evolution determined that a TLR 7/8-dual antagonist was an optimal profile for lead optimization



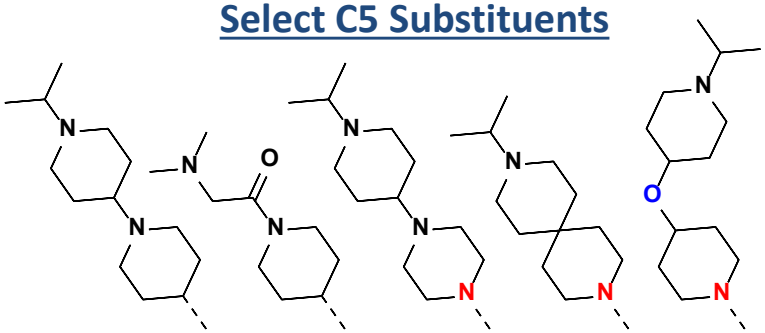
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Indole-based Lead

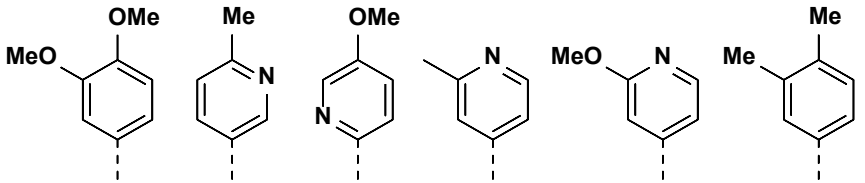
Initial Indole SAR:



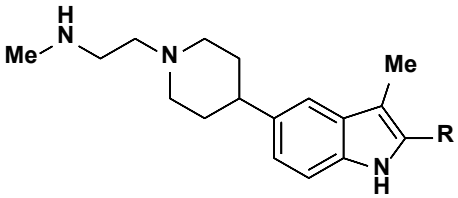
Select C5 Substituents

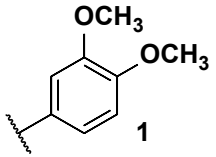


Select C2 Substituents



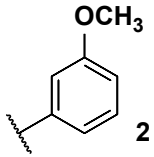
Indole C2 SAR





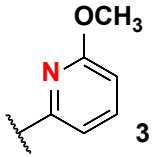
1

TLR 7: 250 nM
TLR 8: 4900 nM
TLR 9: 430 nM



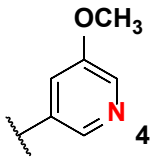
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TLR 7: 6,200 nM
TLR 8: >10,000nM
TLR 9: 1,900 nM



3

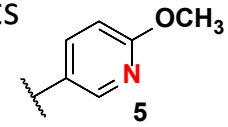
TLR 7: 3,340 nM
TLR 8: 620 nM
TLR 9: 639 nM



4

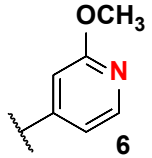
TLR 7: >10,000 nM
TLR 8: >10,000 nM
TLR 9: 4,600 nM

- Initial SAR developed toward TLR 7/8/9 antagonists
- C3-methyl and C5-di-amine moieties important to maintain strong TLR 9 activity



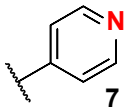
5

TLR 7: >10,000 nM
TLR 8: NT
TLR 9: 820 nM



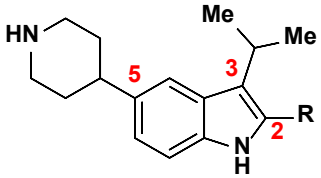
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TLR 7: 520 nM
TLR 8: 1,170 nM
TLR 9: 1,540nM

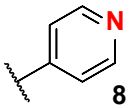


7

TLR 7: 550 nM
TLR 8: 200 nM
TLR 9: 590 nM

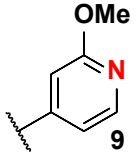


- SAR evolved to emphasize TLR 7/8 dual antagonism profile
- C3-Isopropyl optimal for TLR 7/8 activity; diminished TLR 9 activity
- SAR around 4-pyridyl construct explored at C2;
- 2,6-Dimethyl pyridine (**13**) proved optimal
  - Excellent TLR 7/8 potency and selectivity



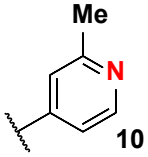
8

TLR 7: 29 nM
TLR 8: 86nM
TLR 9/7 35x



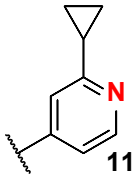
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TLR 7: 74 nM
TLR 8: 93 nM
TLR 9/7 63x



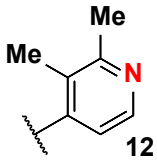
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TLR 7: 6 nM
TLR 8: 4 nM
TLR 9/7 238x



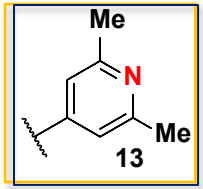
11

TLR 7: 92 nM
TLR 8: 99 nM
TLR 9/7 16x



12

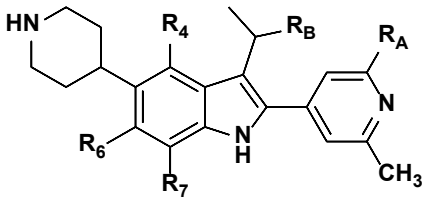
TLR 7: 28 nM
TLR 8: 22 nM
TLR 9/7 25x



13

TLR 7: 0.7 nM
TLR 8: 3 nM
TLR 9/7 350x

Indole Core SAR

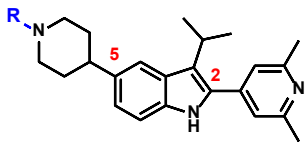


Core I: R_A. R_B = CH_3
Core II: R_A = H, R_B = CH_3
Core III: R_A. R_B = H

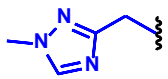
Cmpd	Core	R4	R6	R7	TLR7 IC_{50} (nM)	TLR8 IC_{50} (nM)	TLR9/ TLR7
13	**I**	**H**	**H**	**H**	0.7	3.2	500
10	**II**	**H**	**H**	**H**	6.1	3.9	160
14	**III**	**H**	**H**	**H**	24	15	14
15	**I**	**F**	**H**	**H**	0.9	3.0	1360
16	**I**	**H**	**F**	**H**	0.8	2.7	180
17	**III**	**F**	**F**	**H**	13	2.8	10
18	**III**	**H**	**H**	**F**	610	910	1
19	**III**	**Me**	**H**	**H**	14	27	340
20	**II**	**H**	**Me**	**H**	8.2	130	980
21	**II**	**H**	**CF_3**	**H**	17	420	77

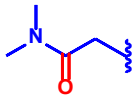
- The impact of Fluoro and alkyl substitution around indole was probed on Cores I, II & III
- Fluorination at C4 (15) or C6 (16) was well tolerated; di-fluorination at C4/C6 provided a boost to TLR 8 activity (17 vs. 14). Fluorination at C7 was detrimental to TLR 7/8 activity (18)
- Methyl group at C4 (19) or C6 (20) showed little impact on TLR 7 activity; TLR 8 activity reduced with C6 substitution

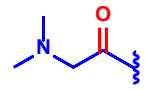
Indole C5 'Capping Group' SAR: Attenuating Basicity



R = H

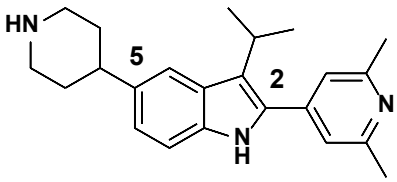




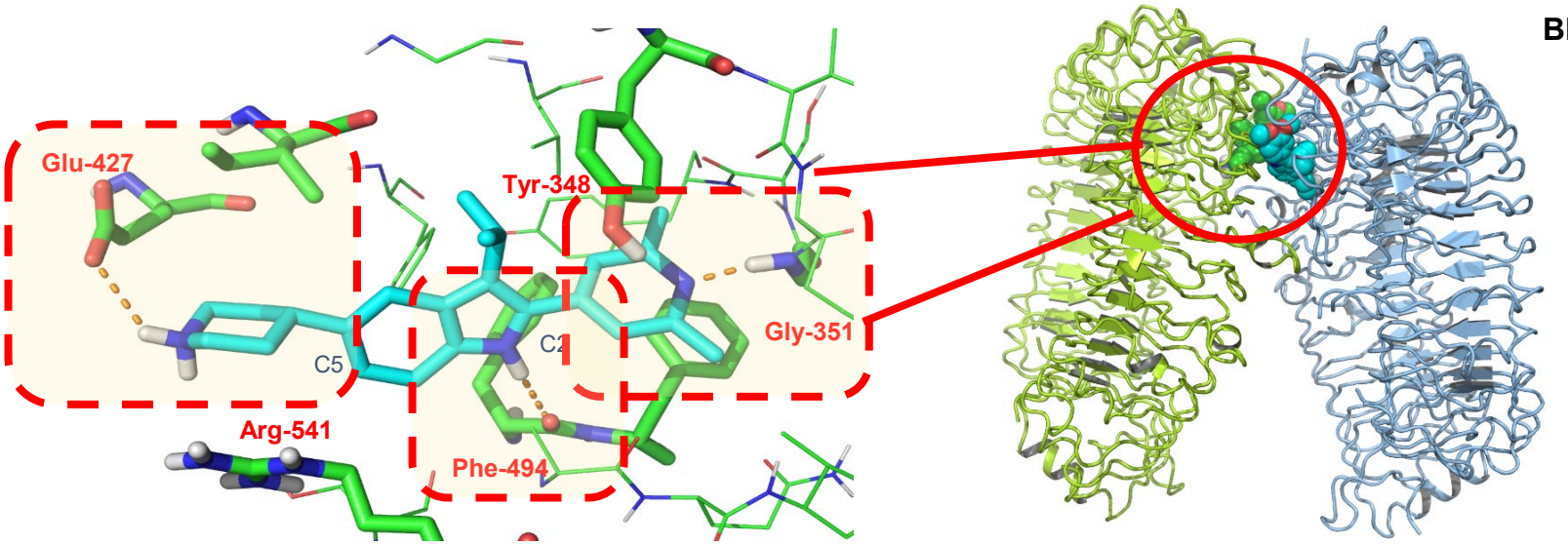
Compound:		13	22	23	24	25	26
hWB TLR7/TLR8 IC_{50} (nM)		1.8 / 2.7	2 / --	69 / 37	63 / --	4 / 3.6	2.6 / 11
C-5 amine pKa (calc.)		9.9	9.9	8.6	7.8	7.1	7.4
hERG PC IC_{50} (mM)		5.6	--	3	<3	2.5	~10
Mouse PK PO: 15 mpk	Bioavail.	58%	22%	61%	60%	82%	80%
	Cmax (nM)	255	227	4853	1980	3,450	788
	AUC (nM)	2,770	823	29,400	20,020	27,800	9720
	Vss (L/kg)	112	94	2.5	7.6	8.5	27
Brain / Blood ratio		20	ND	1.0	2.9	1 (rat)	1.0

- hERG inhibition was not improved with attenuation of piperidine basicity, but did respond to altered placement of the basic amine (**26**)
- Vss was reduced with a corresponding decrease in the basicity of the piperidine nitrogen
- Reduction in CNS exposure was observed with reduced pKa of the basic amine

TLR8 X-ray with BMS-905



BMS-905
(13)



Key Interactions:

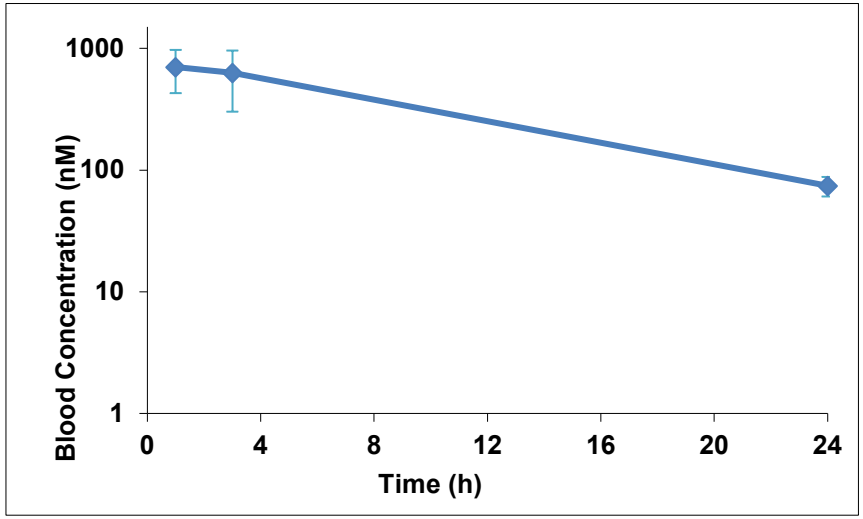
- Hydrogen bond between amide N-H of Gly351 and C-2 pyridine nitrogen
- Methyl groups on pyridine help orient the pyridine in a pi-stacking hydrophobic pocket
- Hydrogen bond between amide oxygen of Phe494 with indole N-H
- Basic piperidine nitrogen (indole C-5 position) makes a salt bridge with Glu427

BMS-905 Profile and PK Results

BMS-905 Select Profiling

Reporter TLR 7 / 8 IC_{50} (nM)	0.7 / 3
Reporter TLR 3 / 4/ 9 IC_{50} (nM)	41,000 / >50,000 / 500
Hum. WB TLR 7 / 8 IC_{50} (nM)	3 / 4
Mouse WB TLR 7 IC_{50} (nM)	1
Caco-2 A-B (nm/sec) (Efflux Ratio)	80 (3.5)
$T_{1/2}$ LM (h/r/m/d) (min)	78 / 99 / 43 / >120
CYP inhibition (mM)	all >20
Prot. Bind. (%free) (h/r/m)	9 / 11 / 5
hERG PC IC_{50} (nM)	5,600

Mouse PO PK Profile BMS-905



Potency

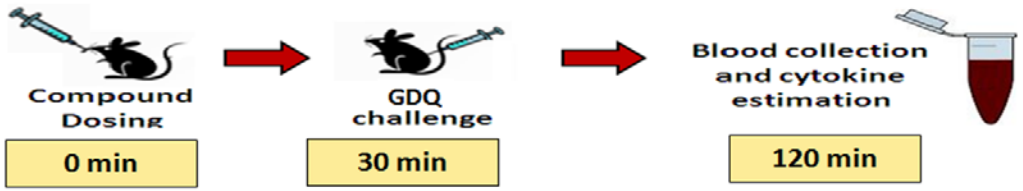
Excellent potency connectivity across species and assays:
Hum → Mouse
Cell → Blood → In Vivo

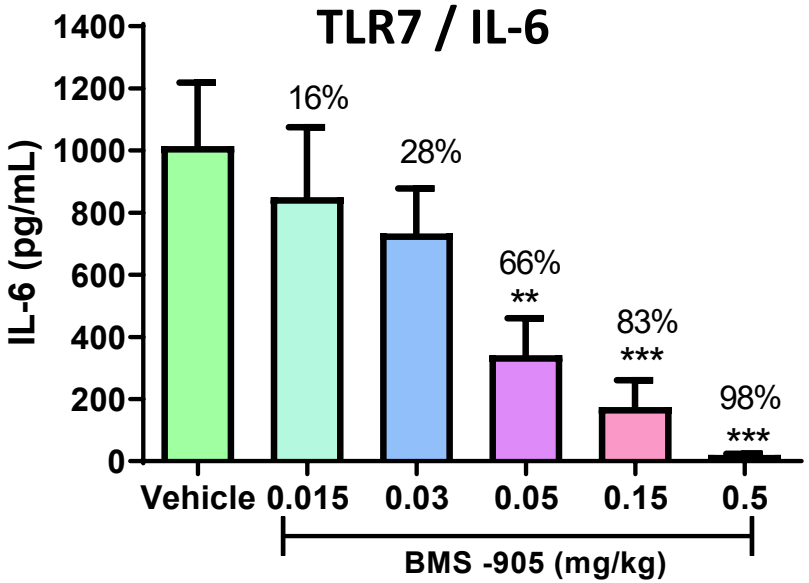
Pharmacokinetics

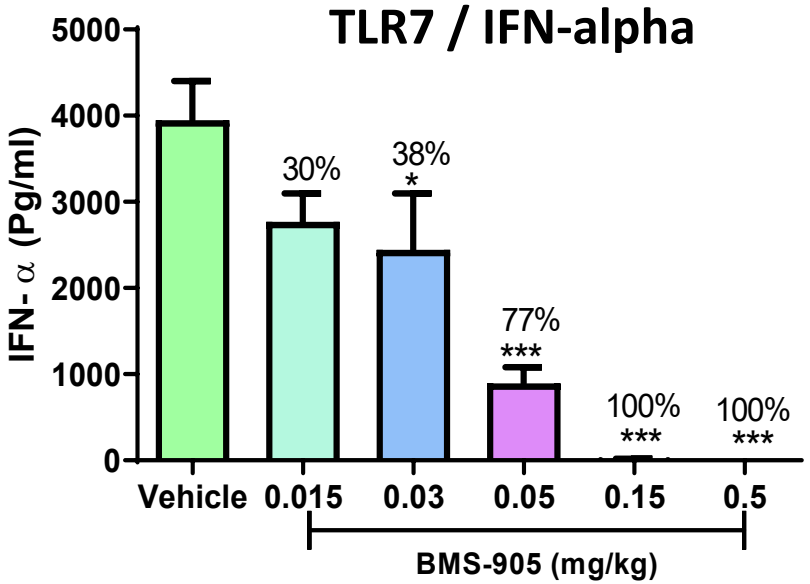
Good bioavailability, low Cl, long $T_{1/2}$
Very large Vss (>100 L/kg in mouse)
High CNS exposure (Brain:Blood ~20 in mouse), not needed for targeted indication

PK Parameters	Mouse		Rat		Dog	
	IV	PO	IV	PO	IV	PO
Dose (mpk)	5	15	2.5	7.5	1	1
AUC_{last} (nM*h)	1583	2773	4058	8323	1402	345
%F	58		>68		>25	
Cl (mL/min/kg)	146		<11		12	
$T_{1/2}$ (h)	9		>64		>112	
V_{ss} (L/kg)	113		<63		>118	
Brain / Blood	20 - 43		---		---	

Pharmacodynamic Evaluation of BMS-905



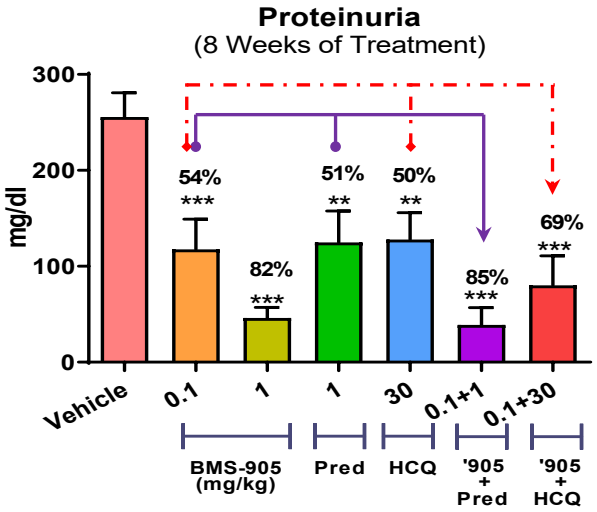


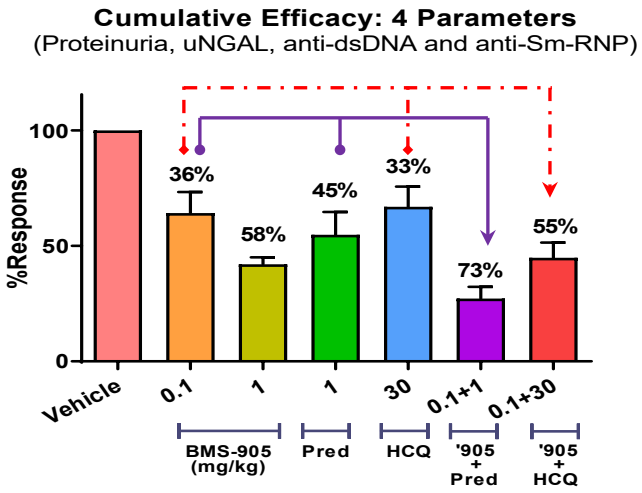


**p<0.01, ***p<0.001 vs. Vehicle control; One way ANOVA with Dunnett's test

- BMS-905 showed robust and dose dependent inhibition of TLR7-driven cytokines in PD model
- *In vitro* mouse Whole Blood TLR7 IC_{50} = 0.30 nM
- *In vivo* TLR7 IC_{50} <0.40 nM (based on blood concentrations from the 0.05 mpk dose)

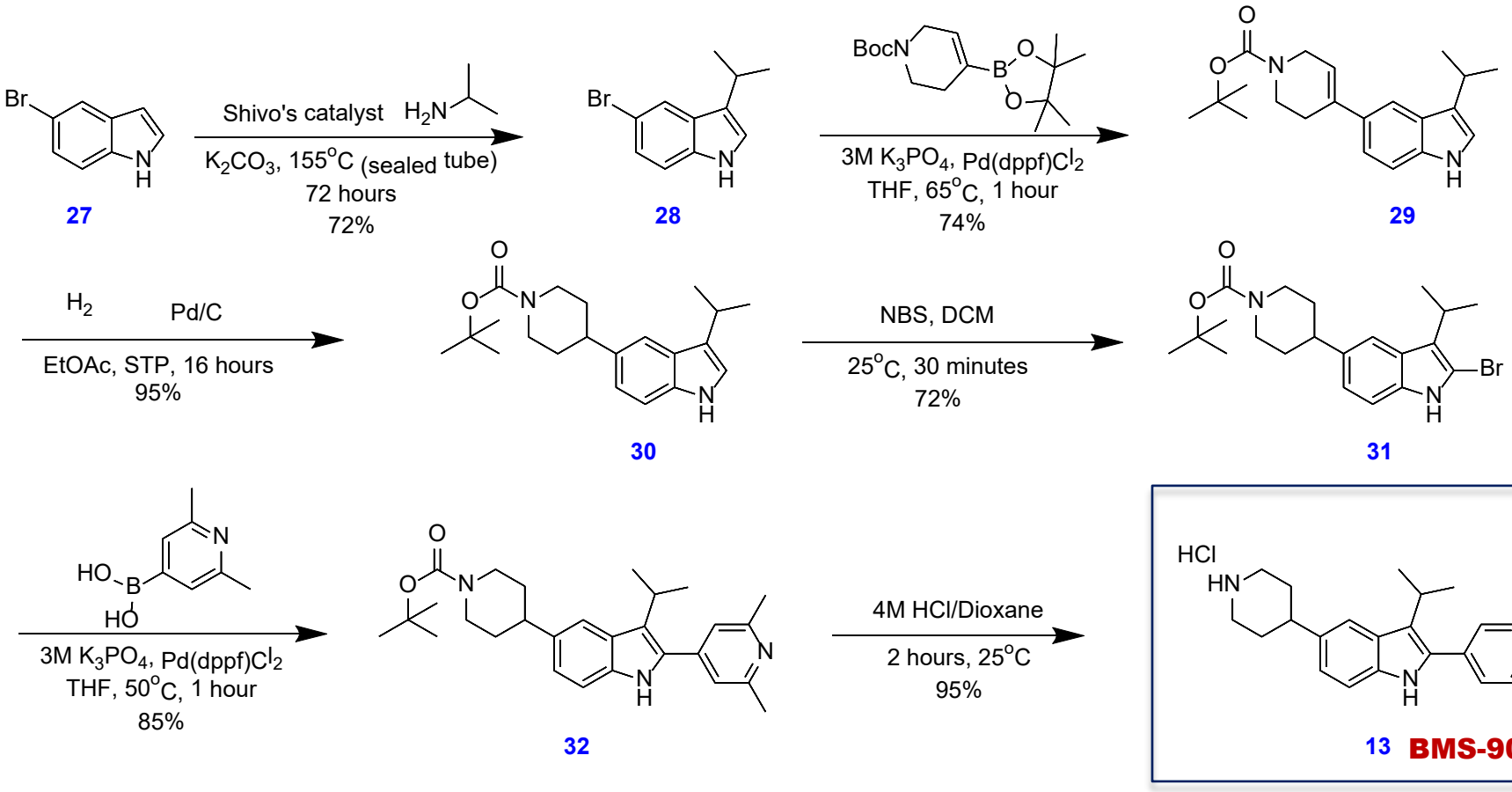
High Efficacy in Mouse Lupus Model as Monotherapy or Combination Therapy





- MRL/lpr mice show spontaneous development of lupus-like symptoms
- BMS-905, Prednisolone (Pred) and Hydroxychloroquine (HCQ) were orally-dosed, once-daily over an 8-week period, as monotherapies and in combination
- Excellent control of proteinuria, auto-antibody levels and other endpoints by BMS-905 as a monotherapy
- Combination of low-dose BMS-905 with either Pred. or HCQ afforded superior efficacy as compared to the monotherapy treatment

Synthetic Scheme for BMS-905



Summary

- Toll-Like Receptors (TLRs) play a key roles in the pathology of lupus
- A High-Throughput Screen for inhibitors of TLR 7/9 activity with selectivity against TLRs 3/4 identified an indole-based series that was optimized for activity against TLR7 and TLR8
- Extensive SAR around the C2 and core positions of the indole core provided a potent and selective TLR 7/8 inhibitor in **BMS-905**
- Co-crystal structure with hTLR8 highlighted the mode of action of this class of antagonists
- **BMS-905** demonstrated excellent potency and good oral PK properties
- **BMS-905** was evaluated in multiple *in vivo* models in order to determine the viability of a TLR driven pharmacodynamic response
- **BMS-905** showed a robust and dose-dependent response as, both, a monotherapy and in combination with Pred. or HCQ
- *In vivo* results established that a small molecule inhibitor of the TLR7/8 pathway serves as a viable and promising approach for the treatment of autoimmune disorders