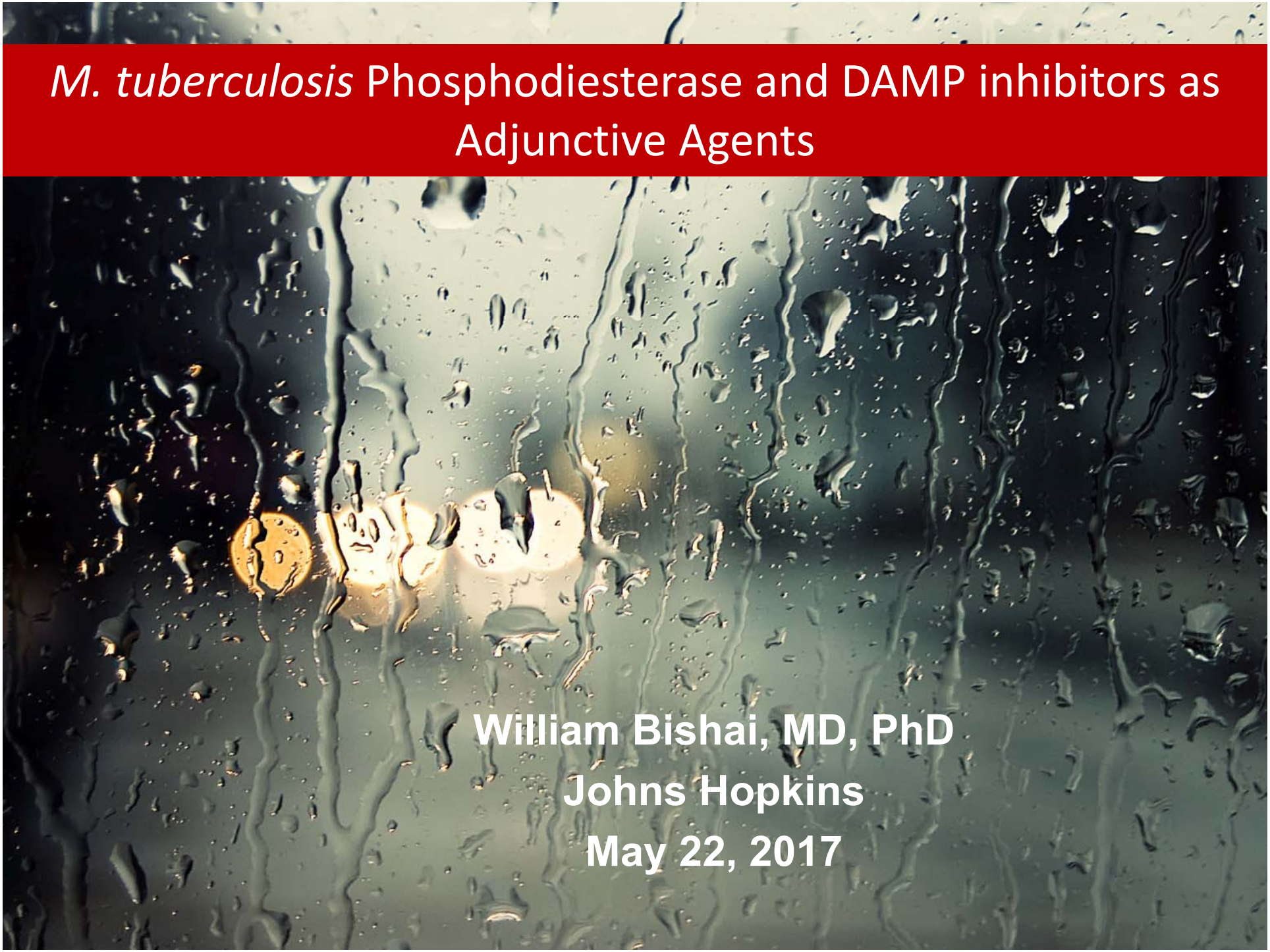
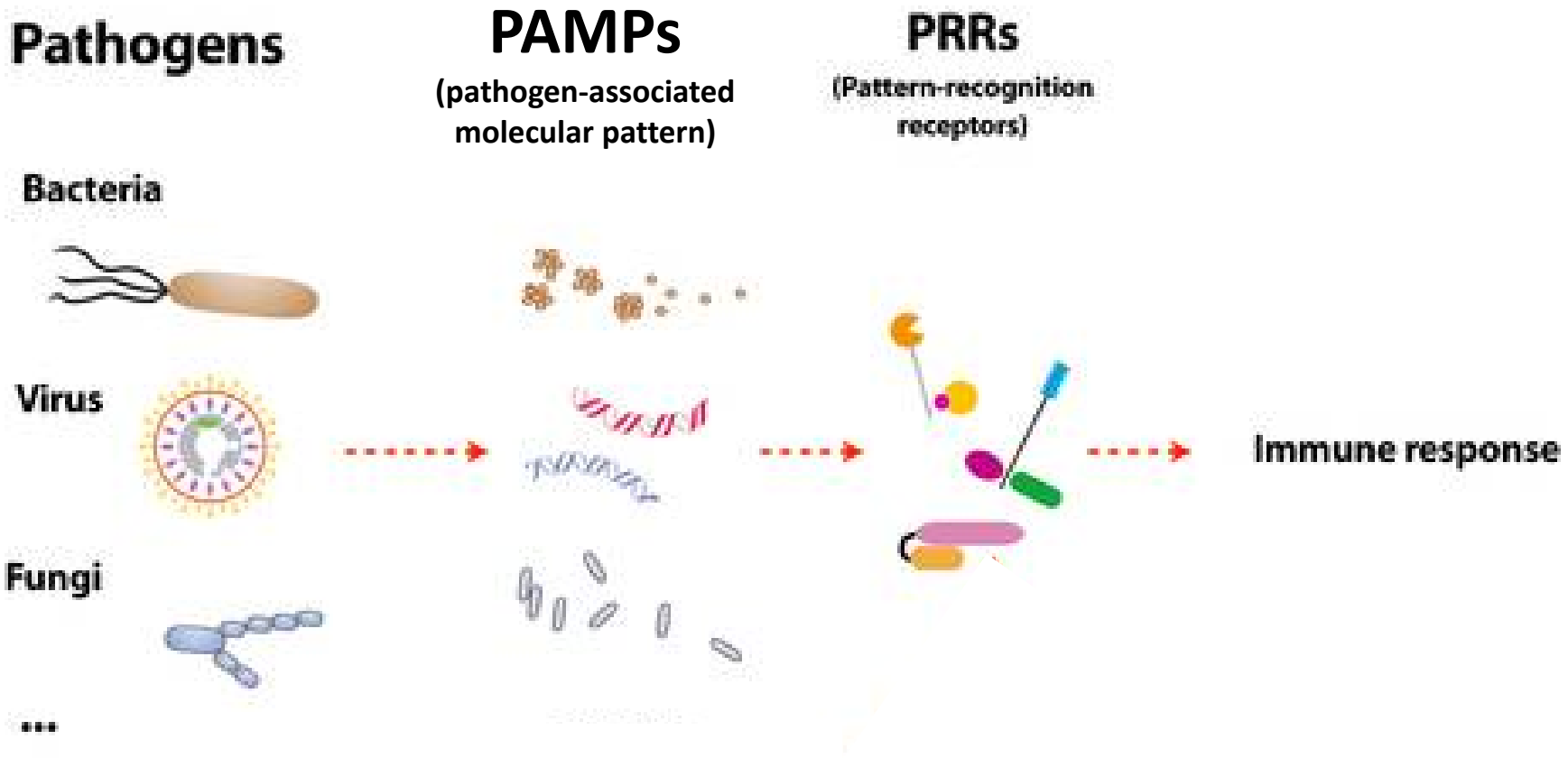
M. tuberculosis Phosphodiesterase and DAMP inhibitors as Adjunctive Agents



William Bishai, MD, PhD
Johns Hopkins
May 22, 2017

What's a DAMP ?


Pathogens
PAMPs
(pathogen-associated molecular pattern)
PRRs
(Pattern-recognition receptors)
Bacteria
Virus
Fungi
...
Immune response

PDE and DAMP Inhibitors as HDT

Strategy

1. **Identify FDA-approved (or nearing approval) drug**
2. **Plausible mechanism of benefit in TB**
3. **Test in mouse or rabbit TB model with Std-Tx**

Bishai Lab HDT Experience

- **Verapamil*** — *Gupta et al. AJRCCM 2013*
- **Cilostazol** — *Maiga et al. JID 2013*
- **Sildenafil** — *Maiga et al. PLoS ONE 2012*
- **Roflimilast** — *Maiga et al. AAC 2015*

  } PDE inhibitors

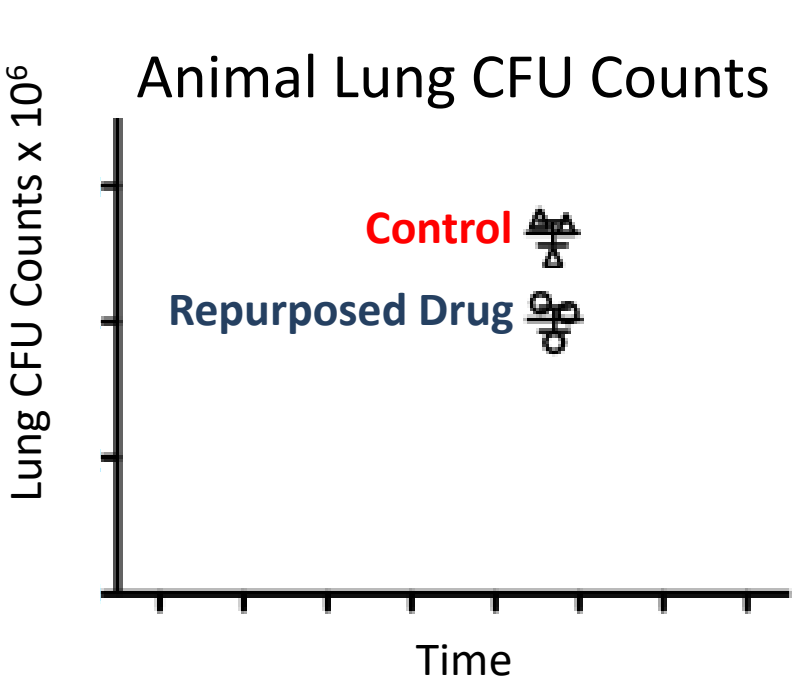
- **Etanercept** — *Skerry et al. PLoS ONE 2012*
- **Tofacitinib** — *Maiga et al. eBiomedicine 2015*
- **Pirfenidone** — *Ahidjo et al. JCI Insight 2016*
- **Cipemastat** — *Urbanowski et al. in preparation*
- **Denileukin diftitox** — *Gupta et al. JID 2017*
- **Tasquinimod** DAMP inhibitor: S100A9 — *Gupta et al. in preparation*
- **Talazoparib** DAMP inhibitor: PARP-1

Effective in multi-time point studies (most tested versus 2HRZ-4HR)
Deleterious
No effect
In progress

Scorecard: 8W 1L 1T

*Human antihypertensive with bacterial target

Observations on HDT



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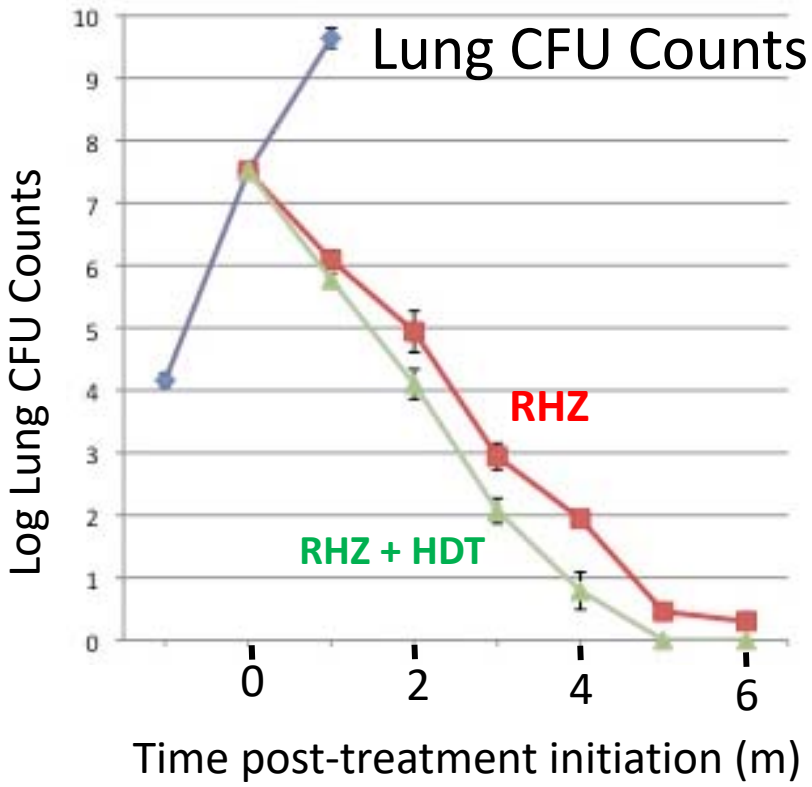


Lessons learned

2HRZ-4HR is hard to beat

Mouse strain matters

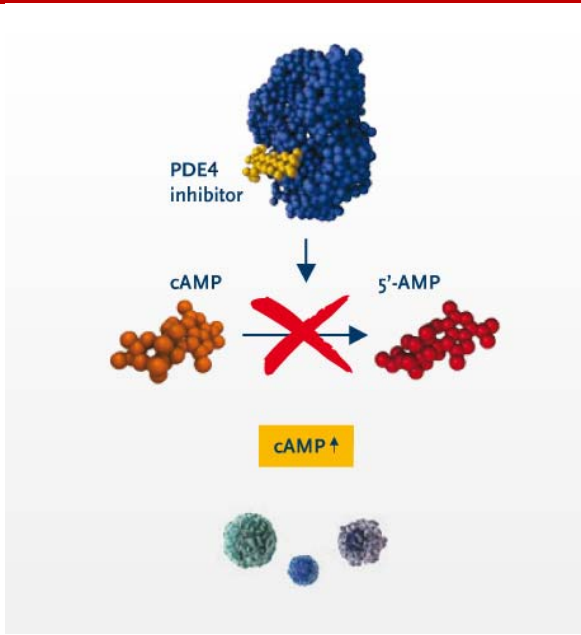
- VER: works in Kramnik, not C3H
- Tofa: works in C3H, not Kramnik

PDE and DAMP Inhibitors as HDT

Outline

1. **PDE inhibitors:** host, microbe, or both
2. **S100A9 inhibitors**
3. **PARP-1 inhibitors**

1. PDE Inhibitors



Well-known PDE-Is

Caffeine	NS	
Theophylline	NS	
Pentoxyifylline	NS	
Amrinone	PDE3	(CHF)
Roflumilast	PDE4	(COPD)
Viagra	PDE5	(ED)

OPEN ACCESS Freely available online PLoS one

Successful Shortening of Tuberculosis Treatment Using Adjuvant Host-Directed Therapy with FDA-Approved Phosphodiesterase Inhibitors in the Mouse Model

Mamoudou Maiga[1], Nisheeth Agarwal[1,2], Nicole C. Ammerman[1], Radhika Gupta[1], Haidan Guo[1], Marama C. Maiga[1], Shichun Lun[1], William R. Bishai[1,3,4]

AAC Journals.ASM.org

Roflumilast, a Type 4 Phosphodiesterase Inhibitor, Shows Promising Adjunctive, Host-Directed Therapeutic Activity in a Mouse Model of Tuberculosis

Mariama C. Maiga,[a,b] Bintou Ahmadou Ahidjo,[a,b] Mamoudou Maiga,[a,c*] William R. Bishai[a,b]

Center for Tuberculosis Research, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA[a]; Howard Hughes Medical Institute, Chevy Chase, Maryland, USA[b]; Université des Sciences, des Techniques et des Technologies de Bamako (USTTB), Bamako, Mali[c]

Adjuvant Host-Directed Therapy with Types 3 and 5 but Not Type 4 Phosphodiesterase Inhibitors Shortens the Duration of Tuberculosis Treatment

The Journal of Infectious Diseases

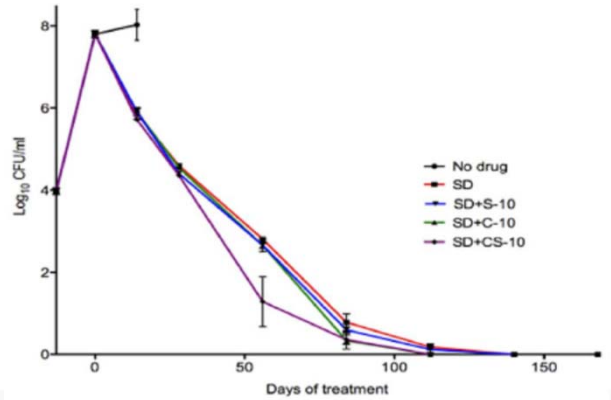
Mamoudou Maiga,[1,5] Nicole C. Ammerman,[1,6] Mariama C. Maiga,[1] Anatole Tounkara,[5] Sophia Siddiqui,[2] Michael Polis,[2] Robert Murphy,[4] and William R. Bishai[1,3,6]

EBioMedicine

Adjunctive Phosphodiesterase-4 Inhibitor Therapy Improves Antibiotic Response to Pulmonary Tuberculosis in a Rabbit Model

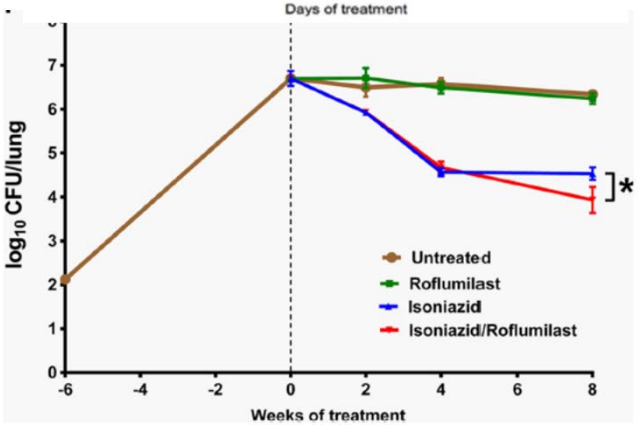
Selvakumar Subbian [a,*], Liana Tsenova [a,b], Jennifer Holloway [c,1], Blas Peixoto [a,1], Paul O'Brien [a,1], Véronique Dartois [a], Vikram Khetani [d], Jerome B. Zeldis [d], Gilla Kaplan [a,2]

1. PDE Inhibitors



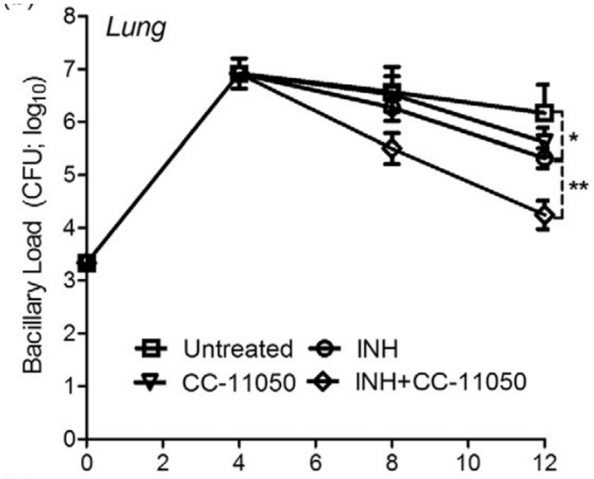
Sildenafil (PDE5-I) + Cilostazol (PDE3-I)
Mice, 6 months
With full Std-Tx

Maiga et al. JID 2013



Roflumilast (PDE4-I)
Mice, 2 months
With INH

Maiga et al. AAC 2015



CC-11050 (PDE4-I)
Rabbits, 3 months
With INH

Subbian et al. eBiomedicine 2016

What PDE are we trying to inhibit?

nature medicine

A bacterial cyclic dinucleotide activates the cytosolic surveillance pathway and mediates innate resistance to tuberculosis

Bappaditya Dey[1,2], Ruchi Jain Dey[1], Laurene S Cheung[1], Supriya Pokkali[1,3], Haidan Guo[1], Jong-Hee Lee[1] & William R Bishai[1,2]

nature chemical biology

ARTICLE

PUBLISHED ONLINE: 12 DECEMBER 2016 | DOI: 10.1038/NCHEMBIO.2254

Inhibition of innate immune cytosolic surveillance by an *M. tuberculosis* phosphodiesterase

Ruchi Jain Dey[1,2,7], Bappaditya Dey[1,2,6,7], Yue Zheng[3,4,6,7], Laurene S Cheung[1], Jie Zhou[3,4], David Sayre[3], Pankaj Kumar[1], Haidan Guo[1], Gyanu Lamichhane[1], Herman O Sintim[3-5]* & William R Bishai[1,2]*

nature chemical biology

ARTICLE

PUBLISHED ONLINE: 26 OCTOBER 2014 | DOI: 10.1038/NCHEMBIO.1661

Hydrolysis of 2′3′-cGAMP by ENPP1 and design of nonhydrolyzable analogs

Lingyin Li[1]*, Qian Yin[2], Pia Kuss[3], Zoltan Maliga[1], José L Millán[3], Hao Wu[2] & Timothy J Mitchison[1]

Host-beneficial cyclic dinucleotides

c-di-AMP:

bacterial PAMP

↑ autophagy

cGAMP

Host DAMP

↑ autophagy

Host-detrimental PDEs

CdnP

Bacterial

inactivates both c-di-AMP & cGAMP

CdnP inhibitors give ↑ autophagy

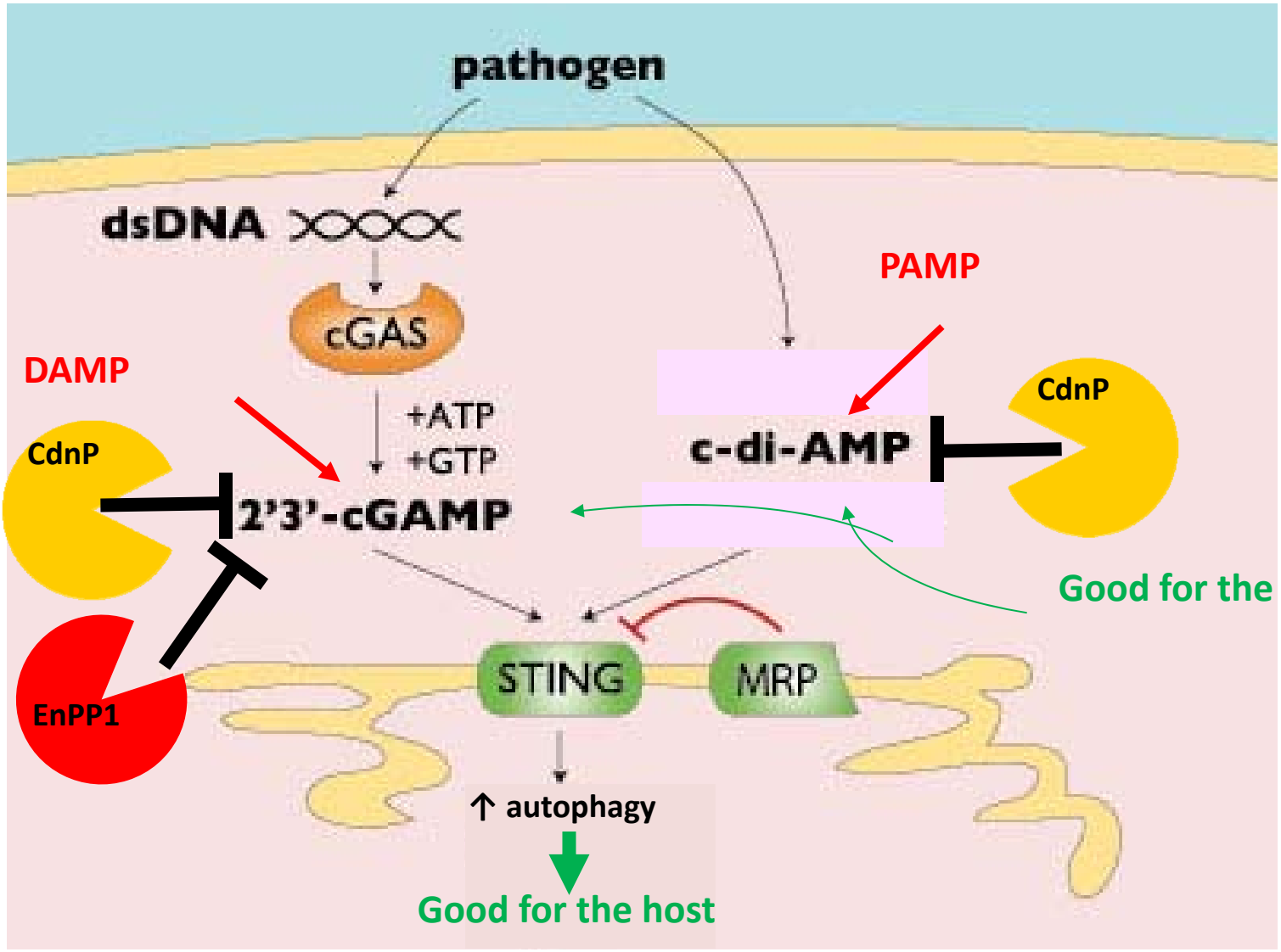
ENPP1

Host

inactivates cGAMP

ENPP1 inhibitors give ↑ autophagy

What PDE are we trying to inhibit?



Rational targets for PDE inhibitors: bacterial CdnP & host ENPP1

Jain-Dey et al. Nat Chem Biol 2016

What PDE are we trying to inhibit?

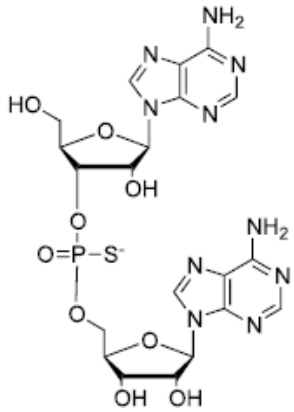
CdnP inhibitors-1

Ap(S)A

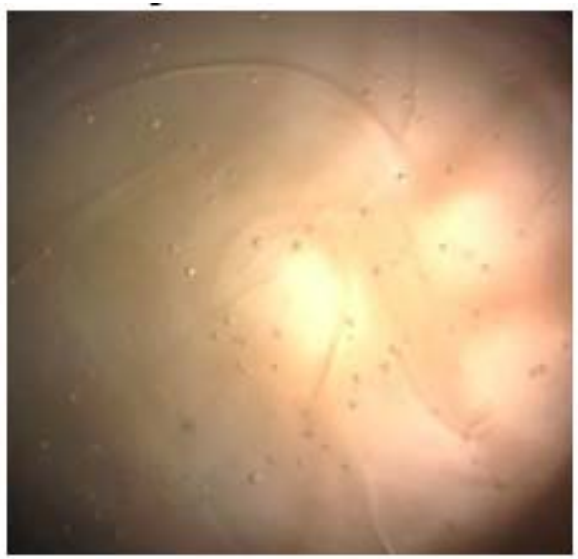
- Non-hydrolyzable c-di-AMP analogue
- Ki 65 μM
- Active in vivo in Mtb cell infection

Jain-Dey et al. NCB 2016

Ap(S)A



CdnP inhibitors-2



CdnP crystals
3D structure available
Virtual screen (Schrödinger, Inc)

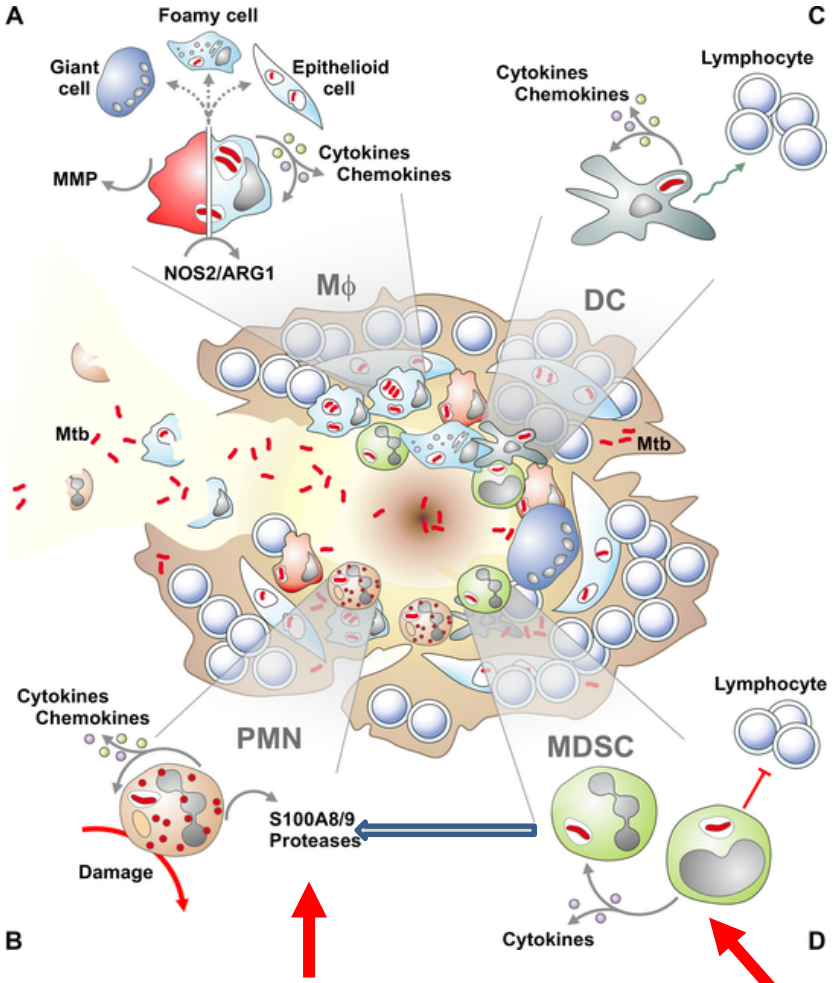
Small molecule docking to CdnP:

- 304 high probability hits
- identified in the structure-based virtual screen of 6 million drug-, lead-, and fragment-like commercially available compounds.

Outline

1. **PDE inhibitors:** host, microbe, or both
2. **S100A9 inhibitors**
3. **PARP-1 inhibitors**

2. S100A9 Inhibitors as HDT

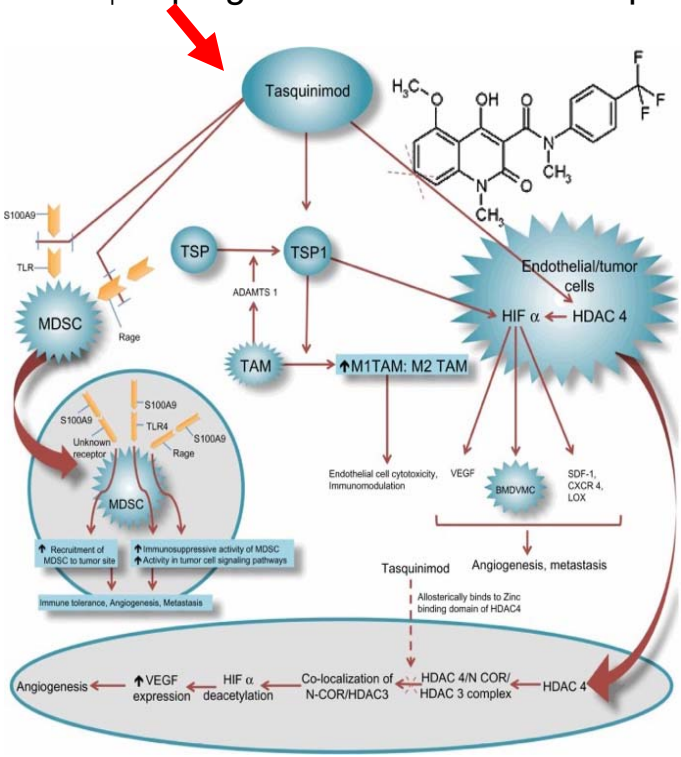


S100A8/9 proteins

- DAMPs expressed by MDSCs & PMNs
- Autocrine role with further activation of MDSCs
- Abundant in human TB granuloma

Tasquinimod

- S100A9 inhibitor (nM affinity)
- Developed by Active Biotech, Inc, Sweden
- Reduces MDSC infiltration in tumors
- ↑ in progression-free survival in prostate CA



MDSCs (myeloid-derived suppressor cells):

- induced during *M.tb* infection
- potent immunosuppressive effects
  - -↓ T cell activation, proliferation, & migration
  - -↑ T regulatory cells (immunosuppressive)

2. S100A9 Inhibitors as HDT: Immunology

Tasquinimod currently being tested in mice

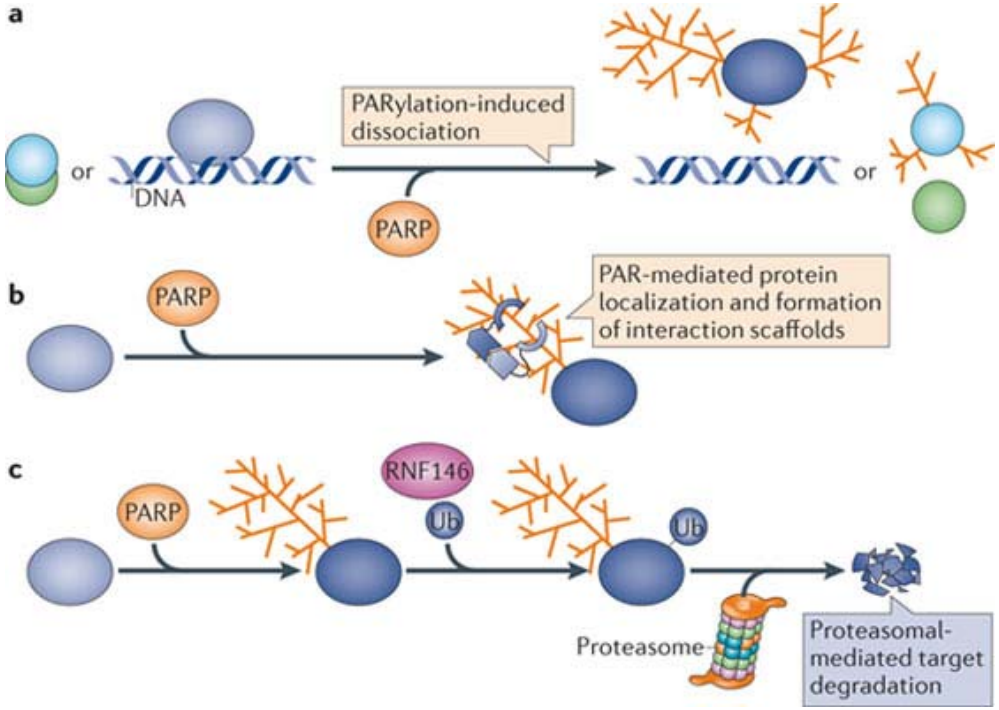
Outline

1. PDE inhibitors
2. S100A9 inhibitors
3. **PARP-1 inhibitors**

3. PARP-1 Inhibitors

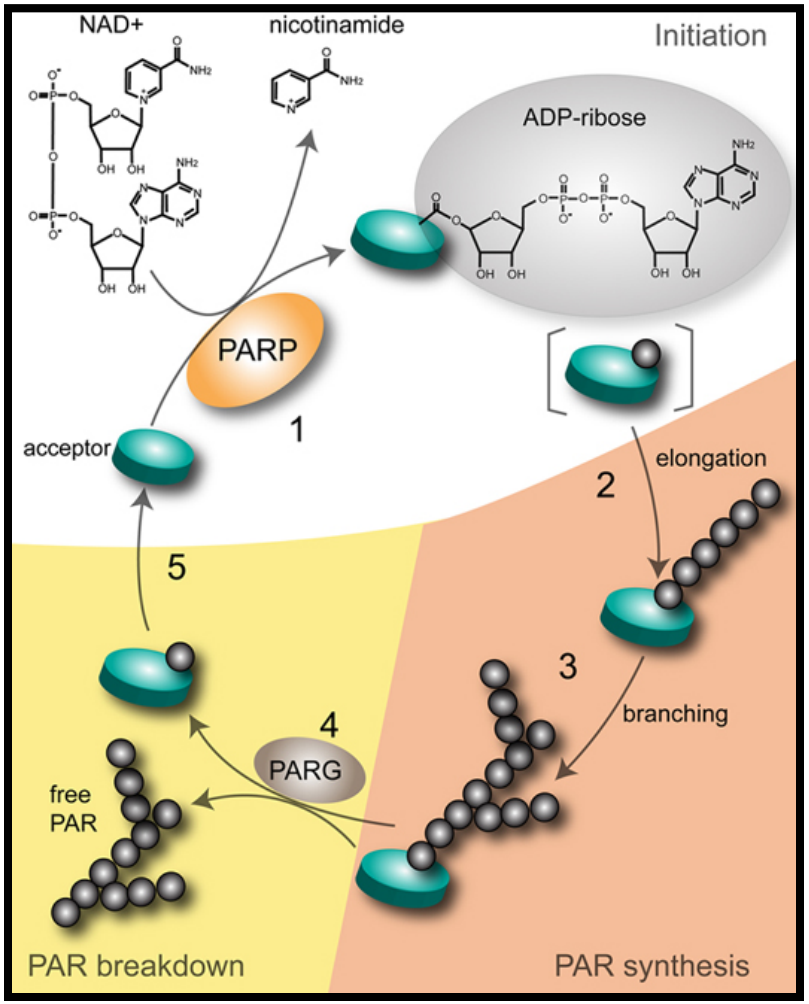
PARP-1 (Poly[ADP-Ribose] Polymerase-1)

- DAMP protein
- ADP-ribosylates nuclear proteins after DNA damage
- Acts in conjunction with BRCA
- Plays role in
  -Tumor transformation
  -Fanconi's anemia
  -Type 1 diabetes



Gibson & Kraus (2012)

PAR Polymer Metabolism:



David *et al.* (2009)

PARP-1 inhibitors

Talazoparib currently being tested in mice

SUMMARY

1. Some HDTs can make TB worse

2. Animal proof of principle should include HDT+Std Tx (6 mo)

3. PDE inhibitors for TB
   - Sildenafil (PDE5-I) + Cilostazol (PDE3-I)
   - Roflumilast (PDE4-I)
   - CG-11050 (PDE4-I)
   - CdnP and ENPP1 are key targets for novel PDE inhibitors for TB

4. S100A9 inhibitors for TB
   - Tasquinimod in progress

5. PARP-1 inhibition:
   - Talazoparib in progress

Contributors

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