CIDARA THERAPEUTICS

In-vitro characterization of antibacterial activity, cidality and spontaneous resistance potential of CD201, a novel lipopolysaccharide-binding antibacterial immunotherapy

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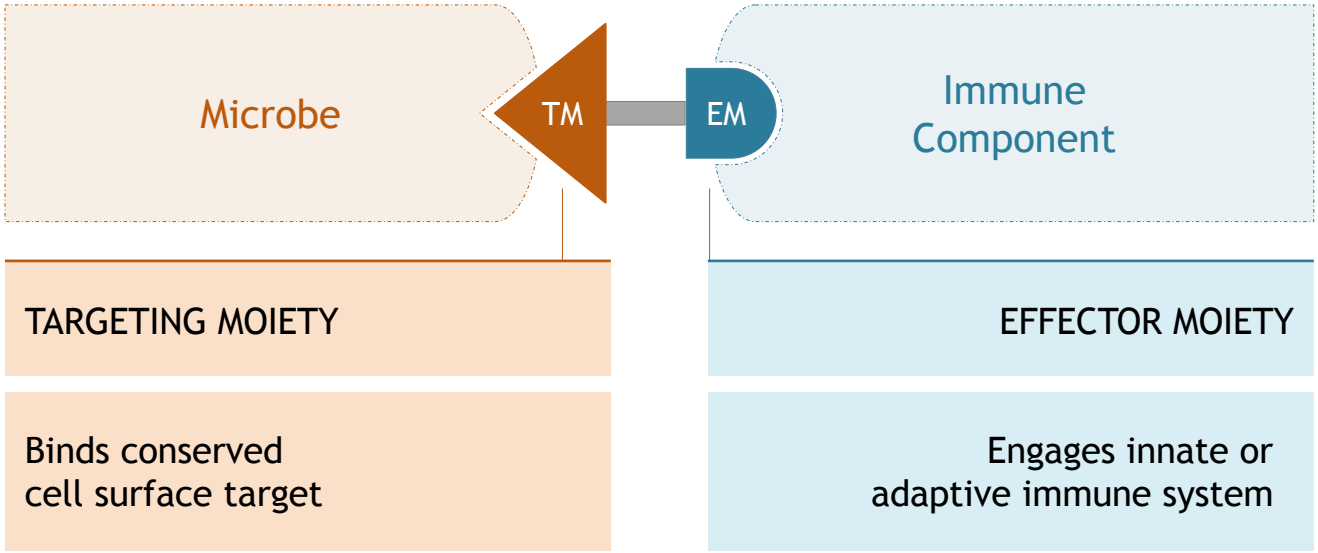
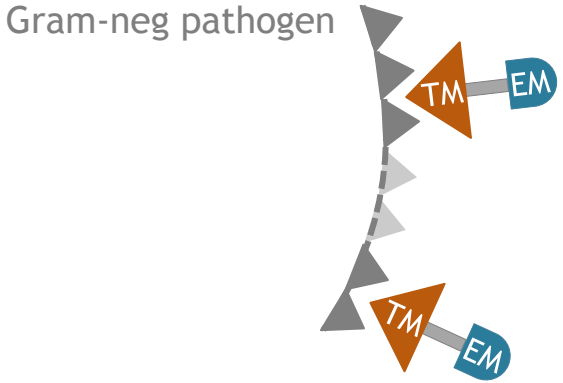
Disclosures / Acknowledgements

- The authors are employees and shareholders of Cidara Therapeutics, Inc. (San Diego, CA, USA)
- Bacterial isolates used in MIC_{90} panels were obtained from Micromyx, LLC (Kalamazoo, MI, USA)

Cloudbreak: inspired by cancer immunotherapy



CD201 bimodal mechanism



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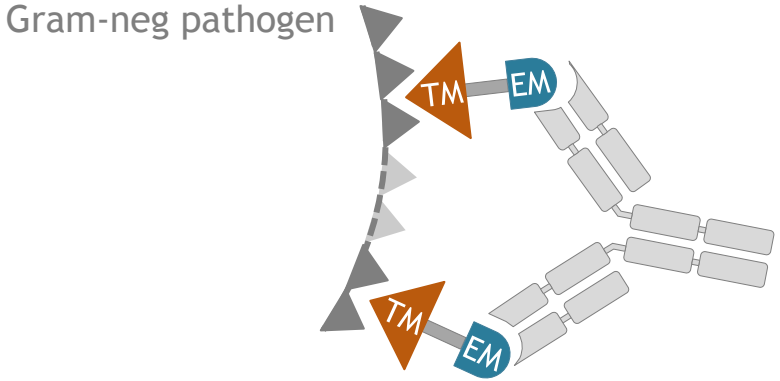
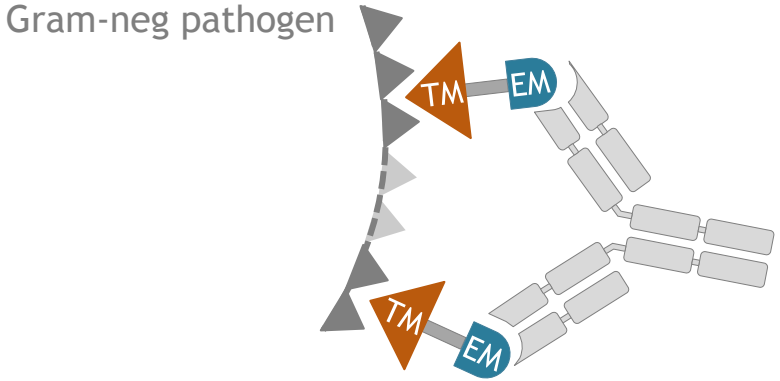
CD201 bimodal mechanism



1. ***Direct kill:*** novel TM tightly binds LPS on Gram-negative pathogens and kills bacteria
2. ***Immunomodulatory:*** EM recruits and initiates an innate immune system response

CD201 bimodal mechanism



1. ***Direct kill:*** novel TM tightly binds LPS on Gram-negative pathogens and kills bacteria
2. ***Immunomodulatory:*** EM recruits and initiates an innate immune system response

Objectives

- The antimicrobial properties of CD201, independent of its immunotherapeutic activity, were characterized through:

    1) **Intrinsic antibacterial activity** (MIC assays)

    2) **Killing kinetics** (time-kill assays)

    3) **Resistance potential** (spontaneous resistance plating)

1) Intrinsic antibacterial activity: MIC assays

			MIC (µg/mL)					
Species	Background	Characteristics/Notes	CD201	COL	PMB	MERO	TIG	LEVO
E. coli	BW25113	WT	2	0.25	0.5	0.03	0.25	0.03
E. coli	BW25113	COL^R (*mcr-1*)	2	4	4	0.03	0.25	0.03
E. coli	BW25113	COL^R (*mcr-2*)	2	4	4	0.03	0.25	0.03
E. coli	BW25113	4X-12, COL^R spont. mut.	2	8	8	0.03	0.125	0.03
E. coli	ATCC 25922	WT	2	0.5	0.5	0.03	0.125	0.03
E. coli	ATCC 25922	WT, + 50% FBS	1	0.125	0.125	0.03	0.5	0.03
E. coli	BAA-2469	CRE (*ndm-1*)	2	0.5	0.5	>8	0.25	>8
K. pneumoniae	ATCC 10031	WT	4	0.5	1	0.03	≤0.06	0.03
P. aeruginosa	PAO1	WT	2	1	2	1	>8	0.5
A. baumannii	ATCC 19606	WT	2	1	1	2	1	0.5

CD201

- MIC values of 2 µg/mL across majority of G-neg strains
- Unaffected by Mcr-1 or Mcr-2 LPS modification or by the uncharacterized, chromosomal resistance mechanism in the 4X-12 COL^R *E. coli* mutant
- Reverse serum shift of 2-fold compared to 4-fold for COL

*According to CLSI broth microdilution guidelines (M07-A10); plates were incubated for 24 h at 37°C and read at 100% inhibition.

1) Intrinsic antibacterial activity: MIC_{90} panels (COL^S)

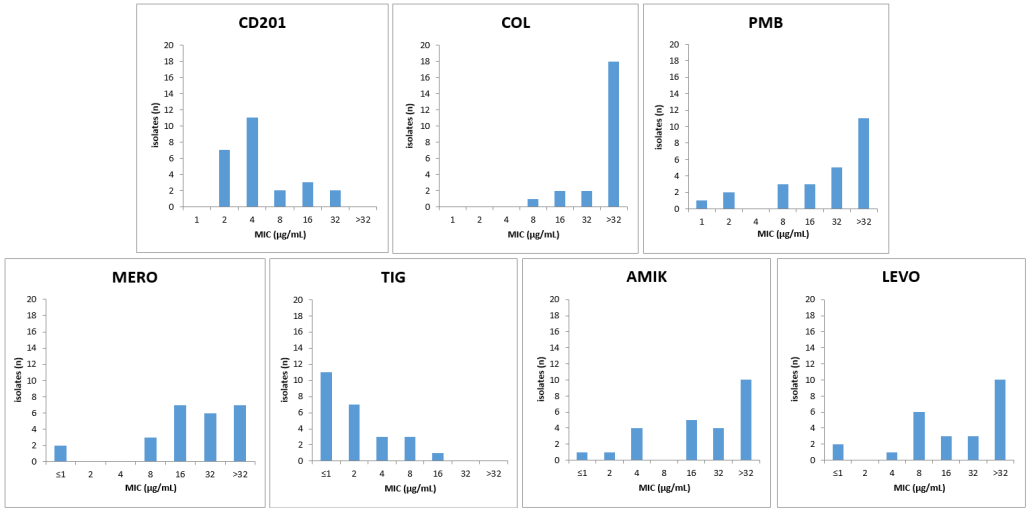
Species (n=20 each)	MIC_{90} (µg/mL) (range)						
	CD201	COL	PMB	MERO	TIG	AMIK	LEVO
E. coli	2 (2 - 2)	0.5 (0.25 - 0.5)	0.5 (0.25 - 1)	0.03 (0.015 - 0.03)	0.5 (0.125 - 0.5)	8 (1 - 8)	>8 (0.03 - >8)
K. pneumoniae	4 (2 - 4)	0.5 (0.25 - 0.5)	0.5 (0.5 - 1)	0.5 (0.03 - 8)	2 (1 - 8)	8 (≤0.5 - 32)	4 (0.03 - >8)
P. aeruginosa	4 (2 - 4)	2 (0.5 - 4)	1 (0.5 - 1)	>8 (0.125 - >8)	>8 (>8 - >8)	16 (2 - 16)	4 (0.5 - >8)
A. baumannii	4 (2 - 4)	2 (0.5 - 4)	1 (0.5 - 2)	>8 (0.125 - >8)	4 (0.5 - >8)	>64 (1 - >64)	>8 (0.06 - >8)

- CD201 has consistent and potent activity vs. COL^S clinical isolates of key G-neg species

*Micromyx clinical isolates collected from US sites between 2012 and 2016; MIC data from ECCMID 2017 abstract #7345/ePoster #P0475

1) Intrinsic antibacterial activity: MIC_{90} panel (COL^R)

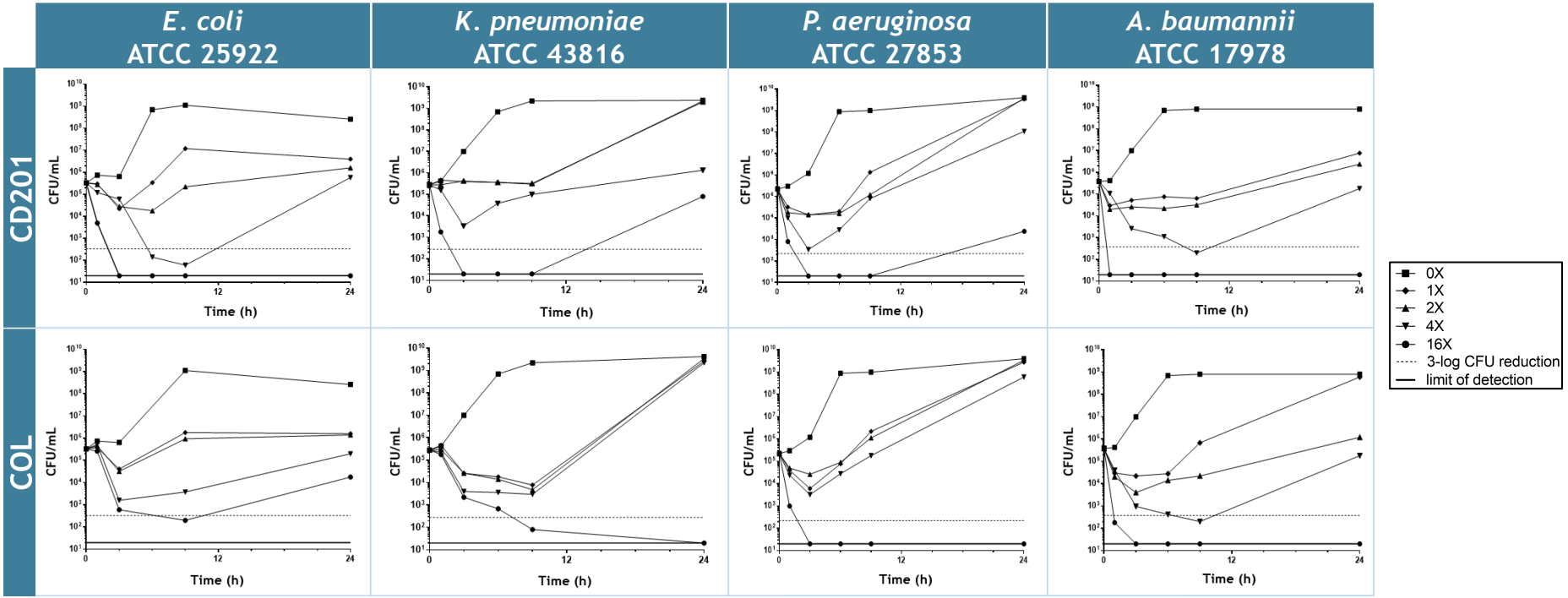
Species	MIC_{90} (µg/mL) (range)						
	CD201	COL	PMB	MERO	TIG	AMIK	LEVO
Kp*, *Pa*, *Ab **(n=25 total)**	16 (2 - 32)	>1024 (8 - >1024)	256 (1 - >1024)	128 (0.03 - 256)	8 (0.5 - >8)	>256 (1 - >256)	128 (0.06 - >256)



- CD201 retains >64-fold more potent activity over COL across chromosomal COL^R MDR isolates

*Micromyx clinical isolates collected from US sites between 2012 and 2016; MIC data from ECCMID 2017 abstract #7345/ePoster #P0475

2) Killing kinetics: Time-kill assays



- CD201 demonstrates similar bactericidal activity vs. *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *A. baumannii* to COL at 4 to 16X MIC

*For CD201 and COL at 0, 1, 2, 4 and 16X MIC; changes in CFU from a starting mid-10^5 inoculum were assessed over 24 h

3) Resistance potential: Spontaneous mutant plating

	CD201				COL			
	1X agar MIC		2X agar MIC		1X agar MIC		2X agar MIC	
Plate #	Colonies	Freq.	Colonies	Freq.	Colonies	Freq.	Colonies	Freq.
1	3	5.0E-09	0	<1.7E-09	15	2.5E-08	1	1.7E-09
2	1	1.7E-09	0	<1.7E-09	9	1.5E-08	1	1.7E-09
3	10	1.3E-08	0	<1.3E-09	16	2.0E-08	5	6.3E-09
AVG.	**4.7**	**6.4E-09**	**0**	**<1.5E-09**	**13.3**	**2.0E-08**	**2.3**	**3.2E-09**

- All 14 of the 1X CD201-selected colonies failed to demonstrate ≥2-fold increases in MIC
- No mutants were selected for CD201 at 2X MIC
- 45 out of the 47 total COL-selected colonies demonstrated ≥2-fold shifts in MIC

*Assessed by triplicate plating of ~$7x10^8$ CFU on agar plates containing 1 or 2X MIC for CD201 and COL; putative mutant colonies isolated after 48 h and evaluated by MIC

3) Resistance potential: Activity vs. COL^R mutants

Strain		MIC (µg/mL) CD201	COL	PMB	MERO
E. coli ATCC 25922 WT		2	0.25	0.25	0.015
COL^R spontaneous mutants (n=45)	MIC_{50}	4	16	8	0.03
	MIC_{90}	4	16	8	0.03
	range	2-8	8-16	4-16	0.015-0.03
Avg. fold-shift change in MIC		**1.7**	**49.1**	**31.6**	**1.8**

- Cross-resistance analysis of CD201 vs. the 45 COL-selected mutants resulted in <2-fold shifts in MIC on average compared to 49-fold for COL

Conclusions

- These in vitro data reflect the intrinsic LPS-binding–based antibacterial activity of CD201
  - Consistent MICs of 2-4 µg/mL across WT G-neg species of interest
  - Bactericidal mechanism of action
  - Lower potential for resistance development in *E. coli* than COL
  - Cross-resistance only observed vs. a subset of chromosomal COL^R mutants, and no cross-resistance observed vs. *mcr-1/2* strains
- This promising in vitro activity is independent of the potential added benefits from CD201 immunotherapeutic activity in vivo