Pseudomonas aeruginosa PcrV and Psl, the Molecular Targets of mAb MEDI3902, are Conserved Among Diverse Hospital Isolates Collected from an International Surveillance Study


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Abstract

Background: Pseudomonas aeruginosa (Pa) is a frequent cause of life-threatening infections in mechanically ventilated patients and is associated with high mortality rates. Bispecific monoclonal antibodies (MEDI3902 targeting T3S injectisome and the Psl exopolysaccharide) is currently under phase 2b development for the prevention of pneumonia in mechanically ventilated subjects with Pa colonization in the lower respiratory tract. In this study, we sought to survey a vast collection of global Pa clinical isolates for presence of pcrV and psl loci and MEDI3902 epitope conservation to evaluate the magnitude of Pb strain coverage by MEDI3902.

Methods: 913 Pb clinical isolates were collected from diverse patients and geographical locations in 2004-2014. Whole genome sequencing of the full collection was performed via MiSeq 2 x 250 runs (Illumina®). PcrV and Psl expression was determined by immunodotting and ELISA, respectively. The crystal structure of anti-PcRV and anti-Psl fragment was solved at 2.8 Å resolution. MEDI3902 activity against representative isolates was tested in cytostaticity and opsonophagocytosis assays and in a murine pneumonia model.

Results: Whole genome sequencing revealed intact pcrV and psl genetic elements in 99% and 94% of isolates, respectively. We identified 46 variants of pcrV (+100 ng) and anti-PcRV (11.4 mpk) and anti-Psl killing activity. Similarly, anti-PcRV activity against representative strains in vitro and in vivo.

Conclusions: Our results indicate PcrV and Psl are highly prevalent in recent clinical isolates from around the world, suggesting MEDI3902 can mediate broad coverage against Pa.

Introduction

• We have described mAbs targeting the P. aeruginosa T3S protein PcrV (Warner et al., 2014) and Psl Eps (Oganesyan et al., 2014), which are components of bispecific clinical candidate MEDI3902 (DiGiandomenico et al., 2014)
• Psl Eps is an abundant, serotype-independent surface polymer that is important in tissue colonization, biofilm formation (persistence) and immune evasion

Methods

PcrV

Results

• Among Diverse Hospital Isolates Collected from an International Surveillance Study (n=913) were collected from diverse patients and geographic locations (depicted) between 2003-2014. Reference genomes PAO1, PA7, and PA14 are marked. Presence (green) or absence (red). Isolates are colored according to their region and highlighted with year and geographical region of collection along with psl operon and PcrV-1 expression. A neighbor-joining tree with ignored branch lengths is decorated with variable substitutions for 100% (10/11) expressed the Psl exopolysaccharide.

Figure 1: P. aeruginosa Psl Eps and the T3S injectisome

• MEDI3902 protects P. aeruginosa cytotoxicity against anti-PcRV and promotes Psl opsonophagocytic killing activity (anti-Psl)

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Figure 3: Summary of psl operon and pcrV surveillance data from 913 P. aeruginosa clinical isolates from worldwide patients.

• Psl expression was determined by immunodotting and ELISA, respectively. The crystal structure of anti-PcRV and anti-Psl fragment was solved at 2.8 Å resolution.

Figure 4: MEDI3902 maintains binding to all 46 PcrV variant sequences. Western immunodotting with anti-PcRV (V2L2MD) against representative strains from each PcrV variant sequence. (V2L2MD) reconformed PcrV (10 ng), anti-PcRV (11.4 mpk) raised in rabbits.

Figure 6: Identification of T3S-1axose and exounnate negative (PA7-like) isolates. Core conserved gene of 913 P. aeruginosa genomes. While the majority of strains are T3S+ a small proportion are T3S-

Figure 7: T3S-1axose negative isolates are susceptible to anti-Psl-mediated opsonophagocytic killing activity. (A) PA7-like isolates are cytotoxic to an epithelial cell line. (B) Opsonophagocytic killing activity assay.

Conclusions

• Whole genome sequencing analysis of 913 geographically and infection type diverse clinical isolates revealed high prevalence of T3S PaV and Psl exopolysaccharide (99.9% of isolates harbor genetically intact pcrV and psl loci; 95.2% of isolates contain the required psl genetic elements; 96.7% contain full-length pcrV); 1.2% (n=11) of isolates were T3S-deficient (PA7-like) but 91% (10/11) expressed the Psl exopolysaccharide.

• Anti-Psl and MEDI3902 mediated potent OPK and in vivo activity against PA7-like isolates

• MEDI3902 should provide broad coverage against P. aeruginosa, even in the absence of T3S

References

1. Byrd et al., 2009 Mol. Micro. 73:622