Phylogenetic and alpha toxin variant analyses of *Staphylococcus aureus* strains isolated from patients during the SAATELLITE study

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Abstract

Introduction

Suvratoxumab (formerly MEDI4893) is a human monoclonal antibody that neutralizes *S. aureus* (SA) alpha toxin (AT). SAATELLITE, a phase 2 study of safety and efficacy of suvratoxumab for reducing incidence of SA pneumonia (NCT02296320), was conducted and recently completed within the consortium for Combatting Bacterial Resistance in Europe. Through whole genome sequencing and analysis we investigated the conservation of the suvratoxumab target region in AT, the activity of survratoxumab against AT variants, that suvratoxumab did not induce escape mutants and that the presence of stop codons in AT was not associated with pneumonia incidence or suvratoxumab treatment.

Materials and Methods

A total of 304 SA isolates (baseline, onset and last available isolates from suspected serious bacterial infections, SSBIs) collected from the lower respiratory tract samples from 165 subjects during SAATELLITE were subjected to whole genome sequencing.

AT gene (*hla*) sequences were translated and amino acid variation was identified in comparison to the reference SA USA300 FPR3757. Phylogenetic analysis, genomic annotation and ST analysis were performed.

AT expression in SA culture supernatants was performed by ELISA. Representative isolates with novel AT subtypes that had not been identified in previous studies were tested for hemolytic activity and suvratoxumab neutralizing activity.

Wilcoxon rank sum test and Fisher's exact test were performed, respectively: a) to compare difference in baseline AT expression in relation to SA pneumonia incidence; b) to evaluate the association between occurrence of AT stop codons and incidence of SA pneumonia at baseline, as well as the association between occurrence of AT stop codons and treatment arms at post baseline.

Results

We identified a total of 44 sequence types (STs) and 21 unique AT subtypes, 7 of which have not been described previously. No substitutions were located in the suvratoxumab binding region and all novel AT subtypes displaying lytic activity were neutralized by suvratoxumab.

We detected stop codons Q113B and W205B in AT sequences in 53 and 2 SA isolates, respectively. We uncovered no significant associations of: 1) baseline AT expression with SA pneumonia incidence [p=0.967]; 2) occurrence of AT gene stop codon with either SA pneumonia incidence [p>0.999] or suvratoxumab treatment [p=0.103; lower frequency of stop codons in suvratoxumab arm versus placebo].

Conclusion

Our data indicated that: 1) suvratoxumab target region in (AT) remains conserved; 2) suvratoxumab is active against all AT variants identified to date; 3) suvratoxumab did not exert pressure on SA clinical isolates for selection of escape mutants.

Background

- *Staphylococcus aureus* causes serious infections that increase morbidity and mortality including ventilator-associated pneumonia (VAP)[1].
- Alpha-toxin (AT) is a pore-forming toxin, encoded by the *hla* gene, that plays a key role in *S. aureus* pathogenesis[2].
- Suvratoxumab (MEDI4893) is a monoclonal antibody targeting *S. aureus* AT[3].
- Safety and efficacy of suvratoxumab (MEDI4893) for reducing incidence of SA pneumonia was evaluated in a phase 2 randomized SAATELLITE study[4].
- In this study we tested neutralizing activity of suvratoxumab against novel AT subtypes and examined difference in baseline AT expression in relation to SA pneumonia incidence, the association between occurrence of AT stop codons and incidence of SA pneumonia at baseline, as well as the association between occurrence of AT stop codons and treatment arms at post baseline.

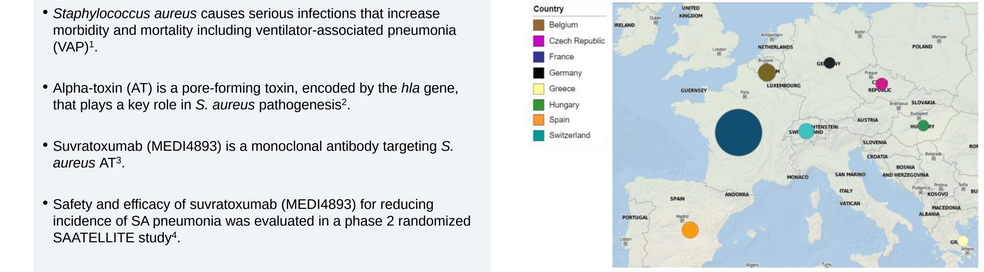
Results

Table 1. Sample collection and *hla* sequencing metrics

Metrics	Number
Number of patients	165
hla (AT gene) sequences obtained	304
AT subtypes	21
Novel subtypes	7 (13 isolates; 10 patients)
Isolates with stop codon in *hla*	55 (36 patients)
Stop codon types	2

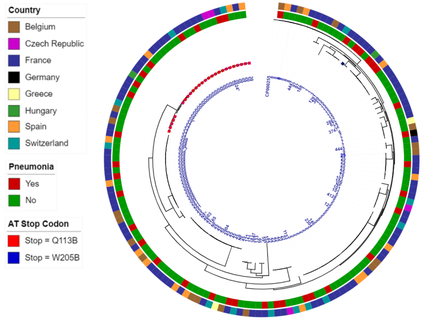
- *S. aureus* isolates were collected from 165 patients
  - 304 *hla* sequences were obtained from all isolates including baseline and later timepoints
    - 21 AT protein subtypes were identified
    - 7 AT protein subtypes were novel and not described previously
  - *hla* genes containing stop codons were identified in 36 patients
    - Q113B has been described previously
    - W205B is novel

Figure 1. Countries of sample collection



- Samples were collected from 8 countries
- Collection counts are shown relative to plot area

Figure 2. Whole genome-based phylogenetic analysis



- The phylogeny of baseline *S. aureus* isolates was analyzed in the context of country of collection, progression to pneumonia and presence of AT stop codons
- There was no apparent association of country, lineage or presence of AT stop codons with progression to pneumoniae

Table 2. Variation in the AT protein (Subtypes) observed in baseline *S. aureus* isolates

AT AA substitutions vs. USA300 reference	Count	Stop Codon	AT Subtype[5]	Lytic[5]	Neut[5]
Identical to Reference CP000255_1058_AT	30		1	NA	NA
Q90R	1		novel	Yes	Yes
D234E	2		3	Yes	Yes
I301T	31		4	Yes	Yes
D234E:I301T	68		11	Yes	Yes
-9.1T:D234E:I301T	1		novel	Yes	Yes
D234E:I301T:K314N	8		23	Yes	Yes
N100K:D234E:I301T	1		28	No	No
L78I:P117S:T155S:I301T	35		33	Yes	Yes
L78I:T155S:H285Y:I301T	5		34	Yes	Yes
L78I:T155S:S265T:I301T	7		39	Yes	Yes
L78I:P117S:T155S:V273I:I301T	1		novel	Yes	Yes
L78I:T155S:D234E:S265T:I301T	6		42	Yes	Yes
L78I:T155S:S265T:I301T:S304C	28		43	Yes	Yes
L78I:D154Y:T155S:D234E:S265T:I301T	1		novel	Yes	Yes
L78I:T155S:S265T:I301T:D302N:S304C	3		novel	Yes	Yes
T45I:L78I:T155S:S265T:T269S:I301T	14		51	Yes	Yes
D39N:T45I:L78I:T155S:S265T:T269S:I301T	4		novel	Yes	Yes
M20I:N25K:S29T:L78I:T155S:N165S:V201M:S265F:I301T	3		53	Yes	Yes
W205B	2	Y	novel	Yes	NA*
L78I:Q113B	53	Y	45	No	NA
SUM:	**304**				

- 21 unique AT protein Subtypes were observed in baseline isolates
  - 7 novel AT protein Subtypes were identified (red)
  - Subtypes were tested for their lytic activity and all were shown to be neutralized by suvratoxumab

Table 3. Fisher's Exact Test of AT stop codon presence and patient progression to pneumonia

	SA Pneumonia		
Stop codon (Y/N)	No (n=98)	Yes (n=36)	P-value
Y	18 (18.4%)	7 (19.4%)	>0.999
N	80 (81.6%)	29 (80.6%)	

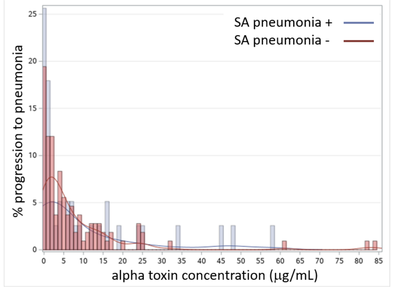
	Treatment		
Stop codon (Y/N)	MEDI4893 5000 mg (n=24)	Placebo (n=37)	P-value
Y	2 (8.3%)	10 (27.0%)	0.103
N	22 (91.7%)	27 (73.0%)	

- There was no evidence of association between the occurrence of AT gene stop codons and:
  - SA pneumonia incidence
  - suvratoxumab treatment (lower frequency of stop codons in suvratoxumab arm versus placebo)

Table 4. Wilcoxon Rank Sum test of baseline AT *in vitro* expression level and patient progression to pneumonia

SA pneumonia	N	Mean	Median	Min	Max	Q1	Q3	SDEV	P-value
Yes	39	9.67	3.20	0.05	58.00	0.40	13.70	14.50	0.9667
No	108	7.96	3.60	0.05	84.10	0.63	9.75	13.50	

Figure 3. Plot of baseline AT *in vitro* expression levels and % patient progression to pneumonia.



- There was no statistically significant relationship between baseline AT
- *in vitro* expression levels and patient progression to pneumonia

Conclusions

- Phylogenetic analysis did not suggest relationships between lineage and country of collection
- Novel AT Subtypes were tested for their lytic activity and all were shown to be neutralized by suvratoxumab
- Suvratoxumab target region in (AT) remains conserved
- Statistical analysis did not reveal significant relationships between patient progression to pneumonia, AT stop codon presence and baseline AT *in vitro* expression levels.

References

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