Understanding how azithromycin reduces asthma exacerbations and the underlying mechanisms of macrolides

Lisa M Jurak[1], Ian A Yang[1, 5], Jodie L Simpson[3], Michelle M Hill[1,4] and John W Upham[1,2]

1. The University of Queensland Diamantina institute, Woolloongabba, QLD 2. Princess Alexandra Hospital, Woolloongabba, QLD 3. Hunter Medical Research Institute, Newcastle, NSW 4. QIMR Berghofer Medical Research Institute, Brisbane, QLD 5. The Prince Charles Hospital, Brisbane, QLD.

Introduction

- Exacerbations contribute significantly to the disease burden of asthma. Our ability to identify people at risk is limited to non-specific clinical variables.

- Macrolide antibiotics are recommended as add-on therapy due to their unique anti-inflammatory and anti-microbial properties.

-Randomised control trials such as the AMAZES study have successfully shown the benefit of azithromycin (AZM) in reducing exacerbations in those with severe, persistent asthma (Figure 1).

This study aims:

1) To elucidate proteomic changes due to AZM treatment in sputum samples collected during the AMAZES

2) To understand the mechanisms of how AZM reduces exacerbations

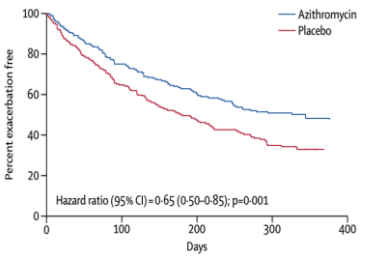

Figure 1: Proportion of patients free from an asthma exacerbation for one year according to treatment group during the AMAZES study.

Methods

52 Samples (balancing clinical variables)

Data dependent acquisition (Discovery proteomics)

Data comparison to human proteome database

Statistical analysis:
Univariate- Differentially enrichment of proteins
Multivariate- sparse partial least squares discriminate analysis (sPLS-DA)

Results

- Univariate analysis identified 1400 proteins common proteins among all conditions, 240 were unique to azithromycin treatment.
- 32 proteins were differentially enriched due to AZM treatment and a further 90 that were enriched between AZM and placebo-treated samples.
- No differences observed between baseline and placebo treated samples.

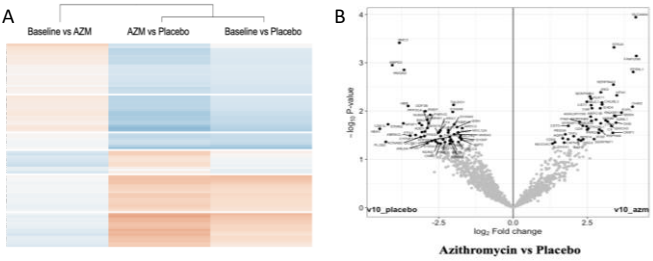

Figure 2: Differentially expressed proteins that explain changes in the sputum proteome due to azithromycin treatment. A) Heatmap of direct contrasts as a result of treatment B) Volcano plot identifying significantly enriched proteins.

- Multivariate modelling partially explains the variance of the data associated with exacerbations and AZM treatment (ROC = 0.65, error rate 30%).

-The model captures the complexity of exacerbations and highlights potential biomarkers that may indicate response to AZM treatment.

-Pathway analysis shows overexpression of both known and unknown pathways associated with AZM.

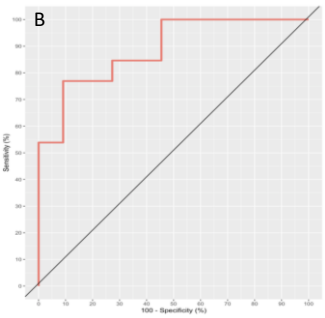

Figure 3: A multivariate signature that explains the variance between exacerbations and AZM treatment A) sPLS-DA model B) ROC curve of sPLS-DA model.

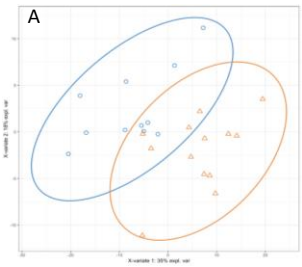

Table 1: Summary of preliminary pathway analysis of azithromycin treatment based on univariate and multivariate modelling.

Pathway changes due to azithromycin	
Pathway name	**P-value**
Remodelling of Epithelial junctions	3.44E-05
Signalling in Neutrophils	6.52E-05
IL-1 signalling	1.29E-04
Phagosome Maturation	1.53E-04
Apoptosis and Phagocytosis	2.14E-3
Nitric Oxide and Reactive oxygen species in Macrophages	5.09E-04
CCR3 Signalling in Eosinophils	5.15E-04
Toll-like receptor signalling	7.07E-04
Chemokine signalling	8.58E-04
Natural Killer cell signalling	3.97E-03

Conclusion

The sputum proteome provides a unique insight into the mechanisms of AZM. Statistical modelling identified potential biomarkers for validation that are representative of both azithromycin treatment and a reduction in exacerbations.

Pathways associated with these signatures appear to be phenotype specific. This knowledge will facilitate development of novel treatment options for severe persistent asthma.

Lung and Allergy Research Centre
l.jurak@uq.edu.au