**O107**

**2-hour Oral Session**

## Different potency of azithromycin and clarithromycin in the inhibition of mTOR signalling in CD4+ T-cells

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### Background

Macrolides are a group of antimicrobial agents with immunomodulatory properties, showing beneficial clinical effects in inflammatory disorders including chronic obstructive pulmonary disorder, cystic fibrosis, diffuse panbronchiolitis and bronchiolitis obliterans. It can be assumed that these effects also occur in patients without any need of immunomodulation. Thus, definition of the immunosuppressive effects on diverse immune cell subsets is of clinical importance. Along those lines we aimed to define the effects of AZM and CLM on $CD4^+$ T-cell function.

### Material and Methods

Upon exposure to AZM and CLM (0.6-40 mg/L), peripheral blood $CD4^+$ T-cells from healthy volunteers were stimulated with agonistic anti-CD3/anti-CD28 monoclonal antibodies. Cell proliferation was measured using thymidine incorporation assays and fluorescent tracers. Secretion of effector cytokines was assessed after 24h (IL-2) and 72h (IL-4, IL-10, IL-13, IL-17 and IFN-gamma) from the corresponding supernatants using multiplex analysis. Cell viability was quantified by annexin V/propidium iodide staining and measurement of caspase 3/7 activity. Intracellular signaling was evaluated using flow cytometry, immunoblotting, reporter cell lines and in vitro kinase assays to identify the mechanism of action.

### Results

Exposure to AZM resulted in a dose-dependent inhibition of T-cell proliferaton. In contrast, the immunosuppressive capacity of CLM was about four-fold lower. Concomitantly, AZM led to a significant suppression of IL-2, IL-10, IL17 and IFN-gamma secretion at a concentration of 10 mg/L and 40 mg/L. Similarly, a significant suppression of cytokine secretion could be observed upon exposure to 40 mg/L CLM but not at 10 mg/L CLM. At AZM concentrations up to 10 mg/L and CLM concentrations up to 40 mg/L, no significant decrease in cellular viability or increase in caspase 3/7 activity could be detected. In line with previous reports, AZM led to a sharp decrease in cellular viability at 40 mg/L, which was also reflected by a two-fold increase in caspase 3/7 activity. Analysis of intracellular signaling revealed that AZM and CLM did not affect NFAT, NF-kB and MAPK signaling. Using the mTOR downstream target S6RP, we showed that AZM inhibited mTOR signaling in a dose-dependent manner. Similarly, at 40 mg/L, CLM significantly inhibited S6RP phosphorylation. Using in vitro kinase assays, we observed that AZM suppresses mTOR activity independently of the presence of FKBP12.

### Conclusion

These data describe for the first time the immunosuppressive activity of macrolides on $CD4^+$ T-cells, showing a higher potency for AZM than for CLM. This effect is mediated by the FKBP12-independent inhibition of mTOR signaling. These finding should give impetus to conduct further basic and clinical studies.