

Title: Novel-generation adjuvants for TB vaccine based on the effective combination of delivery systems and TLR- and NON-TLR immunomodulators

It is becoming increasingly clear that for peptide- or protein subunit vaccines, amplification of T cell priming through the fine tuned combination of antigen uptake and dendritic cell (DC) activation is necessary to elicit optimal protective immune responses for a large range of diseases. This is most efficiently obtained with adjuvant systems that are based on a delivery vesicle that serves to promote uptake and presentation of the vaccine antigen in the relevant subset of antigen-presenting cells (APC), whereas the immunomodulator activate the APC. In our search for adjuvants for use in TB vaccines, we have identified two very effective formulations building on this two component principles.

The IC31 adjuvant consists of a vehicle based on the cationic peptide KLKL(5)KLK and the immunostimulatory oligodeoxynucleotide ODN1a signalling through the TLR9 receptor. In combination with the TB vaccine candidate, Ag85B-ESAT-6, a Phase I trial performed in healthy BCG-naïve volunteers obtained promising safety and immunogenicity data with strong T-cell responses in humans that confirmed all pre-clinical data showing similar prominent Th1 responses.

The CAF01 adjuvant, consisting of DDA as a delivery vehicle and synthetic mycobacterial cordfactor (TDB) as immunomodulator, was found to promote similar high levels of Th1 responses. Recently, TDB was shown to induce very efficient activation of murine macrophages *in vitro* with a distinct activation pattern compared to a conventional TLR9 ligand, CpG. This TDB-mediated activation was found to involve the kinase Syk-Card9 signalling pathway. *In vivo*, the adjuvant containing TDB was found to induce strong and complex immune responses characterized by the simultaneous induction of both Th1 and Th17 responses which are found protective in a mouse model of TB.