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# Blocking IL1, IL33 and IL36 signaling by an anti-IL1RAP antibody is an efficient anti-inflammatory treatment that improves heart function in a model of autoimmune myocarditis.

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## INTRODUCTION

The IL1 receptor accessory protein (IL1RAP) is a coreceptor for the IL1, IL33, and IL36 receptors. We have developed a fully humanized IgG1-LALA antibody (CAN10) that binds IL1RAP with high affinity (Kd=2.53 nM) and disrupts IL1α, IL1β, IL33, IL36α, IL36β and IL36γ signaling, without inducing ADCC. CAN10 is currently undergoing preclinical development in preparation for clinical studies.

IL1, IL33 and IL36 may have disease promoting roles in myocarditis, an inflammatory heart disease with no available treatment. The studies here aim to describe the potential of IL1RAP blockade in inflammation in general and in myocarditis in particular.

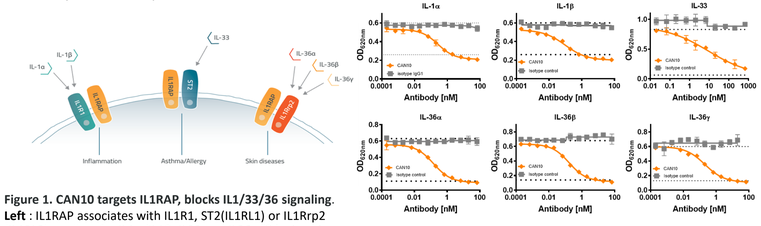



**Figure 1. CAN10 targets IL1RAP, blocks IL1/33/36 signaling.**
**Left** : IL1RAP associates with IL1R1, ST2(IL1RL1) or IL1Rrp2 (IL1RL2) to allow for IL1α/IL1β, IL33 and IL36α/β/γ signaling.
**Right :** CAN10 inhibits signaling from IL1α and IL1β ($IC_{50}$ = 0.268 and 0.097 nM), IL33 ($IC_{50}$= 7.508 nM) and IL36α/β/γ ($IC_{50}$ = 0.147, 0.169, 0.206 nM) in HEK cells transfected with the different receptors and a NF-kB/AP-1 inducible SEAP reporter gene. CAN10 was added in increasing concentrations after stimulation with the indicated cytokines and reporter gene activation was measured.

## STUDY OBJECTIVES

- To study the potency and benefit of IL1RAP-blockade as an anti-inflammatory strategy
- To study the effect of IL1RAP-targeting in experimental autoimmune myocarditis on inflammation, fibrosis and heart function

## RESULTS — Mode of Action

### IL1RAP inhibition is a potent strategy to block inflammation in a way that is not recapitulated by IL1α/β-blockade alone

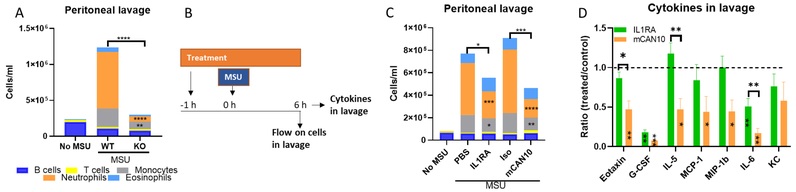



**Figure 2. IL1RAP blockade decreased inflammation in a model of monosodium urate crystal (MSU) induced acute peritonitis.** MSU was given i.p. at 0 hours and mice were terminated after 6 hours. Infiltrating cells and cytokines were measured in the peritoneal lavage. **A**. IL1RAP KO mice display a significant decreased response vs WT mice, with the largest effect on infiltrating neutrophils and monocytes. **B,C:** Treatment with mCAN10 (anti-mouse IL1RAP surrogate of CAN10, 20 mg/kg at -1h) or an equimolar concentration of IL1RA (IL1 receptor antagonist, blocking IL1α and IL1β, 2.3 mg/kg at -1h) significantly reduced the number of infiltrating neutrophils and monocytes in the peritoneal cavity. **D:** mCAN10 and IL1RA significantly decreased IL-6 and G-CSF compared to control. mCAN10 had a more potent effect on IL6 and also reduced eotaxin, IL-5, MCP-1 and MIP-1β. *p<0.05, **p<0.01, ***p<0.005, ****p<0.001.

### IL1RAP inhibition modulates local and systemic inflammation

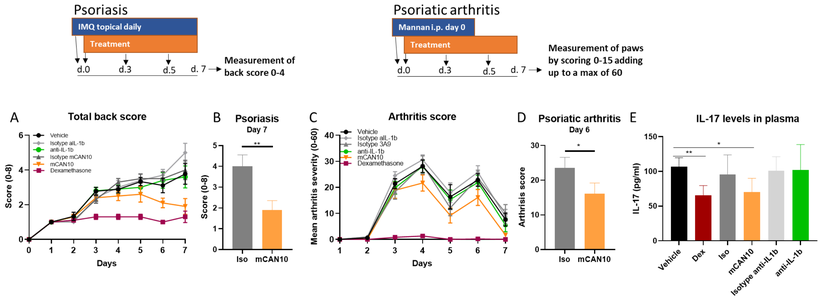
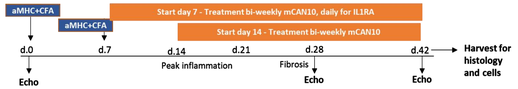



**Figure 3. mCAN10 decreases the inflammation in psoriasis and psoriatic arthritis. A-B:** mCAN10, but not anti-IL1β, reduced the skin inflammation In Imiquimod (IMQ)–induced Psoriasis. IMQ was administered daily to the back skin, and mice were scored for scaling and skin erythema. Antibodies were administered bi-weekly and daily topical dexamethasone was used as positive control. **C-E:** mCAN10, but not anti-IL1β, reduced disease severity in mannan-induced psoriatic arthritis (C,D) as well as circulating IL-17 levels compared to vehicle. (E). Mannan was injected i.p. and arthritis was scored in the paws, daily i.p. dexamethasone was used as positive control. Blood samples were taken at termination. N=10 Balb/c mice (psoriasis) or B6N.Q.NCF1 mice (psoriatic arthritis). *p<0.05, **p<0.01.

## Experimental Autoimmune Myocarditis

### Anti-IL1RAP treatment inhibits inflammation and fibrosis in the heart muscle in experimental autoimmune myocarditis (EAM)



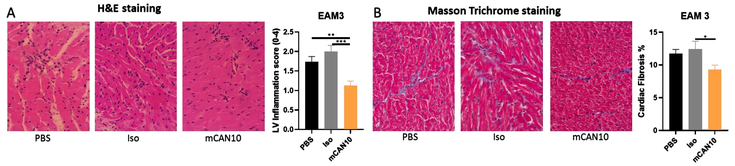




**Figure 4. mCAN10 reduces cardiac inflammation and fibrosis in experimental autoimmune myocarditis (EAM).** EAM was induced by immunizing Balb/C mice with αMHC peptide on day 0 and 7. Treatment with mCAN10 or isotype control started day 7, or day 14, and heart function was analyzed by echocardiography at days 0, 28 and 42. Hearts from mice treated from day 14 were stained with H&E, to assess the degree of inflammation, and with Masson Trichrome, to quantify the collagen percentage. **A:** mCAN10 significantly decreased the infiltrating inflammatory cells in the myocardium. **B:** mCAN10 decreased the cardiac fibrosis in the myocardium as depicted in blue stained area (collagen) and in percentage compared to isotype control. Balb/c n=10, αMHC: alpha myosin heavy chain peptide (100 ug), CFA: Freund's complete adjuvance *p<0.05, **p<0.01, ***p<0.005

### IL1RAP blockade, but not anti-IL1β, IL1RA or prednisone, has a therapeutic effect on heart function in EAM

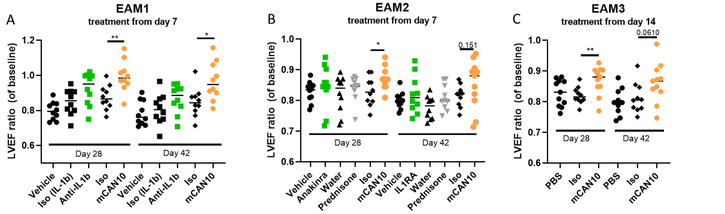
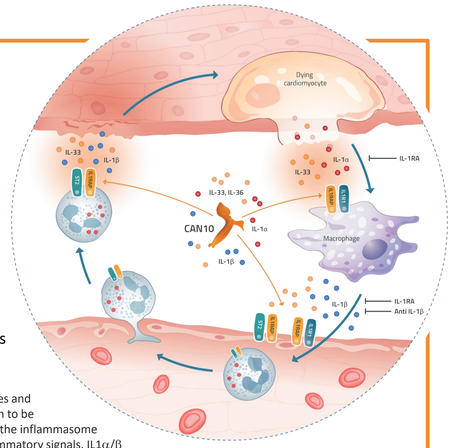



**Figure 5. mCAN10 counteracts deterioration in heart function in experimental autoimmune myocarditis at both early and late treatment start.** LVEF (left ventricular ejection fraction) was measured by echo cardiography at day 28 and 42 after the first αMHC immunization. **A:** mCAN10 significantly preserves heart function compared to isotype, while anti-IL1β treatment does not. **B:** mCAN10 significantly preserves heart function compared to isotype, whereas IL1RA and prednisone does not. **C:** Late treatment start with mCAN10, day 14 compared to day 7, still rescues cardiac function.

## SUMMARY

- CAN10 blocks IL1, IL33 and IL36 signaling through targeting of IL1RAP and is in late-stage preclinical development for clinical trials
- Blocking IL1RAP is a potent anti-inflammatory strategy that is qualitatively different from pure IL1α/β blockade.
- IL1RAP-blockade counteracts inflammation, fibrosis and the decrease in heart function in experimental autoimmune myocarditis
- These studies highlight the potential of CAN10 to treat inflammatory diseases, including myocarditis




**Myocarditis model: IL1RAP blockade by CAN10 decreases inflammation and improves cardiac function in mycoarditis.**
Inflammation in the myocardium causes cell death in cardiomyocytes and subsequent release of alarmins such as IL1α and IL33. IL1α is known to be involved in the pathogenesis of myocarditis leading to activation of the inflammasome in macrophages, release of IL1β, and further downstream pro-inflammatory signals. IL1α/β and IL33 can also activate the endothelia and increase the influx and activation of immune cells. IL36 is less described may also have a role in myocarditis since blockade of IL36 improves cardiomyocyte survival in a model of ischemia/reperfusion. As the cardiac inflammation progresses, fibrosis will build up and the myocardium will lose contractile function, which can eventually lead to heart failure. Blocking IL1RAP in myocarditis patients may protect against disease development at several points, by blocking the function of alarmins released from dying cells, by blocking inflammasome-induced inflammation and by decreasing infiltration and activation of immune cells to the heart.

*References: 1) De Luca et al, 2018, Front. Immunol. 9;1355 2) Abbate et al, 2020, Circ. Res;126, 3) Choi et al., 2020, Cell Reports;30. 4) Luo et al., 2020, Med Sci Monit;26.*

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