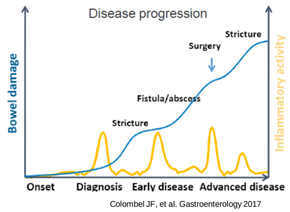
Poster #P201

IL23R-CAR-Tregs – a novel approach to treat Crohn's disease (CD) patients

J. Gertner-Dardenne, T. Abel, S. Boulakirba, D. Fenard, C. Dumont, M. David, C. Gendrin, M. Ayrault, G. Lara, S. Rouquier, Y. Cui, M. de la Rosa

1- Crohn's Disease (CD) and IL-23/ IL23-receptor (IL23R) axis

- CD is an Inflammatory Bowel Disease (IBD) which is characterized by a chronic relapsing and remitting inflammation of the gastrointestinal tract.
- Symptoms include diarrhea, abdominal pain, weight loss and bloody stools.
- Standard of care is targeting the active inflammatory pathways in CD:
  - chronic anti-TNFs+/- steroid are treatment mainstay.
  - 2nd line MoA include anti-α4β7 integrin and anti-IL-12/23.

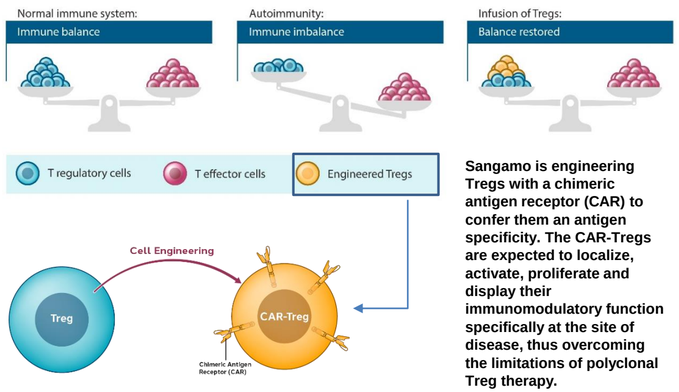


Colombel JF, et al. Gastroenterology 2017

- **Preclinical and clinical data suggest a critical role of the IL-23/Th17 axis in disease pathogenesis :**
  - Several studies have demonstrated a genetic linkage of the IL23R to susceptibility to human autoimmune diseases, including psoriasis, ankylosing spondylitis, and Crohn's disease[1-3]. This suggests that IL-23 is crucial in the pathogenesis of autoimmune diseases.
  - Generation of pathogenic Th17 responses *in vivo* is impaired in both IL-23 and IL23R deficient mice[4-6].
  - Antibody blocking IL-12 and IL-23 have shown promising results in treating IBD patients [7].

2- IL23R-CAR-Treg concept: restore immune homeostasis in CD

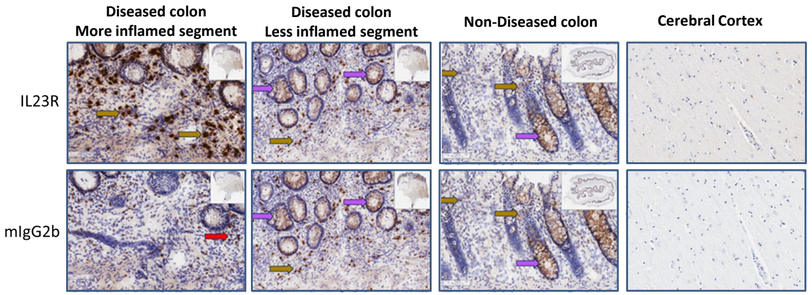
- Regulatory T cells (Tregs) are a subset of immune cells with potent **immunomodulatory** functions, such as maintaining immune homeostasis and immune tolerance, as well as regulating pathogenic proinflammatory immune responses.
- Tregs can be used as a **cell therapy** across various applications where induction of immune tolerance can restore homeostasis and counter disease-state.
  - ➔ Polyclonal Treg have already been used in the clinic and showed a **good safety profile** but a limited efficacy due to a lack of specificity [8-11].



Sangamo is engineering Tregs with a chimeric antigen receptor (CAR) to confer them an antigen specificity. The CAR-Tregs are expected to localize, activate, proliferate and display their immunomodulatory function specifically at the site of disease, thus overcoming the limitations of polyclonal Treg therapy.

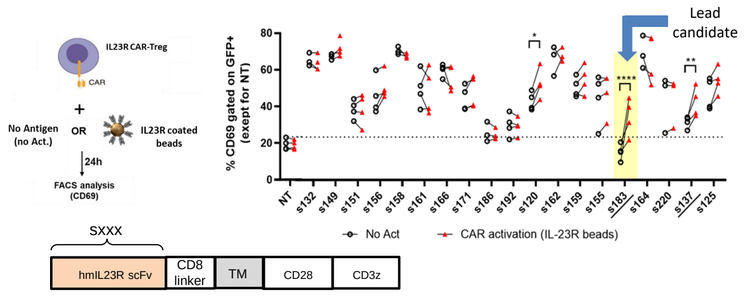
3- IL23R is overexpressed in the gut of CD patients

- Clear evidence of the presence of IL23R (in brown) at the surface of infiltrating cells in diseased colon compared to non-diseased colon (in lamina propria).
- In CD patient, expression of IL-23R is increased in the more inflamed segments compared to the less inflamed segment.
- High number of inflammatory cells in lamina propria with very strong IL-23R staining.



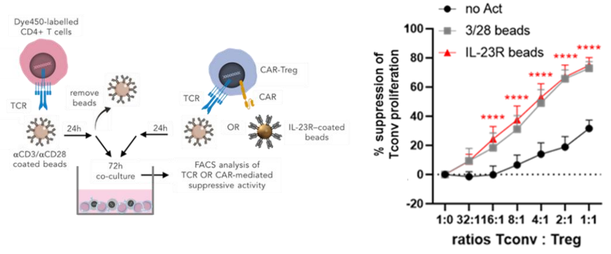
4- *In vitro* selection of an scFv with the lowest background of activation for IL23R-CAR-Treg product candidate

- Selection criteria : (i) binding to IL23R ; (ii) lowest background of activation; (iii) significant CAR-mediated activation



➔ **Sangamo's IL-23R-CAR lead candidate is a second-generation CAR, composed of an anti-IL-23R-scFv identified through a large library screening which obtained 120 potential hits.**

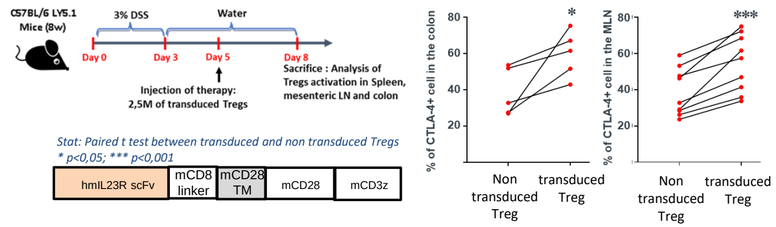
5- *In vitro* IL23R-CAR mediated suppressive activity



➔ **Tregs transduced with the IL23R-CAR lead candidate mediate efficiently and significantly their suppressive function through their CAR.**

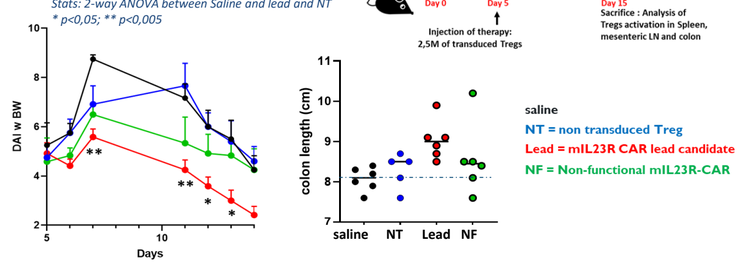
6- Mouse IL23R-CAR-Tregs are found and activated in the targeted organs (colon / MLN)

- A short dextran sodium sulfate (DSS) mouse model was developed to prove that IL-23R-CAR-Tregs migrate to the site of inflammation, engage the target and get activated upon target encounter.
- Three days after injection IL23R-CAR-Tregs were found in the colon and mesenteric lymph nodes (MLN) and showed a significantly higher activation than the controls.



7- IL23R-CAR-Tregs significantly reduce peak of disease, DAI and intestinal inflammation

- Evaluation of efficacy was measured in a DSS induced mouse model of IBD.
- IL23R-CAR-Treg (in red) induced a higher reduction of the peak of the disease compared to other groups. Reduction was maintained until sacrifice.
- The impact of IL-23R CAR-Tregs on the intestinal inflammation was assessed through the measurement of colon length (longer length indicates less inflammation); IL-23R CAR-Tregs had a better beneficial effect on colon length compared to polyclonal Tregs (NT or NF) or saline control.



Conclusions

- IL23R was overexpressed in diseased colon of CD patients compared to healthy controls.
- IL23R-CAR showed high specificity to IL-23R with an undetectable background of activation in comparison to controls.
- IL23R-CAR-Tregs showed a specific and significant CAR-dependent suppressive activity *in vitro*.
- *In vivo*, IL-23R CAR-Tregs were able to locate to targeted organs, activate and show biological activity on DAI and colon length in DSS mouse models.
- **Overall, these data highlight that an IL-23R-CAR-Treg cell therapy could represent a promising therapeutic option for the treatment of CD. This mode of action may also pave the way for treating other chronic diseases involving IL-23R.**

References

1- Duerr et al., 2006; 2- Cargill et al., 2007; 3- Liu et al., 2008; 4- Langrish et al., 2005; 5- Awasthi et al., 2009; 6- McGeachy et al., 2009; 7- Sandborn et al, 2012; 8- Brunstein et al., 2011; 9- Edinger et al. 2011; 10- Marek-Trzonkowska et al. 2012; 11- Trzonkowski et al. 2009.

Disclosures

All authors are employees of Sangamo Therapeutics

Presented at ESGCT – 19-22 October 2021