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


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

- CD is an Inflammatory Bowel Disease (IBD) which results in 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-TNFα-steroids are treatment mainstay.
  - 2nd line includes anti-α4β7 integrin and anti-IL-12/23.

- Preclinical and clinical data support 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.
  - This suggests that IL-23 is crucial in the pathogenesis of autoimmune diseases.
  - Generation of pathogenic Th17-responders in vivo is impaired in both IL-23 and IL-23R deficient-mice.
  - Antibody blocking IL-23 and IL-23R have shown promising results in treating BD patients.

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

- Regulatory T-cells (Tregs) are a subset of immune cells with potent immunoregulatory functions, such as maintaining immune homeostasis and immune tolerance, as well as regulating pathogenic pro-inflammatory 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.

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 IL23R is increased in the more inflamed segments compared to the less inflamed segment.
- High number of inflammatory cells in lamina propria with very strong IL23R staining.

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

- Selection criteria: (a) binding to IL23R; (b) lowest background of activation; (c) significant CAR-mediated activation

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 IL23R-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 (MNL) 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-Tregs (in red) induced a higher reduction of the peak of the disease compared to other groups.
- Reduction was maintained until sacrifice.
- The impact of IL23R-CAR-Tregs on the intestinal inflammation was assessed through the measurement of colon length (longer length indicates less inflammation).

References
1- Duer et al., 2005; 2- Capgall et al., 2007; 3- Liu et al., 2008; 4- Langrish et al., 2005; 5- Awasthi et al., 2009; 6- McGeehy et al., 2000; 7- Darnell et al., 2012; 8- Shokunbi et al., 2011; 9- Edinger et al., 2011; 10- Marei-Trzonkowski et al., 2012; 11- Trzonkowski et al., 2000.

Disclosures
All authors are employees of Sangamo Therapeutics