IL21-expressing T follicular (Tfh) and peripheral helper (Tph) cells measured by FC. These generated T cells were also cultured in the presence of memory B cells. After 5 days of co-culture, plasmablast generation and Ig levels were assessed by FC and ELISA, respectively. Inhibition of OX40-OX40L interaction in vitro was achieved using ISB 830, a novel anti-OX40 mAb currently used in clinical trials.

Results: Among the co-stimulatory molecules tested, percentages of OX40L⁺ neutrophils in SLE (n=54) were increased compared to HD (n=25)(mean + SD: HD = 1.34%±1.62 vs SLE = 4.53%±8.1; p=0.29). OX40L expression positively correlated with SLE disease activity score (SLEDAI) (p = 0.04; r = 0.31) and with anti-DNA antibodies (p=0.04, r = 0.33). Of note, the percentage of OX40L⁺ neutrophils was higher in anti-sm-RNP⁺ patients (n=16, mean= 9%±9.8), compared to anti-sm-RNP⁻ patients (n=27, mean = 1.4%±2.5; p = 0.02). The percentage of OX40L⁺ neutrophils was higher in patients with class III or IV lupus nephritis, and inflammatory infiltrate within the kidney biopsy disclosed OX40L⁺ neutrophils, in close contact with T cells. Neutrophils from HD express OX40L with TLR8 agonist, or IFN α priming followed by TLR7 agonist. When memory CD4 T cells were cultured in the presence of TLR8-stimulated neutrophils, the proportion of IL21-expressing Tfh (CXCR5⁺PD1⁺) and Tph (CXCR5⁺PD1^{hi}) were increased, compared to culture with unstimulated neutrophils. This process was dependent on OX40-OX40L interactions, since in vitro treatment with the anti-OX40 blocking antibody ISB 830, inhibited the differentiation of memory T cells into Tfh and Tph. Both generated Tfh and Tph were able to promote the differentiation of memory B cells into Ig-secreting plasmablasts.

Conclusion: Our results disclose an unprecedented phenomenon where cross-talk between TLR7/8-activated neutrophils and CD4 lymphocytes operates through OX40L-OX40 costimulation, and neutrophils promote the differentiation of pro-inflammatory Tfh and Tph, as well as IL21 production. Therefore, OX40L/OX40 should be considered as a potentially therapeutic axis in SLE patients.

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THU0036 FIRST-IN-HUMAN TRIAL OF BCMA-CD19 COMPOUND CAR IN THE TREATMENT OF AUTOANTIBODY MEDIATED DISORDERS

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Background: Donor-specific anti-HLA antibodies (DSAs) are antibodies in the recipient directed against donor class I/II HLA antigens. The existence of DSAs before allogeneic hematopoietic stem cell transplantation (AHSCT) are known to cause primary graft failure. Currently there's no established method of DSA desensitization due to the long half-life of plasma cells.

Systemic lupus erythematosus (SLE) is a heterogeneous autoimmune disease involving in multiple organ systems mediated by numerous autoantibodies. Recent results have shown that depletion of B cells by CD19 CAR-T cells effectively reversed some manifestations in two SLE mouse models. However, plasma cells could be spared with single CD19 CAR-T cells, and peripheral circulating anti-DNA IgG and IgM autoantibodies remain elevated or increased in treated mice.

Objectives: We present the efficacy of BCMA-CD19 compound CAR (cCAR), which target on antibody-producing "root", both B cells and plasma cells in pre-clinical study and in our first-in-human phase 1 clinical trial.

Methods: We constructed a BCMA-CD19 cCAR composed of a complete BCMA-CAR fused to a complete CD19 CAR, separated by a self-cleaving P2A peptide. We assessed the functional activity of cCAR in co-culture assay with multiple cell lines. We also verified cCAR efficacy with two mouse models, injected with either BCMA-expressing MM.1S cells or CD19-expressing REH cells. In our phase 1 clinical trial, we enrolled patients with hematologic malignancies with antibody mediated disorders.

Results: BCMA-CD19 cCAR exhibited robust cytotoxic activity against the K562 cells engineered to express either CD19 or BCMA in co-culture assays, indicating the ability of each complete CAR domain to specifically lyse target

cells. In mouse model study, cCAR-T cells were able to eliminate tumor cells in mice injected with MM.1S cells and REH cells, indicating that both BCMA and CD19 are specifically and equally lysing B cells and plasma cells in vivo, making BCMA-CD19 cCAR a candidate for clinical use.

In our first-in-human clinical trial, the first case is a 48-year-old female patient having resistant B-ALL with high DSA titers. She exhibited complete remission of B-ALL at day 14 post-CAR T treatment. MFI of DSA dropped from 7800 to 1400 at 8 weeks post cCAR treatment, the reduction percentage was approximately 80% (Figure 1). The patient had no CRS, and no neurotoxicity was observed.

Figure 1. 1. A) MFI of DSA and other HLA antibodies before and at different time points after cCAR T infusion. B) the percent reduction post-transfusion of cCAR T cells at different time points.

The second case is a 41-year-old female patient having a refractory diffuse large B cell lymphoma with bone marrow (BM) involvement. Furthermore, she has a 20 years of SLE, with manifestation of fever dependent of corticosteroids. On day 28 after cCAR treatment, PET/CT scan showed CR, and BM turned negative. In addition, she is independent of steroids, has no fever and other manifestations, C3/C4 are within normal ranges, and all the ANA dropped significantly, especially the nuclear type ANA, which turned from > 1:1000 to be negative at day 64. She had Grade 1 CRS but with no neurotoxicity observed. The absence of B cells and plasma cells persisted more than 5 months post CAR therapy.

Conclusion: Our first in human clinical trial on BCMA-CD19 cCAR demonstrated profound efficacy in reducing DSA levels in an AHSCT candidate and ANA titer in a SLE patient. There was strong clinical evidence of depletion of antibody-producing roots, B-cells and plasma cells in both patients. Our results further suggested that BCMA-CD19 cCAR has the potential to benefit patients receiving solid organ transplants or those with other antibody-mediated diseases.

Figure 2. Reduction of different type of ANA titer at different time points.

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THU0037 SURVIVIN INHIBITS TRANSCRIPTION OF PBX1 AND SUPPORTS THE EFFECTOR PHENOTYPE OF THE MEMORY CD4 T CELLS IN RHEUMATOID ARTHRITIS

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Background: The oncogenic protein survivin is a marker of severe rheumatoid arthritis (RA). High serum levels of Survivin predict progressive joint damage¹ and poor treatment response².

Objectives: To study the role of survivin in the transcriptional regulation of phenotype in CD4⁺ T cells.

Methods: CD4⁺ T cells of RA female patients were isolated from the peripheral blood. Activated CD4⁺ cells were treated with survivin inhibitor YM155. Transcriptional analysis was done by RNAseq (Illumina) and conventional qPCR. Chromatin of CD4 cells was immunoprecipitated using polyclonal antibodies to survivin and subjected to deep sequencing (survivin ChIPseq, Hiseq2000, Illumina) and aligned to GRCh38. Statistical analysis of differentially expressed genes (DEG) was done in R-studio using Benjamini-Hochberg adjustment for multiple testing (Bioconductor, DESeq2 package).

Results: Survivin ChIPseq of the activated CD4⁺ T cells was enriched with the genes engaged in regulatory transcription factor specific DNA binding (GO:0000987, adj p=0.0005) and RNA polymerase II regulatory transcription (GO:0000978, adj p = 0.0004). Among survivin targets were the genes of HOX-B cluster and TALE family proteins MEIS, PKNOX and PBX1 controlling early leukopoiesis and T cell maturation. Inhibition of survivin in PBMC resulted in significant upregulation of PBX1 (p=0.023), MEIS3 (p=0.0036), similar tendency was observed for HOXB6 and HOXC4 genes. RNAseq analysis CD4 cells of RA patients with different transcription of PBX1, identified 1636 genes (adj p<10⁻⁵). BIRC5, coding for survivin, was 8.3 folds higher in CD4⁺ cells with low PBX1 (p=0.0005). Among the core transcription factors of T helper cell differentiation, we identified NF-κB1 and NF-κB2, TBX21, IRF4, IRF8 and STAT3, BATF and BATF3. This followed by significantly higher TNF, IFNγ and IL17A and IL17F in PBX1lo CD4 T cells. The pathway enrichment analysis of DEG identified strong over-representation of cytokine-specific genes (GO:005125, GO:0005126, GO:0048018, GO:0030545, FDR q-values 10⁻¹²-10⁻⁹). The genes of IL4, IL5, IL13, IL9, IL3 and CSF2 located within the chromosome 5 were common for all GO-lists, and were higher in PBX1lo, but none of those genes was identified by survivin-ChIPseq or PBX1-ChIPseq. Analysis of ChIPseq data identified the genes of STAT3, IRF4, IRF8 and BATF as common targets for PBX1 and survivin.

Conclusion: This genome-wide analysis indicates that survivin regulates transcription of the TALE family protein PBX1 in CD4⁺ T cells, which has essential effect for differentiation and phenotype of Th subsets. Low PBX1 in RA patients is associated with terminally differentiated effector CD4⁺ T cells.

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THU0038 DIFFERENTIAL ROLES OF TNFRI AND TNFRII IN THE MORPHOLOGY OF SECONDARY LYMPHOID ORGANS

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Background: Tumour necrosis factor (TNF) induced signaling events are important in lymphoid organ development and function, both in health and in immune-mediated inflammatory diseases such as arthritis. Important receptors involved in this process include TNF receptors (R) I and II that exert distinct functions. Mice overexpressing transmembrane (tm)TNF develop various features of chronic inflammation, including arthritis, that are mediated via TNFRI and/or TNFRII.

Objectives: To investigate the importance of TNFRI and TNFRII in spleen and peripheral lymph node (PLN) morphology in mice overexpressing tmTNF.

Methods: Spleen and PLN were collected from 13 week old mice with the following genotypes: WT, tmTNF transgenic heterozygotes (tmTNF tg HET), WT x TNFRI^{-/-}, tmTNF tg HET x TNFRI^{-/-}, WT x TNFRII^{-/-} and tmTNF tg HET x TNFRII^{-/-}. Spleens were cut and stained by immunofluorescence for B and T cell markers, imaged by confocal microscopy and analyzed using FIJI software. PLN were used for whole mount tissue staining for B and T cell markers,

cleared, 3D imaged by light sheet microscopy and analyzed using Imaris software.

Results: Although no macroscopic differences were observed, spleens of tmTNF tg HET animals exhibited an altered morphology characterized by smaller follicles (69,73 ± 72,25 μm²) compared to spleens of WT mice (139,3 ± 71,36 μm²), which was accompanied by a decrease in central T cell areas as measured by % of the total follicle (15,22 ± 9,685; WT 26,60 ± 10,46). WT mice lacking either TNFRI or TNFRII had a morphology comparable to WT (follicle size: TNFRI 234,0 ± 166,9 μm²; TNFRII 154,2 ± 87,92 μm²; % of T cell area: TNFRI 27,81 ± 11,03; TNFRII 28,14 ± 19,66). tmTNF tg x TNFRI^{-/-} exhibited a normal spleen architecture (follicle size: 239,2 ± 250,5 μm²; % of T cell area: 29,92 ± 11,46), whereas the spleen follicles of tmTNF tg x TNFRII^{-/-} mice had T cell areas (17,31 ± 8,88) that were comparable to tmTNF tg HET mice.

The size of PLN of tmTNF tg HET mice (3,74 ± 1,31 mm³) was increased compared to WT (2,41 ± 0,60 mm³), also in combination with deficiency of TNFRI (3,98 ± 2,07 mm³) or TNFRII (4,01 mm³). tmTNF tg HET mice had an increase in absolute B cell volume (0,86 ± 0,33 mm³) vs WT (0,51 ± 0,07 mm³) but no change in B cell area as % of total PLN volume. The increased B cell volume was critically dependent on TNF-RII (tmTNF tg HET x TNFRII^{-/-} (14,87). Interestingly, whereas B cell area morphology of tmTNF tg HET, tmTNF tg HET x TNFRII^{-/-} and WT x TNFRII^{-/-} PLN was similar to WT, TNFRI deficiency caused profound alterations in the morphology of the B cell areas that appeared as one peripheral layer of B cells covering a central T cell area rather than properly developed follicles.

Conclusion: The current study demonstrates that overexpression of tmTNF leads to an aberrant spleen architecture, characterized by smaller follicles and a decrease in central T cell areas, which is critically dependent on TNFRI. In addition, overexpression of tmTNF results in enlarged PLN and increased total B cell volume, which is dependent on TNFRII, whereas TNFRI is more important in the proper organization of B cell follicles. Overall, this study employing different state-of-the-art (3D) imaging techniques highlights the importance of tmTNF-TNFR-induced signaling events in secondary lymphoid organ morphology and function, and reveals distinct roles for TNFRI and II. Advancing our knowledge in this field might provide a better understanding of the pathophysiology of TNF-associated diseases such as arthritis, which may be important to develop new or improved treatment strategies.

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THU0039 DECIPHERING DISEASE-RELEVANT T CELL SUBSETS IN RHEUMATOID ARTHRITIS IDENTIFIES A NOVEL CELLULAR SUBSET OF PATHOGENETIC IMPORTANCE IN THERAPEUTIC RESISTANCE.

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Background: Aberrant T cell responses are key in driving autoimmunity and are commonly associated with rheumatoid arthritis (RA). Unravelling pathways of importance in therapeutic partial response and failure is of critical importance, as this will potentially provide new insights into key drivers of immune-mediated pathogenesis.

Objectives: To delineate disease-relevant T cell subsets in RA and assess their potential to act as cellular markers amenable to precision medicine approaches, particularly in the context of therapeutic partial or non-response.

Methods: FACS-based immunophenotyping and ex-vivo functional response profiles of CD4⁺CD161⁺CCR2⁺CCR5⁺ T cells were performed in peripheral blood mononuclear cells (PBMC) obtained from patients with RA and healthy controls, using previously characterised methodologies. RA patients fulfilled the 2010 ACR/EULAR criteria for RA. All samples were obtained after written consent, with the appropriate ethical approvals in place.

Results: RA patients harboured a higher frequency of CCR2⁺CCR5⁺ cells within the CD4⁺CD161⁺ T cell compartment compared with healthy controls. In RA patients this T cell subset had a higher proportion of cells that secrete pro-inflammatory cytokines such as IL-17A, GM-CSF, IFN-γ, and TNF. Importantly, the CD4⁺CD161⁺CCR2⁺CCR5⁺ T cell subset was significantly increased in DMARD non-responders compared to both responders and healthy controls. Moreover, in DMARD non-responders, these cells had a propensity to express increased proportions of pro-inflammatory cytokines. Notably, there was also a significant increase in the ratio of effector: regulatory T cell (Teff: Treg) compared to both responders and healthy controls. In addition, the CD4⁺CD161⁺CCR2⁺CCR5⁺ T cell subset was less responsive to suppression by Tregs. In further support of a role for this T cell population in disease pathogenesis, the frequency of CD4⁺CD161⁺CCR2⁺CCR5⁺ T cells significantly correlated with disease activity, as measured by the DAS28 (R² = 0.65; p = 0.003; n=11).