currently under development. We thus studied the therapeutic potential of interference with ROR t activation in biTregs using the model of pristane induced chronic systemic lupus erythematosus (SLE).

**Methods:** Foxp3Cre x RORCfl/fl mice with selective deficiency of ROR t in biTregs were generated (biTregROR-). SLE was induced by i.p. injection of pristane oil. Systemic auto-immunity, renal immune responses and tissue injury were studied between 2 and 8 months after disease induction.

**Results:** Time course analyses showed increasing infiltration of biTregs into kidneys of pristane injected mice with a maximum at around 5 months. In order to study the functional role of ROR t in biTregs, the course of SLE was analyzed in biTregROR- mice. Assessment of renal histology at 8 months showed severe proliferative glomerulonephritis in pristane injected wild type mice. Histological injury and glomerular cell proliferation in the biTregROR- group, in contrast, was significantly ameliorated. In line, renal inflammatory leukocyte infiltration, blood urea nitrogen levels and albuminuria were also reduced by knockout of ROR t in biTregs. As one possible explanation for the improved outcome, we found that biTregs from wild type mice produced pro-inflammatory IL-17 at all observed time points, while biTregs from biTregROR- mice did not. Furthermore, systemic and organ specific immune responses were strikingly skewed towards protective Th2 immunity in biTregROR- mice. Next, effects of biTreg expressed ROR t on development of humoral auto-immunity were studied. Analyses, however, showed unaltered serum anti-U1-RNP auto-antibody levels of all isotypes. Likewise, renal IgG and complement C3 deposit remained unchanged. This indicates that ROR t activation in biTregs directs renal injury by cellular effectors but does not play a major role for generation of humoral auto-immunity.

**Conclusions:** In summary, our data thus provide first evidence for presence and functional importance of biTregs in SLE. Importantly, biTreg expressed ROR t was identified as factor promoting development of renal injury via induction of IL-17 secretion and suppression of Th2 immunity. Our data therefore favor ROR t directed interventions as innovative new therapeutic option for lupus nephritis.

**P25**

**GENERATION, PHENOTYPE AND FUNCTION OF EX VIVO INDUCED HUMAN REGULATORY T CELLS**

**Fierro, A**1, Candia, E2, Covian, C3, Rodriguez, F3,
Wainstein, N4, Morales, J2, Mosso, C4, Rosemblatt, M5
1Clinica Las Condes, Santiago, Chile; 2Clinica Las Condes, Unidad de Trasplantes, Santiago, Chile; 3Universidad Andres Bello, Facultad de Ciencias Biologicas, Santiago, Chile; 4Clinica Las Condes, Departamento de Hemaoncologia, Santiago, Chile; 5Universidad de Chile- Fundacion Cien & Vida- Universidad Andres Bello, Facultad de Ciencias, Santiago, Chile

**Introduction:** Adoptive transfer of regulatory T cells (Tregs) represents a promising strategy to modulate autoimmun diseases including renal diseases, as well as bone marrow and organ transplantation. Studies performed in mice and humans have shown the feasibility of generating Treg ex vivo from naive T cells (nT) in the presence of IL-2 / TGF, all-trans retinoic acid (atRA) and rapamycin (RAPA). This study reports the expansion, differential expression of homing receptors; methylation of the conserved non-coding sequence 2 (CNCS2) of foxp3, and function of polyclonal human Treg induced from nT in short-term cultures.

**Methods:** nT were isolated from healthy volunteers by negative selection, polyclonally stimulated with anti-CD3 plus anti-CD28 antibodies and cultured in the presence of IL-2 and TGF for 5 days. RAPA and/or atRA were added at different concentrations. The induced Tregs were characterized by flow cytometry and suppression assays in vitro, methylation of the CNCS2 was evaluated by bisulphite conversion and pyro-sequencing.

**Results:** Results show that atRA improves while Rapa reduces the generation of Treg in this short-term setting. atRA enhanced the expression of 7, while the expression of CXCR4 is increased by RAPA. Functional assessment showed that Treg generated in the presence of RAPA had the highest suppressor effect. Methylation assays showed that atRA and RAPA did not lower the high methylation of the induced Treg, however polyclonal re-stimulation in the presence of atRA and RAPA was related to diminished methylation of the CNCS2.

**Conclusions:** These results show that the presence of atRA and RAPA contributes to the generation and function of induced Treg, providing each a differential expression of homing receptors. Repeated polyclonal stimulation in the presence of atRA and RAPA may contribute to decrease the methylation of the critical CNCS2. These results open the possibility of modulating the phenotype and function of Treg generated ex vivo, thus manufacturing Treg with diverse characteristics depending on the context and clinical purpose.

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**P26**

**RESPONSE TO USE OF CNI IN STEROID RESISTANT AND FREQUENTLY RELAPSING NEPHROTIC SYNDROME IN WESTERN INDIA**

**Baluwani, M1, Godhani, U1**
1IKDRC-ITS- Ahmedabad, Department of Nephrology, Ahmedabad, India

**Introduction:** Approximately 5% of children with MCD and even more number of MCD in adults are steroid resistant. Of those patients who are found to have FSGS, about 15% will respond to conventional oral steroid therapy, remaining 85% of patients are steroid resistant and are at substantial risk to progress to ESRD. Thus, present study was undertaken to know underlying different etiologies with special emphasis to calcineurin inhibitors as a treatment options for frequent relapsing and steroid resistant nephrotic syndrome in both children and adults.

**Methods:** The present study was an observational analysis of 30 patients with frequently relapsing or steroid resistant nephrotic syndrome treated with calcineurin inhibitors in addition to low dose steroid therapy presenting to the IKDRC-ITS, during the period from September 2012 to March 2015. Inclusion criteria: FRNS & SRNS were defined by KDIGO guidelines. Only patients undergone renal biopsy to define morphology were included. FRNS or SRNS patients treated with calcineurin inhibitors (Cyclosporine or Tacrolimus) at least for 6 months to define response were included. Exclusion criteria: 1. Children < 1 year of age (to exclude congenital nephrotic syndrome). 2. Patients with systemic disease like lupus, diabetes and other causes of secondary nephrotic syndrome were excluded. As per recommendations of KDIGO, patients with FRNS & SRNS were treated with cyclosporine or tacrolimus for a minimum of 6 month period.

**Results:** Overall FSGS was the most common etiology in 13 (44%) patients followed by IgM nephropathy in 8 (26%) patients, MePGN in 5 (17%) and MCD in 4 (13%) patients. After 3 months of treatment with CNI in majority of patients, proteinuria reduced to <2.5 gm/dl. Renal function remained stable during CNI treatment. Overall
cyclosporine was successful in 75% of patients in treating frequent relapses. Mean time to achieve remission was 3.2 months. Overall Tacrolimus was successful in 77% of patients in treating frequent relapses. Mean time to achieve remission was 2.1 months. Out of 15 patients with steroid resistant nephrotic syndrome, 3 patients treated with cyclosporine, 1 achieved partial remission and still continued on CSA where as 2 patients did not respond. Time to achieve remission was 5 months. 2 patients resistant to cyclosporine and 12 steroid resistant patients were treated with tacrolimus (total 14 patients). 11 of 14 (79%) patients achieved complete remission and 2(14%) achieved partial remission. Mean time to achieve remission was 2.5 months. In SRNS patients, tacrolimus was more effective than cyclosporine and was also effective in 1 pt resistant to CSA.

Conclusions: In frequent relapsing nephrotic syndrome patients, both cyclosporine and tacrolimus has comparable efficacy and no significant difference. Overall success rate (CR, PR and infrequent relapses) was 75% and 77% respectively. In steroid resistant nephrotic syndrome patients, tacrolimus was more effective than cyclosporine and also effective in CSA resistant patients. Combined remission rate (CR & PR) of 93% with tacrolimus as compared to only 33% with cyclosporine was achieved in SRNS. Late steroid resistance is associated with better response to CNI therapy as compared to primary CNI resistance.

P27
ROLE OF CORTICOSTEROID THERAPY IN IgA NEPHROPATHY - WHERE DO WE STAND?
Nagaraju, SP1, Mareddy, AS1, Prabhu, AR1,
Rangaswamyy, D1, madken, M1, Rao, S1, Kaza, S1,
Mateti, UV2, Guddattu, V3, Koulmane Laxminarayana, SL4
1Kasturba medical college- Manipal University- MANIPAL, Department of nephrology, Manipal, India; 2Kasturba medical college- Manipal UniversityMANIPAL, Department of Pharmacy, Manipal, India; 3Kasturba medical college- Manipal University- MANIPAL, Department of statistics, Manipal, India; 4Kasturba medical college- Manipal University- MANIPAL, Department of Pathology, Manipal, India

Introduction: Current guidelines suggest treatment with corticosteroids (CS) in IgA nephropathy (IgAN) with persistent proteinuria >1 g/day despite 3–6 months of supportive care and eGFR >50 ml/min/1.73m2. Whether the benefits of this treatment extend to patients with an eGFR <50 ml/min/1.73m2 is unclear. We retrospectively studied the effect of steroids on disease progression and proteinuria in IgA N patients with initial eGFR <50 ml/min/1.73m2 compared to those with >50ml/min/1.73m2.

Methods: A cohort of biopsy proven primary IgA N diagnosed between March 2010 and February 2015 who received oral corticosteroids and followed-up for a minimum of six months were included. They were categorized into two groups as per eGFR. (Group 1 – eGFR>50 ml/min/1.73m2, Group 2 – eGFR <50 ml/min/ 1.73m2). Baseline characteristics were compared between the groups. The eGFR and urine protein creatinine ratio (UPCR) were follow up at entry time, 6 months, 12 months and at the end of follow-up. Outcomes studied were change in eGFR, proteinuria and progression to end stage renal disease (ESRD). Statistical analysis was done using SPSS version 16.

Results: Out of 44 patients, 23(52%) had eGFR <50 ml/min/1.73m2 (Group1) and 21(48%) had eGFR >50ml/min/1.73m2 (Group 2). The baseline clinical, histopathological, and treatment characteristics of both the groups are shown in table 1. At the end of follow-up, similar reduction of proteinuria (UPCR) (p=0.62) was seen in both the groups. But there was significant difference in change in median eGFR/month (p=0.004) (Table 2). Patients in Group 2 had a median fall in eGFR of -0.46 ml/min/1.73m2 / month, whereas Group 1 had an increase in median eGFR by +0.38ml/min/1.73m2/ month (Table 2). One in each group has reached CKD stage 5(p=0.73). Limitations in our study were retrospective in nature, small cohort and short duration of follow-up.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Group 1 (N=23) (%)</th>
<th>Group 2 (N=21) (%)</th>
<th>‘p’ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>eGFR at end of follow-up</td>
<td>20.94(9.38,37.12)</td>
<td>94.43(77.48,129.91)</td>
<td>0.004</td>
</tr>
<tr>
<td>Change in eGFR/month#</td>
<td>-0.46(-0.1, -1.08)</td>
<td>+0.38(+0.06, 0.00)</td>
<td>0.004</td>
</tr>
<tr>
<td>Initial UPCR#</td>
<td>2.6(1.8,3.3)</td>
<td>2(1.7,3.1)</td>
<td>0.61</td>
</tr>
<tr>
<td>upCR at the end of follow-up#</td>
<td>1.4(0.3,2.0)</td>
<td>1.5(0.55,2.75)</td>
<td>0.73</td>
</tr>
<tr>
<td>Patients reaching ESRD</td>
<td>1(4.5)</td>
<td>1(4.7)</td>
<td>0.24</td>
</tr>
</tbody>
</table>

#- Median with interquartile range, *- Mean with standard deviation, estimated glomerular filtration rate (eGFR), Urine protein-to-creatinine ratio (UPCR)