Prospective long-term analysis of B- and plasma cell subsets in renal transplant patients after treatment with rituximab and bortezomib in antibody mediated rejection

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Introduction:

Treatment of antibody-mediated rejection (AMR) in renal transplant (Tx) patients (pts) is still challenging and long-term outcome remain poor. Our present treatment strategy with bortezomib (BZ) and rituximab display a synergistic approach targeting precursor and mature HLA antibody (DSA) producing plasma blasts (PB) and plasma cells (PC). The aim of this study was to investigate the impact of this combined therapy on different B-cell subsets inclusive PC and PB in a prospective long-term approach over 2 years.

Objective:

The aim of this study was to investigate the impact of this combined therapy on different B-cell subsets inclusive PC and PB in a prospective long-term approach over 2 years.

Methods:

AMR therapy was introduced in 10 Tx pts with DSA and biopsy proven AMR according to BANFF classification. Therapy included steroid pulse, PPH (6x), BZ (1.3.mg/m2; one cycle), 500mg Rituximab and IVIG 1.5g/kg). Phenotype of PCs (CD19dimCD27+CD38+HLA-DRneg) and PBs (CD19dimCD27+CD38+HLA-DR+) were characterized by flow cytometry at baseline (BL), month (M) 1, 3, 6, 12, 18 and 24. DSA MFI were evaluated with Luminex technique.

Results:

CD19B-cells were depleted sustainable (BL: 161.4; M1:0.9; M24: 7.4µl; $p<0.01$). CD20CD27+ memory B-cells are the most frequent recovering population in contrast to naïve B-cells. Memory B-cells recovered within 12M completely, whereas naïve B-cells were still affected 24M post treatment.
MFI of DSAmax were reduced 3M after therapy compared to baseline (MFI: 2500±2453 vs. 4865±2082; p=0.018). 1 year post treatment DSAmax recovered back to baseline.

AMR therapy

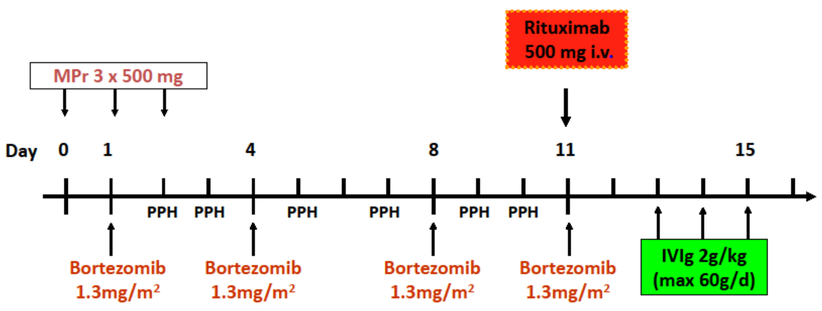

Figure 1 - overview of AMR treatment regiment–
10 kidney transplant patients were diagnosed with AMR due to BANFF criteria and detected de novo donor specific antibodies. For treatment of AMR, therapy was introduced with 3x500mg Methylprednisolon (MPr) bolus and following Bortezomib 1.3mg/m² injections on day 1,4, 8 and 11 after baseline. After Bortezomib-cycle 500mg Rituximab was given at day 11. Finally, 2g/kg IVIg were administered in al patients. A total of 6 PPH treatment sessions are performed over the AMR therapy.

CD19⁺ B-cells were depleted persistend after AMR therapy

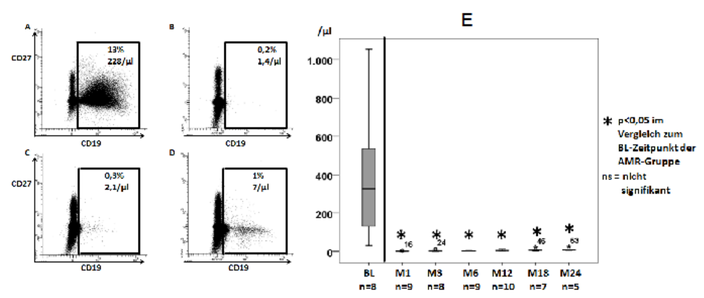

Figure 2
Colour flow cytometry analysis of CD19+ B-cells over the observation period of 24 months after AMR-therapy.
A-D shows representative CD19 B-cells detection in one patient before (A) at month 1 (B) at month 12 (C) and month 24 (D) after AMR therapy.
Numbers of CD19+ B-cells are reduced significantly after combined therapy with rituximab and bortezomib (BL: 327/µl to 1.4/µl M1 $p<0.005$; see E). This effect was persistent over the total observation period.
Still, 24 months after AMR therapy total numbers of B-cells remain at low levels (7.2/µl $p<0.005$).

CD19⁺CD20⁺CD27⁺ memory B-cells are the predominant recovering subpopulation after Rituximab + Bortezomib

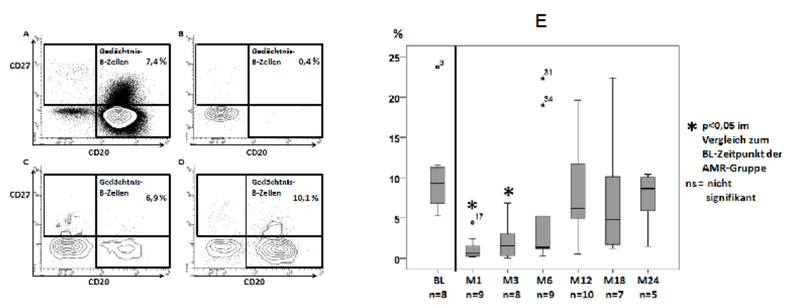

Figure 3
Colour flow cytometry analysis of CD19+CD20+CD27+ memory B-cells over the observation period of 24 months after AMR-therapy.
A-D shows representative memory B-cells detection in one patient before (A) at month 1 (B) at month 12 (C) and month 24 (D) after AMR therapy.
Numbers of memory B-cells are reduced significantly after combined therapy with rituximab and bortezomib (BL: 9.3% to M1:0.6% $p<0.005$; see E). This effect was not persistent. Memory B-cells recover back to baseline values 12 months after initiation of AMR therapy.

Patient characteristics at baseline

	AMR-group (n=10)
Months after Tx and detection of AMR (mean) ± SD	67±78,9
AMR + ACR:	6 (60%)
Banff 1A	1 (10%)
Banff 1B	1 (10%)
Banff 2A	2 (20%)
Banff 2B	0 (0%)
Borderline-Rejektion	2 (20%)
C4d-Positivität	8 (80%)
cg < 2 bzw. cg ≥ 2	5 (50%) bzw. 5 (50%)
g < 2 bzw. g ≥ 2	3 (30%) bzw. 7 (70%)
ptc < 2 bzw. ptc ≥ 2	1 (10%) bzw. 9 (90%)
g + ptc ≥ 2	7 (70%)
ci ≥ 2	3 (30%)
ct ≥ 2	3 (30%)
cv ≥ 2	6 (60%)
Pat. with follw-up biopsy after AMR-therapy	5 (50%)

CD19⁺CD20⁺CD27⁺ memory B-cells are the predominant recovering subpopulation after Rituximab + Bortezomib

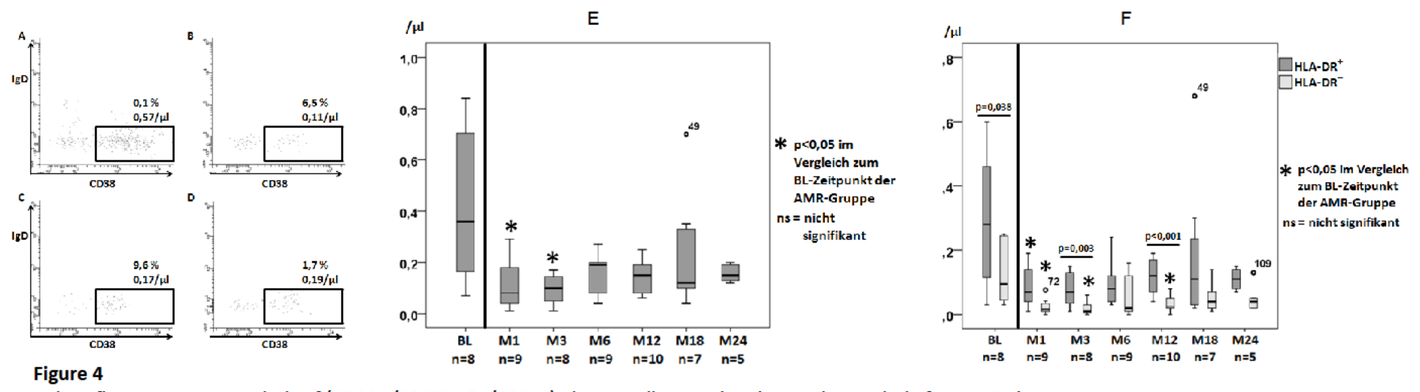

Figure 4
Colour flow cytometry analysis of ($CD20^-$ /$CD27^+IgD^-CD38^+$) plasma cells over the observation period after AMR-therapy.
A-D shows representative plasma cell detection in one patient before (A) at month 1 (B) at month 12 (C) and month 24 (D) after AMR therapy.
Numbers of plasma cells were depleted significantly after combined therapy with rituximab and bortezomib (BL: 0.36/µl to 0.08/µl M1 $p<0.005$; see E). This effect was persistent over the total observation period. Interstingly, there were no differences beteween HLA-DR+ plasma blasts or HLA-DR- plasma cells regarding recovery of poplations.

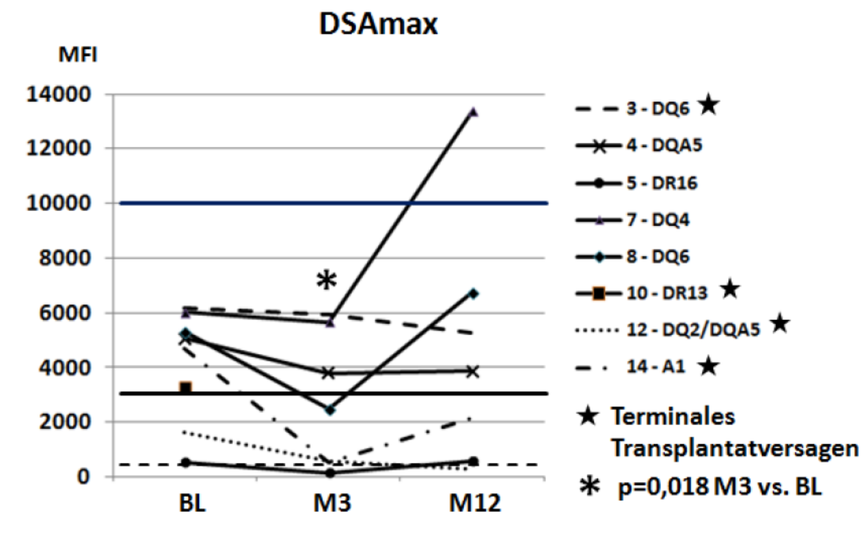

Figure 5
Overview of the DSAmax course covering 12 month after AMR therapy stratified by patient. We found a significant decrease of the DSA max between baseline and month 3 (BL: 4865 MFI; IQA=3824 MFI vs. M3: 2500 MFI; IQA=5125 MFI; p=0.018) after therapy. This effect was not sustainable in the further follow-up. One year after therap there wer no detectable differences between baseline and month 12.

Conclusion:

The present study observed a sustained reduction of peripheral PCs, PBs and B-cells after a combined AMR therapy with BZ + Rituximab. However, PBs and memory B-cells are the predominant B-cell subsets that recovered completely within 12M.
Interestingly, beside these cell reductions in the periphery, MFI of DSAmax were affected only short-term. Thus, the effect of BZ and Rituximab on tissue-based long-lived PCs may be limited. This present data may be helpful in the understanding of B-cell biology in context of AMR.

53 ERA
633--SP
Renal transplantation. Experimental, immune-tolerance of allogenic and xenogenic transplants.
Michael Duerr
DOI: 10.3252/pso.eu.53era.2016
ePosters supported by F. Hoffmann- La Roche Ltd.
53rd CONGRESS ERA-EDTA VIENNA AUSTRIA MAY 21-24 2016
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