**Objective:** To test the hypothesis that low or absent ERAP1 activity alters CD8 T cell immunogenicity through changes in the HLA-B51 peptidome and shapes the CD8 T cell immune response in affected subjects.

**Methods:** We generated HLA-B*51:1 ERAP1 KO LCL clones using CRISPR-Cas9 performed mass spectrometry of the immunoprecipitated MHC-class I peptideosome with subsequent computational deconvolution for HLA-B51-binding peptides. We then assessed single cell (ICS), bulk (ELISA) and proliferative (CFSE) CD8 effector (IFNγ, granzyme B, perforin) T cell responses through stimulation of allogeneic donor cells with WT vs KO LCL and determined ERAP1 haplotypes in 49 untreated TUC patients and controls for HLA-B51 allele and non-HLA-B51 allele susceptibility donors (HD) whose PBMC were profiled using 6 multicolour flow cytometry panels.

**Results:** WT and KO peptides differed significantly (p<0.0005) Fisher’s exact test with a distinctive shift of peptide length frequencies exceeding 9-mer (binding motif) in the KO vs WT. This held true for computationally deconvoluted HLA-B51 binders. IFNγ secretion from CD8 T cells stimulated with KO LCL was significantly different from WT (p<0.0005 ELISA, p<0.005) as were CD8 T cell proliferation of perforin/granzyme B+ CD8 T cell analyses. Of 133 T3 B, NK and monocyte cell populations revealed predominance of CD8 T and NK cell subset in HLA-B51+Hap10-BD vs HLA-B51+Hap10-BD and HD, accounting for 80% of all populations reaching significance (p<0.05, Mann-Whitney). Naïve and effector memory CD8 T cell subsets were inversely correlated. Cohort effect sizes were large (>0.8) or very large (>1.2).

**Conclusion:** We show that absence of functional ERAP1 alters human CD8 T cell immunogenicity. This is mediated by an HLA-class I peptide with propensity for longer peptides above 9mer and suggests loss or de-novo presentation of peptide-HLA-B51 complexes to cognate CD8 TCR. The reciprocal changes in antigen-experienced vs naive CD8 T cell subsets in affected subjects point to biologic significance of HLA-B51:Hap10 in BD. Collectively, our findings suggest that an altered HLA-B51 peptidome modulates immunogenicity of CD8 effector T cells in ERAP1-Hap10 carriers with BD and identify targets for future drug development.

**References:**
3. Basal features at TCZ onset.

**Optimized-TCZ**

- Age, years, mean SD: 68.9±8.7
- Sex, female/male n(%): 71.4±8.5
- Time from GTA diagnosis to TCZ onset (months), median (IQR): 19.5[7.75-45]
- Follow-up on TCZ therapy (MONTHS), MEDIAN (IQR): 15.1[11.1-21.7]
- Relapses, n(%): 5[1.16]
- Severe side effects, n(%): 14[3.24]
- Serious infections, n(%): 6(1.4)
- Dose, (mean) euros per year: IV 7 538.4, SC 7 329.0

**Non-optimized-TCZ**

- Age, years, mean SD: 68.8±8.7
- Sex, female/male n(%): 71.4±8.5
- Time from GTA diagnosis to TCZ onset (months), median (IQR): 19.5[7.75-45]
- Follow-up on TCZ therapy (MONTHS), MEDIAN (IQR): 15.1[11.1-21.7]
- Relapses, n(%): 5[1.16]
- Severe side effects, n(%): 14[3.24]
- Serious infections, n(%): 6(1.4)
- Dose, (mean) euros per year: IV 7 538.4, SC 7 329.0

**Conclusion:** The hypothesis that low or absent ERAP1 activity alters human CD8 T cell immunogenicity through changes in the HLA-B51 peptidome and shapes the CD8 T cell immune response in affected subjects was confirmed. The reciprocal changes in antigen-experienced vs naive CD8 T cell subsets in affected subjects point to biologic significance of HLA-B51:Hap10 in BD. Collectively, our findings suggest that an altered HLA-B51 peptidome modulates immunogenicity of CD8 effector T cells in ERAP1-Hap10 carriers with BD and identify targets for future drug development.