Abstract #23 | Poster

# Autonomous B-cell receptor signaling in activated B-cell DLBCL: A lymphomagenic driver with functional equivalence to an activating CARD11 variant

J. A. Eken[1], M. T. Koning[1], K. Kupcova[2], J. H. Sepúlveda Yáñez[1, 3], R. A. de Groen[1], E. Quinten[1], J. Janssen[4], C. A. van Bergen[1], J. S. Vermaat[1], A. Cleven[5], M. A. Navarrete[3], B. Ylstra[4], D. de Jong[4], O. Havranek[2], H. Jumaa[6], **H. Veelken**[1]

[1] *Leiden University Medical Center, Hematology, Leiden, Netherlands*
[2] *Charles University, BIOCEV, Prague, Czech Republic*
[3] *Universidad de Magallanes, School of Medicine, Punta Arenas, Chile*
[4] *Amsterdam University Medical Center, Pathology, Amsterdam, Netherlands*
[5] *Leiden University Medical Center, Pathology, Leiden, Netherlands*
[6] *Ulm University, Immunology, Ulm, Germany*

Diffuse large B-cell lymphoma of activated B-cell type (ABC-DLBCL) is characterized by chronic active B-cell receptor (BCR) signaling and NF-kB activation. This phenotype can be explained by activating mutations of the BCR signaling cascade in a minority of cases. We investigated the hypothesis that antigen-independent, autonomous BCR signaling acts as an alternative driver mechanism in DLBCL, akin to its essential pathogenetic role in chronic lymphocytic leukemia (CLL).

BCR transcripts from the CARD11 wild-type, IgM-expressing TMD8 and the IgG-expressing, CARD11$^{L251P/L251P}$-mutated OCI-Ly3 ABC-DLBCL cell lines were sequenced and transduced into the murine TKO pre-B cell line. TKO cells lack the *rag2* and *λ5* genes and are therefore unable to express an endogenous BCR. In addition, the BCR signal transduction molecule slp65 is replaced with a tamoxifen-inducible version. TKO cells transduced with the OCI-Ly3 BCR showed calcium flux in the presence of tamoxifen after BCR crosslinking only. In contrast, the TMD8 BCR induced sustained calcium flux and increased CD79 phosphorylation without crosslinking. To demonstrate alternative but equivalent function of the CARD11$^{L251P}$ mutation and autonomous BCR signaling, we introduced a hemizygous L251P variant into the wild-type CARD11 gene in TMD8 cells by CRISPR/Cas-mediated gene editing. CARD11$^{L251P/-}$ but not CARD11$^{wt/wt}$ TMD8 remained viable upon replacement of their BCR with the non-signaling OCI-Ly3 BCR. Vice versa, OCI-Ly3 cells transduced with the TMD8 BCR and a CARD11$^{wt}$ gene survived CRISPR/Cas-mediated knock-out directed at the CARD11$^{L251P}$ allele.

Functional BCR testing of 18 primary DLBCL showed autonomous BCR signaling in 13 cases, in particular in IgM-expressing DLBCL of non-GCB type. Isotype switching to IgG, reversal of somatic BCR mutations to germ-line, and HCDR3 exchange severely reduced autonomous BCR signaling. Probabilistic assignment to genetic DLBCL clusters based on whole exome sequencing revealed autonomous BCR signaling for all five DLBCL assigned with a high likelihood to consensus clusters C5 and C1.

Autonomous BCR signaling represents a novel immunological driver mechanism acquired during somatic

hypermutation. Autonomous BCR signaling and the BCR signaling-mimicking L251P mutation of CARD11 are alternative, functionally equivalent lymphomagenic drivers in ABC-DLBCL. Autonomous BCR signaling adds a new dimension to currently proposed genetics- and transcriptomics-based DLBCL classifications.