Identification of the atypically modified autoantigen Ars2 as the target of B-cell receptors from activated B-cell-type diffuse large B-cell lymphoma

Lorenz Thurner,1 Sylvia Hartmann,2 Moritz Bewarder,1 Natalie Fadle,2 Evi Reglitz,1 Claudia Schormann,1 Natalia Quiroga,1 Maria Kemele,2 Wolfram Klapper,1 Andreas Rosenwald,1 Lorenz Trümper,3 Rainer Maria Bohle,1 Anna Nimmegsgerm,7 Christina Körbel,9 Matthias W. Laschke,6 Michael D. Menger,8 Stefan Barth,4 Boris Kubuschok,10 Anja Mottok,10 Dominic Kaddu-Mulindwa,1 Martin-Leo Hansmann,2 Viola Pöschel,1 Gerhard Held,6 Niels Murawski,1 Stephan Stilgenbauer,1 Frank Neumann,1 Klaus-Dieter Preuss1 and Michael Pfreundschuh1

1Saarland University Medical School, José Carreras Center for Immuno- and Gene Therapy and Internal Medicine I, Homburg/Saar, Germany; 2Dr. Senckenberg Institute of Pathology, Goethe University Hospital of Frankfurt am Main, Frankfurt am Main, Germany; 3Institute of Pathology, University of Kiel, Kiel, Germany; 4Institute of Pathology, University of Würzburg and CCC Mainfranken, Würzburg, Germany; 5Department of Hematology and Medical Oncology, University Hospital Göttingen, Göttingen, Germany; 6Saarland University Medical School, Institute of Pathology, Homburg/Saar, Germany; 7Institute of Medical Microbiology and Hygiene, University of Saarland, Homburg, Germany; 8Institute for Clinical & Experimental Surgery, University of Saarland, Homburg/Saar, Germany; 9Institute for Infectious disease & Molecular Medicine, University of Cape Town, Cape Town, South Africa; 10Department of Internal Medicine II, Augsburg University Medical Center, Augsburg, Germany; 11Institute of Human Genetics, Ulm University and Ulm University Medical Center, Ulm, Germany and 12Department of Hematology/Oncology, Westpfalzklinikum, Kaiserslautern, Germany

ABSTRACT

Introduction

Diffuse large B-cell lymphoma (DLBCL) is the most common aggressive B-cell non-Hodgkin lymphoma. According to the World Health Organization (WHO) classification, DLBCL can be classified based on gene expression profiling (GEP) into an activated B-cell (ABC)-like type, a germinal center B-cell (GCB)-like type and primary mediastinal B-cell lymphoma.11 In contrast to relatively well-studied genetic or epigenetic pathway alterations, little is known about specific and complementary external

It has been suggested that stimulation of B-cell receptors (BCR) by specific antigens plays a pathogenic role in diffuse large B-cell lymphoma (DLBCL). Here, it was the aim to screen for specific reactivities of DLBCL-BCR in the spectrum of autoantigens and antigens of infectious origin. Arsenite resistance protein 2 (Ars2) was identified as the BCR target of three of five activated B-cell type DLBCL cell lines and two of 11 primary DLBCL cases. Compared to controls, Ars2 was hypophosphorylated exclusively in cases and cell lines with Ars2-specific BCR. In a validation cohort, hypophosphorylated Ars2 was found in eight of 31 activated B-cell type DLBCL, but in only one of 20 germinal center B-cell like type DLBCL. Incubation with Ars2 induced BCR-pathway activation and increased proliferation, while an Ars2/ETA’ toxin conjugate induced killing of cell lines with Ars2-reactive BCR. Ars2 appears to play a role in a subgroup of activated B-cell-type DLBCL. Moreover, transformed DLBCL lines with Ars2-reactive BCR still showed growth advantage after incubation with Ars2. These results provide knowledge about the pathogenic role of a specific antigen stimulating the BCR pathway in DLBCL.
studies of different subgroups of DLBCL. In particular, DLBCL of the ABC-type or the recently identified MCD-type or Cluster 5 harbor recurrent mutations in MYD88 and CD79B genes with dependency on constitutive BCR signaling. For primary central nervous system lymphoma, which represents a specific extranodal subtype of DLBCL, with molecular similarities to MCD type or C5 with frequent mutations in MYD88 and CD79B, SAMD14/neurabin-I were recently identified as antigens of BCR, and were hyper-N-glycosylated specifically in patients with SAMD14/neurabin-I-reactive BCR. In systemic DLBCL, a cis and trans stimulation of the BCR by a so far uncharacterized autoantigen was reported for the HBL1 line. Moreover, an anti-idiotypic reactivity of complementarity determining region 3 of the BCR of the TMD8 line against an epitope of a B-cell receptor (BCR) of systemic DLBCL against variously post-translationally modified UniPEx 1 and 2 protein macroarrays, including sumoylation, ubiquitination, citrullination, and acetylation. Protein macroarrays were sumoylated as reported elsewhere. Recombinantly expressed directly. Recombinantly expressed immunotoxins, consisting of Ars2 amino acids 342-375 conjugated to ETA were either obtained from the Fraunhofer Institute of Experimental Medicine and Immunotherapy (Aachen, Germany) or recombinantly expressed in our laboratory in E. coli BL21 and purified by the His-Tag, as described by Nachreiner et al.

### Methods

The study was approved by the local ethics committee (Arztekammer des Saarlandes 12/13). For expression cloning of DLBCL-BCR, patients’ snap-frozen specimens were obtained from the Dr. Senckenberg Institute of Pathology (Frankfurt am Main, Germany). Sera of a second cohort of patients with DLBCL were obtained from the DSHNHL RICOVER-60 trial. DLBCL cryospecimens, of a third cohort of patients, of which the cell-of-origin had been determined by GEP, were obtained from the Institutes of Pathology of Würzburg and Kiel Universities.

### B-cell receptor screening for autoantigens

BCR from nine DLBCL cell lines were prepared by digestion with papain. Moreover, expression cloning of recombinant BCR derived from primary DLBCL cryospecimens was performed, as described in the Online Supplementary Material. These DLBCL line-derived BCR and the pooled recombinantly expressed BCR (each at a concentration of 10 µg/ml) were screened on protein macroarrays containing clones of UniPEx 1 and 2 cDNA expression libraries (Bioscience, Dublin, Ireland), as previously described. To search for further antigens, all recombinant DLBCL-derived antigen-binding fragments (Fab) without reactivity against arsenite resistance protein 2 (Ars2) were screened against variously post-translationally modified UniPEx 1 and 2 protein macroarrays, including sumoylation, ubiquitination, citrullination, and acetylation. Protein macroarrays were sumoylated as described elsewhere and ubiquitination was performed with synchronized HeLa cell extracts. The screening for antigens of infectious origin is described in the Online Supplementary Material.

### Expression of target antigens and immunotoxins

The expression clone of Ars2 and subsequently the epitope-containing region consisting of amino acids 342-375 of Ars2 were recombinantly expressed with a C-terminal FLAG tag by a pSFI vector in HEK293 cells. Additionally, C-terminally FLAG-tagged full-length Ars2 was transfected by electroporation into U2932 and TMD8 via a pRTS vector. C-terminally FLAG-tagged FamH83 and JmJD4 were recombinantly expressed in HEK293 cells. Site-directed mutagenesis of Ars2 and secondary modification of antigens is described in the Online Supplementary Material. Immunotoxins with monomethyl auristatin E (MMAE) are effective in vivo and established in the clinics, but the synthesis of toxin conjugates with MMAE requires enzyme-cleavable dipeptide linkers and is therefore challenging for academic laboratories. Hence, a truncated form of Pseudomonas aeruginosa exotoxin A (ETA) was used, as the ETA conjugate can be recombinantly expressed directly. Recombinantly expressed immunotoxins, consisting of Ars2 amino acids 342-375 conjugated to ETA were either obtained from the Fraunhofer Institute of Experimental Medicine and Immunotherapy (Aachen, Germany) or recombinantly expressed in our laboratory in E. coli BL21 and purified by the His-Tag, as described by Nachreiner et al.

### Enzyme-linked immunosorbent assay (ELISA) for B-cell receptor and serum reactivity against target antigens and competition ELISA with apoptotic debris

Ars2, ubiquitinated FamH83, and sumoylated JmJD4 were confirmed as BCR antigens by ELISA, as previously described. ELISA and competition assays with apoptotic debris are described in detail in the Online Supplementary Material.

### Western blot and isoelectric focusing

Lysates of DLBCL lines or of whole blood from patients were loaded and separated by 10% sodium dodecylsulfate polyacrylamide gel electrophoresis and transferred to a polyvinylidene fluoride membrane using a transblot semidry transfer cell (Bio Rad). After blocking overnight at 4°C in phosphate-buffered saline/10% nonfat dry milk, a recombinant Ars2-reactive His-tagged Fab was incubated at a concentration of 2 µg/ml for 1 h at room temperature, followed by incubation for 1 h at room temperature with murine-anti-his antibody at a ratio of 1:2,000 (Qiagen), with horseradish peroxidase-labeled anti-mouse IgG antibody (Bio Rad). Chemiluminescence reagent (New England BioLabs) was used for immunoblot detection. Isoelectric focusing was performed as previously described. Proliferation, BCR pathway activation assays, cytotoxicity and apoptosis assays are described in the Online Supplementary Material.

### Results

Recombinant BCR in the form of Fab were successfully synthesized from 11 DLBCL cases. Moreover, Fab of “natural” BCR were obtained by papain digestion from nine DLBCL cell lines. From three of these cell lines, recombinant Fab were generated (Online Supplementary Table S1).

### Screening of protein macroarrays and a library of infective agents

The screening of DLBCL Fab identified an expression clone of Ars2 transcript variant 2 (RZPDp828K0526 from Unipex 2, UnigenetID: Hs.118101) spanning from amino acids 253 to 416 as the candidate antigenic target. The screening of the Fab of DLBCL cases and cell lines on post-translationally modified protein macroarrays revealed sumoylated JmJD4 (RZPDp9027E0216D from Unipex 1; UnigenetID: Hs.555974) and ubiquitinylated FamH83 (RZPDp828G0528 from Unipex 2; UnigenetID: Hs.676336) as candidate antigens. The screening against bacterial lysates of 11 bacterial strains did not reveal any specific reactivity. Screening of an Infectious Disease Epitope Microarray (PEPperCHIP Heidelberg, Germany) consisting of 3,760 database-derived B-cell epitopes associated with 196 pathogens, including various bacterial, fungal, parasitic, 

---

**Ars2 in the pathogenesis of DLBCL**
and viral pathogens, revealed no significant binding of the pooled DLBCL-BCR.

**Confirmation of Ars2, sumoylated JmjD4, and ubiquitinated FamH3 as targets of diffuse large B-cell lymphoma B-cell receptors and determination of the B-cell receptor-binding epitope**

ELISA with recombinant Ars2 (UnigeneID: Hs.111801) expressed with a C-terminal FLAG-tag in HEK293 confirmed Ars2 as the BCR target antigen from three of five (60%) ABC-derived cell lines (OCI-Ly3, OCI-Ly10, and U2932; but not HBL1 and TMD8) and none of four GCB-DLBCL cell lines (Figure 1A). Recombinant BCR from two of 11 DLBCL cases (with unknown cell of origin), but none of nine mantle cell lymphomas and none of 11 primary central nervous system lymphomas were reactive with Ars2 (Figure 1B). Of the two Ars2-reactive DLBCL, one was a non-GCB type and one was unclassified according to immunohistochemistry using the Hans classifier.21 ELISA with fragments of different lengths of Ars2 as the coat identified a region spanning amino acids 350 to 416 as the BCR-binding epitope (Figure 1C), and all

![Figure 1](https://example.com/figure1.png)
Ars2-specific BCR derived from cell lines and cryospecimens bound to this epitope. ELISA with recombinant sumoylated JmjD4 or ubiquitylated FamH83 expressed with a C-terminal FLAG-tag in HEK293 confirmed each as target antigens of one DLBCL-derived recombinant Fab and ubiquitinated FamH83 in addition as a target of a recombinant mantle-cell lymphoma–derived Fab (Online Supplementary Figures S1 and S2). Fab did not bind to non-modified JmjD4 and FamH83. Binding of Ars2 to membrane BCR was demonstrated by flow cytometry of DLBCL cell lines with Ars2-reactive BCR, but not for the TMD8 line without Ars2-reactive BCR. Internalization of C-terminally FLAG-tagged Ars2 into U2932 cells was observed after 60 min (Figure 1D). For OCI-Ly10, mutagenesis of K98A of the BCR heavy chain gene had been reported to result in loss of autoreactivity.\(^\text{6}\) OCI-Ly10 K98A resulted in loss of affinity against Ars2 (Figure 1E).

Characterization of Ars2 in diffuse large B-cell lymphoma with Ars2-specific B-cell receptors

No obvious differences in molecular weight of Ars2 from DLBCL cases with Ars2-reactive and Ars2-non-reactive BCR and controls were observed in western blots; similarly, Sanger sequencing revealed identical DNA sequences, excluding mutations in the coding sequence as an explanation for the immunogenicity of Ars2. However, isoelectric focusing of cell lines (Figure 2A) and DLBCL cases (Figure 2B) with an Ars2-reactive BCR revealed a less negatively charged Ars2 isoform. Dephosphorylation with alkaline phosphatase treatment led to a stronger reduction of the negative charges of Ars2 from DLBCL cases and cell lines without Ars2-specific BCR than in cases with Ars2-reactive BCR and resulted in the disappearance of the differences in electric charge between both isoforms of Ars2 (Figure 2C), demonstrating that the different isoelectric focusing pattern of Ars2 was due to hypophosphorylation in cases with Ars2-specific BCR. The hypophosphorylated Ars2 isoform was detected in all of the three DLBCL cell lines with Ars2-reactive BCR (i.e., OCI-Ly3, OCI-Ly10, and U2932), but in none of six DLBCL lines without Ars2-reactive BCR. This association in the nine analyzed DLBCL cell lines between BCR reactivity against Ars2 and the presence of the hypophosphorylated isoform of Ars2 was statistically significant (Fisher exact \(t\)-test: two-tailed \(P=0.0119\)).

Regarding the 11 DLBCL cases with recombinantly expressed Fab (Online Supplementary Table S1) derived from cryospecimens, only the two cases with Ars2-reactive Fab (\#3 and \#11) showed hypophosphorylation of Ars2. In this cohort of 11 cases of cryospecimens and recombinantly expressed Fab, the association between Ars2 reactivity and presence of hypophosphorylated Ars2 isoform was also statistically significant (Fisher exact \(t\)-test: two-tailed \(P=0.0182\)). Looking at the association between BCR reactivity of DLBCL cells and the presence of hypophosphorylated Ars2 isoform overall, considering data from both cell lines and cases with cryospecimen-derived recombinant Fab, the association was statistically highly significant (Fisher exact \(t\)-test: two-tailed \(P<0.0001\)).

Hypophosphorylated Ars2 was detected in the biopsies of eight of 31 (26%) ABC-type DLBCL cases characterized by GEP, but in only one of 20 (5%) GCB-type DLBCL and in the peripheral blood from one in 100 healthy controls.

The hypophosphorylated sites were identified as serine 328 and serine 341 by site-directed point mutagenesis of various predicted sites in Ars2 transfected with a C-termin-
nal FLAG tag in OCI-Ly10, OCI-Ly3 and, as a control, HBL1 (Figure 2D and Online Supplementary Figure S4).

Frequency, titers, and IgG subclasses of Ars2 serum antibodies

Ars2 antibodies were detected by ELISA in the sera of four of 98 patients with DLBCL, with titers ranging from 1:800 to 1:1600, and in one of 400 healthy controls. All four patients with Ars2 antibodies in their sera (#22, #27, #41, #73) were carriers of hypophosphorylated Ars2 in the cells of their peripheral blood (Figure 2B), but this isoform was not detected in the peripheral blood of any of the 94 other patients, resulting in a statistically significant relationship between serum Ars2-autoantibodies and the presence of the hypophosphorylated Ars2 in peripheral blood (Fisher exact t-test: two-tailed \( P < 0.0001 \)).

Effects of Ars2 on diffuse large B-cell lymphoma lines

Western blot analysis of BCR pathway activation after addition of recombinant Ars2 revealed a strong activation in the U2932 cell line with Ars2-reactive BCR, demonstrated by a strong upregulation of pTyr525/526 SYK, pTyr96 BLNK, pTyr759 PLC\( \gamma \)2, and pTyr223 BTK. Moreover, this BCR stimulation by Ars2 led to increases in MYC expression. However, regarding MYC, two cases with Ars2-reactive BCR (#4 and #10) did not have MYC-overexpression, as determined by immunohistochemical analysis (data not shown). No BCR pathway activation was induced by the control antigen MAZ in the U2932 line or by addition of Ars2 to the HBL1 line (Figure 3A). Addition of recombinant Ars2 induced proliferation of U2932 and OCI-Ly3 cells, but not of DLBCL cell lines without Ars2-reactive BCR, such as TMD8, analyzed by the tetrazolium/formazan EZ4U assay (Figure 3B). This Ars2-induced growth stimulus could be reverted by addition of Ars2-neutralizing recombinant Fab derived from patient #4 (Figure 3C). Furthermore, flow cytometry analysis of U2932 cells showed a strong increase of cytoplasmic calcium levels after incubation with the Ars2 epitope, but not the control antigen (Figure 3D).

Cytotoxicity of the Ars2/ETA’ conjugate

Addition of Ars2-ETA’ resulted in inhibition of growth analyzed in proliferation assays. This inhibition could be reverted by preincubation of Ars2/ETA’ toxin with the Ars2-reactive recombinant Fab derived from case #4 (Figure 4A). The Ars2-ETA’ conjugate exerted a specific and dose-dependent toxicity against the Ars2-reactive
BCR-expressing cell lines, but had no observable effect on DLBCL lines lacking Ars2-reactive BCR (Figure 4B). No toxic effect was observed with the control toxin LRPAP1-ETA' against OCI-Ly3. Trypan blue staining after addition of 5 μg/mL Ars2-ETA' showed that 35%, 2% and 0% of OCI-Ly3 cells were alive after 24 h, 48 h and 72 h, respectively, contrasting with findings for the wild-type HBL1 cell line without BCR reactivity against Ars2 (97% viable cells at 24 h; 96% at 48 h; and 97% at 72 h) (Figure 4B). In accordance with this, an increase of apoptotic cells was detected after incubation with Ars2-ETA' in U2932 cells expressing Ars2-reactive BCR (Figure 4C), as demonstrated in the annexin V/propidium iodide assay.

Discussion

Beside the two relatively rare target antigens, ubiquitinated FamH83 and sumoylated JmJD4, in the present study, hypophosphorylated Ars2 was identified as a more frequent antigen of BCR from DLBCL lines and recombinant BCR from primary DLBCL cryospecimens. Ars2 is also known as serrate RNA effector molecule (SRRT). Its gene is located on chromosome 7q21 and the protein is a zinc finger protein consisting of 875 amino acids with a molecular weight of around 100 kDa. Ars2 was described as being involved in miRNA silencing by interacting with the nuclear cap binding complex,22 and as being involved in the innate immune response against RNA viruses by...
Figure 4. Targeting Ars2-reactive diffuse large B-cell lymphoma with Ars2-containing immunotoxins. (A) Growth inhibition by B-cell receptor (BCR)-antigen/immunotoxins. Growth of U2932 cells was inhibited by addition of Ars2/ETA', an immunotoxin of the epitope of the cognate antigen Ars2 fused to a truncated form of Pseudomonas aeruginosa exotoxin A. This growth inhibition could be prevented by co-incubation with neutralizing Ars2-reactive Fab but not by LRPAP1-reactive Fab. Columns (formazan formation detected at an optical density of 450 nm) represent cell proliferation (mean and standard deviation). (B) BCR-specific lysis of DLBCL cell lines by Ars2/ETA' immunotoxin. Above: cytotoxic effect after 24 h, 48 h and 72 h of incubation with 5 μg/mL of recombinant Ars2/ETA' or LRPAP1/ETA' immunotoxins. Cell viability of HBL1 (left) and OCI-LY3 (right) cell lines determined by trypan blue staining. Below: Dose-dependent cytotoxic effect of Ars2/ETA' determined in a lactate dehydrogenase (LDH) release assay. Curves indicate percent specific lysis of the HBL1 line (left) or OCI Ly3 line (right) with and without Ars2-reactive BCR, after incubation with doses from 0 μg/mL to 10 μg/mL Ars2/ETA', LRPAP1/ETA' or phosphate-buffered saline. (C) Induction of apoptosis by addition of Ars2/ETA' immunotoxins. Flow cytometric characterization of apoptotic U2932 or HBL1 cells 24 h after addition of Ars2/ETA' or MAZ/ETA' by annexin-V/propidium iodide staining. U2932 cells have Ars2-reactive BCR resulting in a strong increase of early and late apoptotic cells after addition of Ars2/ETA'.
Ars2 expression was shown to be linked to proliferative states and different roles were described in malignant diseases. All three target antigens of DLBCL-BCR identified in this study share the characteristic of being atypically post-translationally modified, which represents the most likely reason for their immunogenicity. For ubiquitinated FamH83 and sumoylated JmJD4, the lymphoma BCR were specific for the secondary modified isoforms. In contrast, Ars2-reactive BCR of DLBCL bound both the hypophosphorylated and the normally phosphorylated isoforms of Ars2. However, the hypophosphorylated isoform of Ars2 was only observed in cell lines or cryosections of DLBCL with BCR reactivity for Ars2 (P<0.0001). Similarly, regarding peripheral blood, the hypophosphorylated isoform of Ars2 was only observed in lysates of peripheral blood cells of patients seropositive for Ars2 autoantibodies (P<0.0001). These statistically significant associations between Ars2-reactivity and the presence of hypophosphorylated Ars2 indicate that this post-translational modification is involved in the immunogenicity.

Bringing this into the context of other B-cell neoplasias, the hypophosphorylated isoform of Ars2 was only observed in lymphomas of peripheral blood cells of patients seropositive for Ars2 autoantibodies (P<0.0001). These statistically significant associations between Ars2-reactivity and the presence of hypophosphorylated Ars2 indicate that this post-translational modification is involved in the immunogenicity. Physiologically it is the major task of a surface immunoglobulin to bind its cognate antigen and then internalize it, enabling processing and antigen presentation via MHC class II molecules. Targeting Ars2-reactive BCR of DLBCL cell lines resulted in a specific and efficacious killing. Beside this, the Ars2 epitope could be used for bispecific constructs for T- or NK-cell engaging (e.g., CD3/Ars2 or CD16/Ars2), or as an additional ectodomain for chimeric antigen receptor T cells. Secondly, regarding the high relative risk of carriers of atypically hypophosphorylated Ars2, investigating ways of modulating this post-translational modification might be worthwhile in the future.

Disclosures
No conflicts of interest to disclose. MP died during the preparation of the manuscript. Saarland University has applied for a relevant patent.

Contributions
LTh, SH, KDP, BK, and MP designed the study. SH and MLH performed microdissection of DLBCL cases and interpreted data. WK, AW YJK, RMB, BK, FvB, LTr, MZ, WM, DWM, VP, and GH were of great help in the acquisition of DLBCL samples and clinical data. NF performed the protein array. Of interest, Ars2 hypophosphorylation and reactivity of DLBCL BCR against Ars2 was nearly exclusively detected in DLBCL of the ABC type. All the cell lines with anti-Ars2 reactivity were of this cell of origin, and in a validation cohort of DLBCL with cell of origin characterized by GEP, hypophosphorylated Ars2 was detected in eight of 31 cases (26%) of the ABC type of DLBCL, but in only one of 20 cases (5%) of GCB-type DLBCL. In a combined analysis of GEP-typed cryosections and analyzed cell lines, the hypophosphorylated Ars2 isoform was statistically significantly associated with ABC type (P=0.0188).

Considering possible functional effects of Ars2, we observed that its addition stimulated growth of DLBCL lines with Ars2-specific BCR (Figure 3), indicating that these lines still depend to some extent on BCR stimulation by their cognate antigen Ars2. Regarding the mutational background of these cell lines with Ars2-reactive BCR and expression of hypophosphorylated Ars2, OCI-Ly10 has mutated MYD88 (L265P) and a truncating mutation of CD79A, U2932 has a mutated NFκB-pathway by TAK1 mutation, but wild-type CARD11, and wild-type MYD88, and OCI-Ly3 has a mutated CARD11 and mutated MYD88 (L265P). This demonstrates that, despite pathway-activating mutations, the proliferation of these cell lines might still benefit from an upstream BCR pathway stimulation by a cognate antigen.

From a therapeutic point of view, two hypothetical approaches arise from these data. Firstly, BCR antigens might be used as baits to target lymphoma cells in a specific way (i.e., targeting of a cell-bound antibody by an antigen), similarly to anti-idiotype antibodies or peptides, with the advantage of not having to be selected and synthesized individually for each patient, since all Ars2-reactive DLBCL BCR bind the same epitope. From a therapeutic point of view, two hypothetical approaches arise from these data. Firstly, BCR antigens might be used as baits to target lymphoma cells in a specific way (i.e., targeting of a cell-bound antibody by an antigen), similarly to anti-idiotype antibodies or peptides, with the advantage of not having to be selected and synthesized individually for each patient, since all Ars2-reactive DLBCL BCR bind the same epitope. Secondly, the high relative risk of carriers of atypically hypophosphorylated Ars2, investigating ways of modulating this post-translational modification might be worthwhile in the future.

Acknowledgments
We are grateful to the entire team of the José-Carreras-Center for Immuno- and Gene Therapy, the DSHNHL and GLA, the Department of Internal Medicine I of Saarland University Medical School for continuous logistic and intellectual support. We also thank Hans Drexler from DSMZ for constant support.

Funding
This work was supported by a grant from Wilhelm-Sander-Stiftung.
References

1. Rosenwald A, Staudt LM. Gene expression profiling of diffuse large B-cell lymphoma. Leuk Lymphoma. 2003;44(Suppl 3):541-47.

2. Alizadeh AA, Eisen MB, Davis RE, et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature. 2000;406(6797):503-11.

3. Reddy A, Zhang J, Davis NS, et al. Genetic and functional drivers of diffuse large B-cell lymphoma identified by gene expression profiling. Nature. 2000;406(6797):503-11.

4. Lenz G, Wright G, Dave SS, et al. Stromal gene signatures in large-B-cell lymphomas. N Engl J Med. 2008;359(22):2315-2323.

5. Schmitz R, Wright GW, Huang DW, et al. Genetics and pathogenesis of diffuse large B-cell lymphoma. N Engl J Med. 2012;367(15):1496-1507.

6. Chapuy B, Stewart C, Dunford AJ, et al. Molecular subtypes of diffuse large B-cell lymphoma are associated with distinct pathogenic mechanisms and outcomes. Nat Med. 2016;22(4):679-80.

7. Davids RE, Nigo VN, Lenz G, et al. Chronic active B-cell-receptor signalling in diffuse large B-cell lymphoma. Nature. 2010;465(7277):88-92.

8. Young RM, Wu T, Schmitz R, et al. Survival of human lymphoma cells requires B-cell receptor engagement by self-antigens. Proc Natl Acad Sci U S A. 2011;108(14):5347-5354.

9. Montesinos-Rongen M, Godlew ska E, Brunn A, Wiestler OD, Siebert R, Deckert D. Activating L265F mutations of the MYD88 gene are common in primary central nervous system lymphoma. Acta Neuropathol. 2011;122(6):791-792.

10. Montesinos-Rongen M, Schafer E, Siebert R, Deckert D. Genes regulating the B cell receptor pathway are recurrently mutated in primary central nervous system lymphoma. Acta Neuropathol. 2012;124(6):905-916.

11. Thurner L, Freuess K-D, Bewarder M, et al. Hyper N-glycosylated SAMDI4 and neurabin-I as driver CNS autoantigens of FCNSL. Blood. 2015;126(27):2744-2753.

12. Cepok S, Zhou D, Srivastava R, et al. Development of potent monoclonal antibody auristatin conjugates for cancer therapy. Nat Biotechnol. 2008;26(7):778-84.

13. Oflazoglu E, Kissler KM, Sievers EL, et al. Hyperphosphorylated paratarg-7: a new molecularly defined risk factor for monoclonal gammopathy of undetermined significance of the IgM type and Waldenstrom macroglubulinemia. Blood. 2011;117(10):2918-2923.

14. Bewarder M, Preuss KD, Fadle N, et al. Circulating autoantibodies to phosphorylated eNOS-eNOS-7 are a hallmark of pancreatic cancer. J Proteome Res. 2011;10(1):105-112.

15. Zhou W, Capello M, Fredolini C, et al. Mass spectrometry analysis of the post-translational modifications of alpha-enolase from pancreatic ductal adenocarcinoma cells. J Proteome Res. 2010;9(6):2929-2936.

16. Grass S, Freuess KD, Wokowicz A, et al. Hyperphosphorylated paratarg-7: a novel molecularly defined risk factor for monoclonal gammopathy of undetermined significance of the IgM type and Waldenstrom macroglubulinemia. Blood. 2011;117(10):2918-2923.

17. Thurner L, Frebbe N, Regitz E, et al. The molecular basis for development of proinflammatory autoantibodies to phosphorylated eNOS-eNOS-7: a novel molecularly defined risk factor for monoclonal gammopathy of undetermined significance of the IgM type and Waldenstrom macroglubulinemia. J Proteome Res. 2011;10(12):3340-3346.

18. Nigo VN, Young RM, Schmitz R, et al. Oncogeneically active MYD88 mutations in human lymphoma. Nature. 2011;470(7352):115-121.

19. Gordon MS, Kanegai CM, Doerr JR, et al. Antigenic epitope of the autoantigen myelin basic protein that induces encephalomyelitis. J Exp Med. 2000;191(5):12.

20. Freuess KD, Frebbe N, Regitz E, et al. Hyperphosphorylation of autoantigenic targets of paraproteins is due to inactivation of PP2A. Blood. 2011;118(12):3340-3346.

21. Arentz-Hansen H, Korner R, Molberg O, et al. The intestinal T cell response to alphagludin in adult celiac disease is focused on a single deamidated glutamine targeted by tissue transglutaminase. J Exp Med. 2001;194(4):603-612.

22. Zamvil SS, Mitchell DJ, Moore AC, et al. Citrulline is an essential constituent of circulating autoantibodies to phosphorylated enolase, a novel biomarker for alcoholic pancreatitis. J Clin Invest. 2007;119(9):2416-2421.

23. Compagno M, Lim WK, Brunn A, et al. Mutations of multiple genes cause deregulation of NF-kappaB in diffuse large B-cell lymphoma. Nature. 2009;459(7247):717-721.

24. Mondello F, Brea EJ, De Stanchina E, et al. Panobinostat acts synergistically with irinotecin in diffuse large B cell lymphoma cells with MyD88 L265F mutations. JCI Insight. 2017;2(6):e90196.

25. Kochenderfer JN, Dudley ME, Kassim SH, et al. Chemotherapy-refractory diffuse large B-cell lymphoma and indolent B-cell malignancies can be effectively treated with autologous T cells expressing an anti-CD19 chimeric antigen receptor. J Clin Oncol. 2015;33(6):540-549.