Cancer Immunology, Immunotherapy (2018) 67:1797–1807
https://doi.org/10.1007/s00262-018-2242-9

FOCUSSED RESEARCH REVIEW

Oncogenic *MYD88* mutations in lymphoma: novel insights and therapeutic possibilities

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Received: 1 May 2018 / Accepted: 5 September 2018 / Published online: 11 September 2018

Abstract

Oncogenic *MYD88* mutations, most notably the Leu 265 Pro (L265P) mutation, were recently identified as potential driver mutations in various B-cell non-Hodgkin Lymphomas (NHLs). The L265P mutation is now thought to be common to virtually all NHLs and occurs in between 4 and 90% of cases, depending on the entity. Since it is tumor-specific, the mutation, and the pathways it regulates, might serve as advantageous therapeutic targets for both conventional chemotherapeutic intervention, as well as immunotherapeutic strategies. Here, we review recent progress on elucidating the molecular and cellular processes affected by the L265P mutation of *MYD88*, describe a new in vivo model for MyD88 L265P-mediated oncogenesis, and summarize how these findings could be exploited therapeutically by specific targeting of signaling pathways. In addition, we summarize current and explore future possibilities for conceivable immunotherapeutic approaches, such as L265P-derived peptide vaccination, adoptive transfer of L265P-restricted T cells, and use of T-cell receptor-engineered T cells. With clinical trials regarding their efficacy rapidly expanding to NHLs, we also discuss potential combinations of immune checkpoint inhibitors with the described targeted chemotherapies of L265P signaling networks, and/or with the above immunological approaches as potential ways of targeting *MYD88*-mutated lymphomas in the future.

Keywords Non-Hodgkin lymphoma · Diffuse large B-cell lymphoma (DLBCL) · Waldenström's macroglobulinemia · MyD88 · Immunotherapy · Toll-like receptor

Abbreviations

BTK	Bruton's tyrosine kinase
CIB	Checkpoint inhibitor blockade
CLL	Chronic lymphocytic leukemia
DD	Death domain
DLBCL	Diffuse large B-cell lymphoma
GMP	Good manufacturing practice
HEK	Human embryonic kidney
HL	Hodgkin lymphoma
HLA	Human leukocyte antigen
IFN	Interferon
IL	Interleukin
IL-1R	IL-1 receptor
iODN	Inhibitory oligodeoxynucleotides
IRAK	IL-1R-associated kinase

This Focussed Research Review is based on a presentation given at the 3rd Symposium on "Advances in Cancer Immunology and Immunotherapy", held in Athens, Greece, November 2nd–4th, 2017. It is part of a Cancer Immunology, Immunotherapy series of papers from this conference.

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JAK	Janus kinase
L265P	Leu 265 Pro
MGUS	Monoclonal gammopathy of undetermined significance
MHC	Major histocompatibility complex
MyD88	Myeloid differentiation 88
NF-κB	Nuclear factor κ B
NHL	Non-Hodgkin lymphoma
PCNSL	Primary central nervous system lymphoma
PMBCL	Primary mediastinal large B-cell lymphoma
PTIT	Peptide-based T-cell-mediated immunotherapy
TAK1	Transforming growth factor beta-activated kinase 1
TCR	T-cell receptor
TIR	Toll/Interleukin-1 (IL-1) receptor
TLR	Toll-like receptor
WM	Waldenström's macroglobulinemia
WT	Wild type

Introduction

In the genomic era, whole exome sequencing has been applied to numerous tumor entities and has unearthed a great multitude of tumor-associated mutations, some of which have emerged as recurrent across multiple entities. One such mutation in B-cell non-Hodgkin lymphomas (NHLs) is the *MYD88* leucine 265 to proline mutation (henceforward referred to simply as L265P) in the Toll-like receptor adaptor molecule, myeloid differentiation 88 (MyD88). Toll-like receptors (TLR) are one of several families of so-called pattern recognition receptors (PRR) that typically activate innate immunity, but also operate in B lymphocytes [1, 2]. TLRs respond to exogenous microbe-associated molecular patterns (MAMPs, e.g., lipopolysaccharide or CpG DNA) or endogenous danger-associated molecular patterns (DAMPs, e.g., HMGB1). First discovered in 2011 by the Staudt laboratory via a screening approach for drivers in diffuse large B-cell lymphoma (DLBCL) [3], L265P has since been identified in numerous other B-NHL subtypes. Several lines of evidence have led to the notion that MyD88 L265P is an oncogenic driver: first, the high mutation frequency in some NHL entities, e.g., 90% of cases in Waldenström's macroglobulinemia (WM) [4] or 50% in its precursor, monoclonal gammopathy of undetermined significance (MGUS) [5]; second, in chronic lymphocytic leukemia (CLL), its occurrence was shown to be an early, clonal event [6]; and third, in >96% of NHL cases in which zygosity of the L265P mutation was analyzed, the $MYD88^{L265P}$ allele was present in a heterozygous state [3, 7], indicating that a single hit is sufficient to exert an oncogenic effect, which we also confirmed on a mechanistic level [8]. L265P-mediated oncogenesis has mainly been attributed to its ability to drive increased activation of nuclear factor κ B (NF-κB), which has long been known to sustain B-cell survival and differentiation [9]. Recent studies, which we review below, have sought to elucidate the molecular mechanisms by which the mutation drives this pathway (summarized in Fig. 1). Additional work has also implicated mitogen-activated kinase (MAPK) and the transcription factor AP-1 [10] in lymphomagenesis. Given that L265P has not been described in any non-B-cell malignancy, benign tumor, or cells derived thereof, it has drawn attention as a strictly tumor-specific therapeutic target, particularly in combination with Bruton's tyrosine kinase (BTK) inhibitors, which hold promise in NHL therapy [11]. Here, we review the latest information on pre-clinical and clinical studies on pharmacological inhibition and the translational potential of L265P for chemotherapeutic approaches. Finally, fueled by the considerable success of immune checkpoint inhibitors as novel therapeutics, we and others have also noted the potential of L265P for immunotherapy which might allow for an even more specific eradication of *MYD88*-mutant B cells exploiting the capabilities of the host immune system. We, therefore, review the basic concepts and recent studies in this direction and flag up remaining questions to be addressed in further characterizing and targeting this intriguing mutational hotspot in NHL.

Advances in the molecular understanding of *MYD88* L265P signaling

Molecular level of MyD88 L265P signaling

The first results on L265P, obtained by RNAi-mediated loss of function experiments, showed that along with MyD88, the canonical pathway components Interleukin-1 receptor-associated kinase (IRAK) 1 and IRAK4 are non-redundant requirements for ABC DLBCL cell line survival [3]. Moreover, IRAK1/4 inhibition was selectively toxic to DLBCL cell lines in an L265P mutation-specific manner [3]. This was recently confirmed using a new class of IRAK4 inhibitors and shown to affect both NF-κB and JAK-STAT3 survival signals [12]. Apparently, L265P caused a stronger recruitment and phosphorylation of IRAK1 in ABC DLBCL cell lines [3] and also in primary CLL cells [13]. MyD88 recruits IRAKs via so-called death domains (DD) not its Toll-Interleukin-1 receptor (TIR) homology domain to which the mutation maps. We, therefore, wondered how a mutation in the MyD88 TIR domain might allosterically influence signal propagation via IRAKs. When overexpressed in HEK293T cells, isolated MyD88 TIR domains block signaling [14]. However, we found the isolated L265P-mutant TIR domain to strongly drive NF-κB activation in HEK293T cells containing endogenous MyD88, but not HEK293T cells lacking endogenous MyD88 [8]. We speculated that L265P TIRs

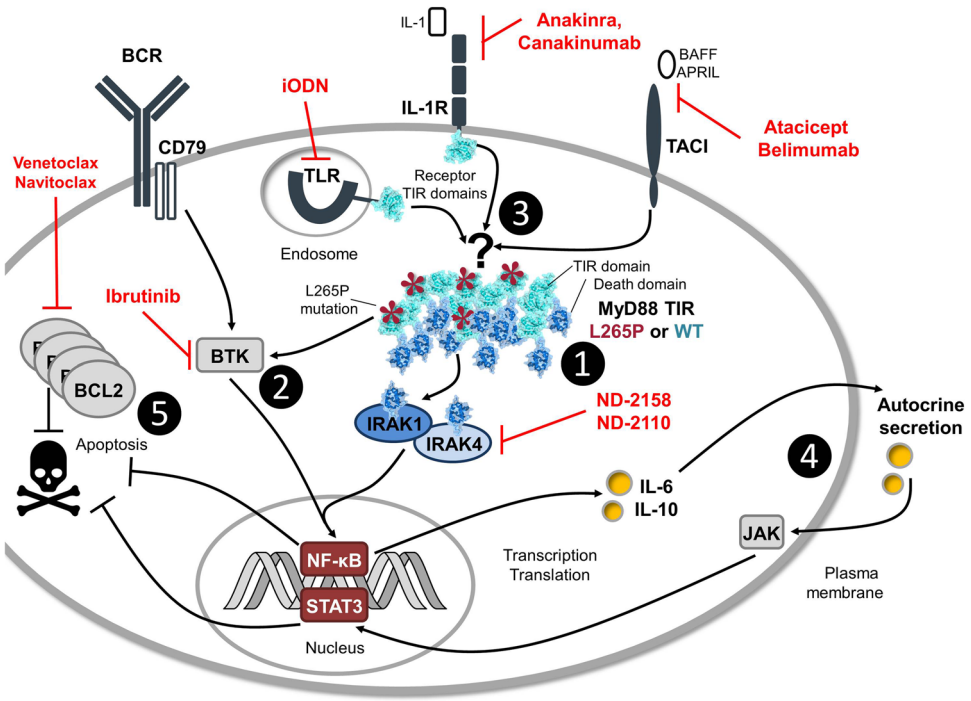

Fig. 1 MyD88 L265P signaling networks and pharmacological targeting. MyD88 L265P, in cooperation with WT MyD88, affects multiple signaling pathways in mutated B-cells, dysregulating gene transcription and promoting survival. L265P Toll/Interleukin-1 receptor (TIR) domains (light blue domains with red asterisks) crosslink with WT MyD88 proteins to high molecular weight complexes, forming a scaffold of death domains (DDs, dark blue) to recruit and activate IRAK kinases (1). MyD88 L265P also binds the proximal BCR kinase, BTK, which activates NF-κB in an IRAK-independent way (2). In accordance, IRAK4 (ND-2158 & ND-2110) and the BTK inhibitor, ibrutinib [see (1) and (2) in red], affect both NF-κB and STAT3 activation. It is not known if L265P acts receptor-independently or whether signals are amplified by TLRs, IL-1R, and TACI (3). L265P-mutated cells may thus be sensitive toward inhibition of endosomal TLRs (via inhibitory oligodeoxynucleotides, iODN), IL-1R (Anakinra, Canakinumab), and TACI (Atacicept, Belimumab) receptor interference, for example, by competition with natural ligands (DNA for TLR9) or by neutralizing the ligands IL-1 and BAFF/April. Furthermore, NF-κB triggers IL-6 and IL-10 release, which might stimulate STAT3 via JAK in an autocrine way (4) and, therefore, reinforce expression of anti-apoptotic genes. High levels of BCL2 prevent apoptosis and potentiate MyD88 L265P effects on tumor cell survival (5)

must, therefore, employ the endogenous MyD88 WT for signaling in this TIR-only setting. Our data suggest that in a heterozygous situation, a L265P-mutated TIR domain is sufficient to engage WT or mutated TIR domains into a scaffold to recruit IRAKs. Indeed, in microscopy and interaction studies of full-length and TIR MyD88 constructs colocalization and binding was strongly increased for all L265P combinations, confirming a key role of L265P TIR domains to seed complexes of higher molecular weight. This L265P-mediated aggregation was confirmed in NHL cell lines harboring the mutation [8]. In agreement with earlier studies showing constitutive binding of MyD88 L265P to phosphorylated IRAK1 [3], the protein aggregates also contained elevated amounts of IRAK1 and thus probably constituted signaling active, so-called "Myddosomes"—TLR post-receptor complexes involving MyD88 [15, 16]. Thus, L265P appears to force TIR-mediated aggregation which is known to promote NF-κB activation [17]. To see whether MyD88 TIR aggregation is required for lymphoma cell survival, TIR aggregation was blocked in DLBCL cell lines, using so-called decoy peptides able to block MyD88 TIR-domain dimerization [18]. Indeed, L265P-mutant DLBCL cell lines were much more sensitive to inhibitory peptides than cell lines with WT MyD88 [8]. Taken together, our studies suggest that L265P causes MyD88 to spontaneously aggregate into Myddosome-like complexes incorporating IRAK1 for increased NF-κB signaling and sustained survival. A recent report indicates that in WM, active Myddosomes may not only affect the B cells that they originate in—when released within extracellular vesicles, these signaling-active complexes may be taken up by bone-marrow macrophages contributing to an inflammatory microenvironment, fueling the pathogenesis of WM [20]. Further work confirming this mechanism in other NHL and charting the composition and other characteristics of L265P-nucleated Myddosomes is clearly warranted.

Regarding the molecular events downstream of the Myddosome, two other kinases, namely, BTK [21] and

Transforming growth factor β-activated kinase 1 (TAK1) [22], were proposed to contribute to MyD88 L265P hyper-signaling. The involvement of BTK has also been corroborated by first results for the treatment of DLBCLs with the BTK inhibitor, ibrutinib [11], and the co-existence of both MyD88 and BCR pathway-activating mutations (e.g., affecting CD79B) in DLBCL [23] suggests a synergy between MyD88 and BCR signaling, at least in certain NHL. Interestingly, in WM cell lines ibrutinib downregulated NF-κB, but did not affect phosphorylation of IRAK1; vice versa, IRAK1/4 inhibition did not affect activation (phosphorylation) of BTK [21], indicating that MyD88 L265P-induced oncogenic signaling may converge with BTK signaling downstream of the canonical MyD88-IRAK Myddosome and involves the IRAK kinases and the BCR–BTK axes independently [24, 25]. However, the report of a MyD88-TLR9-BCR containing "super-complex" by the Staudt lab in ABC DLBCL cell lines and biopsies [19] now suggests that BCR and TLR axis directly converge upon L265P-mutated MyD88. Unexpectedly, this complex directly incorporated the MALT1, CARD11, BCL10 NF-κB pathway mediators previously linked to ABC DLBCL [10], as well as mTOR1. Interestingly, even though BTK was not preferentially bound by TLR9 in mutated vs non-mutated cell lines and was not captured as a MyD88 interactor, the occurrence of the MyD88 super-complex correlated with ibrutinib sensitivity in patients. The mechanistic basis for this correlation and the significance of TLR9 and/or BCR stimulatory signals await further investigation. Clearly, although much progress has been made using model systems, further molecular studies in primary B cells with endogenously mutated MyD88 and using high-resolution imaging are now needed to fully elucidate the molecular switches flicked by this intriguing mutation. Elucidating these molecular details will be vital to fully understand how *MYD88* mutations could be targeted in personalized approaches to NHL treatment. In this regard it has been puzzling that L265P mutated cases or cell lines responded quite differently to BTK inhibitors than non-L265P mutations (e.g., S219C, M232F/T or S243N) [11, 26] and may be associated with better survival [27]. This has been surprising, since they share unifying structural features, hyper-activated NF-κB and appear to be some of the few amino acid positions in MyD88 where mutations are not detrimental to MyD88 function [8]. In general, these non-L265P mutations are not fully understood to date. However, since L265P is the most frequent mutation, a focus on this particular mutation seems well justified.

Transcriptional pathways regulated by oncogenic MYD88 mutations

In terms of the transcriptional changes effected by L265P mutation, most attention has been centered on the canonical NF-κB pathway as a key regulator of development and differentiation in normal B cells [2] and regulator of survival and proliferation of the malignant cells. To investigate how NF-κB activity is regulated in ABC DLBCL, Nogai et al. [28] showed that the atypical nuclear Inhibitor of κ B (IκB) protein, IκB-ζ, a transcriptional regulator, is upregulated and essential for survival of MyD88 L265P ABC DLBCL cell lines and *MYD88* RNAi silencing reduced levels of IκB-ζ. Furthermore, IκB-ζ immunoprecipitated together with both p50 and p52 subunits, indicating that MyD88 L265P might promote both canonical and non-canonical NF-κB pathways. Guo et al. [29] found that MyD88 L265P additionally promotes the BCR-mediated non-canonical NF-κB signaling characterized by p52/p100 via TAK1/IκB kinase (IKK) α in vitro. This squares well with the direct engagement of MALT1, CARD11 and BCL10 pathway members [19] by MyD88. NF-κB itself is known to induce the expression of B lymphocyte-induced maturation protein-1 (Blimp-1), a transcriptional regulator for B-cell terminal maturation to plasma cells [30]. Blimp-1 is inactivated in 24–53% of ABC DLBCL cases by homozygous deletions, truncating or missense mutations, and via transcriptional repression by constitutively active BCL6 and thus a vital tumor repressor gene in ABC DLBCL [31, 32]. Furthermore, a Korean group showed correlations between MyD88 L265P mutation and deletion of 6q, which includes the *PRDM1* gene locus (which encodes for Blimp-1), in WM [33]. Collectively, Blimp-1 may thus represent an important oncogenic regulator in L265P-mutated NHLs.

Furthermore, MyD88 L265P also affects other transcriptional pathways relevant for B cells: Rousseau et al. proposed MAP kinases to contribute to L265P-mediated effects and show that the mutation promotes ERK1/2 phosphorylation in HEK293T cells in the presence of the MKK1/2 substrate TPL2 [34]. The aforementioned direct engagement of CARD11 by MyD88 has also been shown to feed into Jun-mediated AP-1 signaling in ABC DLBCL [10]. A link between L265P and STAT3 signaling has been suggested by synergistic effects of IRAK4 and Janus kinase (JAK) inhibitors [12]. But this may be indirect, e.g., by feed forward loops involving NF-κB regulated pro-inflammatory cytokines like IL-6 or IL-10 which are elevated in ABC DLBCL cell lines [3] and may activate STAT3 in an autocrine way. Interestingly, according to another report, STAT3 directly regulates *MYD88* transcription and STAT3 silencing decreased *MYD88* mRNA levels [35]. Thus the transcriptional effects of L265P mutation appear to go beyond NF-κB signaling; further exploration into this area may thus uncover additional synergies that could be exploited pharmacologically.

Targeting and in vivo models

On this front, those approaches targeting BTK are now clinically most advanced. In a phase 1/2 clinical trial in relapsed or refractory DLBCL, BCR pathway-mutated ABC DLBCLs responded preferentially when the L265P mutation was present, although ABC DLBCLs without BCR or *MYD88* mutations could also be effectively targeted, indicating both genetic and non-genetic mechanisms to affect ibrutinib sensitivity [11]. In addition, IRAK inhibitors have also been discussed as possible L265P-specific intervention tools: Structure-based drug design identified two compounds, ND-2158 and ND-2110, to inhibit IRAK4 at nanomolar concentrations in vitro and to block lymphoma growth in several xenografts with human ABC DLBCL cell lines in NOD/SCID mice in vivo [12]. Interestingly, a pronounced synergy with ibrutinib and a Syk inhibitor, PRT-062607, was also observed in this study. In a follow-up study, the pyrrolopyrimidine scaffold of an IRAK4 inhibitor was optimized [36]. Although off-target effects on members of the CLK kinase family, dual specificity kinases involved in splicing regulation, were also observed, the resulting compound induced inhibition of IκBα phosphorylation in ABC DLBCL cell lines. In in vivo models, IRAK4 inhibition alone only had a modest effect but the inhibitor showed considerable synergistic potential together with ibrutinib [36]. Results for ongoing clinical trials of additional IRAK4 inhibitors, e.g. CA-4948 in relapsed or refractory NHL (NCT03328078) and Pf-06650833 in rheumatoid arthritis (NCT02996500), will be interesting to watch in order to gauge whether these inhibitors may emerge as promising compounds in NHL therapy alone or in combination. In this vein, we noted an intriguing sensitivity of L265P mutant cell lines, as assessed in an earlier study [37], to the BCL2 inhibitor navitoclax (ABT-263). DLBCL often exhibit translocations (more common for GCB) or locus amplifications (more common in the ABC subtype) of the *BCL2* gene, leading to overexpression of BCL2 [38]. Because BCL2 inhibits B-cell apoptosis induced by BIM, BAX and BAK proteins, high protein levels are known to sustain malignant B cell survival [39]. The cooperation between L265P and BCL2 was strikingly demonstrated in the first autochtonous in vivo model for MyD88 L265P mutant DLBCL [40]. CD19-Cre-mediated B-cell-specific expression of an *Myd88p.L252P* allele (the murine orthologue of human *MYD88* L265P) was sufficient for lymphoproliferative disease and occasional transformation into a clonal lymphoma in the spleen, liver and lymph nodes with strong nuclear p65 staining on histological sections resembling transcriptional NF-κB activity [40]. In accordance to gene locus amplifications known in humans, the additional conditional overexpression of *BCL2* led to a significant disease acceleration and to highly penetrant development of aggressive lymphomas resembling human ABC DLBCL with features of plasmablastic lymphomas, such as CD138 positivity [40]. We consider this model to be an ideal tool for both pre-clinical work to test inhibitor strategies, as well as dissecting the open mechanistic questions in vivo. However, it would be desirable to have combinations with additional alleles, such as *CD79* ITAM mutants or oncogenic *Card11* mutants. In addition, it would be very interesting to see whether the phenotype of the *Myd88p.L252P;BCL2* model would shift by additional *Tnfaip3* (encodes for A20, a negative regulator of NF-κB) deletion and/or *Prdm1* (encoding for Blimp-1) deletion, since these factors have been implicated in DLBCL [31, 41]. Given that all previously published in vivo studies employed xenografts, this autochtonous model is ideal to investigate the efficacy of the inhibitors or inhibitor combinations discussed above.

Open questions and opportunities

One critical mechanistic question requiring an answer is whether L265P acts receptor-independently or whether it amplifies signals by the three types of receptors so far proposed to interact with MyD88: TLRs, the IL-1R [42, 43] or TACI [44]. If confirmed, this would open up additional therapeutic options, e.g., via targeting these receptors and their ligands (Fig. 1). Since L265P-mediated NF-κB activation can be observed in the absence of TLRs (e.g., in HEK293T cells, albeit by overexpression) or TLR stimulation (DLBCL cell lines), it is possible that L265P can induce the process completely independently of any upstream receptor. The structural basis has thus far not been elucidated and several computational studies attribute little overall structural changes to the L to P exchange [8, 24, 45]. Nevertheless, computational modeling indicates the mutation to prompt a shift from heteromeric interactions (e.g., with TLRs) to homodimeric interactions (e.g., receptor-independent aggregation) for L265P [24, 45]. On the other hand, the Goodnow laboratory observed spontaneous proliferation of murine B cells with retroviral MyD88 L265P expression in culture without addition of mitogens, but this was reduced by the TLR9 inhibitor, chloroquine [39]. Genetic ablation of the TLR9 chaperone, *Unc93B1* or *Tlr9* itself had a similar effect in this study [39] but also more recently in human ABC DLBCL cell lines [19]. In a follow-up study WT, Unc93B1- or TLR9-deficient B cells were monitored in vivo in Rag1-deficent mice upon activation with anti-IgM/anti-CD40 and transduction with a L265P MyD88-IRES-EGFP retrovirus [46]. Although in vitro Unc93B1 or TLR9-deficient and MyD88 L265P-transduced cells were less proliferative, in vivo they gave rise to an increased $CD19^{low}IgM^{+}$ and IgM-secreting B-cell population. Unfortunately, comparisons to WT constructs were not made and it is difficult to gauge the influence of the $Rag1^{-/-}$ background with a lack of T and healthy B lymphocytes and retroviral transduction in

this system. Based on these results, intriguing pre-σ model [47] and in psoriasis patients [48], a TLR9 inhibitory oligodeoxynucleotide (iODN), IMO-8400, has moved on to a phase 1/2 open label, multi-dose, dose-escalation clinical trial (NCT02092909) in 31 WM patients with relapsed or refractory disease. The results of this trial will be interesting for NHLs from a therapeutic perspective but may also provide clues for or against an additional mechanistic involvement of afferent receptor signaling in L265P mutated lymphomas.

Concept for an immunotherapeutic exploitation of the L265P mutation

The fact that cancer is often a disease in older age has, at least in part, been attributed to the ability of the human immune system to constantly identify and eliminate cells in which mutations accumulate. This is executed via different mechanisms, one of which involves the detection of so-called neo-antigens arising from non-synonymous mutations [49]. Like their non-mutated counterparts, mutated proteins are processed intracellularly by the immuno-proteasome and loaded onto major histocompatibility class (MHC) I complexes (Fig. 2). When these are displayed on the cell surface, CD8 T lymphocytes recognize such MHC–peptide complexes via their T-cell receptors (TCRs) and become activated. Neo-antigens are especially interesting for cancer surveillance, since they arise locally and spontaneously and thus specific T-cell clones would not have been eliminated during the mechanisms enforcing tolerance to self-antigens. Whether a neo-antigen becomes a peptide ligand is dictated by the individual's human leucocyte antigen (HLA)/major histocompatibility class (MHC) type and the nature of the respective mutation [50]. If the mutation affects a so-called

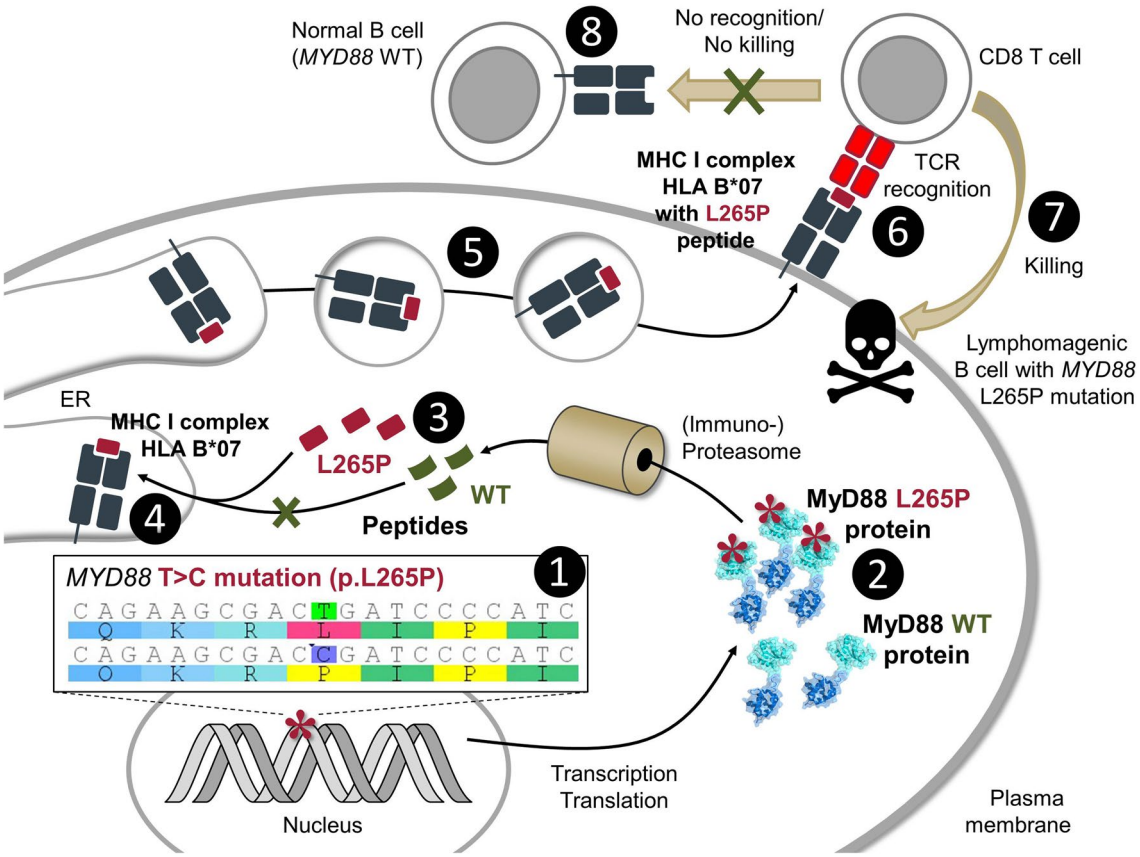

Fig. 2 Presentation of peptides containing L265P mutations as T-cell neo-epitopes. Due to a mostly heterozygous T to C transition (1), L265P-mutated proteins are synthesized alongside WT protein (2). As with most cellular proteins, both MyD88 forms will be subject to immuno-proteosomal degradation into peptides (3) that are typically loaded onto MHC class I (4) and II complexes (not shown) by different cellular pathways (not shown in detail). For certain HLA types, the amino acid sequence of the WT peptide will not be compatible with the requirements for efficient HLA-binding compared to L265P-containing peptides, e.g., when the anchor position is affected. Following trafficking of loaded MHC I-peptide complexes from the ER to the cell surface (5), the MHC I-peptide complex is sampled by T cells via their T-cell receptor (TCR) (6). Since L265P-containing peptides represent neo-antigens that were not negatively selected during T-cell development, certain CD8 T cells with specific TCRs may bind such MHC I-L265P-peptide complexes and be activated for killing (7), whereas non-mutated normal B cells or other tissue cells are not recognized (8)

anchor position [51], the neo-antigen may bind better or worse to the donor's MHC I molecules. The presentation of tumor-associated neo-antigens is exploited by peptide-based T-cell-mediated immunotherapy (PTIT), where T cells are specifically and deliberately raised against certain peptides, e.g., neo-antigen peptides [52]. Although non-mutated tumor-associated antigens also hold promise for therapy, they harbor the potential of causing collateral damage by T-cell responses being targeted to non-cancerous organs or cells displaying these antigens; this is not the case for neo-antigens which are tumor specific. However, a disadvantage of targeting neo-antigens is that they are normally cumbersome to determine and differ greatly even between patients for the same tumor entity. Thus a fully individualized approach is necessary that is difficult to offer to larger cohorts of patients given the process of identification and formulation of suitable peptides is time consuming and elaborate, in addition to regulatory difficulties. Focusing PTIT on recurrent tumor-associated mutations combines the advantages of both an individualized neo-antigen-focused and a tumor-specific approach whilst retaining the applicability to larger patient groups due to recurrence of the mutation(s). Since it is frequent and tumor-specific, the recurrent MyD88 L265P mutation generally represents such an attractive target for PTIT and other immunotherapeutic approaches.

We were the first to analyze the potential of MyD88 L265P peptides to elicit cytotoxic T-cell responses as tumor-specific neo-antigens [53]. Based on *in silico* predictions, we identified potential L265P-containing HLA ligands for several HLA class I restrictions. A set of HLA class I MyD88 L265P -derived ligands—namely, HLA-B*07 peptides RPIPIKYKAM, RPIPIKYKA, and SPGAHQKRPI (L265P mutation underlined) and the -B*15 peptide HQKRPIPIKY, but not the WT equivalent peptides—elicited-specific cytotoxic $CD8^+$ T-cell responses in priming experiments in PBMC from multiple healthy donors. These T cells stained double positive for IFN and TNF in flow cytometry, indicating multi-functionality. For RPIPIKYKAM- and HQKRPIPIKY-specific killing of peptide-loaded target cells by primed polyclonal T cells could also be demonstrated [53]. Interestingly, for RPIPIKYKAM, the score of binding to HLA-B*07 was > 280-fold higher than for the WT peptide using the NetMHC prediction algorithm [54], indicating that such a peptide may be an ideal candidate for PTIT. These data highlight the potential of L265P-specific peptide-based immunotherapy as a novel personalized and, nevertheless, broadly applicable treatment approach for patients with L265P mutation. Nielsen et al. independently confirmed our findings in that the HLA-B*07 peptides RPIPIKYA/M were recognized by $CD8^+$ T cells in 5/6 donors and mutant peptides were able to prime T cells at a 100,000-fold lower concentration than WT peptide [55]. Furthermore, the authors could show that in autologous B cells, expression of MyD88 L265P using a transgene led to T-cell priming, indicating endogenous processing of the translated protein sequence. Thus, it can be expected, but has not been formally shown, that cells harboring the L265P mutation would also present L265P peptides in case of permissive HLA alleles.

From a translational perspective, these studies raise the possibility that T-cell recognition of L265P peptides could be exploited therapeutically in three ways (Fig. 3): (1) by vaccinating patients with L265P peptides (PTIT) [56]; this would be the by far simplest application of this concept as sets of HLA-matched peptides can easily be manufactured under GMP conditions and could be combined to exploit all possible HLA alleles present in the patient, thus combining a personalized and ware-house peptide approach. Nevertheless, since the mutation is recurrent, a defined set of peptide drugs would make the route through toxicology/pharmacology testing, clinical trials, and regulatory approval more straightforward than can be expected for fully personalized approaches; (2) ex vivo priming of L265P-specific patient T cells and adoptive transfer into the patient (see [57] for a general overview of adoptive T-cell transfer strategies in cancer treatment). When priming induced by vaccinating the patient is not sufficient to mount a significant T-cell response in vivo, it is conceivable that mutation-specific T cells can be primed and/or the minor numbers expanded ex vivo from a patient´s blood samples. We showed that even in CLL patients, where T-cell numbers in the blood are comparable low due to leukemic B-cell expansion, priming worked ex vivo [53]. Compared to approach #1, this adoptive transfer would be more cumbersome and rely on a locally available service of GMP manufacture of cell products; (3) transduction of polyclonal patient T cells with genetically engineered TCRs specific for L265P peptide–MHC peptides. In such an attempt [58], the coding sequences of the TCR α and β chains are cloned from MHC-L265P peptide-reactive T-cell clones (e.g., in [53]) and, optionally after affinity optimization in vitro, virally transduced into the patient's T cells (Fig. 3). This means that in addition to their cognate TCR, the transduced pool of $CD8^+$ T cells will express another TCR that is specific for the L265P peptide–MHC complex and could thus be activated to eliminate tumor cells. One single combination of α and β TCR chains identified to effectively work with a given HLA could then be used as a treatment applicable to all L265P-positive patients of a given HLA-type, thus combining features of both a personalized, tumor-specific approach, and a treatment that is identically applicable to entire groups patients. Our preliminary in vitro data show that TCR-gene-modified T cells can effectively recognize cells expressing mutated *MYD88* constructs (Fig. 4a), and kill them (Fig. 4b). Demonstration of killing activity on fresh tumor cells and safety testing to detect any potential cross reactivity with self-antigens needs to be performed before initiating a clinical trial. However, first

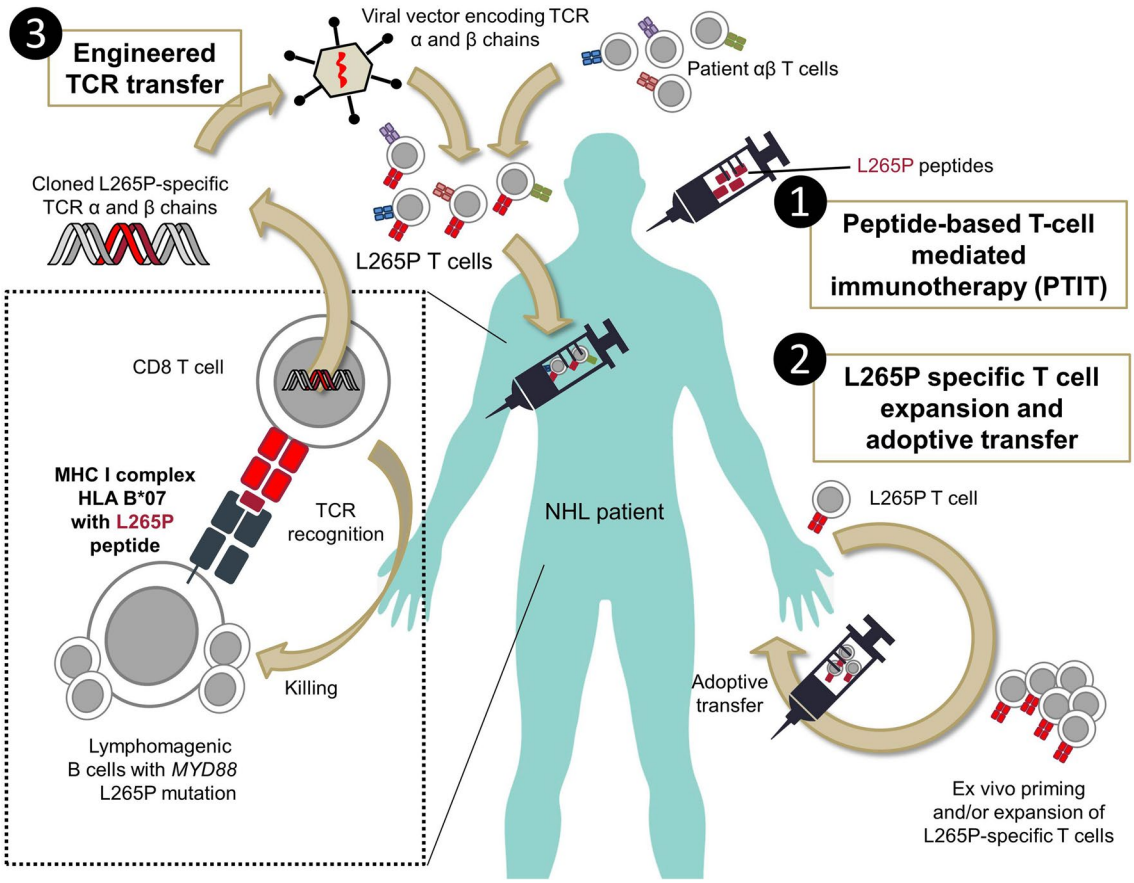

Fig. 3 Exploitation of L265P mutations for T-cell-based immunotherapy. The figure shows three conceivable approaches for exploiting L265P mutations in T-cell-based immunotherapies: (1) patients with the appropriate HLA types and confirmed *MYD88* mutation are vaccinated with MyD88 L265P-derived peptides to prime specific T cells in the patient. (2) Where this is not possible or ineffective, T cells can either be primed from patient PBMC entirely ex vivo, or low numbers of specific T cells be expanded ex vivo under GMP conditions. (3) Alternatively, polyclonal T cells from the patient are virally transduced with TCR sequences. These TCRs could be identified and cloned beforehand from T cells primed with L265P peptides affinity matured in vitro before engraftment into the viral vector. For each HLA type, a single defined TCR sequence pair (for α and β chains) would need to be generated once to be applied to all patients with L265P mutation bearing this HLA-type. A combination of different viruses to exploit all possible L265P-conducive HLA alleles in the patient would be feasible. Following viral transduction of the polyclonal patient T cells, the engineered T cells, which express an HLA-L265P-restricted TCR in addition to their original/cognate TCR will be adoptively transferred back to the patient

clinical trials using such an approach for other antigens are already ongoing [58].

These L265P-focused immunotherapeutic approaches need to be explored as individual treatment options, but they may later also be combined with non-specific checkpoint inhibitor blockade (CIB), to direct the unleashed immune response towards L265P-mutated cells. In this context, it is interesting to review the current clinical results for CIB in NHLs. In general, lymphomas have been considered to harbor an immunosuppressive microenvironment with expression of PD-L1 that reinforces T-cell exhaustion. In addition, in certain lymphoid malignancies, e.g., primary mediastinal large B-cell lymphoma (PMBCL), WM and primary central nervous system lymphoma (PCNSL), PD-L1, and/or PD-L2 expression were increased, in some entities due to amplification and translocation of the genomic region 9p24.1 [59–61]. This has raised hopes that CIB may be effective in NHLs—or at least as effective as it has been for other solid tumors. Recent and more detailed reviews provide a summary of the clinical results for CIB specifically in NHLs [61, 62]. In general, it appears that results for Hodgkin Lymphoma (HL), PMBCL, and PCNSL lymphomas—possibly due to the displayed 9p24.1 alterations—are encouraging. Unfortunately, in other NHLs, the results obtained thus far are less positive, at least for CIB monotherapies, warranting the search for novel combination approaches with pharmacological or other immunotherapies. It will be interesting to determine whether L265P-containing neo-antigens contribute to T-cell-mediated immune control in those entities with high mutation frequency. The attractiveness of tumor immunotherapy directed against neo-antigens such as L265P has the tremendous attractiveness of lack of toxicity (provided TCR

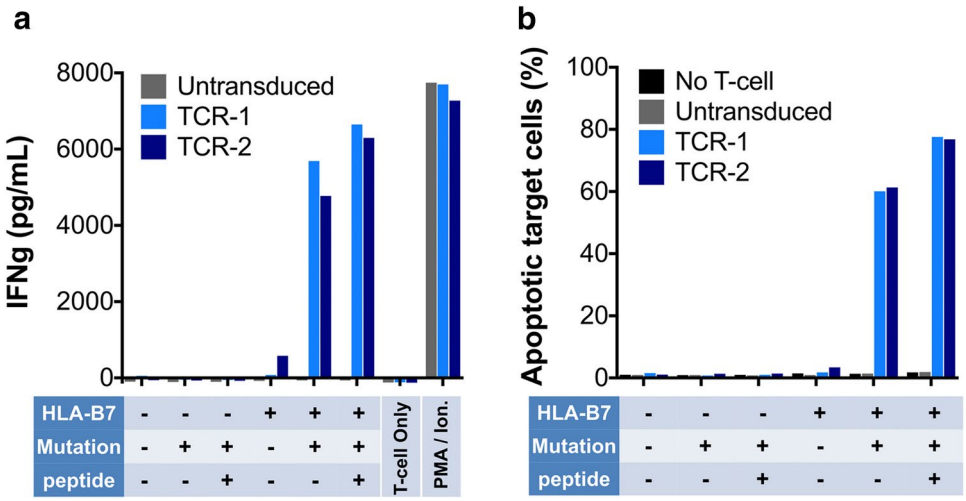

Fig. 4 Selective response of TCR-engineered T-cells against cells expressing the L265P mutation. **a** Peripheral $CD8^+$ T cells isolated from healthy donors and virally transduced to express L265P-specific T-cell receptors (TCRs) were co-cultured with K562 target cells expressing different combinations of WT or mutant *MYD88* and human *HLA-B*07*. Experiments included also cells pulsed with a peptide encompassing the mutation. IFNγ release was analyzed by ELISA. **b** K562 cells transduced with mutant *MYD88* and *HLA-B*07* are specifically killed by TCR-engineered T cells. The proportion of apoptotic target cells is measured by flow cytometry via intracellular staining of activated caspase-3

cross reactivity is excluded), and is substantiated by recent data showing extreme safety with virtual lack of toxicity in a recent vaccination trial [63]. However, lymphomas associated with *MYD88;CD79B* mutations appear to be prone to immunoediting—e.g., mutation or deletion of *HLA-A, HLA-B*, or *HLA-C* [64]—posing potential caveats for immunotherapeutic approaches that need to be considered.

Conclusion

In conclusion, there has been much progress on understanding both the molecular mechanisms for *MYD88* L265P-driven oncogenesis, as well as its translational exploitation from a diagnostic and therapeutic point of view. Undoubtedly, the next years will show which of the approaches of pharmacological inhibition or immunotherapeutic concepts put forward so far will prove applicable clinically.

Acknowledgements We thank Hans-Georg Rammensee, Stefan Stevanović, Juliane Walz and Annika Nelde for helpful discussions. Free clipart was from pngtree (human-body-structure_3520512, cartoon-syringe_1100816) or thenounproject (skull-and-crossbones/9626).

Author contributions All authors collected and analyzed data, A. Weber coordinated the study and drafted the manuscript, and all authors contributed toward and approved the final manuscript.

Funding This work was supported by Deutsche Forschungsgemeinschaft (DFG) Collaborative Research Center (SFB) 685 "Immunotherapy" (to Y. Cardona Gloria, A. Weber and O.-O. Wolz), the Federal State Baden-Württemberg Junior Professor Program (to A. Weber), the University of Tübingen and the Medical Faculty Tübingen (Fortüne Junior Grant to O.-O. Wolz) as well as by DAAD support (Özcan Çınar).

Compliance with ethical standards

Conflict of interest OOW and AW are named inventors on a patent for the immunotherapeutic exploitation of L265P mutations. The authors declare that there are no other potential conflicts of interest.

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