Aus der Neurologischen Klinik und Poliklinik

Klinik der Universität München

Direktorin: Prof. Dr. Marianne Dieterich, FANA, FEAN

The roles of BAFF-R and CAR-TCells in the growth and treatment of primary central nervous system lymphoma

Dissertation

zum Erwerb des Doktorgrades der Humanbiologie

an der Medizinischen Fakultät der

Ludwig-Maximilians-Universität zu München

vorgelegt von

Xiaolan Zhou

aus

Liaoning, China

2022

Mit Genehmigung der Medizinischen

Fakultät der Universität M ünchen

Berichterstatter: Prof. Dr. med. Andreas Straube

PD Dr. Tobias Birnbaum

Prof. Dr. Stylianos Michalakis

Mitberichterstatter: Prof. Dr. med. Louisa vonBaumgarten

Dekan: Prof. Dr.med. Thomas Gudermann

Tag der mündlichen Prüfung: 23.05.2022

LMU LUDWIG-MAXIMILIANS-UNIVERSITÄT MÜNCHEN | Promotionsbüro Medizinische Fakultät | MMRS

Affidavit

Xiaolan Zhou

Surname, first name

I hereby declare, that the submitted thesis entitled:

The roles of BAFF-R and CAR-TCells in the growth and treatment of primary central nervous system lymphoma is my own work. I have only used the sources indicated and have not made unauthorised use of services of a third party. Where the work of others has been quoted or reproduced, the source is always given.

I further declare that the submitted thesis or parts thereof have not been presented as part of an examination degree to any other university.

Shenyang, 04.05.2021

place,date

Zhou, Xiaolan

Signature doctoral candidate

Table of content

List of abbreviations

ABC	activated B cell-like
AID	autoimmune inflammatory disease
ALL	acute lymphoblastic leukemia
APRIL	a proliferation-inducing ligand
BAFF	B cell activating factor of the TNF family
BAFF-R	B cell activating factor receptor
BCMA	B cell maturation antigen
BCR	B cell receptor
BM	bone marrow
BLyS	B lymphocyte stimulator
BR3	BAFF receptor 3
BTK	Bruton tyrosine kinase
CART	Chimeric antigen receptor T
CNS	central nervous system
CR	complete remission
CSF	cerebrospinal fluid
CXCL9	C-X-C motif chemokine 9
CXCL13	C-X-C motif chemokine 13
DLBCL	diffuse large B-cell lymphoma
EBV	Epstein-Barr virus
ELISA	enzyme-linked immunosorbent assay
FACS	Fluorescence-activated cell sorting
FCS	fetal calf serum
GC	germinal center-like
HRP	horse radish peroxidase
LN	lymph node
MBT	metastatic brain tumor

MTX	methotrexate
NHL	non-Hodgkin lymphoma
NID	neuroinfectious disease
OS	overall survival
PB	peripheral blood
PBT	primary brain tumor
PCR	polymerase chain reaction
PCNSL	primary central nervous system lymphoma
TACI	transmembrane activator and CAML interactor
TNF	tumor necrosis factor
TSA	thyramide signal amplification
TWEAK	TNF-like weak inducer of apoptosis
WBRT	whole-brain radiation therapy

List of publications

I. The Role of BAFF-R Signaling in the Growth of Primary Central Nervous System Lymphoma.

Xiaolan Zhou, Matthias Mulazzani, Iven-Alex von Mücke-Heim, Sigrid Langer, Wenlong Zhang, Hellen Ishikawa-Ankerhold, Martin Dreyling, Andreas Straube, Louisa von Baumgarten

Front Oncol. 2020 May 27;10:682. doi: 10.3389/fonc.2020.00682.

II. Long-term in vivo microscopy of CAR T cell dynamics during eradication of CNS lymphoma in mice

MatthiasMulazzani, Simon P Fräßle, Iven von Mücke-Heim, Sigrid Langer, **Xiaolan Zhou**, Hellen Ishikawa-Ankerhold, Justin Leube, Wenlong Zhang, Sarah Dötsch, Mortimer Svec, Martina Rudelius, Martin Dreyling, Michael von Bergwelt-Baildon, Andreas Straube, Veit R Buchholz, Dirk H Busch, Louisa von Baumgarten

ProcNatl Acad Sci U S A. 2019 Nov 26;116(48):24275-24284.doi:10.1073/pnas.1903854116.

Your contribution to the publications (here paper I & II)

1.1 Contribution to paper I

I established and performed all *in vitro* and *in vivo* experiments, analyzed most of the data, prepared all figures and wrote the original draft. The first co-author Matthias Mulazzani gave methodological input, carried out additional statistical analysis, and co-wrote the original draft.

1.2 Contribution to paper II

I established, performed and analyzed apart of the experimental work and prepared the results for publication (e.g. Figure S1 paper II).

2. Introduction

2.1 Primary central nervous system lymphoma

Primary Central Nervous system lymphoma (PCNSL) is a rare extranodal nonHodgkin lymphoma (NHL) that is highly aggressive, and the brain, eyes, spinal cord, and pia meninges are often involved, but there is no evidence of systemic lymphoma(1, 2). More than 90% of PCNSL are diffuse large B-cell lymphoma (DLBCL), expressing CD20, CD19, CD22, CD79a and other cell markers. T-cell lymphoma, Burkitt lymphoma, and other low-grade lymphomas were rare (3). The pathophysiology of PCNSL is not clear, it is more common in immunodeficient patients and is associated with viral infection such as EBV(4). However, in immunocompetent patients, the pathophysiology remains largely obscure. Over the past 20 years, the incidence of PCNSL in immune-healthy patients has been rising(3). Compared with radiotherapy alone, high-dose methotrexate based polychemotherapy has greatly improved the prognosis of PCNSL patients, but the efficacy has not reached a satisfactory point. Only 30%~40% of patients can obtain sustained remission, especially fragile patients that do not qualify for high dose chemotherapy have a dismal prognosis(5). Therefore, our studies aimed to analyze the pathogenesis of PCNSL to find novel targets for treatment.

2.1.1 Epidemiology

PCNSL was initially characterized by Henry and his colleagues in 1974 (2). It accounted for about 4% of all intracranial malignant tumors and 4%~6% of extranodal lymphomas(6) (7). Moreover, it has an incidence of 0.4 per 100,000. and the onset peak is between 60 and 65 years old, and it is more common in males (male to female ratio is 1.2-1.7:1)(8).

2.1.2 Etiology and pathogenesis

The etiology and pathogenesis of PCNSL is not yet clear. PCNSL can occur in people with compromised immune function and normal immune function, although patients with comprised immune function are more prone to develop PCNSL. In

these patients the immune system is compromised by long-term use of immunosuppressive agents after transplantation or in autoimmune disease, or by active human immunodeficiency virus (HIV) infection(9). In immunodeficient patients PCNSL is mainly associated with an infection with Epstein Barr Virus (10). There are several theories that explain the formation of PCNSL. On one hand, transformation of B cells into malignant B-cells may occur in the periphery and these malignant B-cells enter the central nervous system where they are less prone to be eradicated by the immune system(11). On the other hand, malignant B-cells may stem from undifferentiated pluripotent stem cells in intracranial blood vessels(12). Another hypothesis argues that a high expression of bcl-2 in B-cells inhibits apoptotic genes and is associated with a higher risk of PCNSL while low expression of Bax and Bcl-X genes in PCNSL may be related to their important role in improving lymphocyte survival (13, 14). Epigenetic changes including MGMT, DAPK, CDKN2A and RFC caused by the corresponding CpG island methylation have been described in PCNSL(15). Furthermore, dysfunction of several signaling pathways including JAK/STAT and NF-κB signaling pathways may promote tumor proliferation and survival(16-18). Recently our study also identified a possible link between PCNSL and CNS inflammation, which may represent an additional mechanism for the occurrence of PCNSL(19).

PCNSL is tumor that is centered around vessels. 90% to 95% of PCNSL are DLBCL, expressing markers like LCA, CD19, CD20, CD22, CD79a and My-1(20), However, whether malignant B-cells belong to the activated B-cell (ABC) or the germinal center (GC) B-cell type is still controversial(4). The vast majority of PCNSLs express MUM-1 (> 95 %) that is highly suggestive of late germinal center and/or activated B cells(21). As a typical marker of GC B cells, 50-80 % PCNSLs express BCL6(14).

2.1.3 Clinical manifestations

The clinical symptoms of PCNSL depend on its location in the CNS. The vast majority of the lesions of PCNSL are distributed in the cerebral hemispheres, the basal ganglia and the corpus callosum (22). Tumor cells infiltrate the white matter tract and internal capsule of corpus callosum, which results in personality changes

and cognitive decline(22). Mass edema and compression may lead to loss of speech function, limb paresis, headache, nausea and vomiting. Infiltration of the walls of the third ventricle can lead to abnormal secretion of antidiuretic hormones, diabetes insipidus, increased appetite, and decreased libido. The invasion of the fourth ventricle and the brainstem may cause dizziness, ataxia, and intractable vomiting. Tumor invasion of cranial nerves may cause corresponding symptoms, and invasion of the spinal cord may cause myelopathy related manifestations(4). Some patients with ocular involvement present with blurred vision, impaired vision, eye pain, photophobia, floaters, and usually fatigue and binocular involvement(3). Due to the aggressive growth pattern of PCNSL, symptoms are often rapidly progressive (23-25).

2.1.4 Diagnosis

Magnetic resonance imaging (MRI) is the first choice for evaluating PCNSL. PCNSL usually present as unifocal homogenously contrast enhancing tumors. Less frequently, contrast enhancement is patchy or absent (26-29).However, MRI features may be nonspecific and usually cannot be distinguished from other brain tumors like malignant glioma, metastasis or lesions of inflammatory origin(22). Therefore, histopathologic proof is obligatory. Biopsy can be obtained by operative resection or stereotactic biopsy. Steroids are lymphcytoid and may lead to a temporary regression of PCNSL and non-diagnostic biopsy. Therefore, steroids should be avoided before histology is obtained (30).

2.1.5 Prognosis

PCNSL are highly aggressive tumors and the prognosis is poor, without tumor specific treatment, the median survival is weeks to months(31). Age and poor clinical performance are the most important prognostic variables(32).

Currently, Two scoring systems are commonly used, the International extranodal Lymphoma Study Group (IELSG) and the Memorial Sloan Kettering Cancer Center (MSKCC) prognostic score. For the IELSG five variables were independent predictors of poor outcome : the physical state (Eastern Cooperative Oncology Group score) > 1, age > 60 years, increased serum lactate dehydrogenase (LDH)

levels, high CSF protein concentrations, and tumors deep in the brain(33). The total 2-year overall survival rate in the patients who met IELSG variables, with zero to one, two to three, and four to five unfavorable variables were 80%, 48% and 15%, respectively(33). The MSKCC score was divided into three groups by age and Karnowski's performance (KPS): age ≤50 years, age> 50years and KPS≥70, age>50 years and KPS<70 , and the median overall survival were 8.5, 3.2 and 1.1 years respectively(34).

2.1.6 Therapy strategies

PCNSL shows highly invasive growth, therefore surgical resection usually is not generally recommended. In the setting of hydrocephalus, uncontrolled mass effect or a single and safely resectable lesion it can thus be evaluated (3).PCNSL are sensitive to radiation and chemotherapy. It has been reported that local enhanced radiotherapy can prolong the survival time of patients, but the median survival time is only 1 year, and tumor recurrence is inevitable(35). The combination of chemotherapy and radiation led to a longer survival time(36). However, combined treatment was reported with an increased exposure of severe delayed neurotoxicity, leading to profound gait impairment, cognitive decline and incontinence (37, 38). Therefore, radiotherapy is usually deferred or dose reduced radiation protocols are used(39).

High-dose methotrexate (MTX) has evolved as the most effective treatment(40). In modern treatment protocols it is usually combined with other chemotherapeutics like cytarabine, Ifosfamide or temozolomide(41). As relapses are frequent, in young healthy patients, induction chemotherapy can be combined with consolidating myelosuppressive high-dose chemotherapy followed by autologous stem cell transplantation in young and healthy patients(42).

However, especially in older patients satisfactory remission rates are usually not achieved(43). About 30% of PCNSL do not respond to treatment and about half of patients relapse(44). Therefore, novel therapeutic strategies improving patient survival are urgently warranted.

In recent years, a variety of new targeted drugs have emerged, making the treatment of PCNSL leap from traditional chemotherapy to precision therapy. Targeted therapies include the CD20 antibody rituximab(44), the mTor inhitor Temsirolimus(45) or, as PCNSL relies on BCR signaling pathways the BTK inhibitor ibrutinib (46). Furthermore, immune modulatory drugs like pomalidomide or checkpoint inhibitors show promising activity(47).

2.2 The BAFF/BAFF-R Pathway

2.2.1 The introduction of BAFF and BAFF-R

BAFF(B cell activating factor of the TNF family) belongs to the TNF superfamily (TNFSF), also known as BLyS (B lymphocyte stimulator), was identified in 1999 by several groups (48, 49). It shares high homology with two other members of the TNFSF: the TNF-like weak inducer of apoptosis (TWEAK) and a proliferationinducing ligand (APRIL)(50). Human BAFF is a type II membrane-bound protein , consisting of 285 amino acids and available in several splicing variants: membrane-bound or soluble monomer or trimers, homotrimers or heterotrimers(50).Soluble BAFF is released from the cell membrane by cleavage(48) and this process is stimulated by CD40L, IL-10, G-CSF, IFN-γ and IFN-α(51) and EBV infection(10). Up to now soluble BAFF was found to play a relevant part for B-cell biology in vitro and in vivo. There has been evidence that BAFF regulates human B-cell proliferation and that It can induce isotype conversion of immunoglobulin (Ig) by stimulating B cells to secrete IL-10(52). Concurrently, in vitro BAFF stimulated Ig generation upon B-cell receptor (BCR) interaction with anti-IgM(48). Furthermore, BAFF increased the activity of a B-cell specific transcription factor Pax5(Pax5/BSAP)(53). Nonetheless, the differential functions of the other forms of BAFF remain unclear.

BAFF performs its function by binding to three known receptors (54): TACI (transmembrane activator and calcium modulator cyclophilin ligand interactor), BCMA (B-cell maturation antigen) and BAFF-R [also called BR3(BAFF receptor 3), CD268 or TNFRSF17]. Expression of the latter two receptors(BCMA and BAFF-R) are restricted to B-cells, while TACI is expressed on both B-cells and activated

Tcells(55). On the other hand, TACI and BCMA interact with both BAFF and APRIL, while BAFF-R binds only to BAFF(see Figure 1)(56). Additionally, BAFF-R has 100 times more affinity with BAFF than BCMA and TACI, which indicated that the binding of BAFF to multiple B cell lines was strongly correlated with the surface expression of BAFF-R, weakly with another two receptors (57, 58).

BAFF-R is a type III transmembrane protein made up of 184 amino acids. The human BAFF-R gene (TNFRSF13C) is positioned on chromosome 22q13.1–13.31. Except in bone marrow plasma cells, human BAFF-R is diffusely expressed in all B cells(53). However, it has also been shown that a small number of stationary human T cells bind to soluble BAFF through BAFF-R, and BAFF-R secretion increases after in vitro stimulation(58). In addition to the plasma membrane, BAFFR can also be found in the cytoplasm, the nuclear envelope, and the nucleoplasm(59).

Both BAFF-R−/− and BAFF−/− mice were found to suffer a severe decrease in mature B cells(59, 60). By comparison, neither BCMA−/− nor TACI−/− mice display any B cell shortage(61, 62), substantiating BAFF-R as the fundamental receptor for BAFF-dependent B cell survival. In vitro experiments showed that BAFF increased the chemotaxis of B lymphocytes to CCL21, CXCLI2, and CXCLI3, and this effect was attenuated by BAFF-R blocker(63). Meanwhile, the effect of BAFF and BAFF-R on B lymphocytes was highly dependent on the activation of NF-κB signaling pathway(63). Therefore, the NF-κB pathway was found to be the critical downstream mediator of BAFF / BAFF-R in B-cells. BAFF/ BAFF-R induces NF-κB activation through two pathways (canonical pathway and alternative pathway), to regulate transcription of target genes, and to preserve B cells from spontaneous as well as drug-induced apoptosis(64, 65). Mice that are short of elements of the alternative NF-κB pathway exhibit consequences resemble those of BAFF or BAFF-R knockout mice(66).

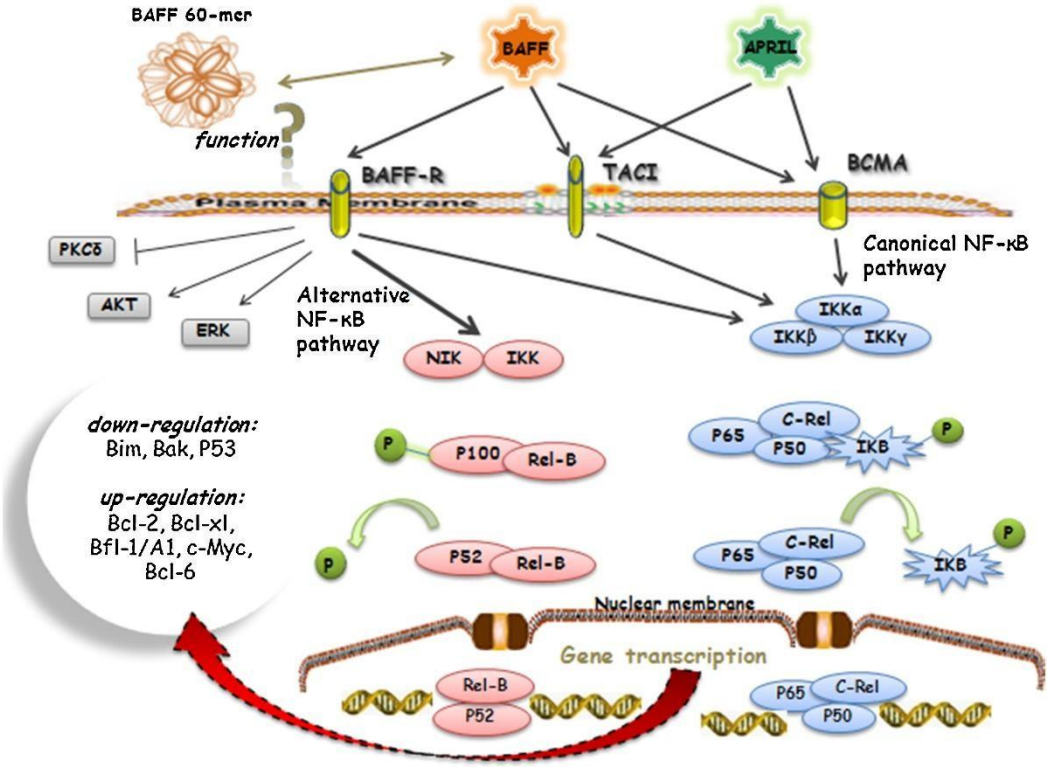

Figure 1 Summary of BAFF/BAFF-R axis in B cells. BAFF has high affinity with BAFF-R and TACI but low affinity (humans) or no affinity (mice) with BCMA, while APRIL binds only to TACI and BCMA. NF-κB is the primary downstream signaling pathway of BAFF receptors in B cells. BAFF/ BAFF-R induces NF-κB activation through two pathways (canonical pathway and alternative pathway), regulates target genes transcription and preserves B cells from spontaneous and drug-induced apoptosis. Adapted from(57).

2.2.2 The function of BAFF/BAFF-R in B-cell malignancies

The effect of BAFF/BAFF-R in B cell development raised the question whether BAFF/BAFF-R was involved in B cell malignancy pathogenesis. BAFF and NF-κB may form a positive feedback loop, the expression of BAFF gene can activate both classical and non-classical NF-κB signaling pathways, and the activated NF-κB pathways can in turn promote the expression of BAFF, thus facilitating the survival and proliferation of malignant B cells(67, 68). BAFF and APRIL also can support the survival of non-Hodgkin lymphoma(NHL) cells in vitro by upregulation of BCL2 and BCLxL but downregulation of BAX (69). The level of BAFF in peripheral blood serum of NHL patients was significantly increased, and was positively correlated with the aggressiveness of the disease, but negatively correlated with the prognosis of patients (70-72). On the other hand, BAFF/BAFF-R is also found to increase proliferation and survival of DLBCL cells(73-77). A cohort of 66 patients

with DLBCL treated with rituximab combined with CHOP (R-CHOP), revealed that serum BAFF can be used to predict overall survival (OS) and progression-free survival (PFS) prognosis after R-CHOP treatment. Higher BAFF serum levels had fewer responses to treatment and the levels correlated with relapse and progression (71). Interestingly, BAFF-R is also found to be dysregulated in some subtypes of aggressive B-NHL like B-lineage acute lymphoblastic leukemia (ALL), in which all the samples of patients tested were positive for high BAFF-R cell surface expression(74).

2.2.3 BAFF/BAFF-R in primary CNS lymphoma

BAFF expression in normal human brain is about ten times lower than in lymphoid tissue expression (tonsils and adenoids), and is mainly produced by astrocytes(78). In the immunohistochemical study of PCNSL patient samples, BAFF transcription and expression has been detected in both malignant B cells and astrocytes(78). Furthermore, BAFF receptors were found to be strongly expressed in situ, indicating that autocrine and paracrine mechanisms of BAFF and its receptors are possibly involved for promoting proliferation and survival of the malignant B cells in PCSNL (76, 79). On the other hand, The level of BAFF in CSF in PCNSL patients was significantly higher than that in other lesional neurological diseases (80). However, CSF levels of soluble TACI and soluble BCMA levels were also higher in PCNSL patients, which may limit the local availability of BAFF(81, 82). Consistent with these findings, our group also confirmed the high expression of BAFF and APRIL as well as their receptors in PCNSL specimens, and APRIL alone or in combination with BAFF may serve as biomarkers for diagnosis and treatment of these patients (83, 84).

2.3 Aims of the Paper I

As mentioned above, In PCNSL, BAFF transcription and expression has been detected in both malignant B cells and astrocytes(78). Patients with PCNSL showed remarkably enhanced BAFF CSF levels when in contrast with other lesional neurological diseases(80). Our previous work has confirmed a high expression of BAFF and BAFF-R in PCNSL specimen(83) and we hypothesized

that there may be a potential role of autocrine BAFF/BAFF-R signaling in the pathophysiology of PCNSL. Since BAFF-R is the most unique BAFF receptor in Bcells, although BAFF-R express characteristics in PCNSL has been described previously, its significance for PCNSL pathophysiology remains unclear. We hypothesized that BAFF-R is critical for the survival and growth of PCNSL.

Consequently, the objectives of this thesis were:

- to construct a BAFF-R knockout DLBCL cell line in vitro
- to determine the particular contribution of BAFF-R to DLBCL proliferation and survival in vitro
- to create a novel animal model of PCNSL
- to combine this animal model with a chronic cranial window and repeated intravital fluorescence microscopy
- to follow in vivo development and progression of orthotopic PCNSL
- to estimate the consequence of BAFF-R absence on PCNSL in vivo.

2.4 Chimeric Antigen Receptor (CAR) T cells and lymphoma

2.4.1 Chimeric Antigen Receptor (CAR) T cells

CAR is a synthetic transmembrane structure consisting mainly of three domains: extracellular domain, transmembrane domain and internal domain(85). Its extracellular domain is a single stranded variable fragment scFv, usually CD3ζ, mainly for antigen recognition. Transmembrane domains mainly guarantee CAR stability. The intracellular domain is a T cell signal transduction domain, which promotes signal transduction and activates T cells during antigen recognition(86). Unlike ordinary T cells, which identify antigens by T cell receptors (TCR), CAR-T cells recognize tumor-associated antigens (TAA) on the surface of tumor cells through extracellular sc Fv without limitation of major histocompatibility complexes (MHC).

Autologous CAR T cells are generated by patient derived T cells that are incubated with viral vectors encoding CAR by gene transfection. Specificity, killing activity and persistence of the transfected T cells were upgraded after purification and large-scale amplification, which could overcome tumor immune escape and immune tolerance, specifically identify and efficiently kill tumor cells(87).

2.4.2 CAR-T in the treatment of CNS lymphoma

CAR-T treatment has brought great changes to the treatment of B lymphocyte tumors(88, 89). Anti - CD19-CAR-Tcells have shown therapeutic success in the treatment of multiple subtypes of B cell lymphomas (including DLBCL, follicular and marginal region lymphoma)(90)and even can induce long -term persistent complete remission in patients with partially recurrent / refractory DLBC(91).

First data indicates, that CAR-T cells can be utilized to treat secondary central nervous system lymphoma. A 68-year-old woman suffered from secondary CNS manifestation of DLBCL progressed after previous chemotherapy and allogeneic hematopoietic stem cell transplantation. When she was served with

CAR-T cells targeting CD19 she got completer remission (CR) after 1- month clinic follow-up (by PET/CT and brain MRI validation), subcutaneous recurrence was confirmed by biopsy at the second month of follow-up, but after biopsy CAR-T cells spontaneously proliferated and she reconfirmed as CR at the third month of follow-up. The remission lasted up to 12 months and there was no neurotoxicity, graft-versus-host disease or other serious side effects. After more than a year of CAR-T treatment, the patient eventually died of tumor recurrence, but the disease never recurred intracranially(92).The results show that CAR-T cells can pass through the blood-brain barrier and can be applied to cure CNS lymphoma. In another retrospective summary of CAR-T therapy treatment SCNSL, a total of 8 patients were included. CAR-T therapy also achieved encouraging therapeutic effects for these patients(93), therefore further evaluation and study should be made for the PCNSL patients in the future.

2.5 Aims of the Paper II

While CAR-T has significant preclinical and clinical therapeutic effects on B cell malignancies, the efficacy of CAR-T cells in PCNSL has never been evaluated. moreover, many important aspects of CAR-T cell biology remain unclear, including the dynamics of their recruitment and positioning inside the tumor, their interaction with tumor cells, CAR-T cell behavior after tumor regression, and prolonged persistence in the brain.

Therefore, the purposes of the project were:

-to assess the therapeutic effects of second-generation anti-CD19CAR-Tcells in PCNSL

-to answer fundamental questions about CAR-T cell biology within the brain, including tumor invasion patterns, migration, positioning and long-term persistence.

3. Summary (English)

Primary lymphoma of the central nervous system (PCNSL) is a rare and aggressive extranodal non-Hodgkin's lymphoma. It is limited to brain, pia meningeal, spinal cord, and eyeball tissue invasion, but no other tissue or lymph

node involved. The etiology and pathogenesis of PCNSL is incompletely understood. Compared to other systemic lymphoma, PCNSL still have a dismal prognosis and limited treatment options, especially in patients with progressive or recurrent PCNSL. Therefore, novel therapeutic strategies are urgently warranted.

3.1 The Function of BAFF/BAFF-R signaling in the growth of PCNSL

BAFF binds to its specific receptor BAFF-R to promote B cell proliferation and survival, and BAFF-R expression can be up-regulated in many B cell malignancies, including DLBCL cells. To date it is unknown whether BAFF/BAFF-R contributes to the pathogenesis of PCNSL. Our study dissects the role of BAFF-R in controlling survival and proliferation of DLBCL and, in particular, its contribution to the development and survival in an orthotopic PCNSL mouse model. We found that autocrine BAFF-R signaling is essential to protect tumor cells against serum deprivation stress. Furthermore, we were able to supervise intracranial tumor growth across time applying a microsurgically implanted chronic cranial window. Thereby, we demonstrate that after knockout of BAFF-R applying CRISPR-Cas9, tumor growth in an orthotopic PCNSL model is reduced, leading to prolonged survival in mice. Additionally, our results showed that anti-BAFF-R has the same effect as BAFF-R knockout in vitro, indicating that anti-BAFF-R may be targeted as a precision therapy, further preclinical investigations are required to confirm this hypothesis.

3.2 The efficacy and biology of CAR T cells in the PCNSL

Although CAR T cell treatment has brought great changes to the treatment of B cell malignancies, there are no reports whether this approach can be used to treat PCNSL. Important questions, like the dynamics of the intracranial and intratumoral CAR T recruitment, their interactions with the tumor cells and their persistence in the CNS and the periphery have not been characterized in detail.

We could show that intracranial injection of a small amount of anti-CD19 CAR T cells can lead to complete regression of large, established intracranial tumors.

We also demonstrated that anti CD19 CAR-T cells invade PCNSL in high numbers. In the first week after CAR-T cell injection, anti-CD19 CAR-T cells migrated at speeds lower than mock CAR-Tcells, preventing tumor progression, suggesting anti-tumor cytotoxicity. At later time points, the number of anti-CD19 CAR-T cells increased, and proliferation could be observed. Anti-CD19 CAR-T cells persisted in the CNS for more than 150 days. Furthermore, anti-CD 19 CAR T cells entered the blood stream and persisted in the draining and undraining lymph nodes. Our research illustrates the high potential of antiCD19 CAR T cell treatment in PCNSL.

4. Zusammenfassung (Deutsch)

Primäre ZNS Lymphome (PZNSL) sindseltene, extranodale Non-HodgkinLymphome, die sich nur im ZNS und dessen Anhangsgebilden manifestieren.Die Pathogenese des PZNSL ist nur unzureichend verstanden. Trotz intensiver Therapien ist die Prognose des PZNSL weiterhin schlecht, insbesondere für ältere Patienten und Patienten im Rezidiv. Neue Therapiestrategien werden daher dringend benötigt.

4.1 Die Rolle der BAFF-R-Signalübertragung beim Wachstum von PCNSL

BAFF-R ist ein Transmembranprotein, das bei vielen B-Zell-Malignomen, einschließlich dem systemischen diffus großzelligen B-Zell Lymphom (DLBCL) hochreguliert ist. Bisher ist nicht bekannt, ob die BAFF/ BAFF-R vermittelte Signalkaskade zur PathogenesedesPZNSL beiträgt. Ein Projekt der kumulativen Doktorarbeit beschäftigen sich daher mit derRolle von BAFF-R für das Überlebens und die Proliferation von DLBCL*in vitro* und*in vivo* im orthotopen Mausmodell des PZNSL. Wir konnten zeigen, dass die autokrine BAFF-R-Signalübertragung wesentlich daran beteiligt ist, das Überleben von Tumorzellen unter Stressbedingungen durch Nährstoffentzug zu regulieren. Darüber hinaus konnten wir das intrakranielle Tumorwachstum mithilfe eines mikrochirurgisch implantierten chronischen Schädelfensters longitudinal im

Zeitverlauf überwachen. Dadurch konnten wir zeigen, dass nach dem Ausschalten von BAFF-R unter Verwendung von CRISPR-Cas9 das Tumorwachstum in einem orthotopen PCNSL-Modell verringert ist, was zu einem verlängerten Überleben im Mausmodellführt. Eine zielgerichtete Inhibiton von BAFF-R stellt somit einen vielversprechenden therapeutischen Angriffspunkt in der Behandlung von PZNSL dar. Weitere präklinische Studien sind erforderlich, um diese Hypothese zu beweisen.

4.2 Die Wirksamkeit und Biologie von CAR-T-Zellen im PCNSL

Chimäre Antigen-Rezeptor T Zellen (CAR-T Zellen) haben in der Behandlung von systemischen B-Zell Malignomen zu eindrucksvollen Behandlungserfolgen geführt. Ob diese Therapie auch zur Behandlung von PZNSL eingesetzt werden kann ist jedoch bislang ungeklärt. Zudem ist die Dynamik einer etwaigen intrazerebralen bzw. intratumoralen Rekrutierung von CAR-T Zellen, deren Interaktion mit Tumorzellen sowie ihre Persistenz bislang nicht auf zellulärer Ebene *in vivo* untersucht.

Wir konnten zeigen, dass die intrakranielle Injektion einer geringen Anzahl von anti-CD19 CAR-T-Zellen zur vollständigen Regression großer, etablierter intrakranieller Tumoren führen kann. CART-Zellen infiltireren solide PZNSL. In der ersten Woche nach der CAR-T Zell injektion migriertenanti-CD19 CAR-T Zellen mit deutlich niedrigerer Geschwindigkeiten als mock CART-Zellen. Zudem kam es zu einer Tumorregression, was antitumorale Zytotoxizität nahelegt. Zu späteren Zeitpunkten kam es zu einer deutlichenintratumoralen Proliferation der anti-CD19 CAR-T Zellen . Anti-CD19 CAR-T Zellen ließen sich mehr als 150 Tage im ZNS nachweisen. Anti-CD19 CAR-T Zellen ließen sich auch im peripheren Blut sowie in den drainierenden und nicht drainierenden Lymphknoten nachweisen.

Unsere Forschung zeigt das hohe Potenzial der Anti-CD19-CAR-T-Zell therapie zur Behandlung des PZNSL.

5. Paper I

Front Oncol. 2020 May 27;10:682. doi: 10.3389/fonc.2020.00682.

The Role of BAFF-R Signaling in the Growth of Primary Central Nervous System Lymphoma.

Xiaolan Zhou, Matthias Mulazzani, Iven-Alex von Mücke-Heim, Sigrid Langer, Wenlong Zhang, Hellen Ishikawa-Ankerhold, Martin Dreyling, Andreas Straube, Louisa von Baumgarten

6. Paper II

Proc Natl Acad Sci U S A. 2019 Nov 26;116(48):24275-24284. doi:10.1073/pnas.1903854116.

Long-term in vivo microscopy of CAR T cell dynamics during eradication of CNS lymphoma in mice

Matthias Mulazzani, Simon P Fräßle, Iven von Mücke-Heim, Sigrid Langer, Xiaolan Zhou, Hellen Ishikawa-Ankerhold, Justin Leube, Wenlong Zhang, Sarah Dötsch, Mortimer Svec, Martina Rudelius, Martin Dreyling, Michael von Bergwelt-Baildon, Andreas Straube, Veit R Buchholz, Dirk H Busch, Louisa von Baumgarten

References

1. Hochberg FH, Miller DC. Primary central nervous system lymphoma. Journal of neurosurgery. 1988;68(6):835-53.
2. Henry JM, Heffner RR, Jr., Dillard SH, Earle KM, Davis RL. Primary malignant lymphomas of the central nervous system. Cancer. 1974;34(4):1293302.
3. Yang H, Xun Y, Yang A, Liu F, You H. Advances and challenges in the treatment of primary central nervous system lymphoma. Journal of cellular physiology. 2020.
4. Chukwueke UN,Nayak L. Central Nervous System Lymphoma. Hematology/oncology clinics of North America. 2019;33(4):597-611.
5. Santambrogio E, Nicolosi M, Vassallo F, Castellino A, Novo M, Chiappella A, et al. Aggressive Non-Hodgkin lymphomas: risk factors and treatment of central nervous system recurrence. Expert review of hematology. 2019;12(9):787-96. 6. Roth P, Korfel A, Martus P, Weller M. Pathogenesis and management of primary CNS lymphoma. Expert review of anticancer therapy. 2012;12(5):62333.
7. Wang CC, Carnevale J, Rubenstein JL. Progress in central nervous system lymphomas. Br J Haematol. 2014;166(3):311-25.
8. Villano JL, Koshy M, Shaikh H, Dolecek TA, McCarthy BJ. Age, gender, and racial differences in incidence and survival in primary CNS lymphoma. British journal of cancer. 2011;105(9):1414-8.
9. Brandsma D, Bromberg JEC. PrimaryCNS lymphoma in HIV infection. Handbook of clinical neurology. 2018;152:177-86.
10. He B, Raab-Traub N, Casali P, Cerutti A. EBV-encoded latent membrane protein 1 cooperates with BAFF/BLyS and APRIL to induce T cell-independent Ig heavy chain class switching. Journal of immunology. 2003;171(10):5215-24.
11. Schabet M. Epidemiology of primary CNS lymphoma. Journal of neurooncology. 1999;43(3):199-201.
12. Bhagavathi S, Wilson JD. Primary central nervous system lymphoma.

Archives of pathology & laboratory medicine. 2008;132(11):1830-4.
13.Camilleri-Broet S, Criniere E, Broet P, Delwail V, Mokhtari K, Moreau A, et al. A uniform activated B-cell-like immunophenotype might explain the poor prognosis of primary central nervous system lymphomas: analysis of 83 cases. Blood. 2006;107(1):190-6.
14.Braaten KM, Betensky RA, de Leval L, Okada Y, Hochberg FH, Louis DN, et al. BCL-6 expression predicts improved survival in patients with primary central nervous system lymphoma. Clinical cancer research : an official journal of the American Association for Cancer Research. 2003;9(3):1063-9.
15.Cai Q, Fang Y, Young KH. Primary Central Nervous System Lymphoma: Molecular Pathogenesis and Advances in Treatment. Translational oncology. 2019;12(3):523-38.
16.Schwindt H, Vater I, Kreuz M, Montesinos-Rongen M, Brunn A, Richter J, et al. Chromosomal imbalances and partial uniparental disomies in primary central nervous system lymphoma. Leukemia. 2009;23(10):1875-84.
17.Montesinos-Rongen M, Schafer E, Siebert R, Deckert M. Genes regulating the B cell receptor pathway are recurrently mutated in primary central nervous system lymphoma. Acta neuropathologica. 2012;124(6):905-6.
18.Gonzalez-Aguilar A, Idbaih A, Boisselier B, Habbita N, Rossetto M, Laurenge A, et al. Recurrent mutations of MYD88 and TBL1XR1 in primary central nervous system lymphomas. Clinical cancer research : an official journal of the American Association for Cancer Research. 2012;18(19):5203-11. 19.O'Connor T, Zhou X, Kosla J, Adili A, Garcia Beccaria M, Kotsiliti E, et al. Age-Related Gliosis Promotes Central Nervous System Lymphoma through CCL19-Mediated Tumor Cell Retention. Cancer cell. 2019;36(3):250-67.e9.
20.Shenkier TN, Blay JY, O'Neill BP, Poortmans P, Thiel E, Jahnke K, et al. Primary CNS lymphoma of T-cell origin: a descriptive analysis from the international primary CNS lymphoma collaborative group. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2005;23(10):2233-9.
21.Bhagavathi S, Sharathkumar A, Hunter S, Sung L, Kanhere R, Venturina MD, et al. Activated B-cell immunophenotype might be associated with poor

prognosis of primary central nervous system lymphomas. Clinical neuropathology. 2008;27(1):13-20.
22.Kuker W, Nagele T, Korfel A, Heckl S, Thiel E, Bamberg M, et al. Primary central nervous system lymphomas (PCNSL): MRI features at presentation in 100 patients. Journal of neuro-oncology. 2005;72(2):169-77.
23.Josephson SA, Papanastassiou AM, Berger MS, Barbaro NM, McDermott MW, Hilton JF, et al. The diagnostic utility of brain biopsy procedures in patients with rapidly deteriorating neurological conditions or dementia. Journal of neurosurgery. 2007;106(1):72-5.
24.Bataille B, Delwail V, Menet E, Vandermarcq P, Ingrand P, Wager M, et al. Primary intracerebral malignant lymphoma: report of 248 cases. Journal of neurosurgery. 2000;92(2):261-6.
25.Chan CC, Rubenstein JL, Coupland SE, Davis JL, Harbour JW, Johnston PB, et al. Primary vitreoretinal lymphoma: a report from an International Primary Central Nervous System Lymphoma Collaborative Group symposium. The oncologist. 2011;16(11):1589-99.
26.da Rocha AJ, Sobreira Guedes BV, da Silveira da Rocha TM, Maia Junior AC, Chiattone CS. Modern techniques of magnetic resonance in the evaluation of primary central nervous system lymphoma: contributions to the diagnosis and differential diagnosis. Revista brasileira de hematologia e hemoterapia. 2016;38(1):44-54.
27.Barajas RF, Jr., Rubenstein JL, Chang JS, Hwang J, Cha S. Diffusionweighted MR imaging derived apparent diffusion coefficient is predictive of clinical outcome in primary central nervous system lymphoma. AJNR American journal of neuroradiology. 2010;31(1):60-6.
28.Haldorsen IS, Krakenes J, Krossnes BK, Mella O, Espeland A. CT and MR imaging features of primary central nervous system lymphoma in Norway, 19892003. AJNR American journal of neuroradiology. 2009;30(4):744-51.
29.Buhring U, Herrlinger U, Krings T, Thiex R, Weller M, Kuker W. MRI features of primary central nervous system lymphomas at presentation. Neurology. 2001;57(3):393-6.

30.Gupta M, Gupta T, Purandare N, Rangarajan V, Puranik A, Moiyadi A, et al. Utility of flouro-deoxy-glucose positron emission tomography/computed tomography in the diagnostic and staging evaluation of patients with primary CNS lymphoma. CNS oncology. 2019;8(4):Cns46.
31.Ostrom QT, Gittleman H, de Blank PM, Finlay JL, Gurney JG, McKeanCowdin R, et al. American Brain Tumor Association Adolescent and Young Adult Primary Brain and Central Nervous System Tumors Diagnosed in the United States in 2008-2012. Neuro-oncology. 2015;18(suppl_1):i1-i50.
32.Shiels MS, Pfeiffer RM, Besson C, Clarke CA, Morton LM, Nogueira L, et al. Trends in primary central nervous system lymphoma incidence and survival in the US. British Journal of Haematology. 2016;174(3):417-24.
33.Ferreri AJ, Blay JY, Reni M, Pasini F, Spina M, Ambrosetti A, et al. Prognostic scoring system for primary CNS lymphomas: the International Extranodal Lymphoma Study Group experience. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2003;21(2):266-72.
34.Abrey LE, Ben-Porat L, Panageas KS, Yahalom J, Berkey B, Curran W, et al. Primary central nervous system lymphoma: the Memorial Sloan-Kettering Cancer Center prognostic model. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2006;24(36):5711-5.
35.Ling SM, Roach M, 3rd, Larson DA, Wara WM. Radiotherapy of primary central nervous system lymphoma in patients with and without human immunodeficiency virus. Ten years of treatment experience at the University of California San Francisco. Cancer. 1994;73(10):2570-82.
36.DeAngelis LM, Yahalom J, Thaler HT, Kher U. Combined modality therapy for primary CNS lymphoma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 1992;10(4):635-43.
37.Shibamoto Y, Ogino H, Hasegawa M, Suzuki K, Nishio M, Fujii T, et al. Results of radiation monotherapy for primary central nervous system lymphoma in the 1990s. International journal of radiation oncology, biology, physics. 2005;62(3):809-13.
38.Nelson DF. Radiotherapy in the treatment of primary central nervous system lymphoma (PCNSL). Journal of neuro-oncology. 1999;43(3):241-7.

39.Seidel S, Schlegel U. Have treatment protocols for primary CNS lymphoma advanced in the past 10 years. Expert review of anticancer therapy. 2019;19(10):909-15.
40.Park DM, Abrey LE. Pharmacotherapy of primary CNS lymphoma. Expert opinion on pharmacotherapy. 2002;3(1):39-49.
41.Fraser E, Gruenberg K, Rubenstein JL. New approaches in primary central nervous system lymphoma. Chinese clinical oncology. 2015;4(1):11. 42.Illerhaus G, Marks R, Ihorst G, Guttenberger R, Ostertag C, Derigs G, et al. High-dose chemotherapy with autologous stem-cell transplantation and hyperfractionated radiotherapy as first-line treatment of primary CNS lymphoma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2006;24(24):3865-70.
43.Roth P, Hoang-Xuan K. Challenges in the treatment of elderly patients with primary central nervous system lymphoma. Current opinion in neurology. 2014;27(6):697-701.
44.Ferreri AJM, Cwynarski K, Pulczynski E, Ponzoni M, Deckert M, Politi LS, et al. Chemoimmunotherapy with methotrexate, cytarabine, thiotepa, and rituximab (MATRix regimen) in patients with primary CNS lymphoma: results of the first randomisation of the International Extranodal Lymphoma Study Group32 (IELSG32) phase 2 trial. The Lancet Haematology. 2016;3(5):e217-e27.
45.Mondello P, Mian M, Bertoni F. Primary central nervous system lymphoma: Novel precision therapies. Critical reviews in oncology/hematology. 2019;141:139-45.
46.Lionakis MS, Dunleavy K, Roschewski M, Widemann BC, Butman JA, Schmitz R, et al. Inhibition of B Cell Receptor Signaling by Ibrutinib in Primary CNS Lymphoma. Cancer cell. 2017;31(6):833-43.e5.
47.Tsang M, Cleveland J, Rubenstein JL. On point in primary CNS lymphoma. Hematological oncology. 2020;38(5):640-7.
48.Schneider P, MacKay F, Steiner V, Hofmann K, Bodmer JL, Holler N, et al. BAFF, a novel ligand of the tumor necrosis factor family, stimulates B cell growth. The Journal of experimental medicine. 1999;189(11):1747-56.

49.Hahne M, Kataoka T, Schroter M, Hofmann K, Irmler M, Bodmer JL, et al. APRIL, a new ligand of the tumor necrosis factor family, stimulates tumor cell growth. The Journal of experimental medicine. 1998;188(6):1185-90.
50.Daridon C, Youinou P, Pers J-O. BAFF, APRIL, TWE-PRIL: Who's who? Autoimmunity Reviews. 2008;7(4):267-71.
51.Litinskiy MB, Nardelli B, Hilbert DM, He B, Schaffer A, Casali P, et al. DCs induce CD40-independent immunoglobulin class switching through BLyS and APRIL. Nature immunology. 2002;3(9):822-9.
52.Xu LG, Wu M, Hu J, Zhai Z, Shu HB. Identification of downstream genes upregulated by the tumor necrosis factor family member TALL-1. Journal of leukocyte biology. 2002;72(2):410-6.
53.Hase H, Kanno Y, Kojima M, Hasegawa K, Sakurai D, Kojima H, et al. BAFF/BLyS can potentiate B-cell selection with the B-cell coreceptor complex. Blood. 2004;103(6):2257-65.
54.Mackay F, Schneider P. Cracking the BAFF code. Nat Rev Immunol. 2009;9.
55.Marsters SA, Yan M, Pitti RM, Haas PE, Dixit VM, Ashkenazi A. Interaction of the TNF homologues BLyS and APRIL with the TNF receptor homologues BCMA and TACI. Current Biology. 2000;10(13):785-8.
56.Thompson JS, Bixler SA, Qian F, Vora K, Scott ML, Cachero TG, et al. BAFF-R, a Newly Identified TNF Receptor That Specifically Interacts with BAFF. Science. 2001;293(5537):2108-11.
57.Yang S, Li JY, Xu W. Role of BAFF/BAFF-R axis in B-cell non-Hodgkin lymphoma. Critical reviews in oncology/hematology. 2014;91(2):113-22.
58.Thompson JS, Bixler SA, Qian F, Vora K, Scott ML, Cachero TG, et al. BAFF-R, a newly identified TNF receptor that specifically interacts with BAFF. Science. 2001;293(5537):2108-11.
59.Sasaki Y, Casola S, Kutok JL, Rajewsky K, Schmidt-Supprian M. TNF family member B cell-activating factor (BAFF) receptor-dependent and -independent roles for BAFF in B cell physiology. Journal of immunology. 2004;173.
60.Shulga-Morskaya S, Dobles M, Walsh ME, Ng LG, MacKay F, Rao SP, et al. B Cell-Activating Factor Belonging to the TNF Family Acts through Separate Receptors to Support B Cell Survival and T Cell-Independent Antibody Formation. The Journal of Immunology. 2004;173(4):2331-41.

61.Xu S, Lam KP. B-cell maturation protein, which binds the tumor necrosis factor family members BAFF and APRIL, is dispensable for humoral immune responses. Molecular and cellular biology. 2001;21(12):4067-74.
62.Yan M, Brady JR, Chan B, Lee WP, Hsu B, Harless S. Identification of a novel receptor for B lymphocyte stimulator that is mutated in a mouse strain with severe B cell deficiency. Curr Biol. 2001;11.
63.Badr G, Borhis G, Lefevre EA, Chaoul N, Deshayes F, Dessirier V, et al. BAFF enhances chemotaxis of primary human B cells: a particular synergy between BAFF and CXCL13 on memory B cells. Blood. 2008;111(5):2744-54.
64.Gardam S, Brink R. Non-canonical NF-kappa B signaling initiated by BAFF influences B cell biology at multiple junctures. Frontiers in Immunology. 2014;4.
65.Tang X, Zhang L, Wei W. Roles of TRAFs in NF-kappa B signaling pathways mediated by BAFF. Immunology Letters. 2018;196:113-8.
66.Beinke S, Ley SC. Functions of NF-kappaB1 and NF-kappaB2 in immune cell biology. The Biochemical journal. 2004;382(Pt 2):393-409.
67.Woodland RT, Schmidt MR, Thompson CB. BLyS and B cell homeostasis. Seminars in immunology. 2006;18(5):318-26.
68.Fu L, Lin-Lee YC, Pham LV, Tamayo A, Yoshimura L, Ford RJ. Constitutive NF-kappaB and NFAT activation leads to stimulation of the BLyS survival pathway in aggressive B-cell lymphomas. Blood. 2006;107(11):4540-8.
69.He B, Chadburn A, Jou E, Schattner EJ, Knowles DM, Cerutti A. Lymphoma B cells evade apoptosis through the TNF family members BAFF/BLyS and APRIL. Journal of immunology. 2004;172(5):3268-79.
70.Novak AJ, Grote DM, Stenson M, Ziesmer SC, Witzig TE, Habermann TM, et al. Expression of BLyS and its receptors in B-cell non-Hodgkin lymphoma: correlation with disease activity and patient outcome. Blood. 2004;104(8):224753.
71.Kim SJ, Lee SJ, Choi IY, Park Y, Choi CW, Kim IS, et al. Serum BAFF predicts prognosis better than APRIL in diffuse large B-cell lymphoma patients treated with rituximab plus CHOP chemotherapy. European journal of haematology. 2008;81(3):177-84.

72.Kuo SH, Yeh PY, Chen LT, Wu MS, Lin CW, Yeh KH, et al. Overexpression of B cell-activating factor of TNF family (BAFF) is associated with Helicobacter pylori-independent growth of gastric diffuse large B-cell lymphoma with histologic evidence of MALT lymphoma. Blood. 2008;112(7):2927-34.
73.Pham LV, Fu L, Tamayo AT, Bueso-Ramos C, Drakos E, Vega F, et al. Constitutive BR3 receptor signaling in diffuse, large B-cell lymphomas stabilizes nuclear factor-κB–inducing kinase while activating both canonical and alternative nuclear factor-κB pathways. Blood. 2011;117(1):200-10.
74.Parameswaran R, Muschen M, Kim YM, Groffen J, Heisterkamp N. A functional receptor for B-cell-activating factor is expressed on human acute lymphoblastic leukemias. Cancer research. 2010;70(11):4346-56.
75.Lyu MA, Cheung LH, Hittelman WN, Marks JW, Aguiar RC, Rosenblum MG. The rGel/BLyS fusion toxin specifically targets malignant B cells expressing the BLyS receptors BAFF-R, TACI, and BCMA. Mol Cancer Ther. 2007;6(2):460-70.
76.Kern C, Cornuel JF, Billard C, Tang R, Rouillard D, Stenou V, et al. Involvement of BAFF and APRIL in the resistance to apoptosis of B-CLL through an autocrine pathway. Blood. 2004;103(2):679-88.
77.Endo T, Nishio M, Enzler T, Cottam HB, Fukuda T, James DF, et al. BAFF and APRIL support chronic lymphocytic leukemia B-cell survival through activation of the canonical NF-kappaB pathway. Blood. 2007;109(2):703-10.
78.Krumbholz M, Theil D, Derfuss T, Rosenwald A, Schrader F, Monoranu CM, et al. BAFF is produced by astrocytes and up-regulated in multiple sclerosis lesions and primary central nervous system lymphoma. The Journal of experimental medicine. 2005;201.
79.Krumbholz M, Theil D, Derfuss T, Rosenwald A, Schrader F, Monoranu CM, et al. BAFF is produced by astrocytes and up-regulated in multiple sclerosis lesions and primary central nervous system lymphoma. J Exp Med. 2005;201(2):195-200.
80.Mizutani H, Nakane S, Ikeda T, Nakamura H, Takamatsu K, Makino K, et al. CSF TACI and BAFF levels in patients with primary CNS lymphoma as novel diagnostic biomarkers. Ann Clin Transl Neurol. 2018;5(12):1611-6.

81.Thaler FS, Laurent SA, Huber M, Mulazzani M, Dreyling M, Kodel U, et al. Soluble TACI and soluble BCMA as biomarkers in primary central nervous system lymphoma. Neuro-oncology. 2017;19(12):1618-27.
82.Laurent SA, Hoffmann FS, Kuhn PH, Cheng Q, Chu Y, Schmidt-Supprian M, et al. gamma-Secretase directly sheds the survival receptor BCMA from plasma cells. Nat Commun. 2015;6:7333.
83.Birnbaum T, Langer S, Roeber S, von Baumgarten L, Straube A. Expression of B-cell activating factor, a proliferating inducing ligand and its receptors in primary central nervous system lymphoma. Neurology International.
2013;5(1):e4.
84.Mulazzani M, Huber M, Borchard S, Langer S, Angele B, Schuh E, et al. APRIL and BAFF: novel biomarkers for central nervous system lymphoma.
Journal of hematology & oncology. 2019;12(1):102.
85.Srivastava S, Riddell SR. Engineering CAR-T cells: Design concepts. Trends in immunology. 2015;36(8):494-502.
86.Pircher M, Schirrmann T, Petrausch U. T Cell Engineering. Progress in tumor research. 2015;42:110-35.
87.Beyar-Katz O, Gill S. Advances in chimeric antigen receptor T cells. Current opinion in hematology. 2020;27(6):368-77.
88.Kochenderfer JN, Dudley ME, Kassim SH, Somerville RP, Carpenter RO, Stetler-Stevenson M, 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. Journal of clinical oncology : official journal of the American Society of Clinical Oncology.
2015;33(6):540-9.
89.Schuster SJ, Svoboda J, Chong EA, Nasta SD, Mato AR, Anak Ö, et al. Chimeric Antigen Receptor T Cells in Refractory B-Cell Lymphomas. The New England journal of medicine. 2017;377(26):2545-54.
90.Brudno JN, Kochenderfer JN. Chimeric antigen receptor T-cell therapies for lymphoma. Nature reviews Clinical oncology. 2018;15(1):31-46.
91.Kochenderfer JN, Somerville RPT, Lu T, Yang JC, Sherry RM, Feldman SA, et al. Long-Duration Complete Remissions of Diffuse Large B Cell Lymphoma

after Anti-CD19 Chimeric Antigen Receptor T Cell Therapy. Molecular therapy : the journal of the American Society of Gene Therapy. 2017;25(10):2245-53.
92.Abramson JS, McGree B, Noyes S, Plummer S, Wong C, Chen YB, et al. Anti-CD19 CAR T Cells in CNS Diffuse Large-B-Cell Lymphoma. The New England journal of medicine. 2017;377(8):783-4.
93.Frigault MJ, Dietrich J, Martinez-Lage M, Leick M, Choi BD, DeFilipp Z, et al. Tisagenlecleucel CAR T-cell therapy in secondary CNS lymphoma. Blood. 2019;134(11):860-6.

Acknowledgements

Before I came to Munich, what runs my life was fear、anxiety、unsatisfaction, I was always in a hurry, always flooded with unfulfilled things and always suffered from the consequences of the burden of these things. However, my life was truly changed after my 3 years' experience in Munich. To everyone I meet in München, it is all of your kind love that make my think 、 my feel 、 my act change. My suffer ends, my pain dissolves and I get my inner peace and joy. Without you I can not acquire these beautiful treasure.

Firstly, I would like to express my great appreciations to PD Dr. Louisa von Baumgarten for supervision of my PhD study. I thank her for her support, patience, advice and trust in my research work. Apart from her brilliant scientific knowledge, I also learned practical skills from her no matter in scientific work or in life out of lab. I admire her focusing on her goals and working hardly towards them, and I am lucky enough to witness her focus on becoming、achieving and attaining her scientific achievements and I am encouraged to follow her steps in my future scienticfic life. in her life out of lab , she is also my role mode and I admire her dedication to her family and friends.

I also would like to acknowlegde many thanks to Prof. Dr. Andreas Straube to give me the opportunity to work in our lab, I am very grateful for being a member in such a supportive and cooperative working team under his leadership. My appreciation also to his kindness and warmness whenever he met me- it is one of the greatest support for me to have courage to adapt to the new environment.

Great thanks to our technician Sigrid Langer, I want to thank her for answering my questions patiently, showing me tiny detailed parts of experiments, and instructing me how to perform experimental techniques; thanks for having happy time together with chatting, sharing stories and practising German; thanks for understanding me and telling me how to relax; thanks for caring about me like a big sister; thanks for being my close friend in my life.

I also really appreciated Matthias Mulazzani, I thank him for for encouraging and supporting me, for teaching me how to do animal experiments and how to image, for revising my manuscripts and instructing on my English writing, for showing me how to balance between life and science, for showing me how excellent a person like him is. Without him my research work can not keep going such smoothly, and particularly, I thank him for enlightening me when I was in a depressive state. To Louisa 、 Sigrid and him ,they are the light that dispels the darkness in my life.

I am also grateful to have my excellent friends (Yeyao, Huan and Yaqun) accompanying with me during my hard time and exploring different things together out of the lab, they are my best friends wherever and whenever and I am proud that we love each other and care about each other.

Many thanks to my other colleagues wenlong 、 Iven-Alex von Mücke-Heim, thank them for encouraging and supporting me in my experiment. Also many thanks to Prof. Dr. Steffen Massberg and all of his lab members, who make me feel staying in the lab is full of joy, warmness and happiness, and the financial supports from China Scholarship Council.

Lastly, I would like to thank my family for loving and supporting me in life, we respect each other's dream and our love is flourishing and can flourish forever.