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Maria del Pilar DOMINGUEZ

CRCN INSERM Researcher

Chair of Excellence Labex SIGNALIFE

Mediterranean Center for Molecular Medicine, C3M

Université Cote d'Azur, / INSERM U1065

151 Route de Saint-Antoine,m06200 Nice, France

SUMMARY

Hematological malignancies are aggressive cancers that have limited treatment options. Despite significant progress in the treatment of these tumors, a considerable proportion of blood cancer patients are refractory/relapse after receiving the standard treatment, which is usually chemotherapy or a bone marrow transplant. Therefore, there is a continued need for the design and development of more efficient therapies. Genomic analysis of lymphoma and leukemia revealed widespread epigenetic dysregulation in these types of tumors, with frequent somatic mutations in genes encoding enzymes with an epigenetic role. Subsequent pre-clinical studies from our group and others showed that mutations in epigenetic-modifying proteins promote tumor formation. Thus, pharmacological inhibition of epigenetic molecules has been the focus of many translational studies over the last decade, with the development of drugs inhibiting histone deacetylases (HDACs), DNA methyl transferases (DNMTs), bromodomain and extra-terminal domain proteins (BETs) and histone acetyl transferases (HATs). However, these epigenetic-based therapies have shown so far limited clinical benefit, mainly because the mechanisms underpinning their molecular effects are poorly understood.

To optimize the use of epigenetic therapies in blood cancer, I am investigating the major epigenetic processes driving the malignant phenotype as well as the anti-tumor immune response. During my first postdoc I established that TET2, an enzyme involved in DNA demethylation, is a tumor suppressor in B-cell lymphomas. Then, during my second postdoc I combined my expertise in epigenetics and immuno-oncology to study how epigenetic mechanisms regulate anti-tumor immunity and how this knowledge can be leveraged to find new therapies to enhance the function of the immune system against tumors. This work revealed a critical immunoregulatory function of HDAC inhibitors (HDACi) in plasmacytoid dendritic cells, an immune cell population that I focused on during my PhD. In my independent team, we are combining relevant human and mouse preclinical models with cutting-edge technologies in epigenomics and transcriptomics to define a new strategy to advance our understanding of hematological malignancies. My final goal is to maximize the efficacy of epigenetic therapies, leading to the design of more targeted therapeutic approaches for blood cancer.

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RESEARCH PROJECTS

PREVIOUS PROJECTS

(2006-2022)

I. Thesis project (2006-2011)

Regulation by lovastatin of the activation of inflammatory dendritic cells

- *Dominguez et al., European Journal of Immunology, 2011 (Dominguez and Ardavin 2010)*
- *Dominguez and Ardavin, Immunological Reviews 2010 (Review) (Dominguez, Lopez-Bravo et al. 2011)*

This project was carried out in the Department of Immunology and Oncology of the National Center for Biotechnology, CSIC (Madrid, Spain), under the direction of Prof. Carlos Fernández-Ardavín Castro.

Statins are drugs with multiple lipid-lowering effects such as the inhibition of endogenous cholesterol synthesis, the increase in uptake and degradation of LDL and the blockade of lipoprotein secretion. Statins are prescribed to around 200 million adults worldwide for treating hypercholesterolemia and reducing the risk of atherosclerosis and heart attack (Chou, Cantor et al. 2022). Since people with or at risk of cardiovascular disease are subjected to statin treatment for long periods, or even during lifetime, the potential side effects of such treatments have to be taken into consideration. A number of reports addressed the influence of statins on the immune system, but they did not lead to definitive conclusions since data derived from these studies were controversial. In this sense, although published data revealed that statins could promote proinflammatory effects on dendritic cells (DC) (Yilmaz, Reiss et al. 2006), they were generally considered anti-inflammatory drugs. Besides, since DCs are responsible for the induction of T cell responses against pathogens and tumors, and for the presentation of self-antigens during autoimmune diseases, an in depth-analysis of the effect of statins on DC function had to be performed.

In this work we investigated the impact of lovastatin on the responsiveness of monocyte-derived DCs (moDCs) to toll-like receptor (TLR)-mediated activation (Dominguez, Lopez-Bravo et al. 2011). Lovastatin caused a positive regulation of TLR-4 signaling that was paralleled by a higher activation of p38 MAP-kinase, leading to an increased proinflammatory cytokine and IFN- β production. In contrast, lovastatin promoted a negative regulation of IFN- β -mediated autocrine signaling, paralleled by a lower expression of the transcription factor IRF-1, leading to the inhibition of the enzymes iNOS and HO-1. Interestingly, iNOS/HO-1 activation and NO production by LPS-stimulated moDCs was strictly controlled by autocrine signalling through the type-I IFN receptor IFNAR (**Figure 1**).

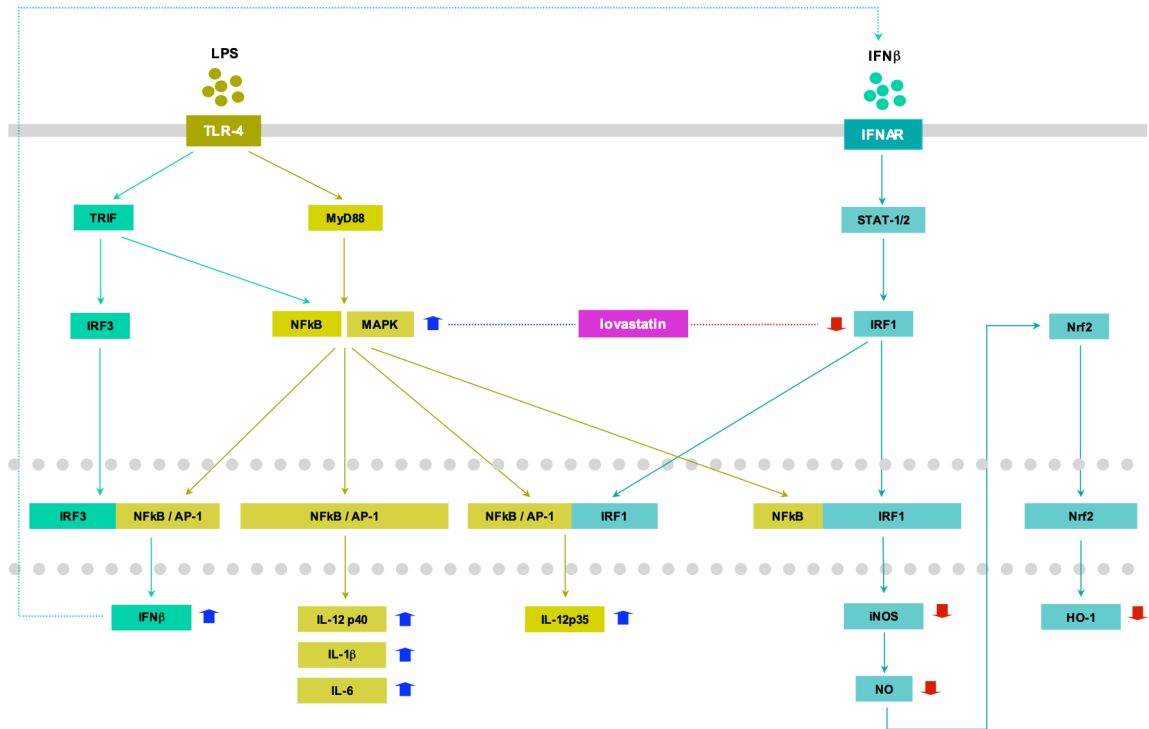


Figure 1. Proposed model for lovastatin regulation of the LPS response in cells with reduced iNOS and HO-1 expression. Lovastatin positively regulates (blue arrows) TLR4 signaling at the p38 level, promoting the synthesis of pro-inflammatory cytokines (IL-12, p40, IL-1 β , and IL-6) and IFN β . Conversely, lovastatin negatively regulates (red arrows) the IFNAR-dependent pathway, inhibiting IRF-1 synthesis, which results in decreased expression of iNOS and HO-1.

Defective iNOS/HO-1 activation on moDCs resulted in limited cytoprotective capacity against ROS and reduced microbicidal potential. These *in vitro* data were validated using an *in vivo* model of *Listeria* infection that revealed that iNOS activation by splenic inflammatory moDCs was strongly reduced in lovastatin treated mice. Our results suggested that statin treatment could therefore have severe implications during immune responses in which defective activation of iNOS/HO-1 metabolism might lead to a failure of immunity.

II. Postdoctoral project (2012-2017)

Epigenetic regulation of germinal center B-cells and its link to lymphomagenesis

This project was carried out at Weill Cornell Medicine (New York, USA), under the direction of Prof. Ari Melnick.

Diffuse large B-cell lymphomas (DLBCL) are a group of aggressive tumors and the most common form of non-Hodgkin lymphoma (Coiffier, Thieblemont et al. 2010). DLBCLs arise from germinal center (GC) and post-GC B cells and are noted for their marked genetic and epigenetic heterogeneity, aggressive behavior, and variable clinical outcomes. Epigenetic modifications, and specifically methylation of cytosine nucleotides within DNA sequence, emerged as important mechanisms of gene regulation (Fazzari and Greally 2004). Published literature unequivocally linked changes in DNA methylation to gene deregulation and carcinogenesis (Klose and Bird 2006). Hypermethylation of CpG islands and shores correlates with inhibition of tumor suppressor genes, while aberrant loss of methylation leads to activation of oncogenes with increases in chromosomal fragility and translocations (Kaneko, Shibuya et al. 1985, Klangby, Okan et al. 1998, Kawano, Miller et al. 1999, Kawamata, Inagaki et al. 2005, Krieger, Grunau et al. 2005, Kulis, Heath et al. 2012). Previous genome-wide studies in DLBCL from my group revealed profound alterations in the cytosine methylation patterning. We also observed that the degree of disruption in DNA methylation as compared to normal B cells correlated with aggressiveness of tumors. In addition GC B-cells experience substantial changes in the methylome during the transition through GCs, suggesting that the cause for the aberrant epigenetic modifications detected in DLBCLs could be the abnormal function of the factors driving heterogeneity in their cells of origin. Cytosine methylation is mediated by DNA methyltransferases (DNMT); however, mutations in DNMT are very rare in lymphomas in contrast to other hematologic malignancies (Jiang, Hatzi et al. 2013). This suggested unknown mechanisms of aberrant DNA methylation that is unique to lymphomas and specifically to DLBCLs. We focused on the role of two molecules: activation-induced deaminase (AID) and ten-eleven translocation 2 (TET2) in the normal function of GC B-cells and during lymphomagenesis.

II-1. AID mediates DNA methylation in B cells during the GC reaction, drives epigenetic heterogeneity and accelerates lymphomagenesis

- Dominguez et al., *Cell Reports* 2015 (Dominguez, Teater et al. 2015)
- Teater*, Dominguez* et al. *Nature Communications* 2018 (*co-first author) (Dominguez, Ghamlouch et al. 2018)
- Dominguez and Shakhovich, *Frontiers in Immunology* 2014 (Review) (Dominguez and Shakhovich 2014)
- Dominguez et. al, *Current Opinion in Hematology* 2017 (Review) (Dominguez, Teater et al. 2017)

The protein AID, encoded by the Activation-Induced Cytidine Deaminase (*AICDA*) locus, is a member of APOBEC family of cytosine deaminases and is expressed in B cells in a stage specific manner during B cell transition through the GCs, playing an essential role in class switch DNA recombination (CSR) and somatic hypermutation (SHM) of immunoglobulin genes in GC B-cells (Muramatsu, Kinoshita et al. 2000). AID deaminates deoxycytosines (dC) into deoxyuracils (dU), producing dU:dG mismatches that are removed by both mismatch repair and base excision repair (Honjo, Nagaoka et al. 2005, Zan and Casali 2013). The enzymatic machinery that mediates these processes is error-prone and may introduce point-mutations and changes in DNA methylation in the genome, resulting in a physiological state of genomic and epigenomic instability (Kuppers 2005, Klein and Dalla-Favera 2008, Shakhovich, Cerchiatti et al. 2011). Importantly, AID is highly expressed in the majority of DLBCL cases and is necessary for lymphomagenesis driven by BCL6 (Pasqualucci, Bhagat et al. 2008), which is the most commonly translocated transcription factor in DLBCLs (Bunting and Melnick 2013). Expression of AID was significantly associated with the loss of methylation in DLBCL patients and was predominantly identified within computationally predicted AID-binding RGYW motifs (Shakhovich, Cerchiatti et al. 2011, De, Shakhovich et al. 2013). Published data indicated that AID functioned as a demethylase during embryonic development (Rai, Huggins et al. 2008, Bhutani, Brady et al. 2010). Therefore, we hypothesized that AID had a role as a demethylase during the transit of B cells through the GCs and its continuous overexpression would disrupt DNA methylation in lymphomas, resulting in aberrant DNA methylation and dysregulated transcriptional programming that would contribute to lymphomagenesis.

We used an *in vivo* AID-deficient mouse model to study changes in DNA methylation in B cells during entry into the GC. We determined that site-specific demethylation and diversification of the methylome in GC B-cells were dependent on the function of AID (**Figure 2A and 2B**) (Dominguez, Teater et al. 2015). Moreover, we found that AID-dependent epigenetic hotspots affected genes important for lymphocyte development and proliferation. Finally, we observed significant conservation of AID-dependent epigenetic reprogramming between mouse and human B cells.

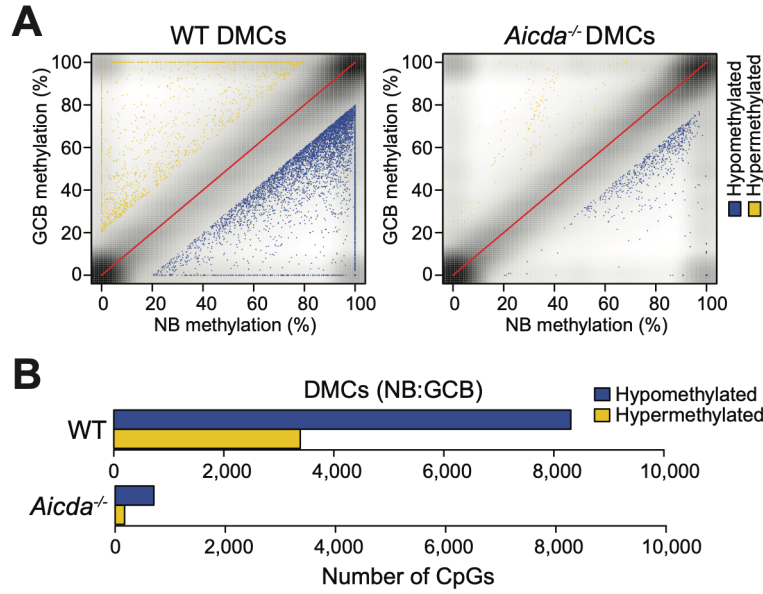


Figure 2. (A) Combined CpG methylation of naïve B cells (NB) and GC B-cells (GCB) from WT mice (seven replicates; left) and *Aicda*^{-/-} mice (six replicates; right), determined by ERRBS using a 20% methylation difference threshold and FDR < 0.001, Fisher's exact test. DMC: differentially methylated cytosine. Hypomethylated-DMCs are indicated in blue, and hypermethylated-DMCs are indicated in yellow. (B) Number of DMCs between GCB and NB from WT and *Aicda*^{-/-} mice determined by ERRBS using a 20% methylation difference threshold and FDR < 0.001, Fisher's exact test. Hypo-DMCs are indicated in blue, and hyper-DMCs are indicated in yellow. (A) and (B) show that the DNA methylation changes after GC transition in WT mice are abrogated in *Aicda*^{-/-} mice.

In a follow-up project, to determine the contribution of AID-mediated aberrant epigenetic modifications to B-cell lymphomagenesis, we studied the effect of AID overexpression in cooperation with the known oncogene *Bcl2*. We demonstrated that overexpression of AID accelerated tumor formation (**Figure 3A**) (Teater, Dominguez et al. 2018). We also assessed the contribution of the epigenetic function of AID in lymphoma formation, and we found that the more aggressive tumor phenotype was associated with increased cytosine methylation heterogeneity (**Figure 3B**).

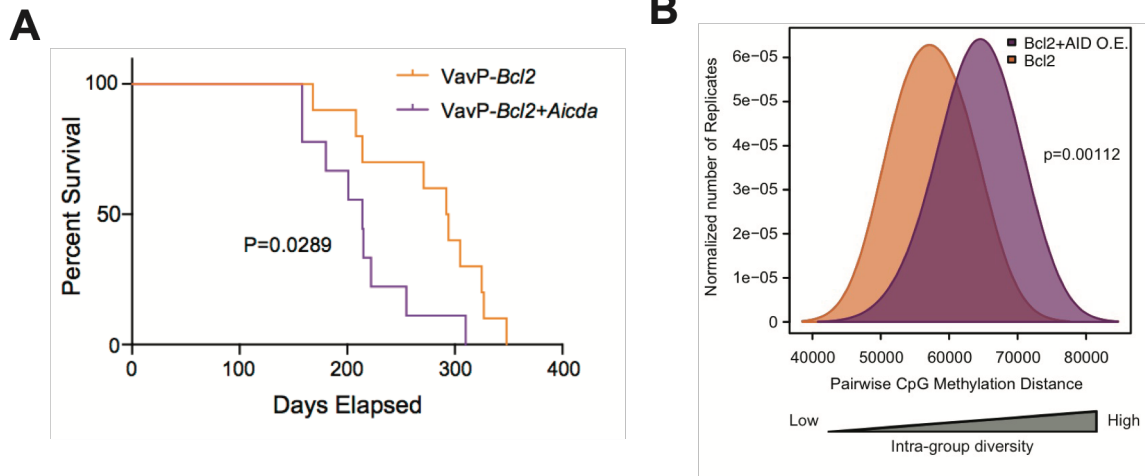


Figure 3. (A) Kaplan–Meier survival curve of VavP-*Bcl2* (n = 10) and VavP-*Bcl2*+*Aicda* (n = 9) mice. Significant differences in survival were evaluated by log-rank (Mantel–Cox) test. (B) Density plot showing the inter-tumor pairwise methylation distance between ERRBS profiles of VavP-*Bcl2* and VavP-*Bcl2*+*Aicda* tumors. VavP-*Bcl2*+*Aicda* tumors have greater pairwise distance, indicating increased inter-tumor heterogeneity among methylation profiles; two-sided Wilcoxon's signed-rank test.

II-2. Role of TET proteins in mature B cells and lymphomagenesis

- Dominguez et al., *Cancer Discovery* 2018 (Dominguez, Ghamlouch et al. 2018)
- Rosikiewicz*, Chen*, Dominguez* et al., *Science Advances* 2020 (*co-first author) (Rosikiewicz, Chen et al. 2020)

DNMT inhibitors (DNMTi) such as 5-azacytidine (5-Aza) had been shown to disrupt the growth of DLBCLs *in vivo* and reverse chemotherapy resistance. Yet very little was known regarding how 5mC became perturbed in DLBCL. Genome sequencing studies showed that ~10% of DLBCLs displayed somatic mutation of TET2, a dioxygenase that converts 5mC to 5-hydroxymethylcytosine (5hmC) (Asmar, Punj et al. 2013). Therefore, we hypothesized that TET2 was a bona fide and unique DLBCL tumor suppressor.

To determine whether TET2 loss of function affected the GC reaction, we generated conditional *Tet2* knockout mice. We found that absence of TET2 in the bone marrow was associated with GC hyperplasia and a decrease in plasma cells in the spleen, two features associated with lymphoma transformation (Dominguez, Ghamlouch et al. 2018). Moreover, we demonstrated that TET2 deficiency accelerated lymphomagenesis, using a mouse model of DLBCL driven by overexpression of the transcription factor BCL6. The transcriptional analysis of GC B-cells revealed that absence of TET2 impaired plasma cell differentiation through repression of genes involved in the exit from the GCs. These GC exit genes, which are regulated by the opposing function of the histone acetyl transferase CREBBP and the histone deacetylase 3 (HDAC3), are also aberrantly repressed in CREBBP-deficient DLBCL (Jiang, Ortega-Molina et al. 2017, Zhang, Vlasevska et al. 2017). To investigate the causes behind this aberrant transcriptional patterning, we analyzed the genome-wide distribution of 5hmC and histone acetylation of GC B-cells. Absence of TET2 resulted in reduced cytosine hydroxymethylation and acetylation of histone H3 in lysine 27 (H3K27ac) at enhancers, and hypermethylation at promoters, of genes required for the differentiation of GC B-cells into plasma cells (**Figure 4**) (Dominguez, Ghamlouch et al. 2018). Finally, we analyzed a cohort of over 100 DLBCL patients and found that samples with TET2 mutations also showed repression of GC exit genes, validating our findings in the mouse models.

In summary, my findings established that TET2 is a tumor suppressor in B-cell lymphomas. My contribution to this work is reflected by two manuscripts as first author in *Cancer Discovery* and *Science Advances* (Dominguez, Ghamlouch et al. 2018, Rosikiewicz, Chen et al. 2020), in collaboration with the groups of Olivier Bernard (Gustave Roussy, France) and Sheng Li (The Jackson Laboratory, USA). These studies led to the design of novel personalized therapies for aggressive lymphomas, a program that I developed in Australia in collaboration with clinical hematologists at the Peter MacCallum Cancer Centre and I have continued in France in collaboration with the Department of Hematology of the CHU of Nice (see below).

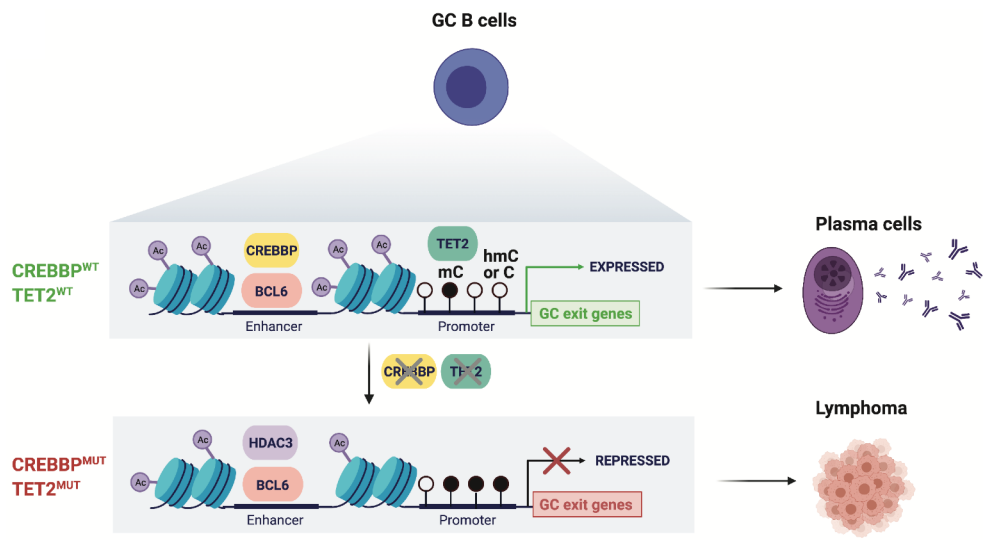


Figure 4. Enhancer/promoter regulation of GC exit genes in GC B-cells. **Top row.** In $TET2^{WT}/CREBBP^{WT}$ GC B-cells, GC exit genes are expressed due to high level of H3K27Ac at enhancers (CREBBP-mediated) and hypomethylation at promoters (TET2-mediated) and GC B-cells differentiate into plasma cells. **Bottom row.** In $TET2^{MUT}$ or $CREBBP^{MUT}$ cells, GC exit genes are aberrantly repressed, which promotes lymphoma.

III. Postdoctoral project (2018-2022)

Immunomodulatory effects of epigenetic therapies in blood cancer

- *Salmon et al., Cancer Discovery 2022 (Salmon, Todorovski et al. 2022)*

This project was carried out at the Peter MacCallum Cancer Centre (Melbourne, Australia), under the direction of Prof. Ricky Johnstone.

This work investigated new therapeutic options to treat acute myeloid leukemia (AML). AML is a type of blood cancer characterized by expansion in the bone marrow of abnormal immature white blood cells called myeloblasts, which interfere with the production of normal blood cells (Shimony, Stahl et al. 2025). It is the second most common leukemia in adults and the 5-year overall survival rate is only 25%. Genes encoding core binding factors (CBFs) including AML1/RUNX1 and CBF β are frequently rearranged in AML, producing AML1-ETO (A/E) and CBF β -SMMHC (C/S) CBF fusion proteins, respectively, and are found in approximately 15% of adult and 25–30% of pediatric patients with AML (Solh, Yohe et al. 2014). A/E and C/S both recruit HDACs to aberrantly repress CBF-target genes, which are involved in differentiation. Although treatment with HDAC inhibitors (HDACi) induces differentiation of leukemic blasts and a robust anti-leukemic response in mouse models of AML (Bots, Verbrugge et al. 2014), their translational potential as single agents has been modest. Apart from affecting tumor cell growth and proliferation, pharmacological inhibition of epigenetic enzymes can mediate anti-tumor immune responses. Previous studies in immune-compromised mice showed impaired efficacy of HDACis, although the exact mechanisms of action of these drugs on immune cells has been less studied (West, Mattarollo et al. 2013). Therefore, we hypothesized that HDACi induced immune-regulatory effects in AML, which could be leveraged to design better treatments.

Using genetically engineered models of AML, we investigated the genes and molecular pathways altered by HDACis. We found that in vivo treatment with the HDACi panobinostat induced a striking upregulation of genes associated with the type I interferon receptor (IFNAR) signaling pathway in leukemia cells (Salmon, Todorovski et al. 2022). Highlighting the importance of this pathway, we demonstrated that the anti-leukemic effect of panobinostat required IFNAR expression on tumor cells, but not on immune cells. We identified plasmacytoid dendritic cells (pDCs), and not tumor cells, as the cells producing type I IFN in response to panobinostat. At the mechanistic level, single cell RNA sequencing (scRNA-seq), total RNA-sequencing (RNA-seq) and chromatin immunoprecipitation sequencing (ChIP-seq) on isolated pDCs revealed transcriptional activation of IFN alpha and IFN beta genes concomitant with increased H3K27 acetylation at these loci (**Figure 5**). This was not observed in the other main subtype of DC, i.e. conventional DCs (cDCs). Notably, we found that depletion of pDCs abrogated the activation of the IFNAR pathway on tumor cells and reduced the effectiveness of the treatment with panobinostat. Based on these results, we combined panobinostat and IFN alpha in vivo in human AML models generated through patient-derived xenotransplantation and found induced myeloid differentiation of leukemia cells and significant reduced tumor burden.

In conclusion, my work offered a new approach with increased therapeutic efficacy to treat AML. In addition, I defined a novel mechanism of immune regulation in AML mediated by pDC activation, providing the rationale to use this cell type as a new target for immunotherapy. This study in which I am senior author was published in Cancer Discovery (Salmon, Todorovski et al. 2022).

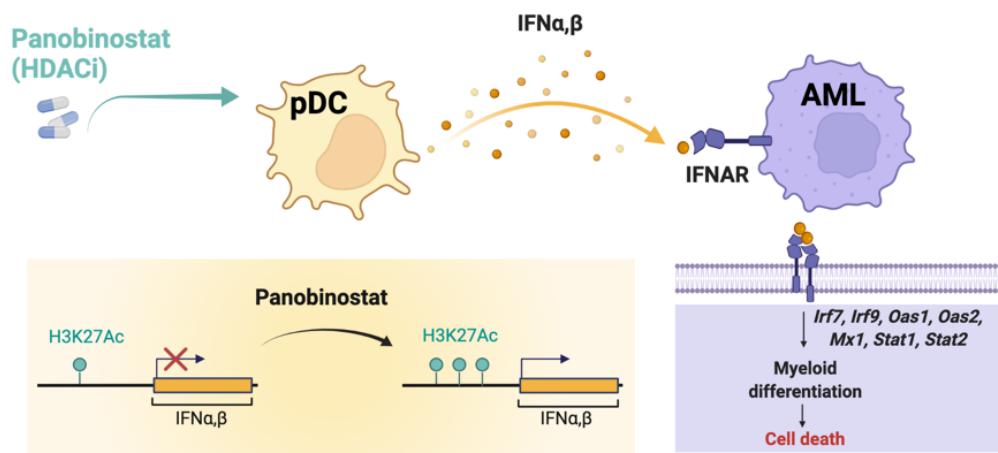


Figure 5. Schematic model of the proposed mechanism of type I IFN expression induced by panobinostat in pDCs, leading to activation of IFNAR in AML cells, which induces their differentiation towards the myeloid lineage and subsequent cell death.

CURRENT PROJECTS

CONTEXT

Hematological malignancies -lymphoma, leukemia and myeloma- are common and aggressive cancers with limited treatment options. Although significant progress has been made in the last two decades to find more targeted therapies for blood cancers, chemotherapy is still the standard treatment for this type of tumors. One main characteristic of lymphoma and leukemia is the presence of mutations in epigenetic proteins, whose function is switching on/off the expression of genes. These epigenetic proteins introduce small chemical tags that result in activation or repression of the associated genes. When epigenetic proteins are mutated, cells do not function properly, leading to tumor formation (Jiang and Melnick 2015). Therefore, therapies aimed at reversing abnormal epigenetic mechanisms are expected to be beneficial for patients with hematological malignancies. While epigenetic therapies are used as anti-cancer treatments, their full potential has not been achieved, mainly because there is a fundamental knowledge gap regarding their underlying molecular effects.

HYPOTHESIS AND STRATEGY

Following up on my previous work, my overarching hypothesis is that understanding how epigenetic mechanisms modulate the biological processes that control both tumor development and anti-tumor immunity will lead to find new immunotherapies and/or epigenetic therapies against hematological cancers. On one hand, my project aims to define the mechanistic events underpinning the development and progression of blood cancers caused by malfunctioning of epigenetic processes. On the other hand, my project focuses on improving the application of epigenetic-based drugs, untangling their effects on heterogeneous tumors as well as the immune compartment.

Specifically, my research project investigates the epigenetic pathways that 1) control tumor formation, 2) the response to anti-cancer drugs and 3) anti-tumor immune responses. The present program is complementary to the existing cancer research groups at C3M working on hematological malignancies, different components of the tumor microenvironment, such as the stroma, and metabolism. In addition, my research adds value to the C3M bringing epigenetic-focused themes as well as human systems, mouse models and innovative methodological approaches to investigate the causes of cancer and enhance the applicability of epigenetic therapies.

FINALIZED PROJECTS

(2023-2026)

I. Enhanced efficacy of a specific HDAC3 inhibitor in combination with 5-Azacitidine against diffuse large B-cell lymphoma

- Calaud, Serrano-Lopez et al., *Blood Neoplasia* 2026 (Calaud 2026)

- Funded by Fondation ARC (Leaders in Oncology 2023)

Our previous studies showed that in TET2- and CREBBP-deficient GC B-cells repression of GC exit genes was due to reduced H3K27ac at enhancers and increased mC at promoters (Dominguez, Ghamlouch et al. 2018, Rosikiewicz, Chen et al. 2020). Therefore, we decided to test a combination of a **histone deacetylase 3 inhibitor (HDAC3i)** with **hypomethylating agents (AZA)**, after establishing a formal agreement with the biotech Melbridge Therapeutics for the use of their HDAC3i.

We first tested the efficacy of 5-Aza plus HDAC3i in human DLBCL cell lines MD901, OCI-Ly7 and SUDHL-4 (**Figure 6A**). Cells were treated with different concentrations of 5-Aza (50, 100, 200nM) or vehicle for 5 days, with HDAC3i (1, 5, 10mM) or vehicle added at day 2 and 4. Analysis at day 7 showed that whereas single agent did not induce cell death based on propidium iodide (PI) staining, the combination induced significantly higher cell death (200nM 5-Aza + 10μM HDAC3i=43.6% PI⁺ cells, **Figure 6B**). We observed similar increased efficacy of 5-Aza + HDAC3i in OCI-Ly7 and SUDHL-4 cells (data not shown). Importantly, we found that 5-Aza + HDAC3i acted synergistically in MD901 cells (combination index (CI)<1, **Figure 6C**), and also in OCI-Ly7 and SUDHL-4 cells (data not shown). We also confirmed that the combinatorial therapy reduced proliferation of DLBCL cells at a higher degree than 5-Aza or HDAC3i alone, based on dilution of cell trace violet over time (**Figure 6D**). Finally, to test the efficacy of the combinatorial therapy *in vivo* we established xenografts. We transplanted lymphoma cells in immuno-deficient mice through intraperitoneal injection to achieve systemic disease. Mice engrafted with OCI-Ly7 cells received daily treatment with 1mg/kg 5-Aza or vehicle for 1 week, and were also treated with 25mg/kg HDAC3i or vehicle for other 3 weeks. 80% mice treated with 5-Aza + HDAC3i survived after 150 days, compared to 50% mice treated with HDAC3i alone; 5-Aza did not induce survival advantage compared to vehicle-treated mice (**Figure 6E**).

These results confirm the superior efficacy of combining 5-Aza and HDAC3i against DLBCL, and they suggest that the enhanced anti-tumor effect of the epigenetic combinatorial treatment is associated with an epigenetic reprogramming that leads to the re-expression of genes involved in GC-B cell differentiation (Calaud 2026).

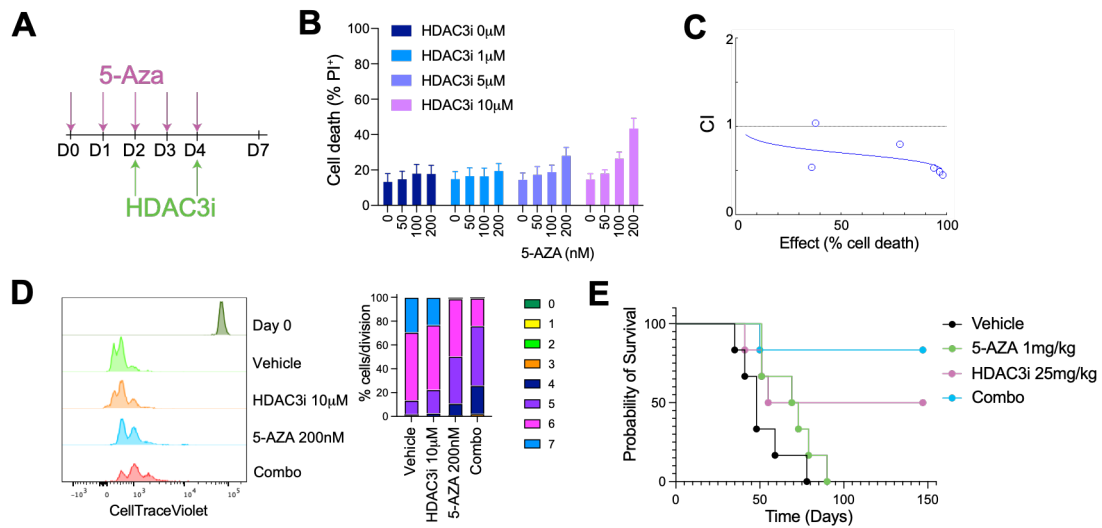


Figure 6. (A-D) Analysis of MD901 cells treated with 5-Aza and HDAC3i: 5-Aza administered every day for 5 days (day 0-day 4); HDAC3i added on days 2 and 4. **A)** Schematic of in vitro treatment of DLBCL cells. **B)** Cell death analysis performed on day 7. **C)** Synergy analysis on day 7. **D)** Cell trace violet analysis on day 4. **E)** Survival curves of NSG mice transplanted with OCI-Ly7 cells (0.1M intraperitoneally) and treated with vehicle, 5-Aza, HDAC3i or combo.

II. Collaborations:

II-1 Epigenetic remodeling via HDAC6 inhibition amplifies anti-tumoral immune responses in myeloid leukemia cells

- *Schliehe-Diecks et al., Cell Death and Disease 2026 (Schliehe-Diecks, Tu et al. 2026)*
- **In collaboration with the group of Dr Sanil Bhatia (University of Dusseldorf)**

Background

AML accounts for ~20% of childhood and ~80% of adult acute leukemia cases. Despite advances, treatment still relies on chemotherapy and stem cell transplantation, with a 5-year survival of only ~30% (Shimony, Stahl et al. 2023). AML has traditionally been viewed as a genetic disease driven by recurrent chromosomal rearrangements and somatic mutations that enhance survival and block myeloid differentiation. More recently, epigenetic dysregulation, including alterations in DNMT and HDAC, has also been implicated in its pathogenesis (Fennell, Bell et al. 2019). HDAC remove acetyl groups from histones and other proteins, thereby regulating gene expression. While HDAC gene mutations have not been reported in AML, oncogenic fusion proteins aberrantly recruit HDACs, leading to transcriptional repression of genes required for hematopoietic differentiation. Consequently, HDAC inhibitors (HDACi) have been explored as potential therapies in AML (Fennell, Bell et al. 2019). However, the results of the clinical trials with these epigenetic drugs have been modest and more research is needed to optimize the use of HDACi as new treatment option against AML. One limitation of currently approved HDACi for hematological malignancies is that they are predominantly pan-HDAC inhibitors, which non-selectively target multiple HDAC isoforms involved in essential cellular processes. This lack of specificity can result in a range of adverse effects (Shah 2019). Moreover, HDACi are rarely used as monotherapy in clinical practice, often being combined with other agents, which adds further complexity. Selective inhibition of specific HDAC isoforms may therefore provide a more targeted therapeutic approach, potentially reducing toxicity while enhancing efficacy. HDAC6 is a distinct member of the family of HDAC, primarily localized in the cytoplasm, where it regulates a variety of cellular functions. Overexpression of HDAC6 is associated with increased tumor aggressiveness and lower survival rates in several malignancies (Asaad, Pepperrell et al. 2025). In this context, the group of Dr Sanil Bhatia employed database-driven analyses to identify HDAC6 as a promising therapeutic target in myeloid lineage-derived leukemia cells (Schliehe-Diecks, Tu et al. 2025). Importantly, Bhatia's team recently developed novel HDAC6 targeting inhibitors that exhibit *ex vivo* and *in vivo* anti-leukemic activity against AML cells, with improved selectivity profile compared to clinically approved pan-HDAC inhibitors (Tretbar, Schliehe-Diecks et al. 2024). We initiated a collaboration, since my recent work demonstrated that combining a HDACi (panobinostat) with IFN α induced myeloid differentiation of leukemic cells and significant reduced tumor burden compared to panobinostat alone in human and mouse AML models (Salmon, Todorovski et al. 2022).

Results

HDAC6 has emerged as a promising therapeutic target in cancer due to its immunomodulatory effects. While its prognostic significance remains debated, we demonstrate that HDAC6 loss significantly impairs myeloid leukemia progression *in vivo*, despite having no functional impact on leukemia cell proliferation *in vitro*. Global proteome and secretome profiling of HDAC6-knockout (KO) cells revealed upregulation of several immune-related modulators, including RNase T2, a tumor suppressor known to modulate the tumor microenvironment. Notably, RNase T2 upregulation upon HDAC6 loss was observed in myeloid leukemia cells but not in lymphoblastic leukemia cells. Moreover, pharmacological inhibition of HDAC6 recapitulated this phenotype, leading to RNase T2 upregulation in myeloid leukemia cells. ATAC-seq revealed increased chromatin accessibility of RNase T2 following HDAC6 loss, highlighting a functionally epigenetic regulatory contribution. My team contributed to the analysis of these genome-wide sequencing experiments in HDAC6-deficient AML cells.

Further functional assays conducted in an immunocompetent setting, both *ex vivo* and *in vivo*, demonstrated that HDAC6 inhibition sensitized murine myeloid leukemia cells to broad CD8⁺ T cell activation as evidenced by increased TNF α and CD107a expression. Consistently, in a syngeneic murine model, HDAC6 inhibition restricted the growth of myeloid leukemia cells. Moreover, an extended drug screening analysis identified Cytarabine and Clofarabine as significantly synergizing with HDAC6 inhibitor (Ricolinostat) in myeloid leukemia cell lines and in patient-derived xenograft (PDX) cells, while showing limited synergy in lymphoid leukemia cell lines, PDX, or healthy control cells. These findings suggest that HDAC6 represents a promising therapeutic target in myeloid lineage-derived leukemia cells (Schliehe-Diecks, Tu et al. 2026).

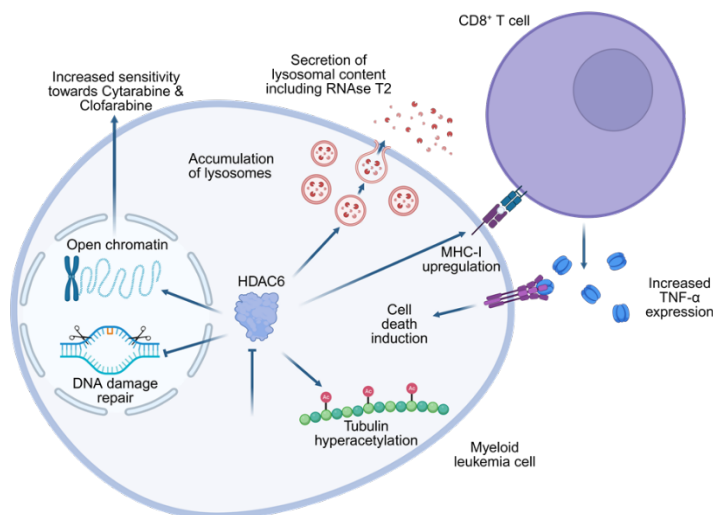


Figure 7. HDAC6 inhibition enhances anti-tumor immunity and improves the response to chemotherapy in myeloid leukemia cells.

II-2 *Tet2* deficiency promotes IgG1+ B-cell expansion and differentiation blockade through deregulation of the *Nfkbia*–c-Rel axis

- Ghamlouch et al., *HemaSphere* 2026 (Ghamlouch, Degaud et al. 2026)
- In collaboration with the group of Olivier Bernard (Gustave Roussy)

The germinal center (GC) reaction is a major step of B-cell differentiation, orchestrating an effective humoral immune response by generating plasma cells and memory B-cells. TET2, an alpha-ketoglutarate-dependent dioxygenase, assumes a central role in this process by regulating isotype switching to IgG1, B-cell exit from the GC, and plasma cell differentiation. I previously demonstrated that TET2 is a tumor suppressor in DLBCL (Dominguez, Ghamlouch et al. 2018). DLBCL classifications incorporated *TET2* mutations into specific subgroups, ST2. The ST2 genetic DLBCL subgroup is characterized by high NF- κ B and PI3K activity and predominant expression of IgG BCRs (Wright, Huang et al. 2020). Using *in vivo* SRBC immunization and an *in vitro* GC-like reaction model, we investigated the consequences of *Tet2*-deficiency on IgG1+ GC B-cell biology. In addition to affecting plasma cell generation, *Tet2* loss impaired IgG1 memory B-cell differentiation and promoted IgG1+ B-cells proliferation. In IgG1+ GC B-cells, disrupted differentiation is associated with *Nfkbia* hypermethylation, activation of c-Rel and sustained high IgG1 levels. In recall immunization, *Tet2*-deficient IgG1+ GC B-cells and MBC showed lower T-bet expression, hindering their differentiation into plasma cells and resulting in their accumulation. These findings underscore the pivotal role of Tet2 in balancing differentiation and proliferation during the humoral response, shedding light on the transformation mechanisms of *TET2*-inactivated DLBCL (Ghamlouch, Degaud et al. 2026).

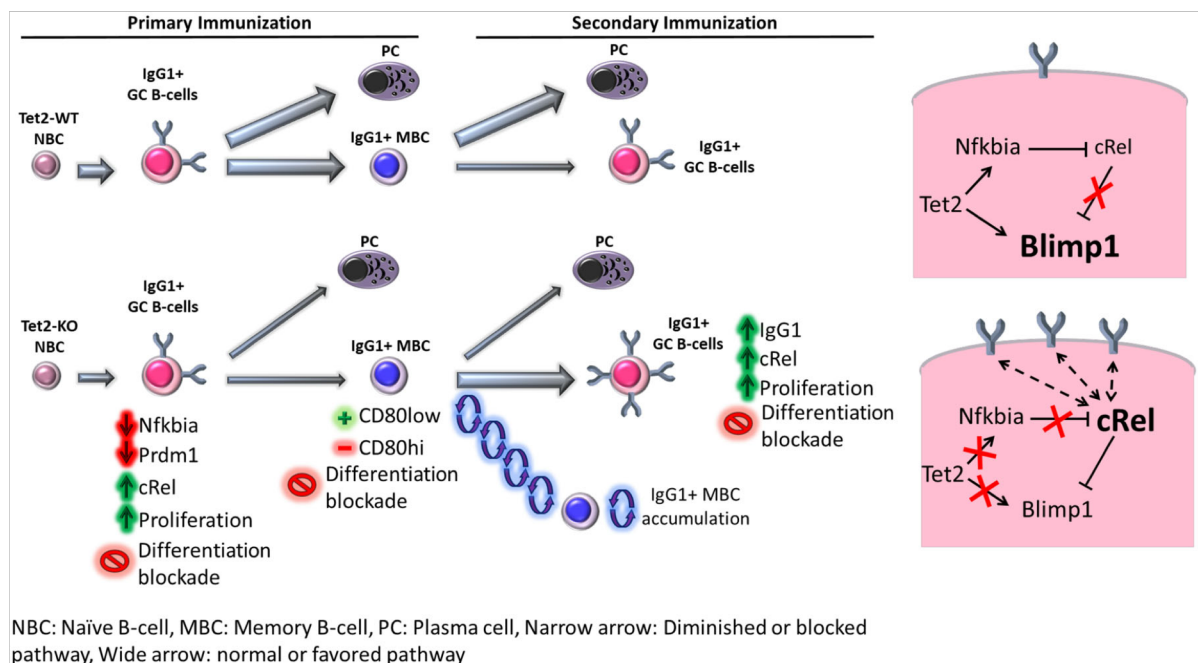


Figure 8. Diagram depicting the effects of Tet2 inactivation on IgG1 + GC B cells during primary and secondary immunizations, alongside a proposed model of the underlying molecular mechanisms. At the cellular level, Tet2-deficient mice exhibit germinal center (GC) hyperplasia and impaired plasma cell differentiation following T-dependent immunization. During primary immunization, Tet2 inactivation reduces the generation of IgG1+ GC B-cells by impairing isotype switching to IgG1 and hindering their differentiation into plasma cells and memory B-cells. This deficiency enhances the proliferative response of IgG1+ GC B-cells to external cues, leading to their accumulation during subsequent immunizations and a persistent differentiation blockade. At the molecular level, Tet2 deficiency directly impacts Prdm1 (encoding Blimp1) expression through hypermethylation of its regulatory elements and induces sustained DNA methylation at the Nfkb1a locus. This results in the downregulation of Nfkb1a, leading to increased c-Rel activity, which further represses Blimp1 expression. Elevated IgG1 BCR expression likely sustains c-Rel activation. Consequently, there is an increased proliferation capacity of IgG1+ GC B-cells, coupled with a blockade in memory and plasma cell differentiation.

ONGOING PROJECTS

CONTEXT, HYPOTHESIS AND AIMS

I. Investigating deregulated epigenetic mechanisms in lymphoma.

- As part of the supervision of Abdelillah Maarad's thesis
- Funded by Labex SIGNALIFE Université Côte d'Azur

Rationale

The presence of epigenetic alterations in GC B-cells, the cells of origin of DLBCL, predisposes these cells to become malignant, but the processes and pathways involved are not clearly defined. In this regard, I previously demonstrated that TET2 is a tumor suppressor in DLBCL (Dominguez, Ghamlouch et al. 2018). However, the lack of TET2 is not sufficient to induce lymphomagenesis, which suggests that cooperation with other mechanisms is required to initiate lymphoma formation. Two genes with frequent co-occurring mutations with *TET2* are *GNA13* and its receptor *P2RY8*. *GNA13* is a member of the G protein a family that transduces signals from the G-coupled protein receptors *P2RY8* and Sphingosine-1-phosphate receptor (*S1PR2*). Around 20% of DLBCL patients with *TET2* mutations also present mutated *GNA13*, based on targeted sequencing of 70 genes in 400 lymphoma samples (Sha, Barrans et al. 2019) (**Figure 9A**). The co-selection of these two oncogenic drivers is higher than the frequency of *TET2* and *SGK1* mutations (15%), which define one of the new genetic subtypes of DLBCL, the subgroup ST2 (Wright, Huang et al. 2020), as mentioned before. This data suggests that TET2 and *GNA13* absence may contribute together to tumor formation and development, but there is no clear link between the function of these molecules (**Figure 9B**), and more research is needed to fully understand the origins of lymphoma in this setting.

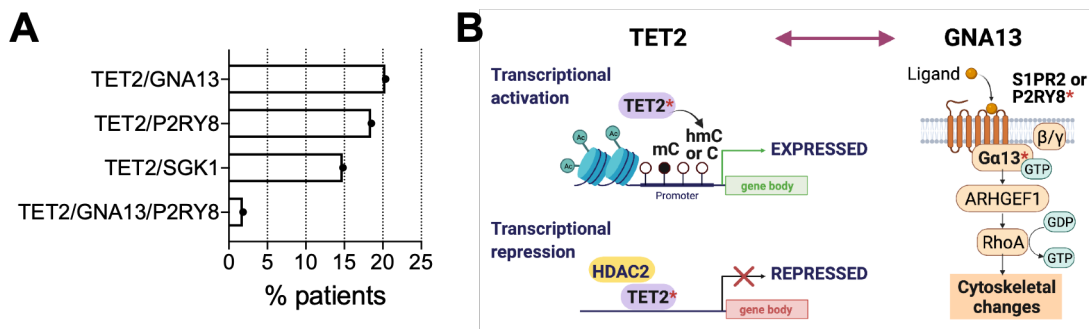


Figure 9. A) Co-occurrence of TET2 and GNA13, P2RY8 or SGK1 mutations in DLBCL patients. Graph shows the percentage of patients with mutations in TET2/GNA13, TET2/P2RY8, TET2/SGK1 or TET2/GNA13/P2RY8 within the 15% DLBCL patients with TET2 mutations. **B)** Schematic representation of TET2 and GNA13 known functions. Genes mutated in DLBCL are marked with an asterisk. HDAC2: Histone deacetylase 2.

Methodology

To uncover how TET2 and GNA13 cooperate to drive lymphoma formation, I have generated genetically engineered mouse models whose B cells lack TET2 and GNA13, breeding CD23-Cre (B-cell specific) or AID-Cre (GC B cell-specific) mice with Tet2^{f/f} mice. After immunization with the antigen NP-CGG, these conditional TET2/GNA13-deficient mice show expansion of GC B-cells and splenomegaly compared to TET2- or GNA13-deficient mice, both features associated with malignant transformation (**Figure 10A**). These preliminary results suggest that the concomitant absence of TET2 and GNA13 in mice mimics human DLBCL and, therefore, these mouse models are an invaluable tool to study the initiation and progression of the human disease.

As a complementary approach, we will utilize CRISPR technology to perform screens with customized epigenetic libraries in TET2-, GNA13- or TET2/GNA13-deficient DLBCL cells, to define the key epigenetic regulators of these populations (**Figure 10B**). **These experiments will help to identify clinically relevant drug targets for patients harboring these mutations.**

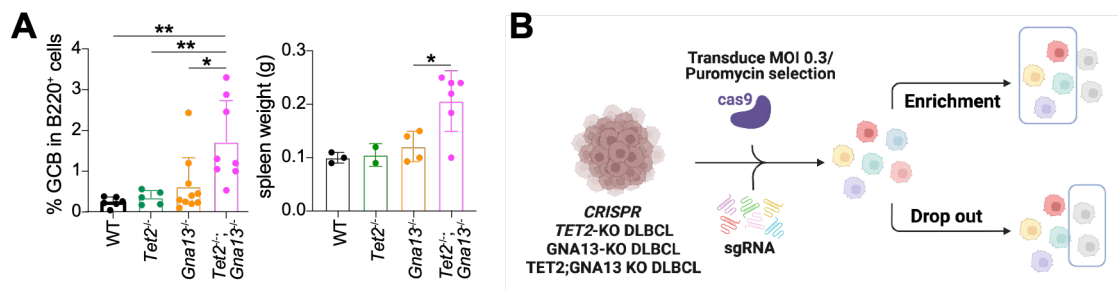


Figure 10. A) Percentage of GC B-cells (GCB) in the spleen of WT, AID-Cre;Tet2^{-/-}, AID-Cre;Gna13^{-/-} or AID-Cre;Tet2^{-/-};Gna13^{-/-} mice at day 8 after immunization with NP-CGG antigen. **B)** Schematic of CRISPR/Cas9 screen. Depleted guides will indicate genes that are essential for survival of TET2 KO, GNA13 KO or TET2;GNA13 KO DLBCL cells. Enriched guides will indicate genes that need to be silenced for survival of TET2 KO, GNA13 KO or TET2;GNA13 KO DLBCL cells.

II. Optimizing epigenetic-based therapies in blood cancer

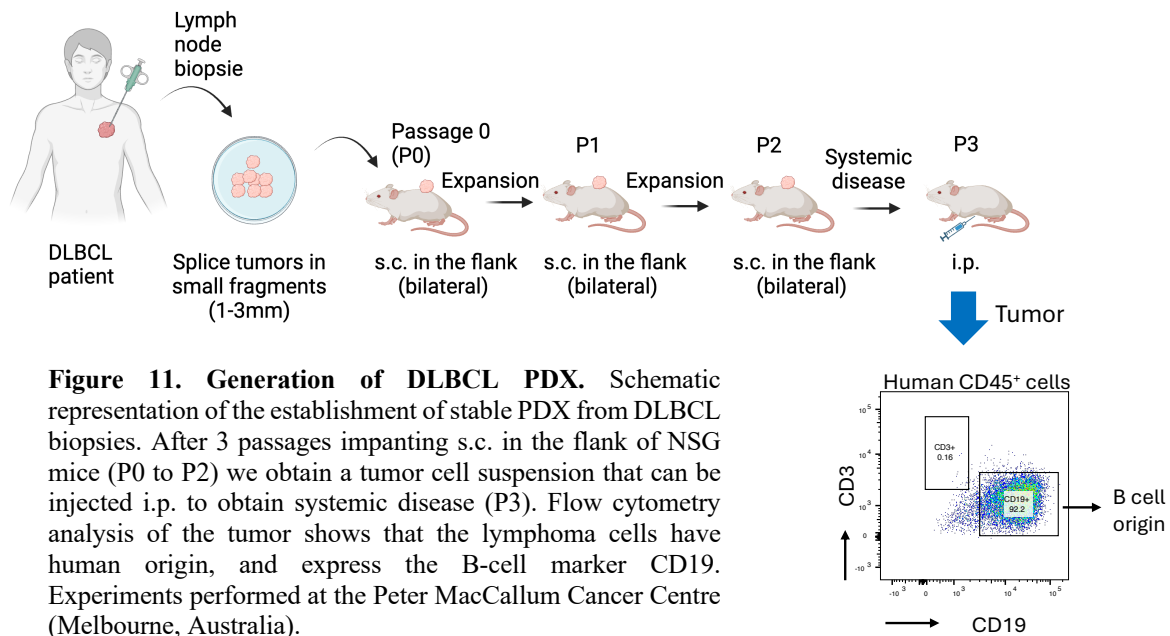
- As part of the supervision of Calixte Calaud's thesis
- Funded by Fondation ARC (Leaders in Oncology 2023) and La Ligue contre le Cancer (Predoctoral fellowship awarded to Calixte Calaud)

Rationale

Since lymphomas and leukemias are characterized by profound alterations in the epigenome (histone modifications and DNA methylation), therapies aimed at reversing abnormal epigenetic mechanisms such as HDACi and hypomethylating agents are expected to be beneficial for patients with blood cancer, resulting in increased patient survival. However, epigenetic therapies are not yet widely used in clinical practice, and more fundamental research is needed to prioritize and develop those treatments that are most likely to be curative for blood cancers and other type of tumors. I hypothesize that **combinatorial therapies** will show enhanced efficacy compared to single agents.

Methodology

My group is using patient-derived xenografts (PDX) and 3D organoids from primary DLBCL samples to evaluate the response to different combinatorial epigenetic therapies in relevant settings for clinical applications. We collected primary DLBCL specimens from the Peter MacCallum Cancer Centre in collaboration with Prof Michale Dickinson to test the combinatorial therapy in patient-derived xenografts (PDX) and in 3D organoid models. Primary samples from relapsed/refractory DLBCL patients at the Peter MacCallum Cancer Centre (Melbourne, Australia) were transplanted into NSG mice, following a protocol previously described that allows the generation of patient-derived lymphoma cells that can be expanded *in vivo* (Cacciapuoti, Cappelli et al. 2021) (**Figure 11**). The location of C3M within the hôpital L'Archet allowed the establishment of collaborations with the clinic to set up these human-relevant experimental settings. I have continued this part of the project at my independent laboratory at the C3M in collaboration with the Department of Hematology of the CHU of Nice (Prof Nicolas Mounier, Prof Thomas Cluzeau).



The 3 main aspects of this theme are summarized in **Figure 12**:

Drug screening: My laboratory will investigate the efficacy of combinatorial therapies using Digital RNA with perturbation of Genes (DRUGseq), a novel high-throughput platform for drug discovery. This technology allows in a cost-effective manner the screening of multiple compounds, analyzing in a single assay their therapeutic effectiveness through flow cytometry (differentiation markers/cell death), and their mechanism of action through transcriptional profiling. Using DRUGseq we will be able to screen a large number of epigenetic inhibitors in vitro, selecting the most promising candidates for further testing in PDX and organoids models. This technique can be broadly applied using other classes of drugs, such as kinases inhibitors, and other types of cancer.

Multi-omics analysis: To investigate the molecular pathways that mediate the anti-cancer effects of the combinatorial therapy, we will integrate the transcriptional data obtained from DRUG-seq (lymphoma/leukemia cell lines) with the analysis of primary lymphomas derived from PDX and organoids after treatment. Due to the heterogeneous nature of the primary tumors, we will rely on single-cell multi-omics technologies to be able to deconvolute the composition and cellular states of the human lymphoma samples: single-cell RNA sequencing (scRNAseq; transcriptome), scCUT&TAG (scC&T; histones/chromatin accessibility) (Bartosovic, Kabbe et al. 2021) and single cell reduced representation bisulfite sequencing (scRRBS). This analysis will reveal how the treatment modulates the epigenome, identifying the pathways targeted by the epigenetic therapy and revealing new targets that could ultimately be exploited therapeutically (epi-genes).

Functional studies: to investigate the role of the epi-genes in mediating the anti-cancer activity of the combinatorial therapy we will apply an in vivo innovative approach using CRISPR-activation (CRISPRa). CRISPRa utilizes single guide RNA (sgRNA) to direct a nuclease deactivated Cas9 (dCas9) to the transcriptional start sites of the genes of interest, resulting in the

transcriptional activation of the endogenous target genes (Heidersbach, Dorigi et al. 2023). Tumor cells expressing dCas9 will be transduced with CRISPRa sgRNA targeting epi-genes and transplanted into NSG mice. These lymphoma cells will also express the luciferase gene, allowing tracking of the lymphoma growth with an imaging system. We will analyze survival to determine whether CRISPR-induced upregulation of the epi-gene reduces tumor growth.

We expect that the results of these pre-clinical studies will provide the rationale to establish clinical trials for DLBCL using new combinatorial treatments.

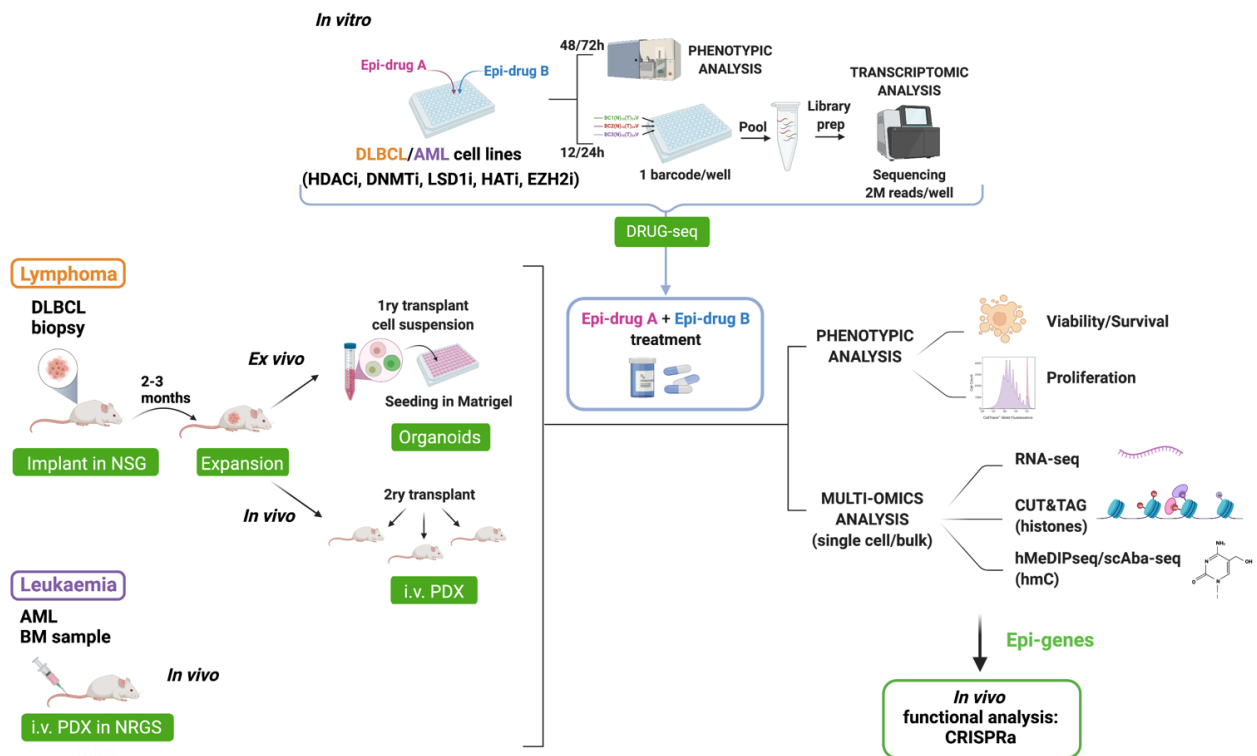


Figure 12. Theme 2 research plan. We will expand in vivo the primary DLBCL specimens through the establishment of PDX, implanting pieces of lymphoma tissue in the flank of NSG mice. 1ry transplants will be collected and used to establish organoids and 2ry transplants, injecting the tumor cells intravenously (i.v.) to generate systemic disease. We will use DRUG-seq to test different combinatorial epi-drugs in vitro in DLBCL cell lines. The most promising epi-drugs combos will be tested in primary samples (organoids and PDX), analysing their efficacy (phenotype/survival) and mechanism of action (multi-omics analysis) to define epi-genes. Finally, the functional relevance of epi-genes will be tested in vivo.

III. Studying the interactions between the tumor and the immune system in lymphoma

- As part of the supervision of Océane Zinsou's master project
- Funded by Fondation ARC (Leaders in Oncology 2023)

Rationale

One of the main problems of studies testing epigenetic-based treatments has been focusing only on the effect of these therapies on the cancer cells, largely ignoring their consequences on the function of the other cells of the tumor microenvironment. A holistic approach that investigates how epigenetic mechanisms regulate the dynamic crosstalk between the tumor cells and the immune cells will lead to the discovery of new strategies to improve the efficacy of epigenetic therapies and develop new immunotherapies. In this regard, I have recently discovered that HDACi mediate their beneficial effect in leukemia through the epigenetic activation of pDC (Salmon, Todorovski et al. 2022). Using a mouse model of leukemia, we demonstrated that treatment with a histone deacetylase inhibitor (HDACi) induces the activation of a type of immune cells (dendritic cells), a process that is required for the full therapeutic effect of this epigenetic drug.

Methodology

I have already established *in vivo* immunocompetent models to study DLBCL which my group is using to analyze, before and after the epigenetic treatment, the frequencies and location of the immune cells through flow cytometry, immunohistochemistry and single cell/bulk sequencing methods, including spatial transcriptomics (**Figure 13**). The most relevant cell types, based on the phenotypic analysis, will be isolated and characterized at the transcriptomic and proteomic level to determine which pathways are modulated by the epigenetic therapy. I have already optimized genome-wide sequencing techniques for the analysis of immune cells, to characterize the transcriptome and epigenome of the cells of the immune system in response to different epigenetic therapies. By comparing the composition of the immune system in non-tumor bearing mice versus lymphoma- or leukemia-bearing mice, we will get a better picture of the immunological changes mediated by the presence of cancer cells and how both the immune compartment and the tumor are regulated by epigenetic therapies. We will use this knowledge to improve the efficacy of epigenetic-based approaches to treat B-cell lymphomas and develop new immunotherapies. In addition, since many types of immune cells can be detected by routine pathology, **this information will be clinically relevant to establish cancer diagnosis and to design biomarkers of response to therapy.**

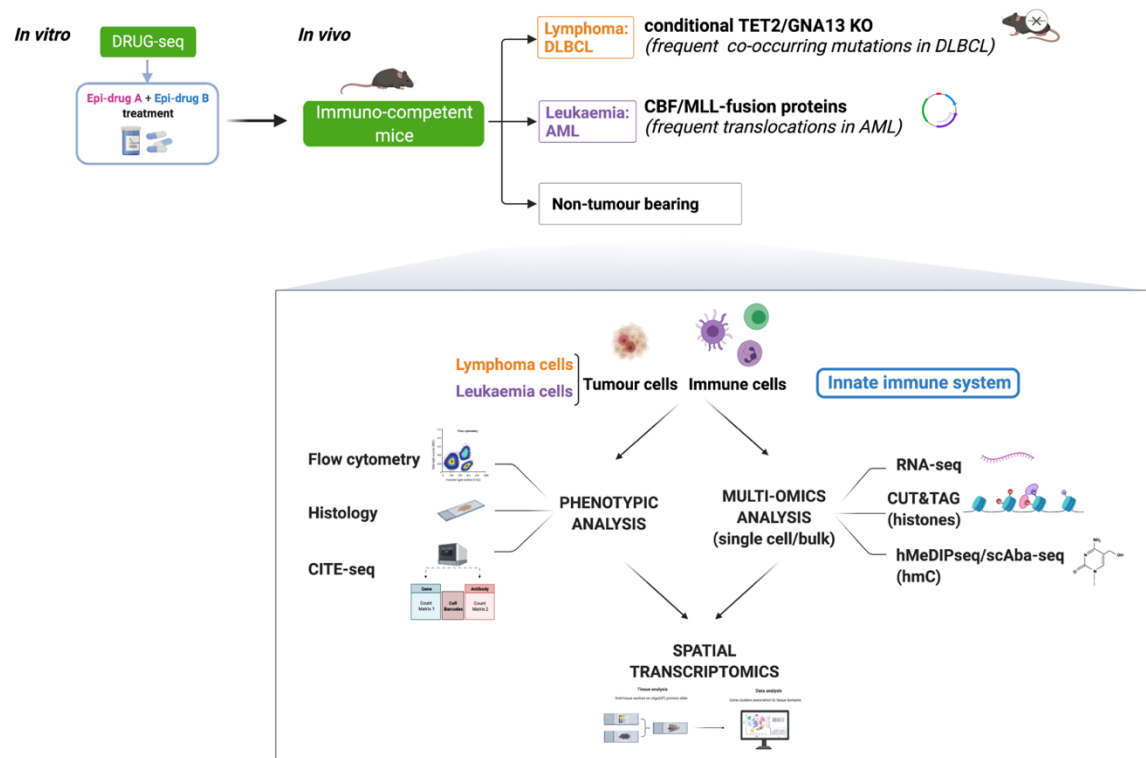


Figure 13. Theme 3 research plan. Immuno-competent mouse models of lymphoma and leukemia (or non-tumour bearing mice as control) will be utilized to characterize the tumour cells and immune cells at the phenotypic and molecular level before and after treatment with the combinatorial treatments selected through DRUGseq

IV. TET2-MAP: Decoding the TET2 interactome in mature B-cell lymphomas

- As part of the supervision of Dr Emilio Serrano-Lopez's project
- Funded by the European Hematology Association, EHA (Kick-Off Grant awarded to Dr Emilio Serrano-Lopez)

Rationale

The GC reaction represents a highly specialized microenvironment, where B cells undergo extensive transcriptional and epigenetic changes that drive their proliferation and survival or apoptosis, to ensure that only those with the highest antigen affinity are positively selected. DLBCLs display aberrant transcriptional and epigenetic programming compared to normal GC B cells, the etiology of which is not completely understood. Therefore, a deeper understanding of the molecular mechanisms governing the GC reaction is essential to elucidate the origins of DLBCL and to identify novel therapeutic opportunities, since only 60% of patients respond to the standard immune-chemotherapy treatment. Genomic analysis of DLBCLs revealed widespread alterations in the epigenome, with frequent somatic mutations in genes encoding enzymes with an epigenetic role (Bakhshi and Georgel 2020). Most studies have focused on enzymes involved in histones modifications while less is known about enzymes regulating DNA methylation. Importantly, higher degree of disruption in DNA methylation in DLBCL patients, as compared to normal B cells, correlates with tumor aggressiveness and poorer survival outcomes for these patients (Shaknovich, Geng et al. 2010, Chambwe, Kormaksson et al. 2014), highlighting the critical role of this process in disease biology and underscoring the need to investigate its underlying mechanisms.

In this regard, ~10% of DLBCL patients display somatic mutations in TET2 (Bakhshi and Georgel 2020), a dioxygenase crucial in DNA demethylation through the sequential oxidation of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC), intermediates that are removed by the base excision repair machinery, reverting to unmethylated cytosines (Zhang, Zhang et al. 2023). Previous work from our group demonstrated that TET2 is required for the correct exit of GC B cells from the GC reaction and differentiation into plasma cells, unveiling a previously unrecognized role as a tumor suppressor in GC-derived lymphomas (Dominguez, Ghamlouch et al. 2018, Rosikiewicz, Chen et al. 2020). TET2 absence in GC B cells resulted in an accelerated formation of DLBCL when in cooperation with the oncogene BCL6. Moreover, TET2 deficiency deregulates the transcriptome and epigenome of GC B-cells. In this regard, my group showed that TET2-deficient B cells exhibit gene expression patterns closely resembling those of cells harboring mutations in the histone methyltransferase CREBBP (Dominguez, Ghamlouch et al. 2018), suggesting a potential functional interplay between these two enzymes. Another study from my group proposed a dependency in GC B cells between TET2 and AID, the deaminase responsible for immunoglobulin diversification processes (Rosikiewicz, Chen et al. 2020). These findings highlight the critical role of TET2 in the correct function of GC B cells. However, the mechanisms that regulate TET2 activity in GC B cells and their molecular partners are not well defined.

Methodology

Although several studies have attempted to elucidate the processes regulating the function of TET2, current knowledge remains largely restricted to specific cellular contexts (Cong, Zhang et al. 2021). In the case of B cells, while the phenotype of TET2 deficiency during the GC reaction are relatively well understood, little is known about how TET2 activity is regulated across the different phases of this process.

This project aims to shed light on how TET2 exerts its activity and is regulated in B cells undergoing the GC reaction, which is essential for their proper function as a tumor suppressor in DLBCL. To this end, two aims are proposed:

AIM 1) To investigate the activity of TET2 during the GC reaction in B cells through the identification of its interactors. Studying the interactome of TET2 in GC B cells (B220⁺GL7⁺, CD95⁺) will uncover its key regulators in B cells, providing mechanistic insights into how it modulates GC dynamics and contributes to lymphomagenesis when altered. To identify the interactors of TET2, we will employ the *BioID* (*Proximity-Dependent Biotin Identification*) technology, which leverages proximity-dependent biotinylation to capture both stable and transient interactions networks within living (Roux, Kim et al. 2018).

AIM 2) Validation and functional assessment of TET2 interactors in B cell differentiation during the GC reaction. Key interactors (2-3) will be validated using complementary approaches such as co-immunoprecipitation and microscopy assays. In addition, to investigate how the interaction between TET2 and its partners influences B cell differentiation, we will selectively inhibit the expression of key interactors and evaluate their impact on GC B cell differentiation capacity. This approach will allow us to define the functional contribution of individual TET2 interactors to the regulation of B cell fate decisions.

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