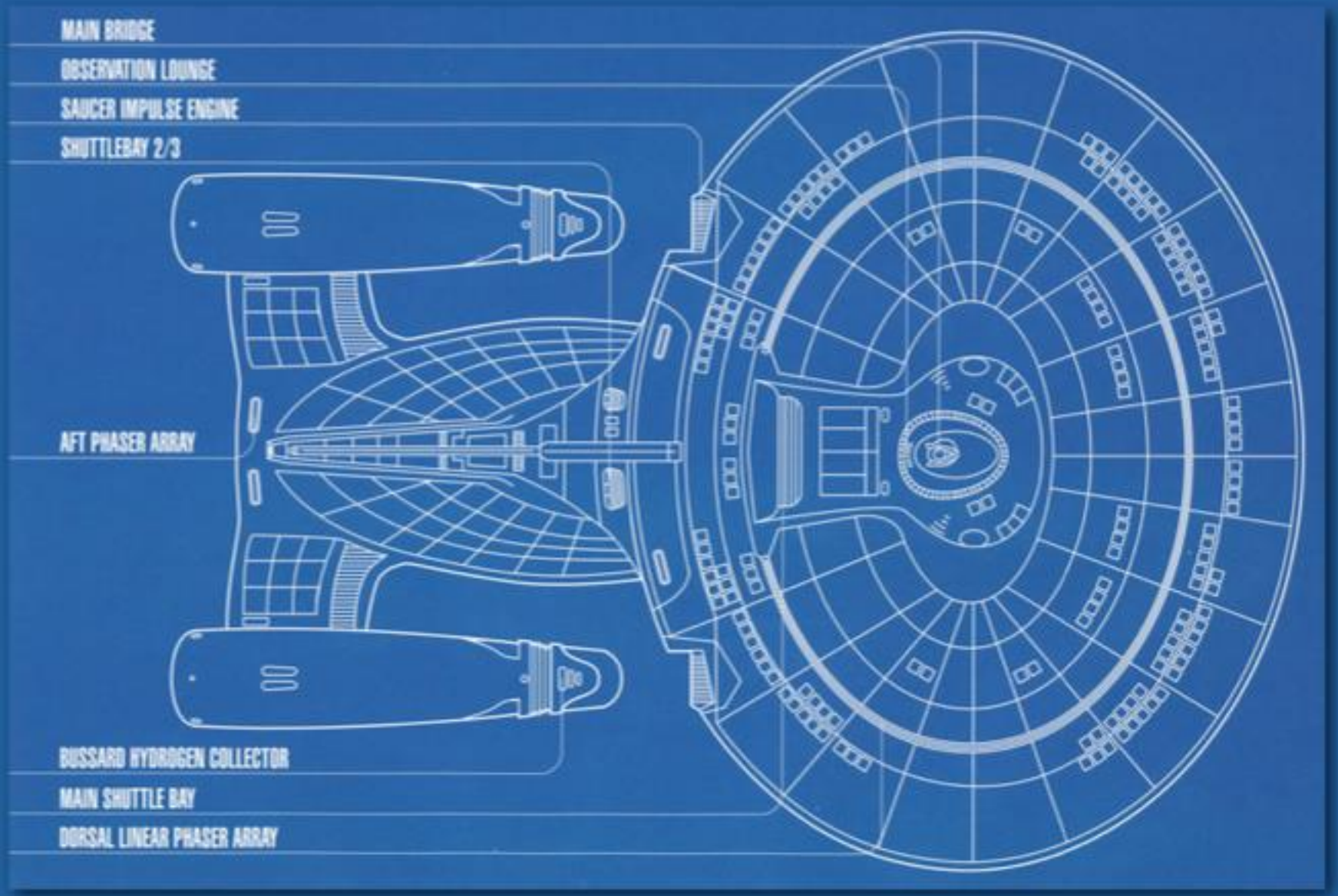
Role of epigenetic switches in B-cell lymphomas

Ari Melnick, MD

Gebroe Family Professor of Hematology/Oncology

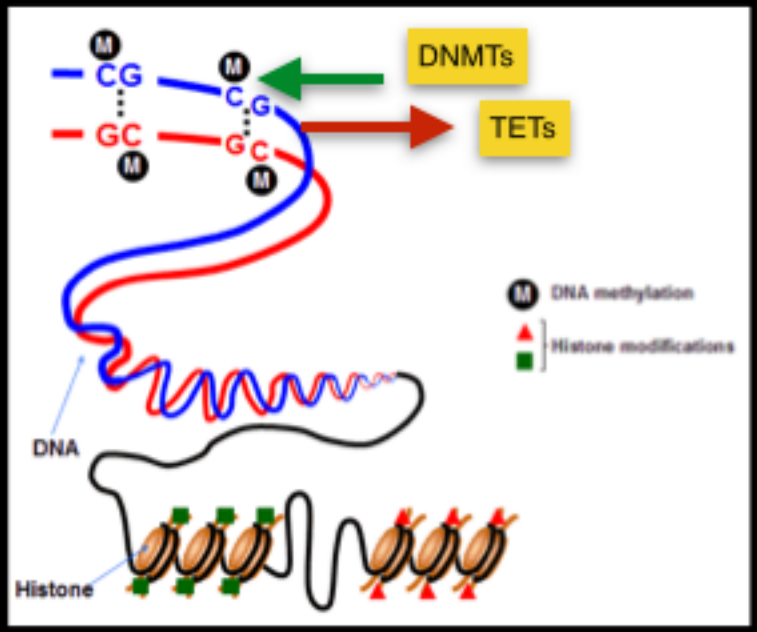
Weill Cornell Medical College

The Epigenome as a Blueprint for the Cancer Cell



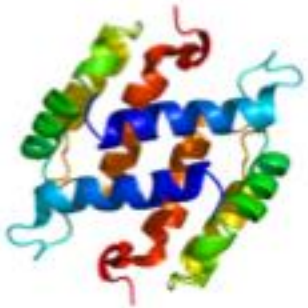
Epigenetic instructions are written in several chemical languages

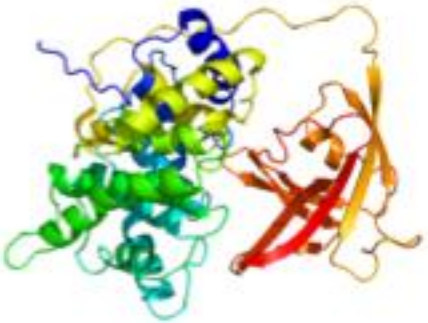
Cytosine mods



Histone mods

The histone code is written, read and erased by specialized proteins



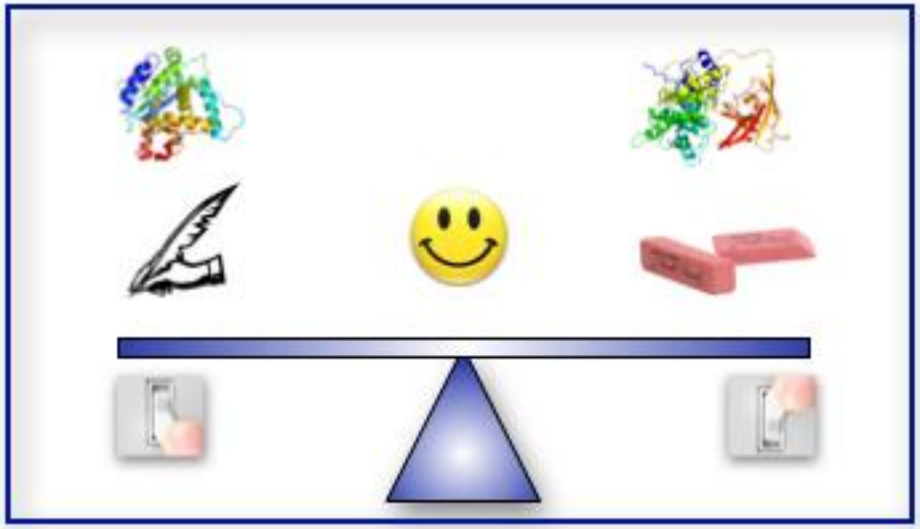


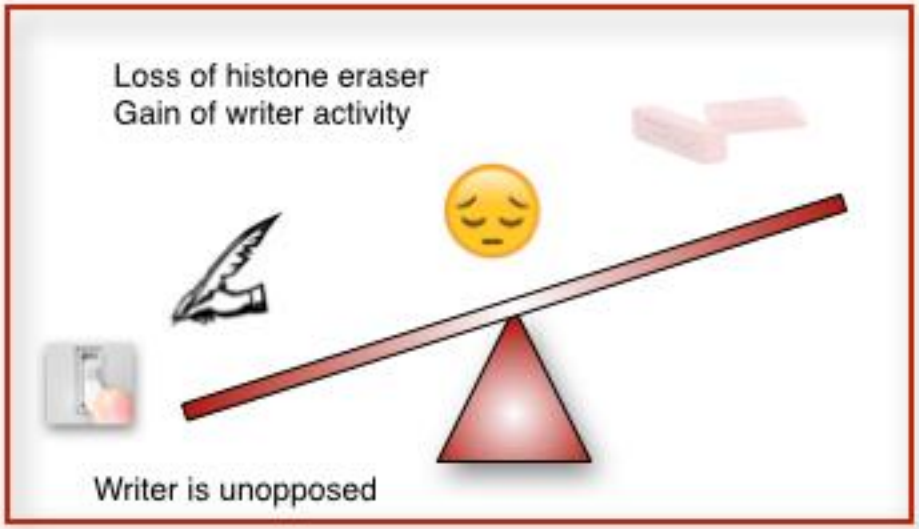
Writers

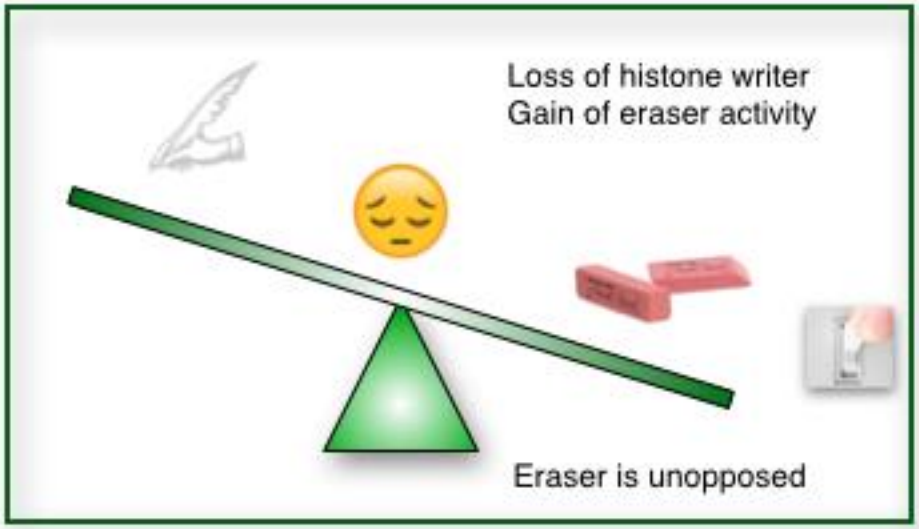
Readers

Erasers

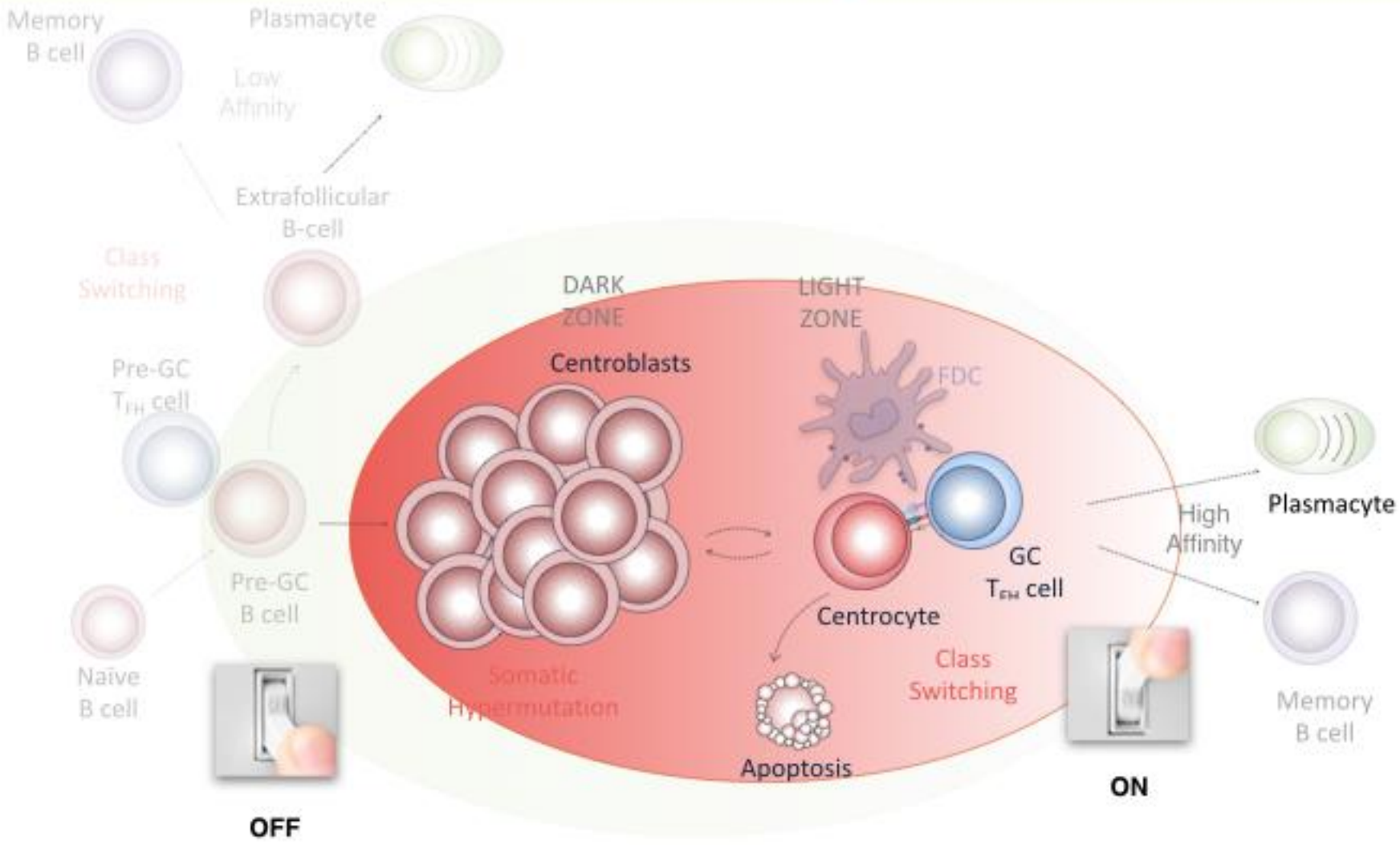
Competition between histone writers and erasers maintains the epigenome in a dynamic and reversible state







How do B-cells transiently deviate from their programmed differentiation path to form germinal centers?



Adapted from Hatzi and Melnick, Trends in Molecular Medicine, 2014

Frequent mutation of histone modifying enzymes in GC derived lymphomas

ARTICLE

doi:10.1038/nature10351

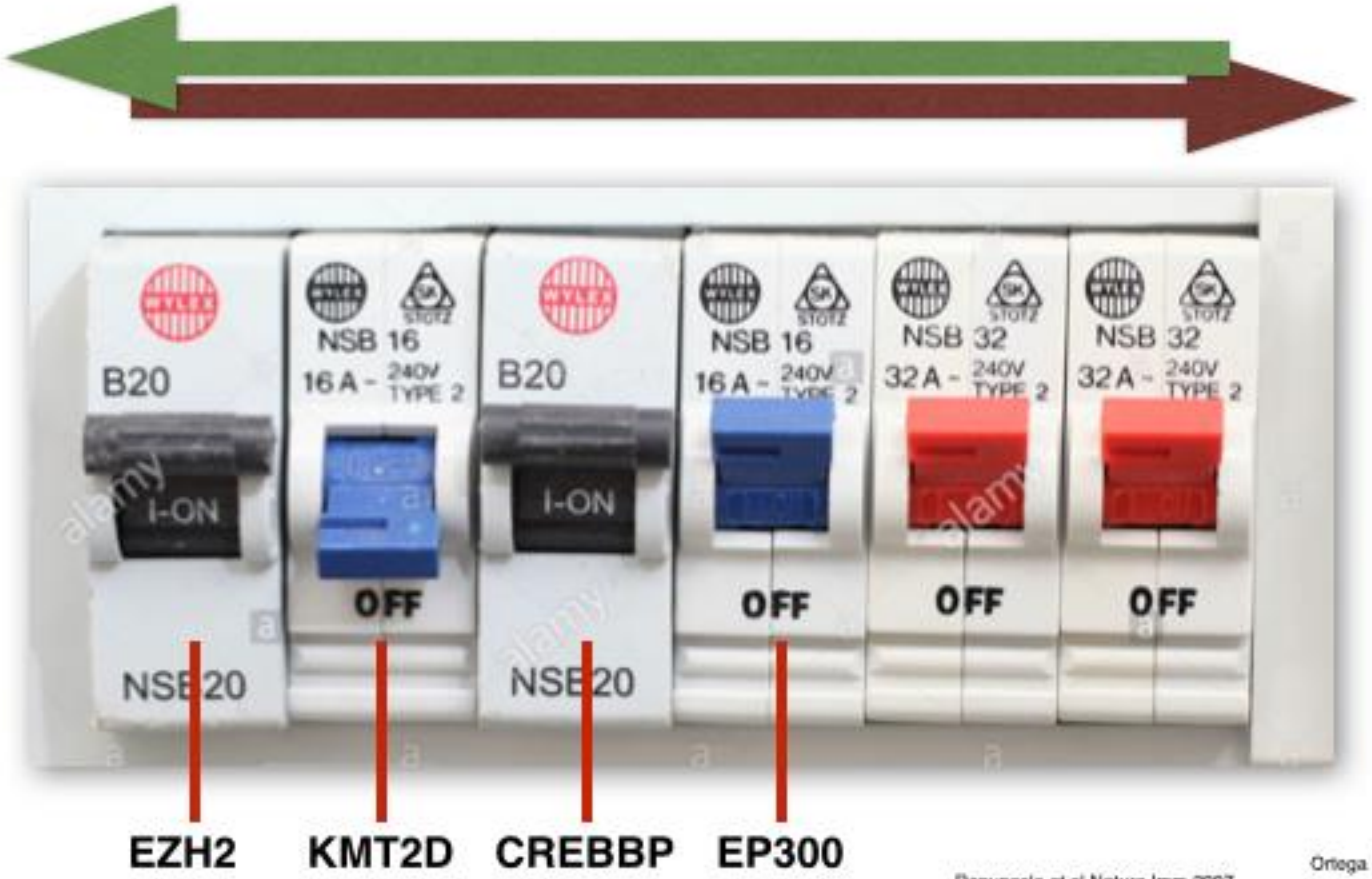
Frequent mutation of histone-modifying genes in non-Hodgkin lymphoma

Ryan D. Morin[1]*, Maria Mendez-Lago[1]*, Andrew J. Mungall[1], Rodrigo Goya[1], Karen L. Mungall[1], Richard D. Corbett[1], Nathalie A. Johnson[2], Tesa M. Severson[1], Readman Chiu[1], Matthew Field[1], Shaun Jackman[1], Martin Krzywinski[1], David W. Scott[2], Diane L. Trinh[1], Jessica Tamura-Wells[1], Sa Li[1], Marlo R. Firme[1], Sanja Rogic[2], Malachi Griffith[1], Susanna Chan[1], Oleksandr Yakovenko[1], Irmtraud M. Meyer[3], Eric Y. Zhao[1], Duane Smailus[1], Michelle Moksa[1], Suganthi Chittaranjan[1], Lisa Rimsza[4], Angela Brooks-Wilson[1,5], John J. Spinelli[6,7], Susana Ben-Neriah[2], Barbara Meissner[2], Bruce Woolcock[2], Merrill Boyle[2], Helen McDonald[1], Angela Tam[1], Yongjun Zhao[1], Allen Delaney[1], Thomas Zeng[1], Kane Tse[1], Yaron Butterfield[1], Inanç Birol[1], Rob Holt[1], Jacqueline Schein[1], Douglas E. Horsman[2], Richard Moore[1], Steven J. M. Jones[1], Joseph M. Connors[2], Martin Hirst[1], Randy D. Gascoyne[2,8] & Marco A. Marra[1,9]

KMT2D	**H3K4me1/2**	**30-80%**
CREBBP	**HAT**	**30-40%**
EP300	**HAT**	**10%**
EZH2	**H3K27me1/2/3**	**~30% in GCB-DLBCL and FL**

GC epigenetic switches linked to lymphomagenesis

B cell

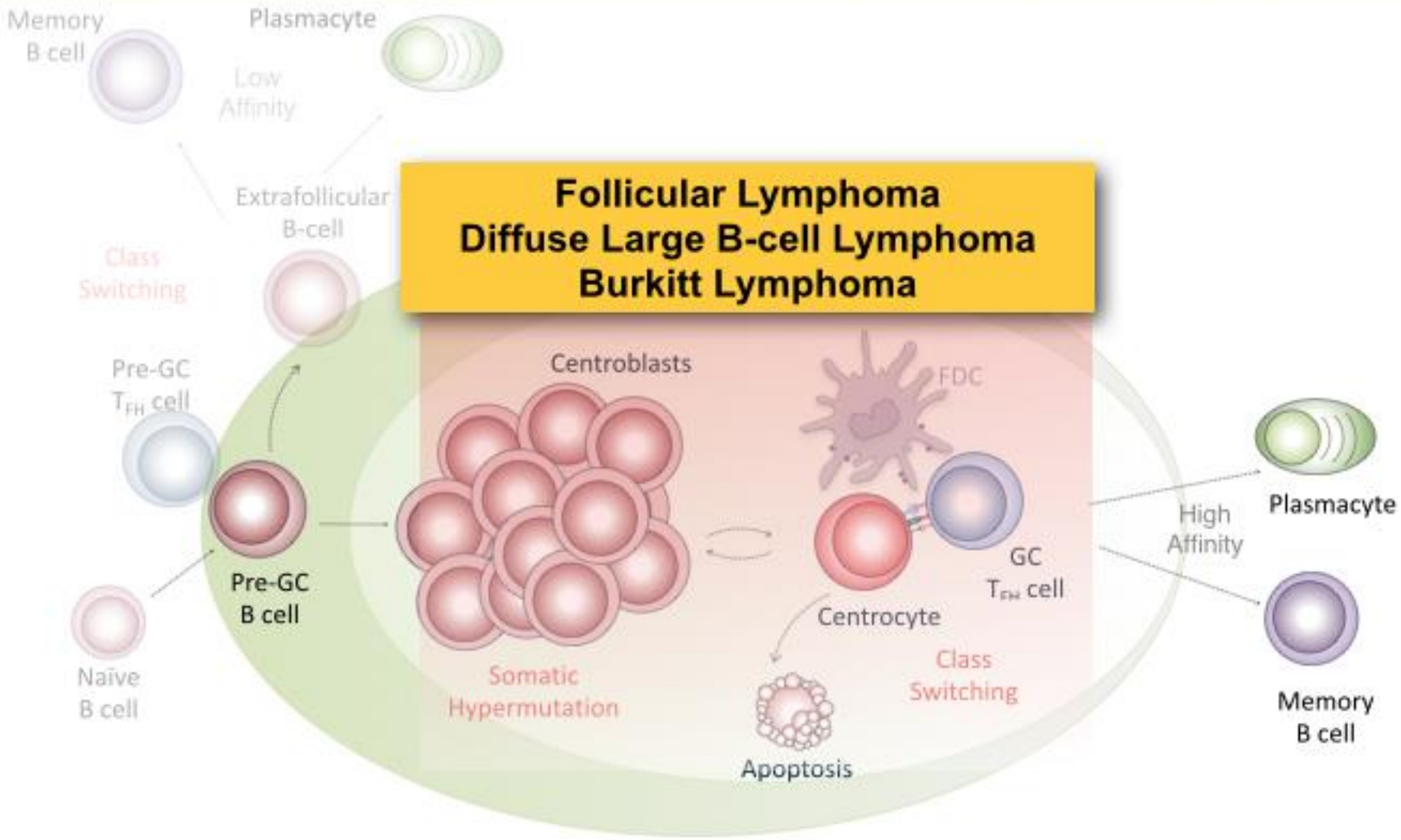


GC
B cell

Ranuncolo et al Nature Imm 2007
Polo et al Blood 2008
Cerchietti et al JCI 2010
Hatzi et al Cell Reports 2013
Huang et al Nature Imm 2013

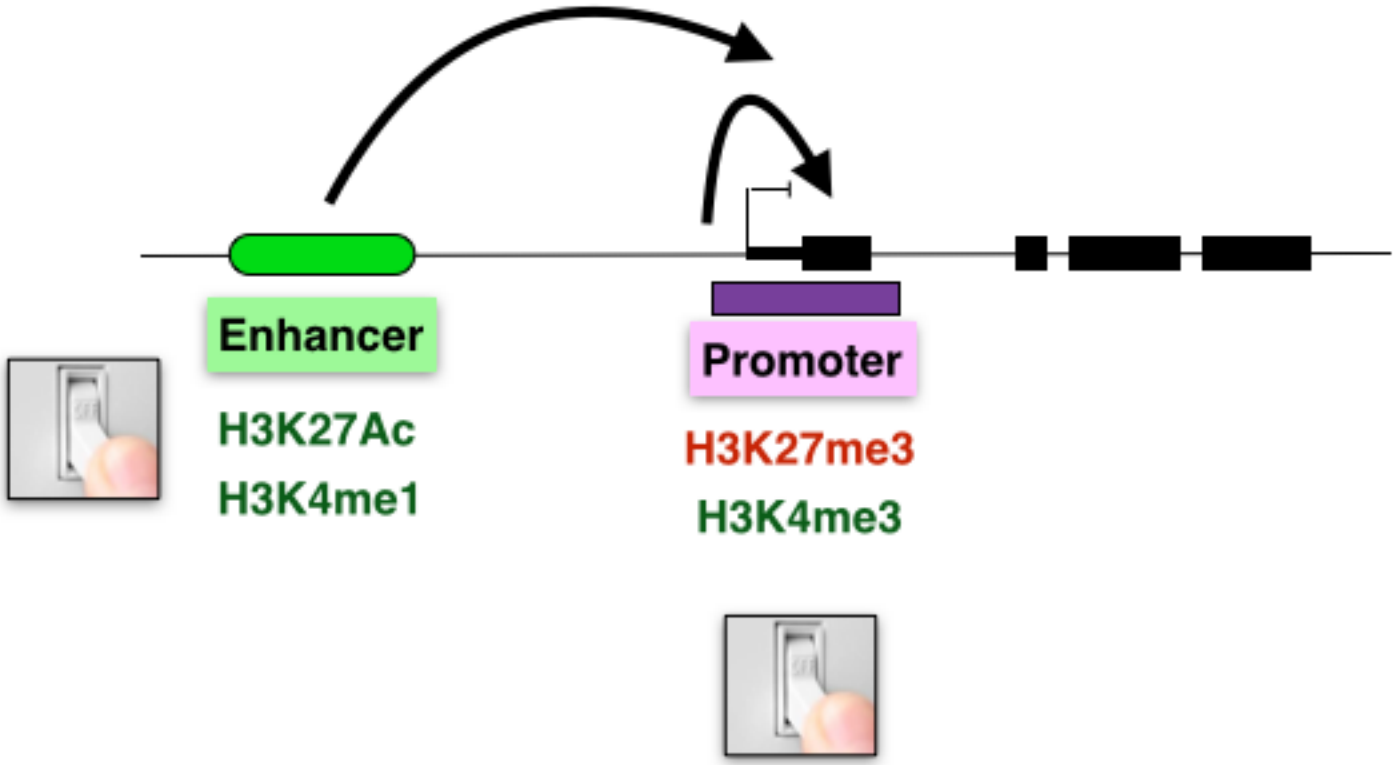
Ortega et al, Nature Medicine, 2015
Ghetu et al Mol Cell 2008
Velichutina et al Blood, 2010
Beguelin et al Cancer Cell 2013
Beguelin et al Cancer Cell 2016
Jiang et al, Cancer Discovery 2016

A majority of B-cell lymphomas arise from GC B-cells



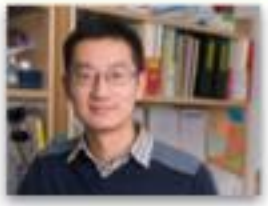
Hatzi and Melnick, Trends in Molecular Medicine, 2014
Shaknovich and Melnick, Curr Opin Hematol, 2011

Histone marks control gene enhancers and promoters



Enhancer switch controlled by BCL6 and CREBBP/EP300

Katerina Hatzi

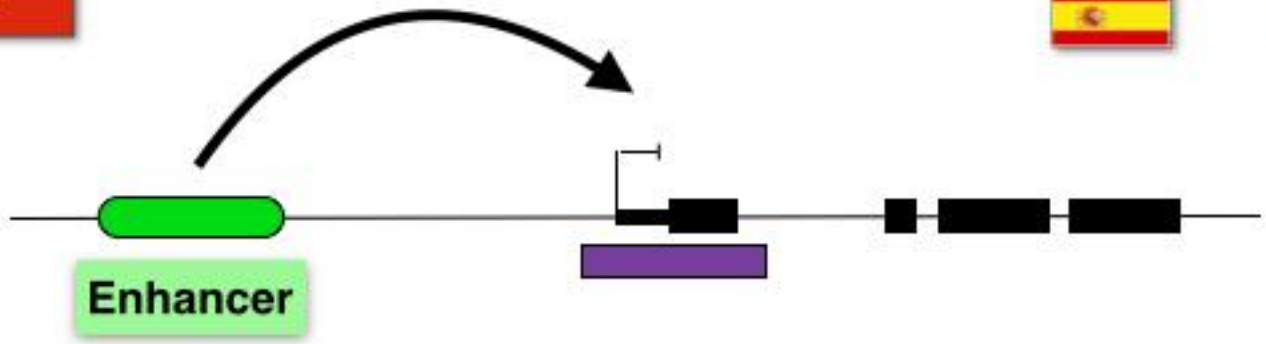


Yanwen Jiang

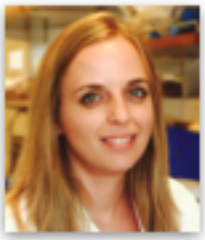
Ranuncolo et al Nat Immunology 2007
Polo et al Blood 2008
Cerchietti et al JCI 2010
Huang et al Nature Immunology 2013
Hatzi et al Cell Reports 2013
Jiang et al Cancer Discovery 2016

Ana Ortega
(Wendel lab)

Hsia-Yuan Ying

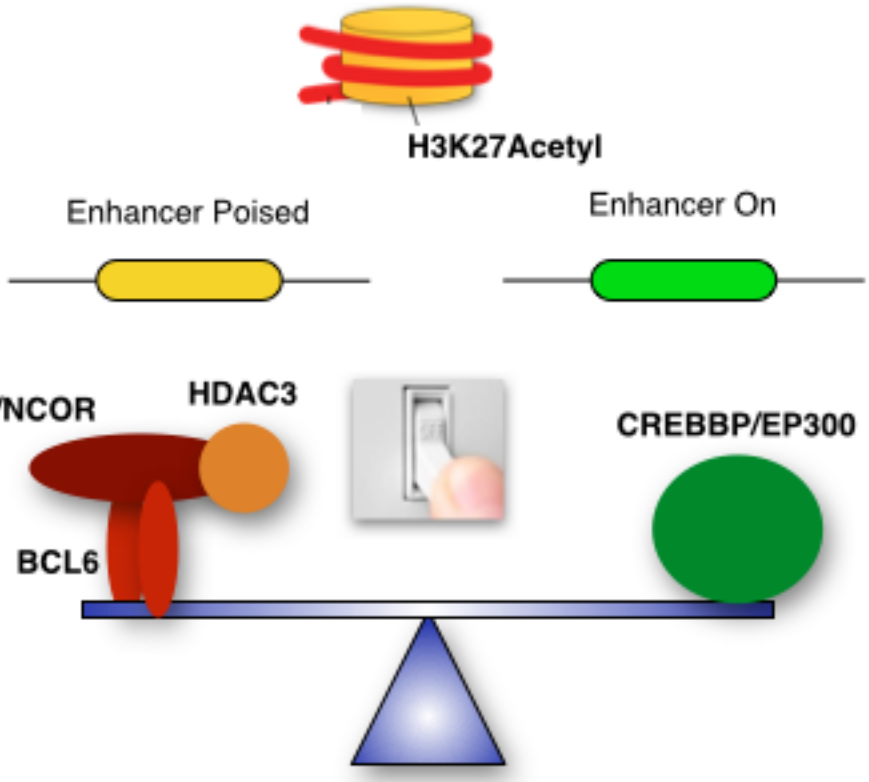


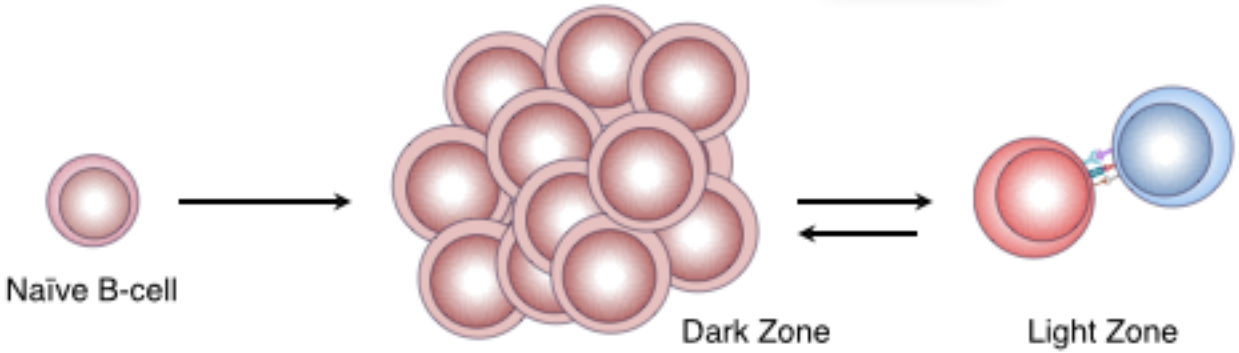
Dynamic "enhancer switching" is required for B-cells to become centroblasts and then exit the GC reaction



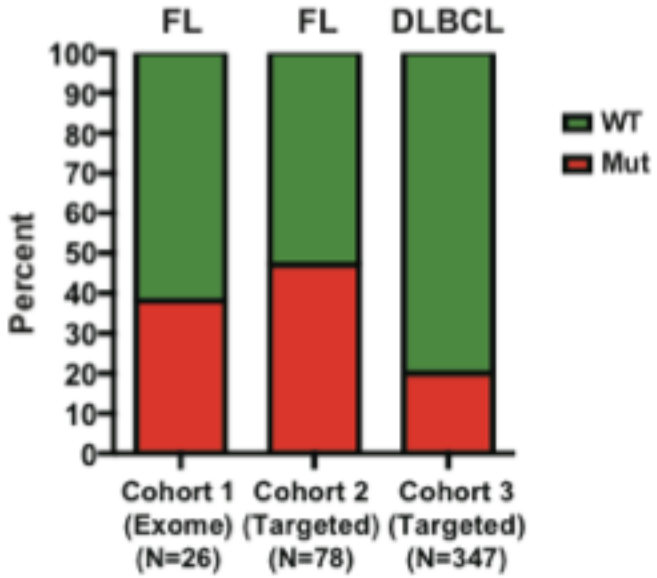
Katerina Hatzi

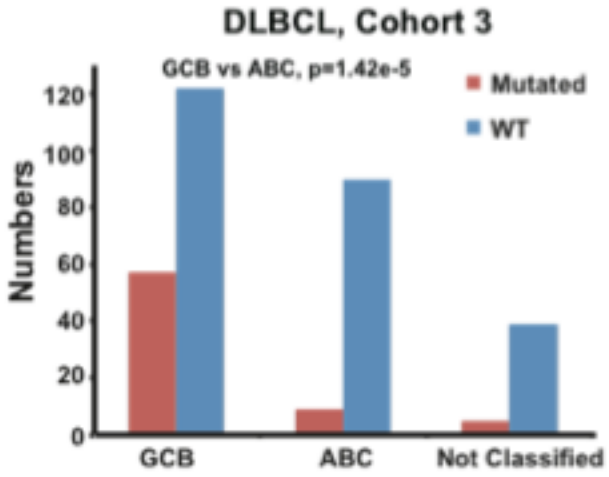
Polo et al Nat Med 2004
Ranuncolo et al Nat Immunol 2007
Polo et al Blood 2008
Cerchietti et al J Clin Investigation 2010
Hatzi et al Cell Reports 2013

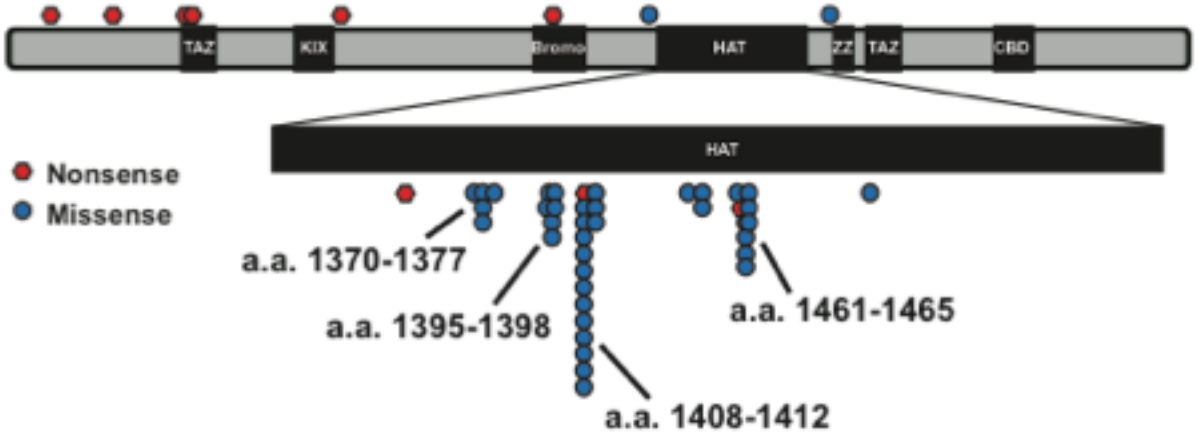




Frequent somatic mutation of CREBBP/EP300 in DLBCL and FL

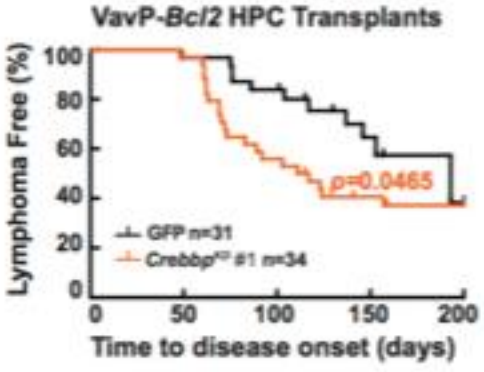


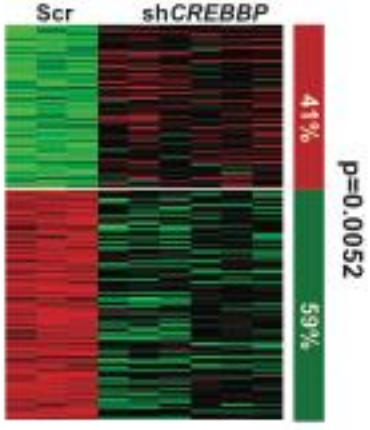


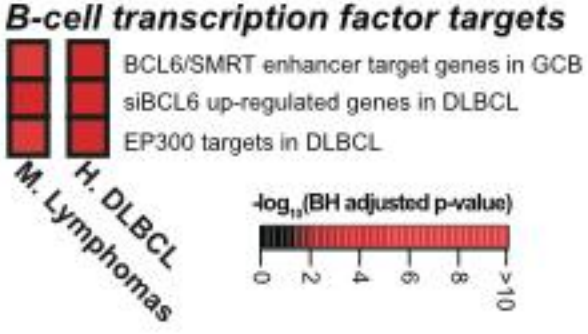


Cerchietti et al J Clin Investigation 2010, Pasqualucci et al Nature 2011, Jiang et al Cancer Discovery 2016

CREBBP mouse models and human patients point to disruption of BCL6 regulated enhancers as a driver mechanism

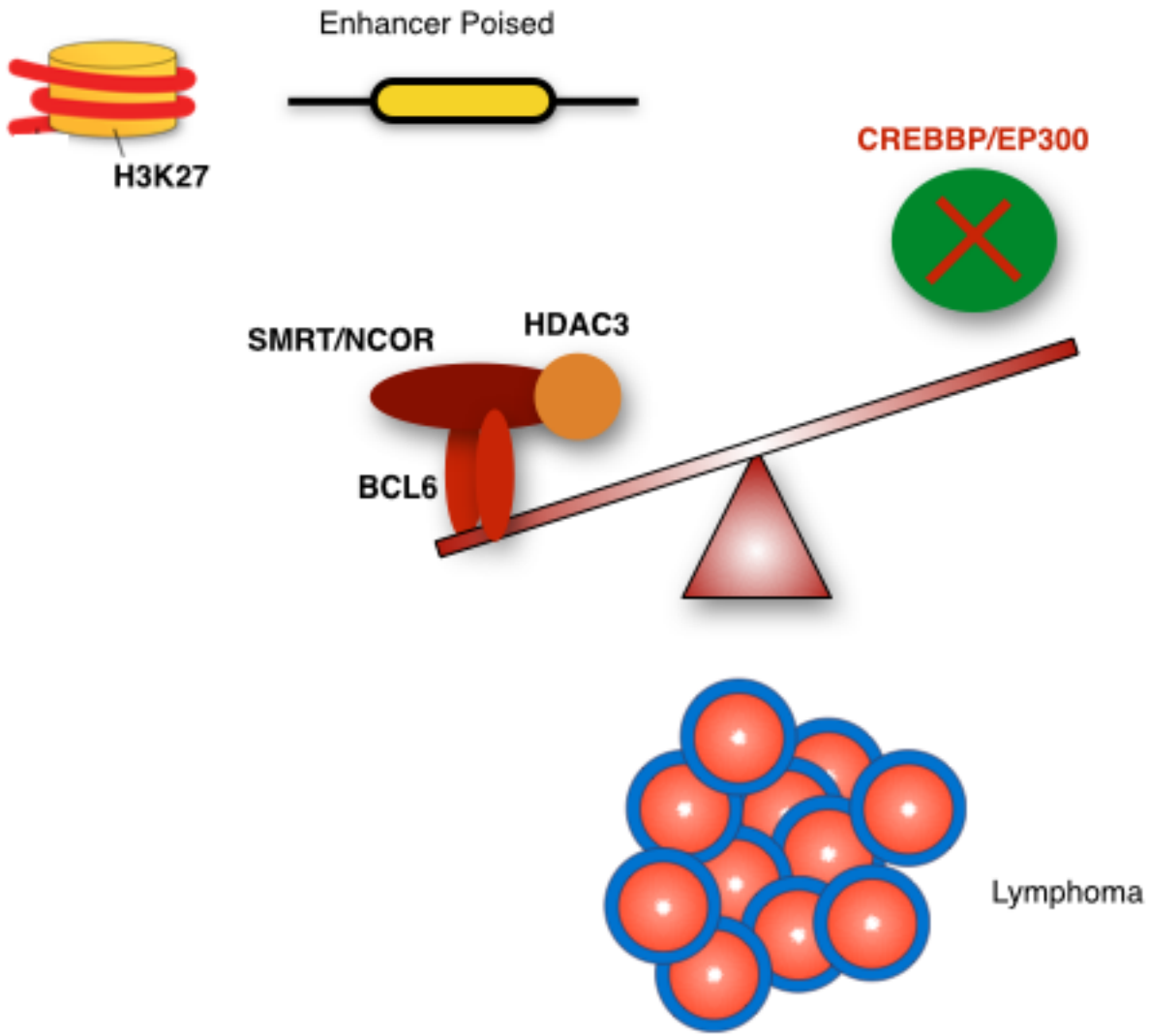






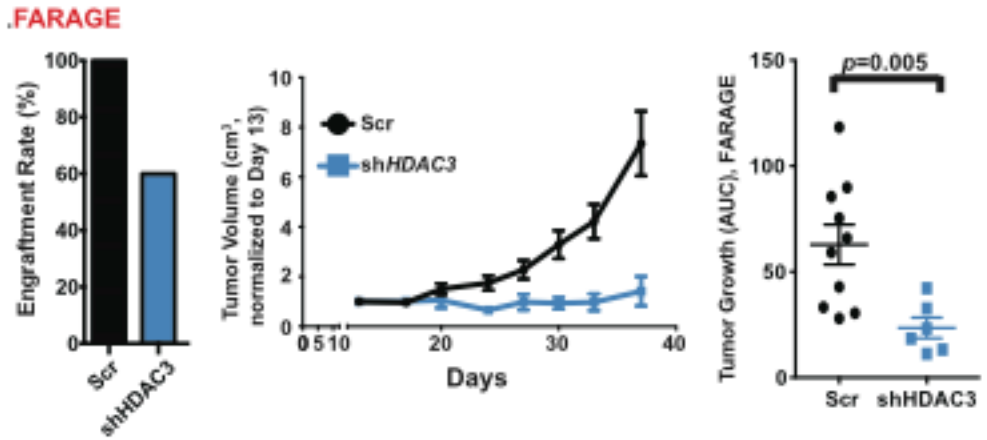
Jiang et al Cancer Discovery 2016

Mutation of CREBBP and EP300 disrupt enhancer switches resulting in **sustained repression** of BCL6 target enhancers

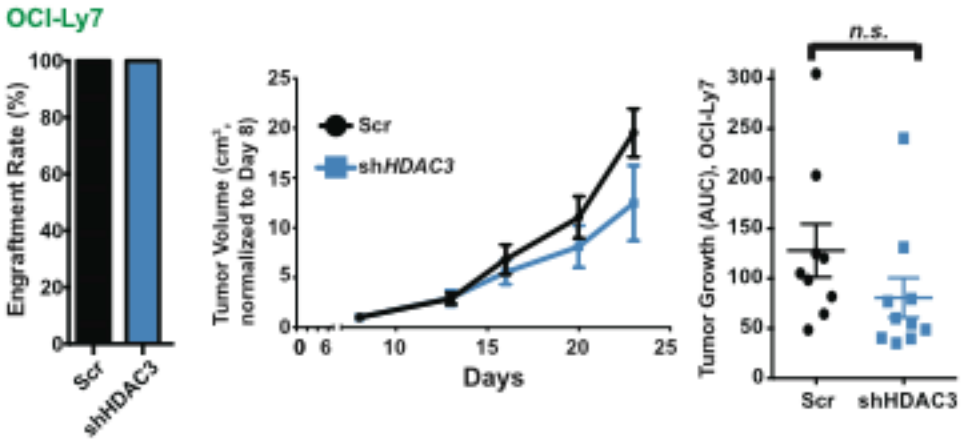


Jiang et al Cancer Discovery 2016

Somatic mutations of CREBBP and EP300 in FL patients



CREBBP MUTANT

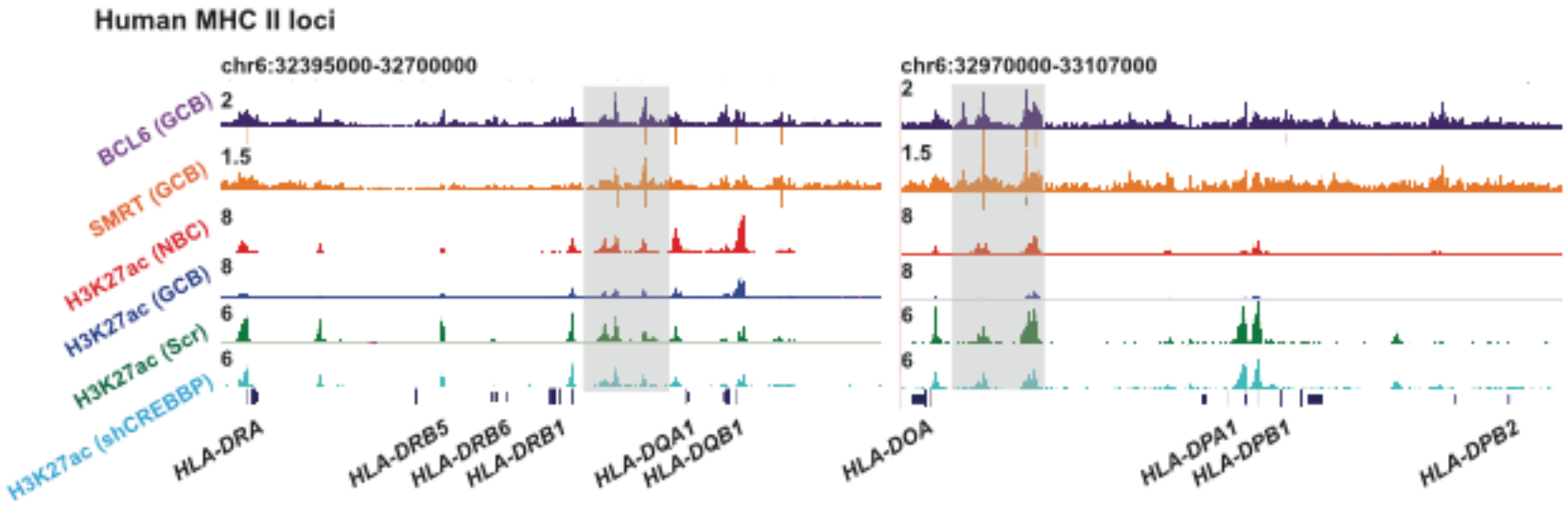


CREBBP WT

Jiang et al Cancer Discovery 2016

BCL6-SMRT and CREBBP counter-regulation of MHC class II loci

Jiang et al Cancer Discovery 2016



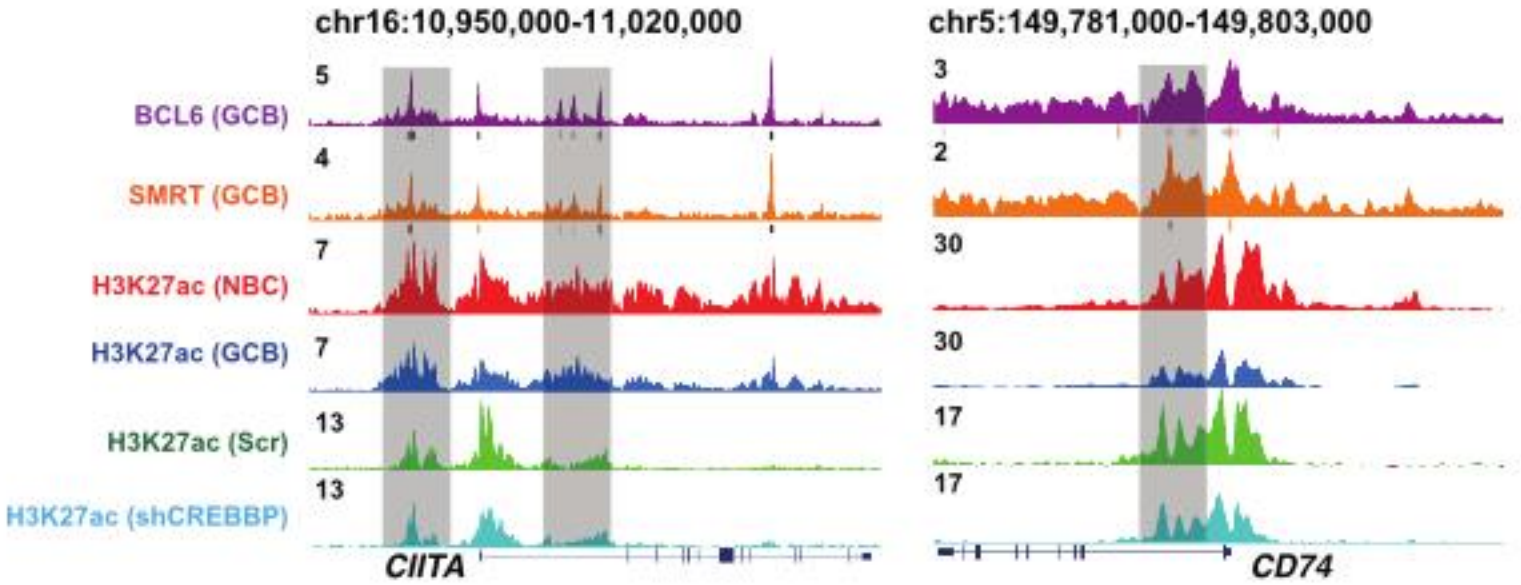
Loss of MHC class II expression associated with inferior outcome in DLBCL
(Rimsza et al, Blood 2004)

CREBBP mutant DLBCL cases feature reduced MHC class II and reduced T-cell interactions
(Green et al PNAS 2015)

HDAC inhibitors can induce MHC class II expression in DLBCL cells
(Cycon et al, Immunology 2013)

CIITA and CD74 are regulated through similar mechanism

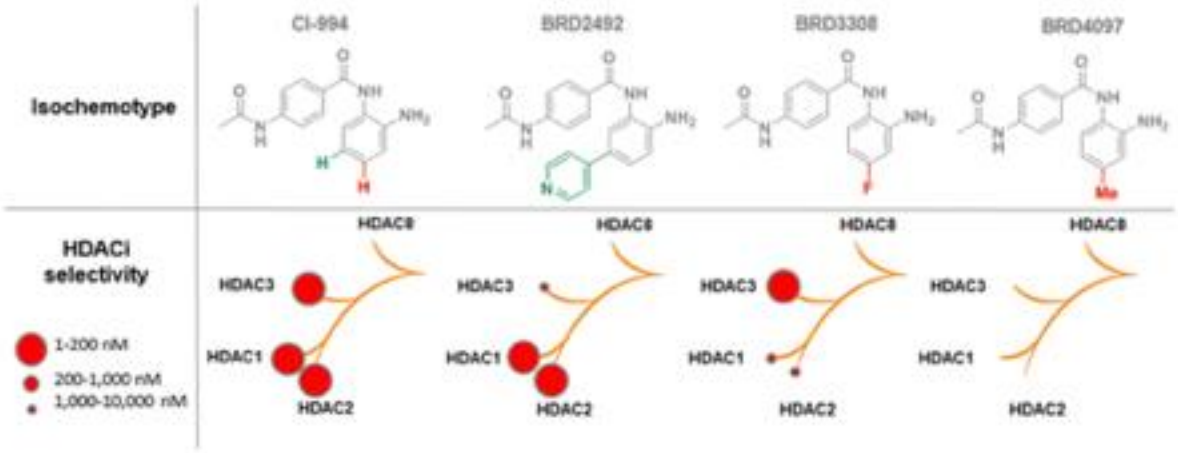
Jiang et al Cancer Discovery 2016



CIITA and CD74 are mutated in DLBCL
(TCGA data)

CIITA mutated in PMBL
(Mottok et al Cell Reports 2015)

Development of HDAC3 specific inhibitors

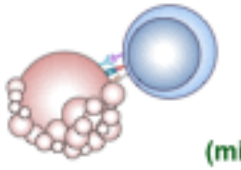


Wagner et al ACS Chem Biol 2016

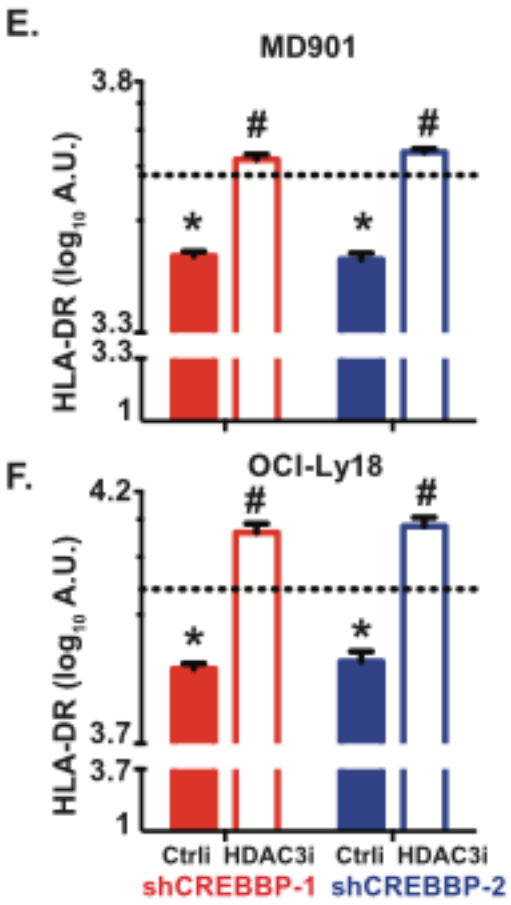
Ed Holson, KDAC pharmaceuticals

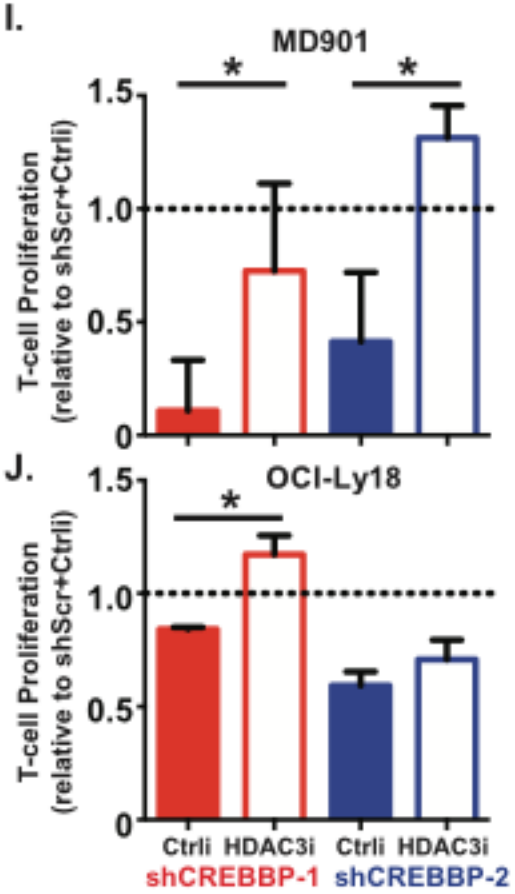
HDAC3 inhibitor restores CREBBP shRNA induced MHC class II H3K27 acetylation and expression

MCH II expression
(flow cytometry)



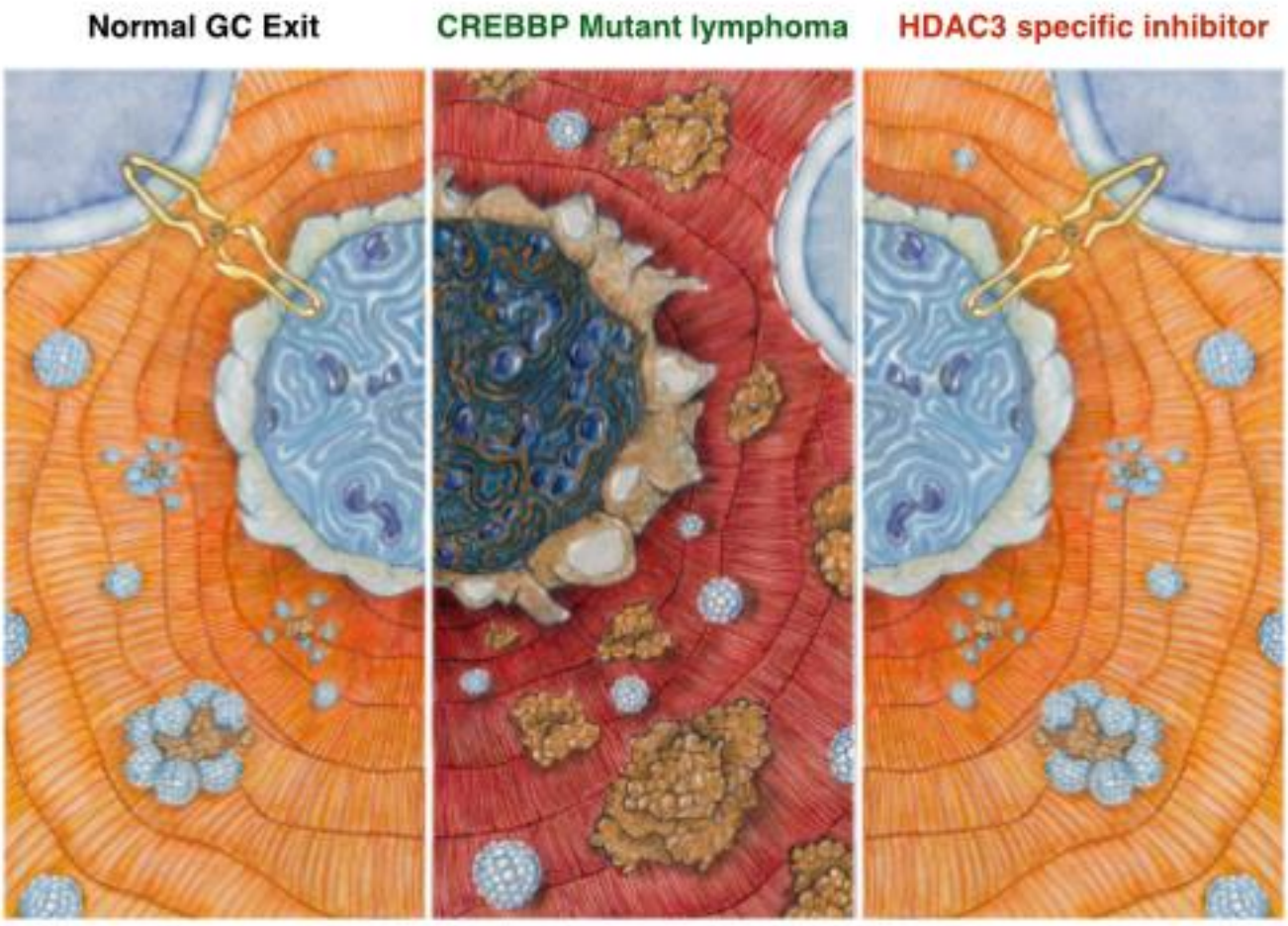
T-cell activation
(mixed lymphocyte reaction)





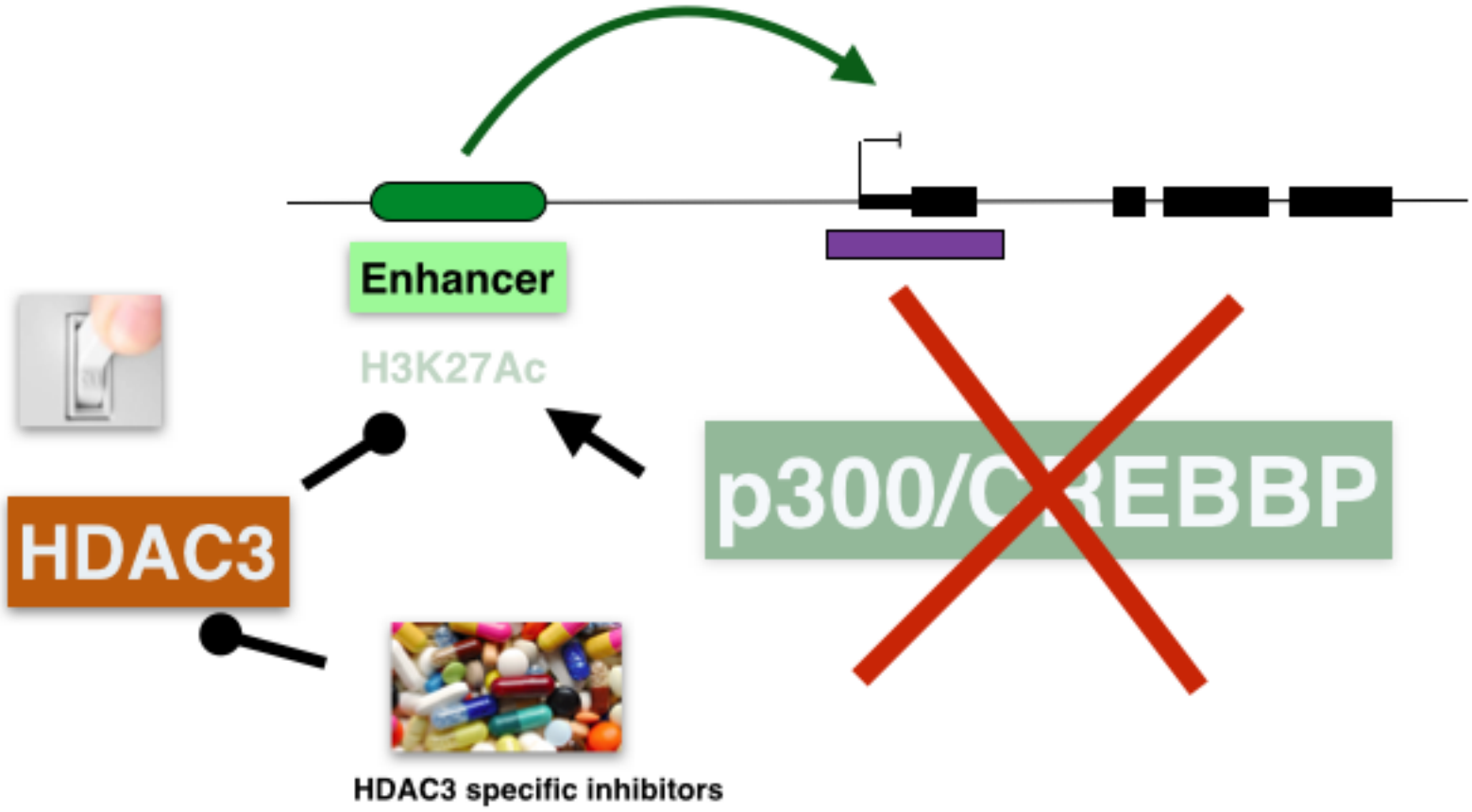
Jiang et al Cancer Discovery 2016

Restoring immuno surveillance in DLBCL using HDAC3 specific epigenetic targeted therapy



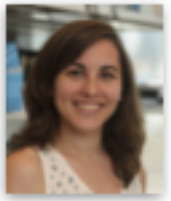
Jiang et al Cancer Discovery 2016

An enhancer switch controlled by BCL6 and HDAC3 vs CREBBP/EP300 is disrupted by CREBPP-EP300 somatic mutations



Jiang et al Cancer Discovery 2016

Targeting KMT2D with histone demethylase or HDAC inhibitors might restore epigenetic programming



Ana Ortega
(Wendel lab)

Isaac Boss

CD40, BCR

Enhancer

H3Kme1/2

KMT2D Mutation

Histone demethylases

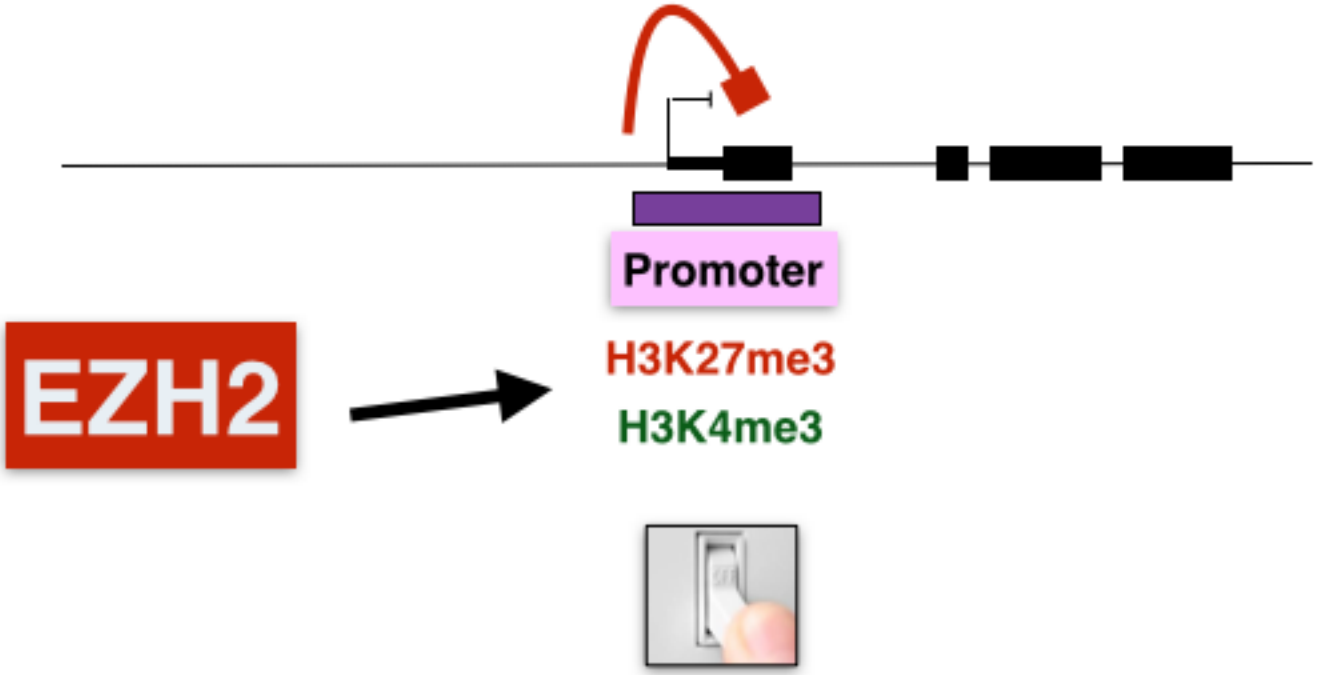
Targeted therapy?

Ortega, Boss et al Nature Medicine 2015

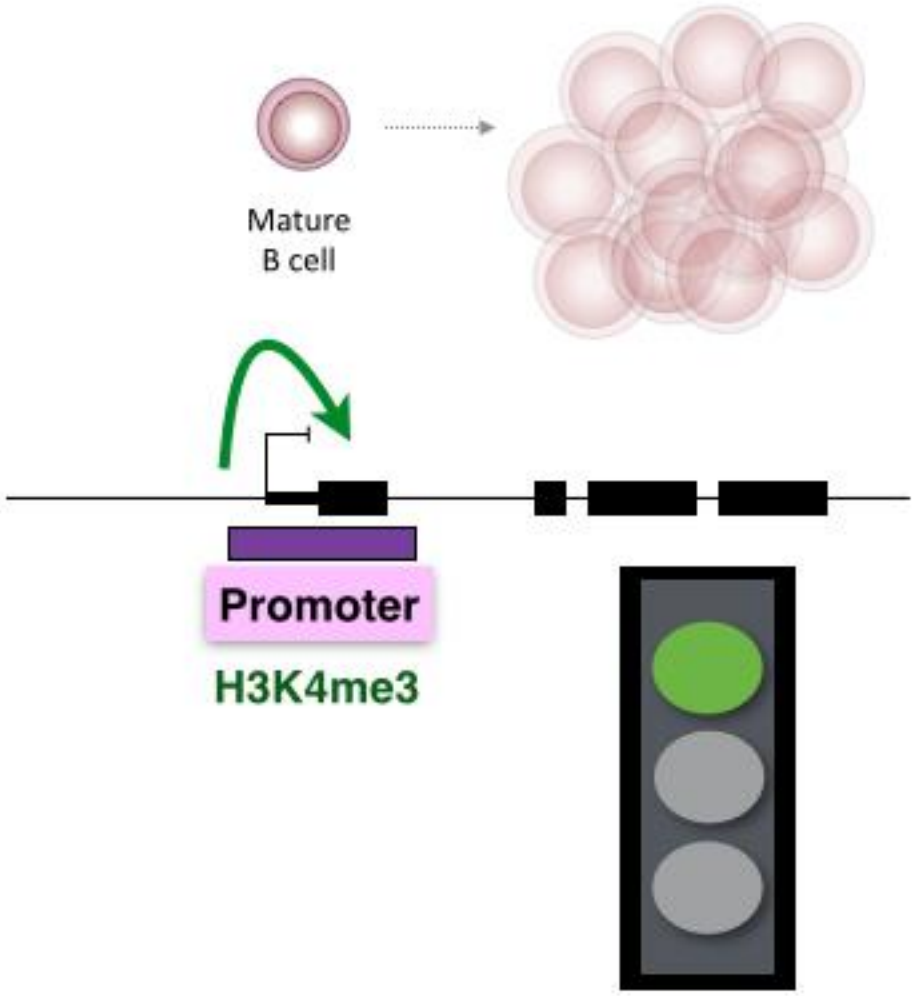
Zhang et al Nature Medicine 2015

EZH2 and BIVALENT CHROMATIN FORMATION

A switch that transiently suppresses active B-cell promoters



B-cell differentiation and checkpoint genes are active in mature B-cells

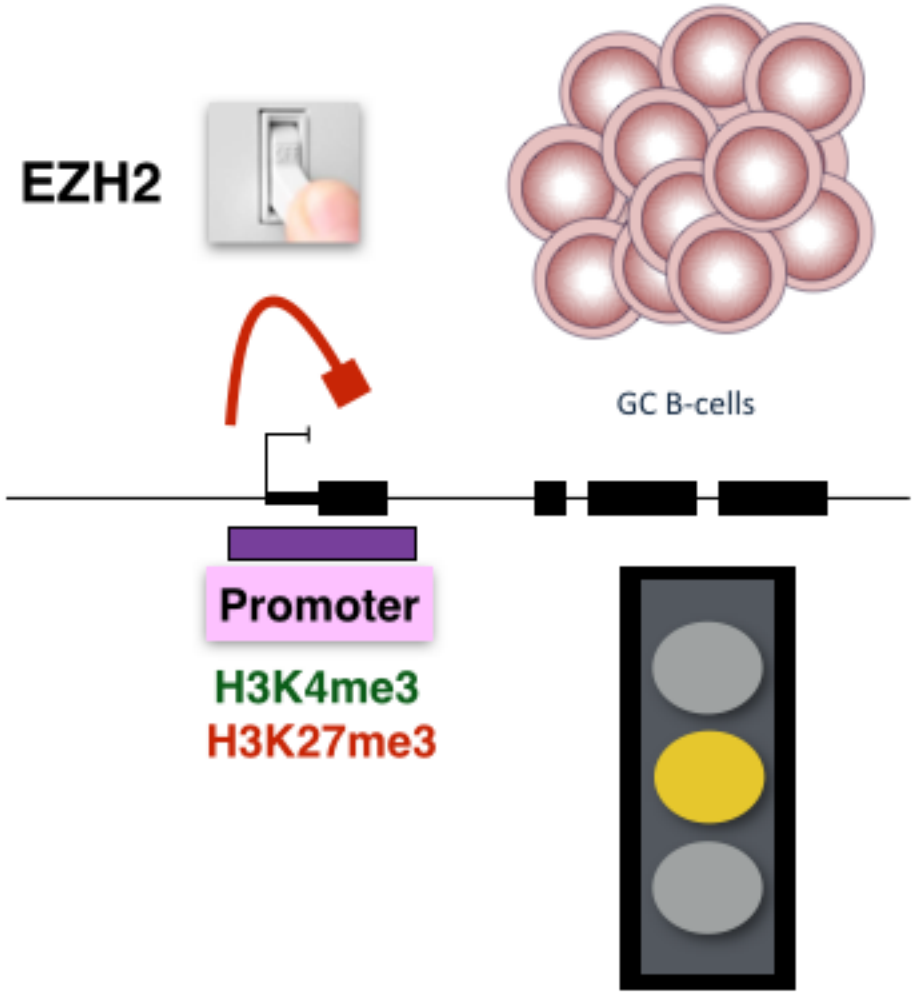


Checkpoints
NFkB
IL10
CD40 induced
IRF4 induced
Apoptosis genes
ABC-DLBCL

Beguelin et al Cancer Cell 2013
Beguelin et al Cancer Cell 2016

ChIP-seq, RNA-seq, animal models and human GC B-cells and DLBCL cells

EZH2 transiently pauses these genes in GC B-cells by generated bivalent chromatin at their promoters

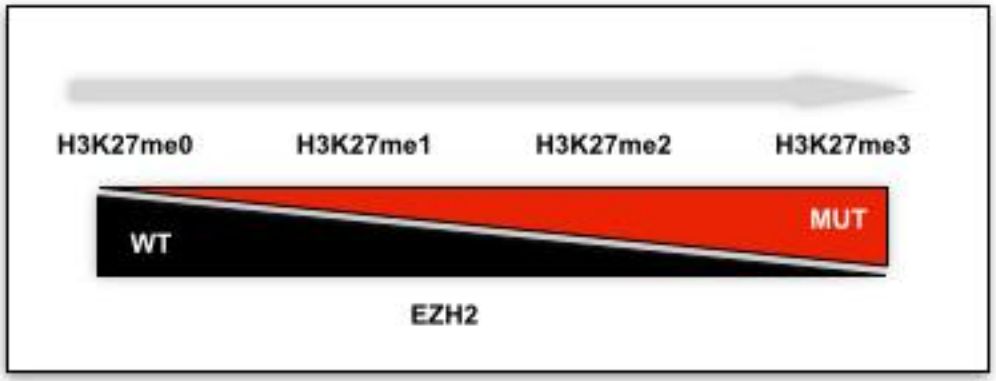


Proliferation
NFkB
IL10
CD40 induced
IRF4 induced
Apoptosis genes
ABC-DLBCL

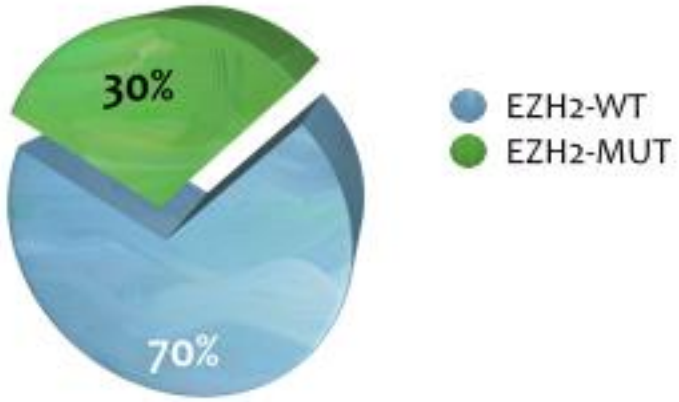
Beguelin et al Cancer Cell 2013
Beguelin et al Cancer Cell 2016

Frequent mutation of EZH2 TYR 641 in DLBCL and FL

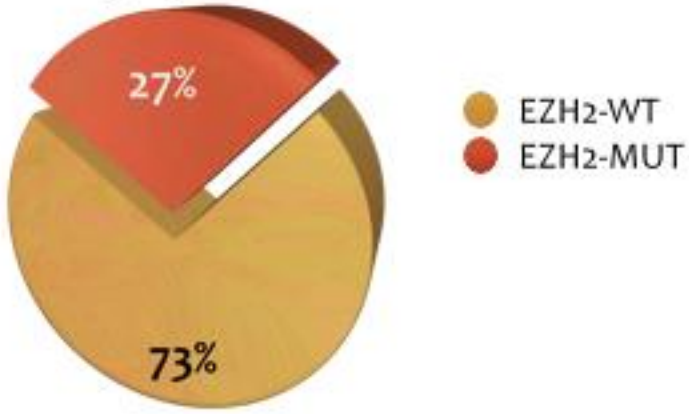
Morin et al, Nat Gen 2010, Bodor et al Blood 2013, Sneeringer, et al, PNAS 2010



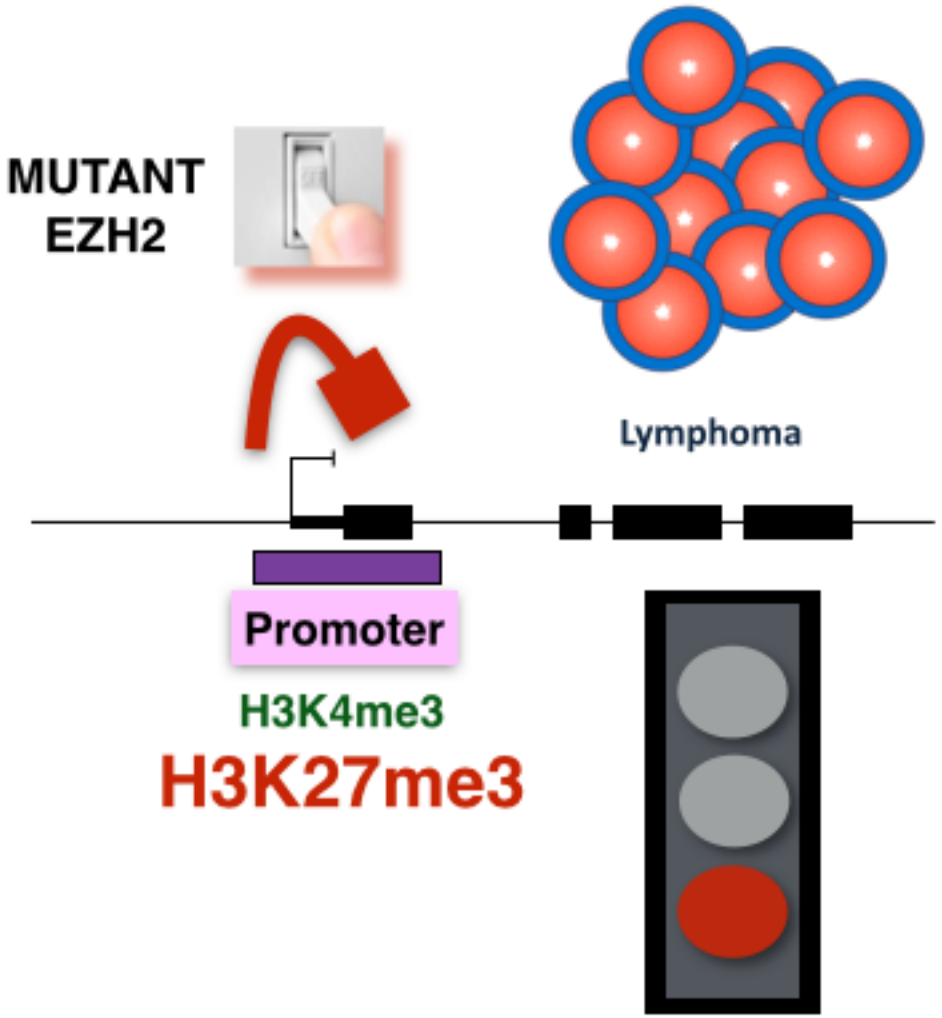
GCB - DLBCL



Follicular Lymphoma



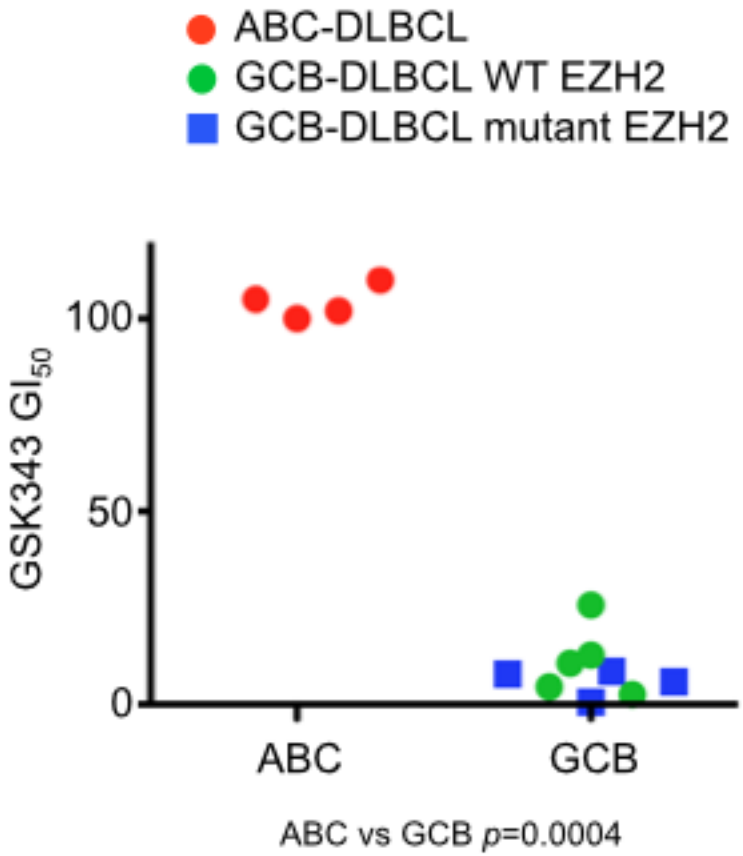
Mutant EZH2 locks genes so that they become irreversibly silenced



Proliferation
NFkB
IL10
CD40 induced
IRF4 induced
Apoptosis genes
ABC-DLBCL

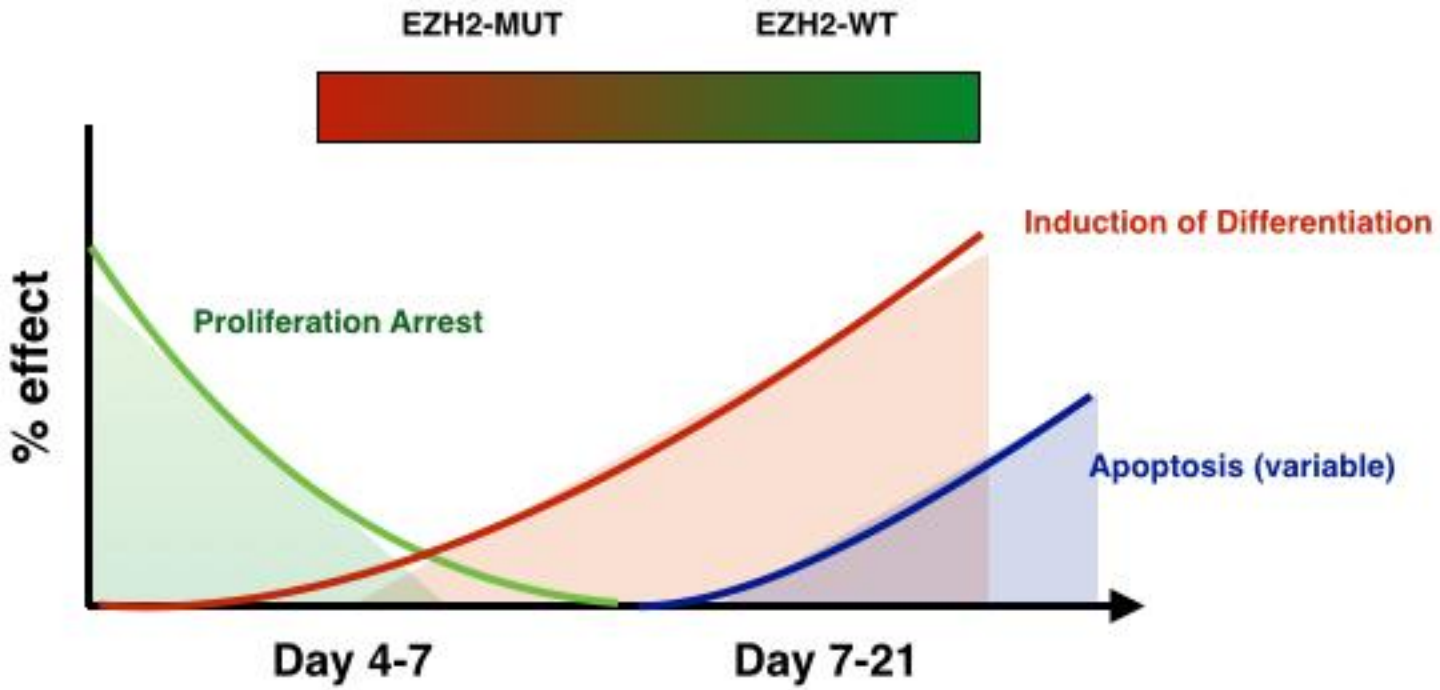
Beguelin et al Cancer Cell 2013
Beguelin et al Cancer Cell 2016

GCB-DLBCL respond regardless of mutation



Beguelin et al Cancer Cell 2013

Tri-Phasic effect of EZH2 inhibitors against WT and MUT DLBCL cells



Who to treat with EZH2 inhibitors?

GCB DLBCL + mutant EZH2

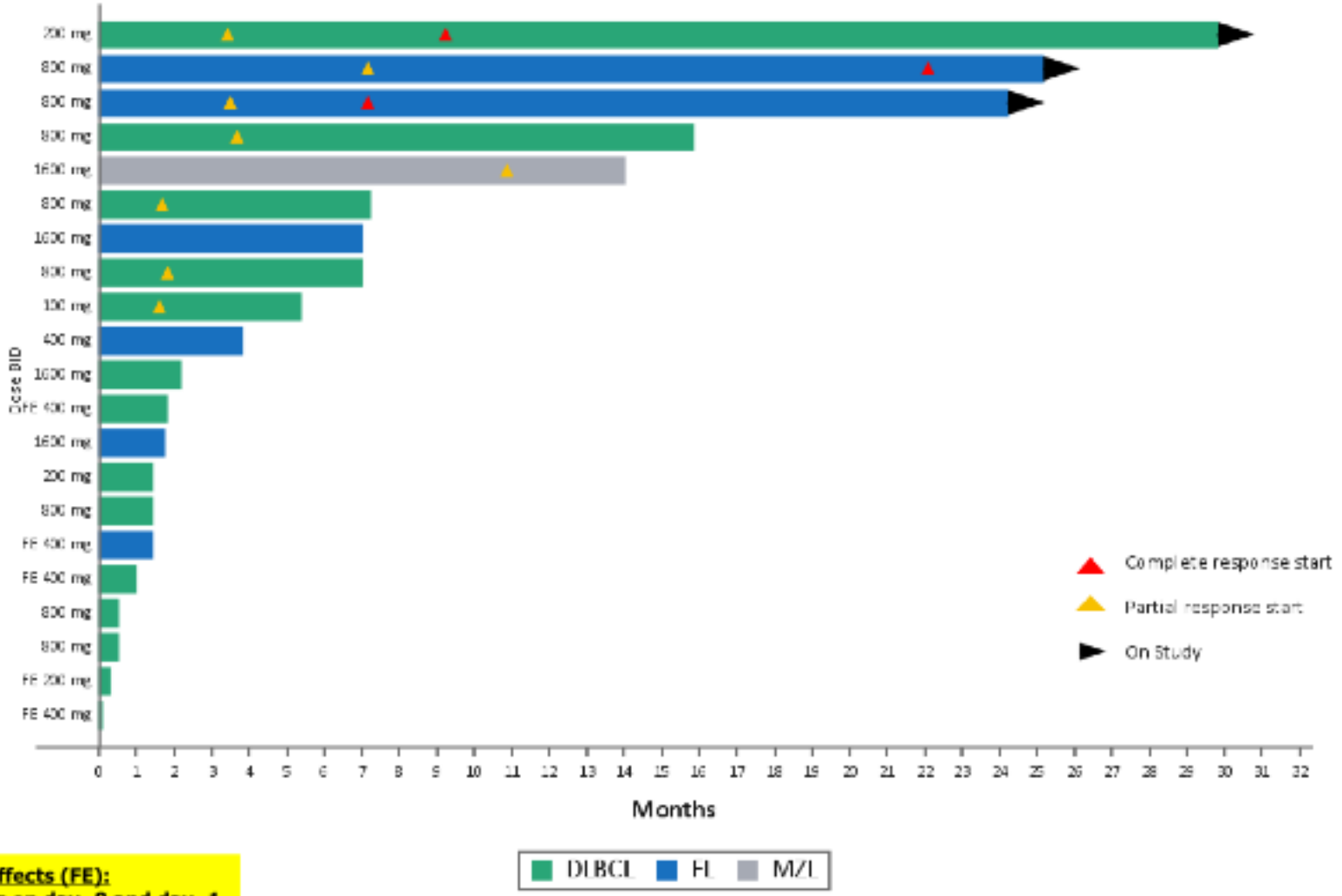
GCB DLBCL + WT EZH2

Follicular lymphoma + mutant EZH2

Follicular lymphoma + WT EZH2

Biomarkers: Bivalent gene signature?
H3K27me3?
EZH2?

Objective Response in NHL
All Patients (n=21)



Food Effects (FE):
200 mg on day -8 and day -1
400 mg BID from day 1

per Cheson/IWG Criteria, 2007

Slide courtesy of Scott Ridich

EZH2 targeted therapy as a **building block** for FL/GCB-DLBCL therapy

Synergy in vitro and enhanced effects in vivo shown for:

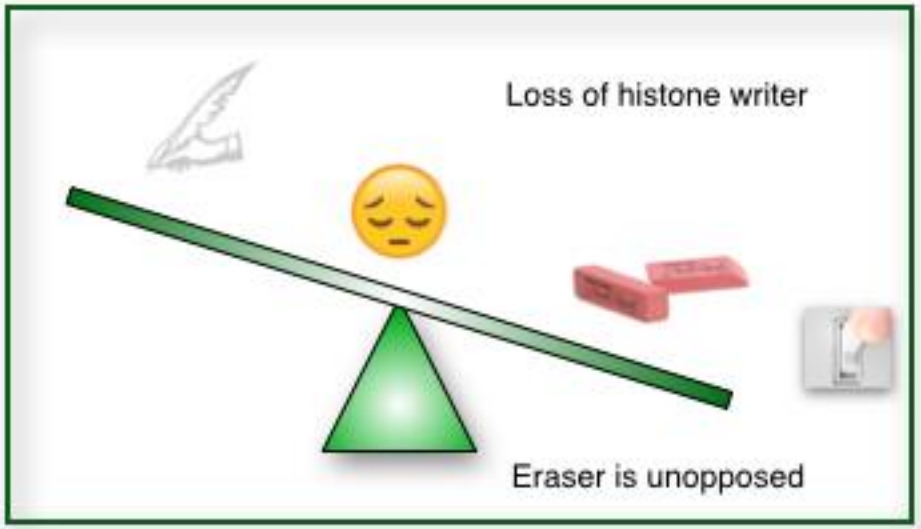
EZH2i **+**

- **Corticosteroid**
- **Chemotherapy**
- **CHOP**

Knutson et al PLoS One 2014

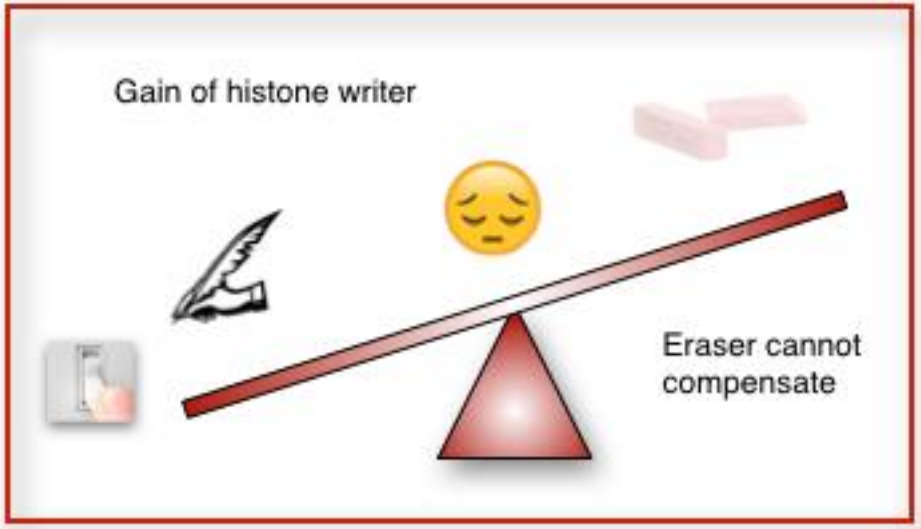
- **BCL2 antagonist** — *Beguelin et al Cancer Cell 2013*
- **BCL6 antagonist** — *Beguelin et al Cancer Cell 2016*

Summary: Epigenetic Circuits in Lymphomagenesis



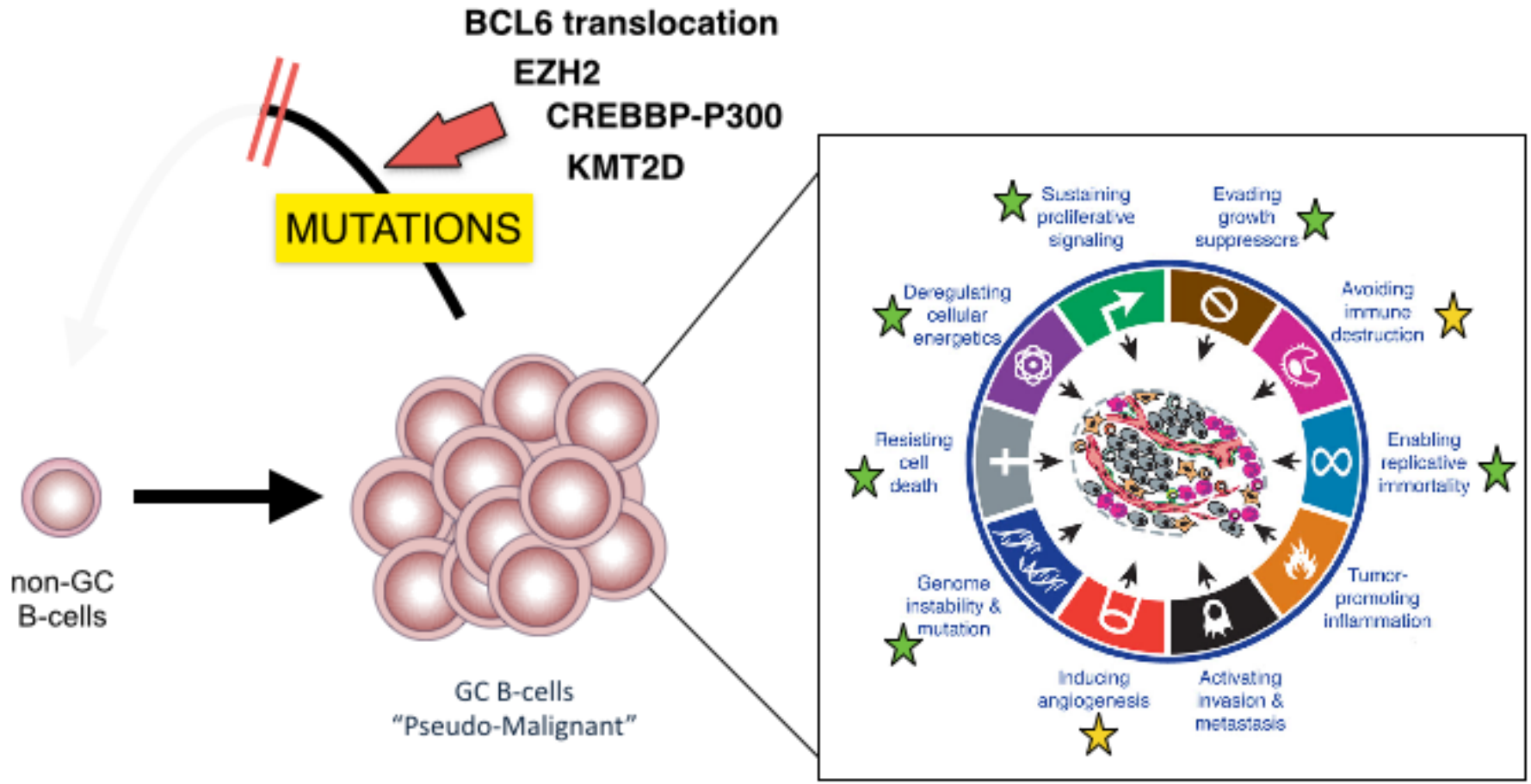
CREBBP
Results in aberrant repression
Linked mostly to enhancers
Results in HDAC3 dependent lymphomas

KMT2D (MLL2)
Linked mostly to enhancers
Results in aberrant repression
Putative histone demethylase dependence



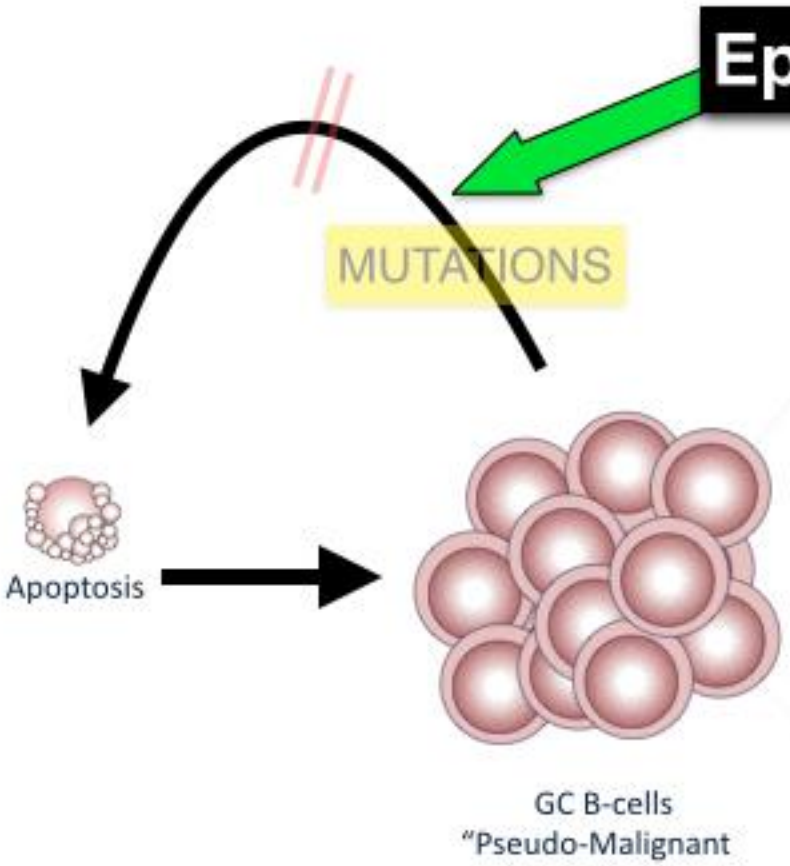
EZH2
Results in aberrant repression
Linked mostly to promoters
Targeted by EZH2 inhibitors
Regardless of mutation
Effective in humans

Epigenetic mutations in DLBCL and FL **MAINTAIN** B-cells in the GC phenotype



Adapted from Hanahan and Weinberg, Cell 2011

Epigenetic therapy can overcome the effect of founder mutations leading to potent anti lymphoma effects



BCL6 inhibitors

EZH2 inhibitors

Specific HDAC isoform inhibitors?

Other histone modifiers…

Adapted from Hanahan and Weinberg, Cell 2011

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