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Cancer types as models in cancer research: T-cell Acute Lymphoblastic Leukemia (T-ALL)

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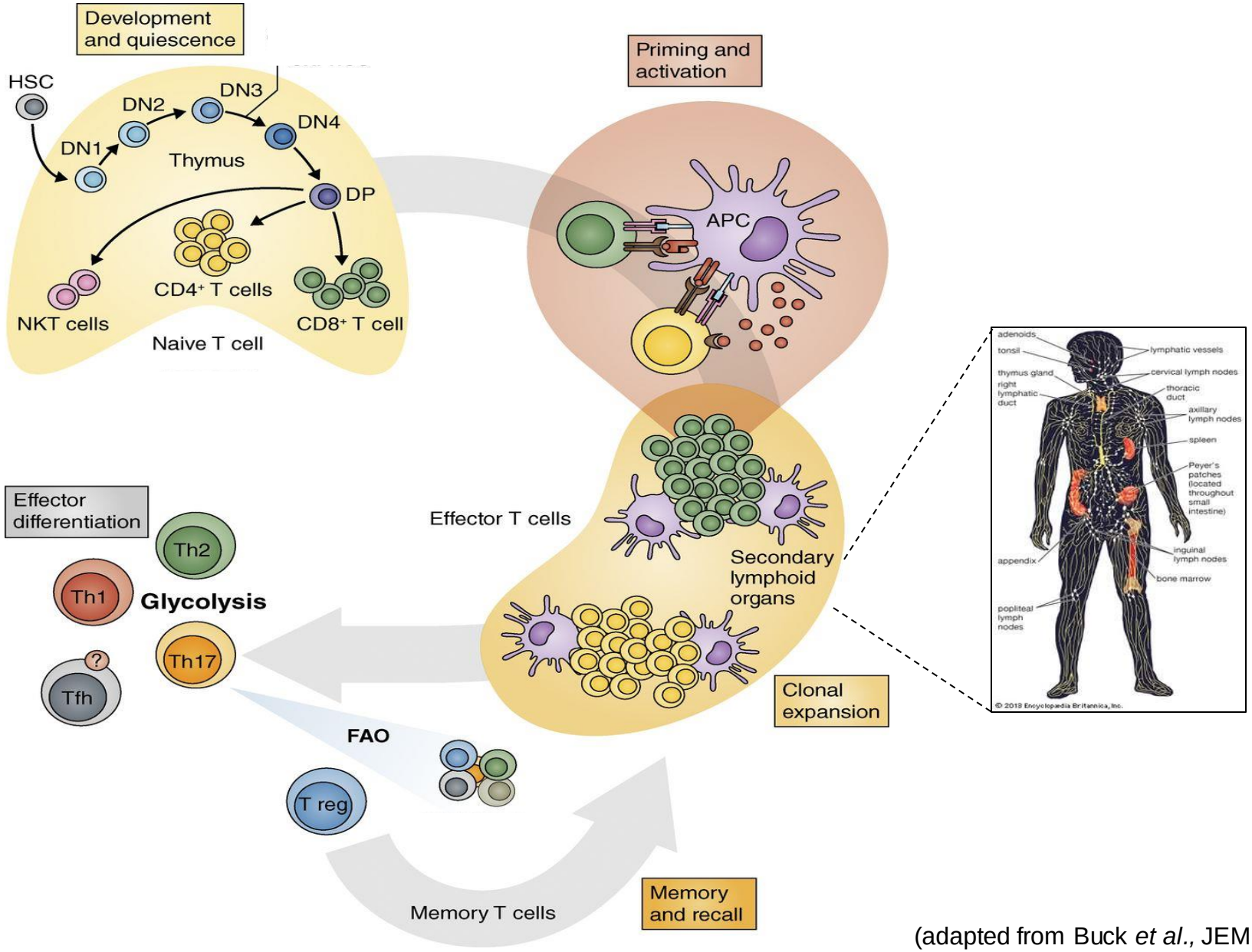
What is T-ALL?

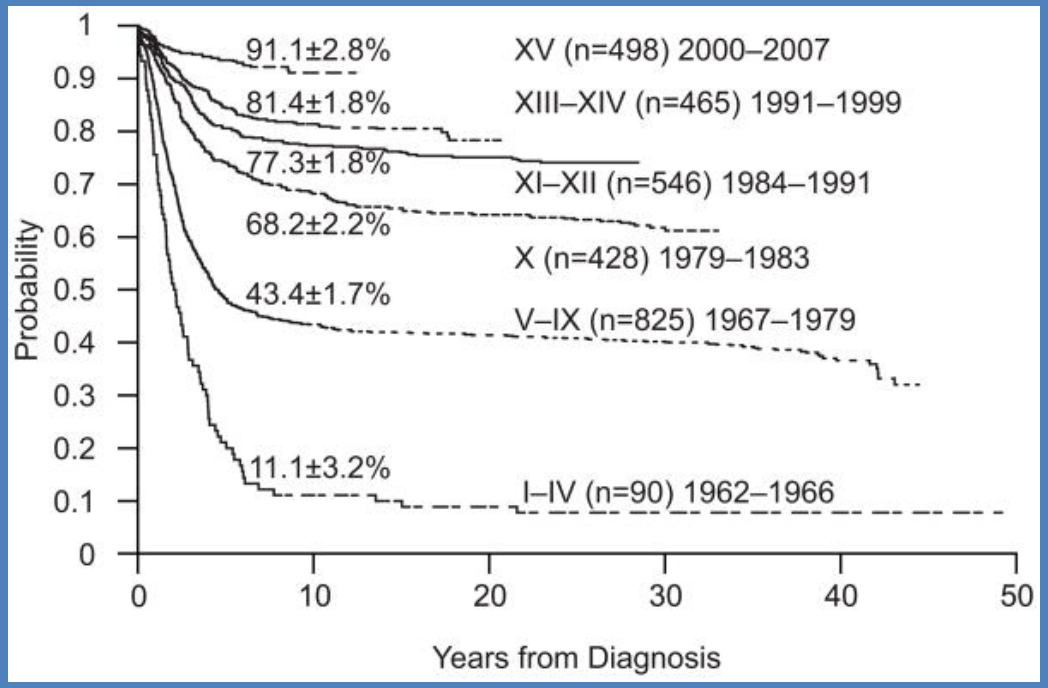
- Acute Lymphoblastic Leukemia (ALL) are hematological tumors derived from the malignant **transformation of immature lymphoid cells**
- ALL is the most common pediatric tumor accounting for 25% of all childhood cancer
- T-ALL is caused by transformation of **immature thymic cells**, while B-ALL derives from B-cell precursors. T-ALL is less frequent than B-ALL, constituting 10-15% of pediatric and 25% of adult cases of ALL
- T-ALL is characterized by the infiltration of the bone marrow of malignant hematopoietic cells expressing immature T-cell markers and generally present clinically with elevated white cell counts, mediastinal masses and frequent CNS infiltration
- Twice as prevalent in males as in females

T-cell differentiation is a complex process



(adapted from Buck *et al.*, JEM 2015)

A 50-year journey to cure childhood ALL



Pui and Evans, 2013

Over time, the survival of T-ALL has significantly improved among younger patients. However, **older patients with T-ALL continue to have poor outcomes** with no significant improvement in survival over time

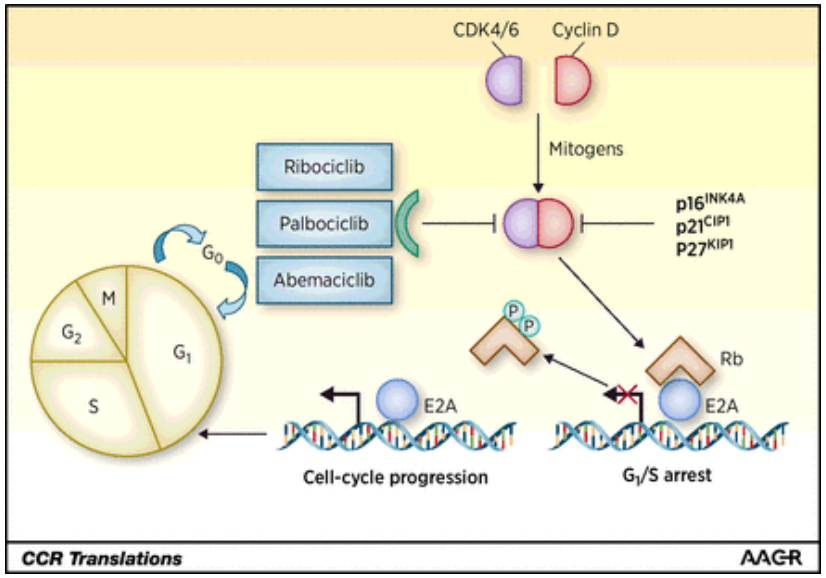
Clinical challenges in T-ALL

- Few new **therapeutic options** and lack of effective targeted therapies
- High prevalence of primary **glucocorticoid resistance** associated with poor prognosis
- Higher rates of **relapse and secondary resistance** to chemotherapy

The mutational landscape of T-ALL

Belver and Ferrando, Nat Rev Cancer 2016
Gianni, Belver and Ferrando, Cold Spring Harb Perspect Med 2020

Cell cycle deregulation in T-ALL



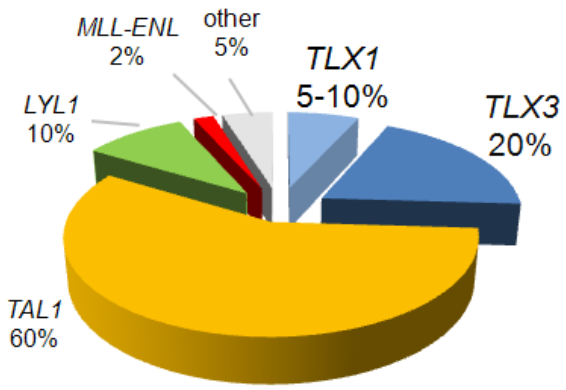
Clin Cancer Res. 2017;23(4):873-875.

- Most frequent event in T-ALL is the loss of CDKN2A/B; mutation or methylation is rare, genomic deletion occurs in over 70% of T-ALL
- Lack of CDKN2 deletion: strong prognostic marker negatively associated with immature/ETP
- Targetable with CDK4/6 inhibitors: highly active and cytotoxic in preclinical models

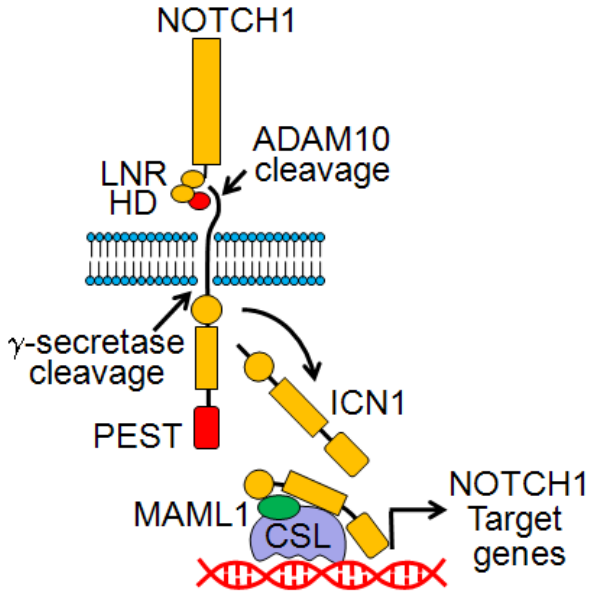
The molecular basis of T-ALL

T-ALL is primarily a NOTCH1 driven disease in which developmentally important transcription factor oncogenes define distinct clinical and biological groups.

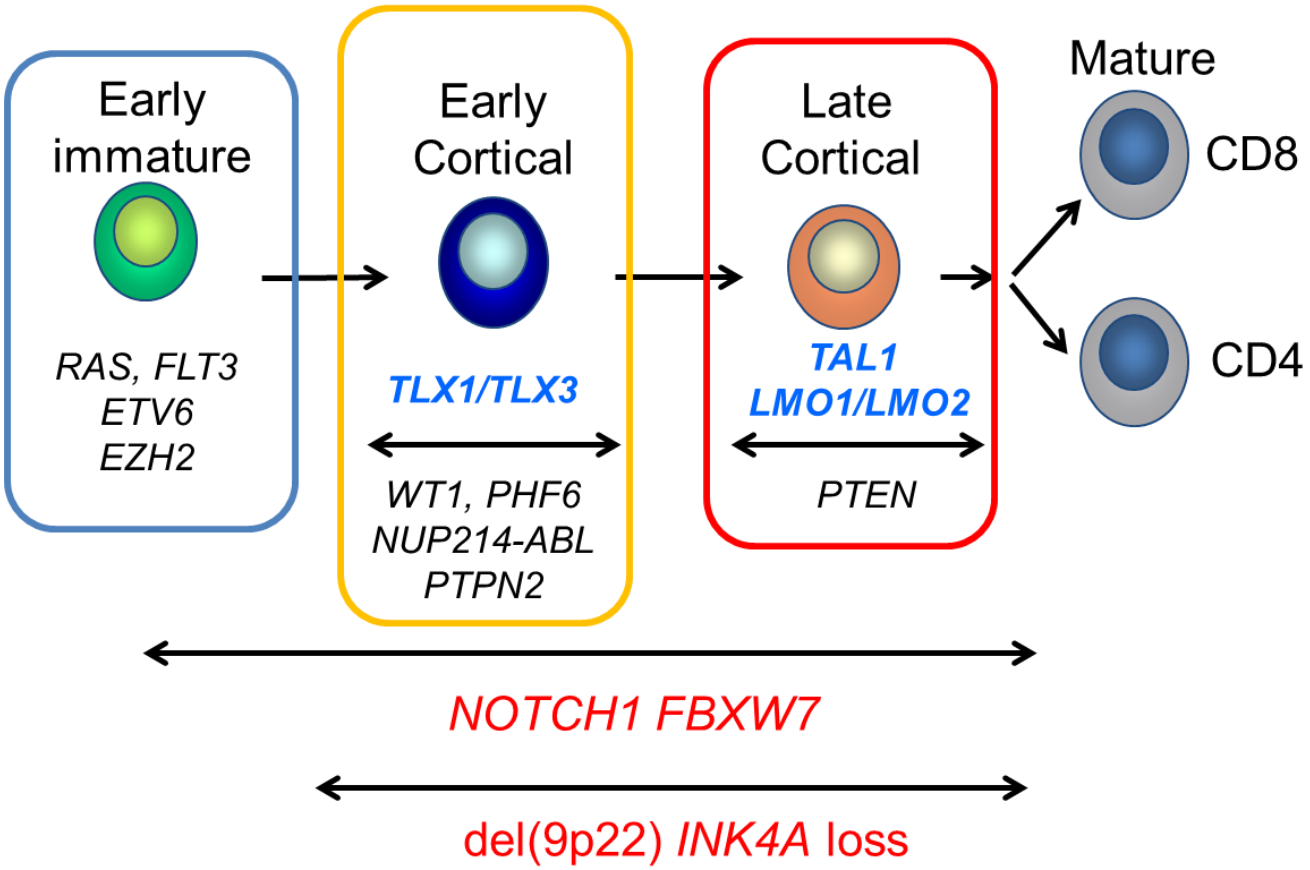
T-ALL transcription factor oncogenes



NOTCH1 mutations in T-ALL

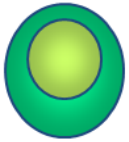


Oncogenic programs and gene expression signatures define molecular groups of T-ALL



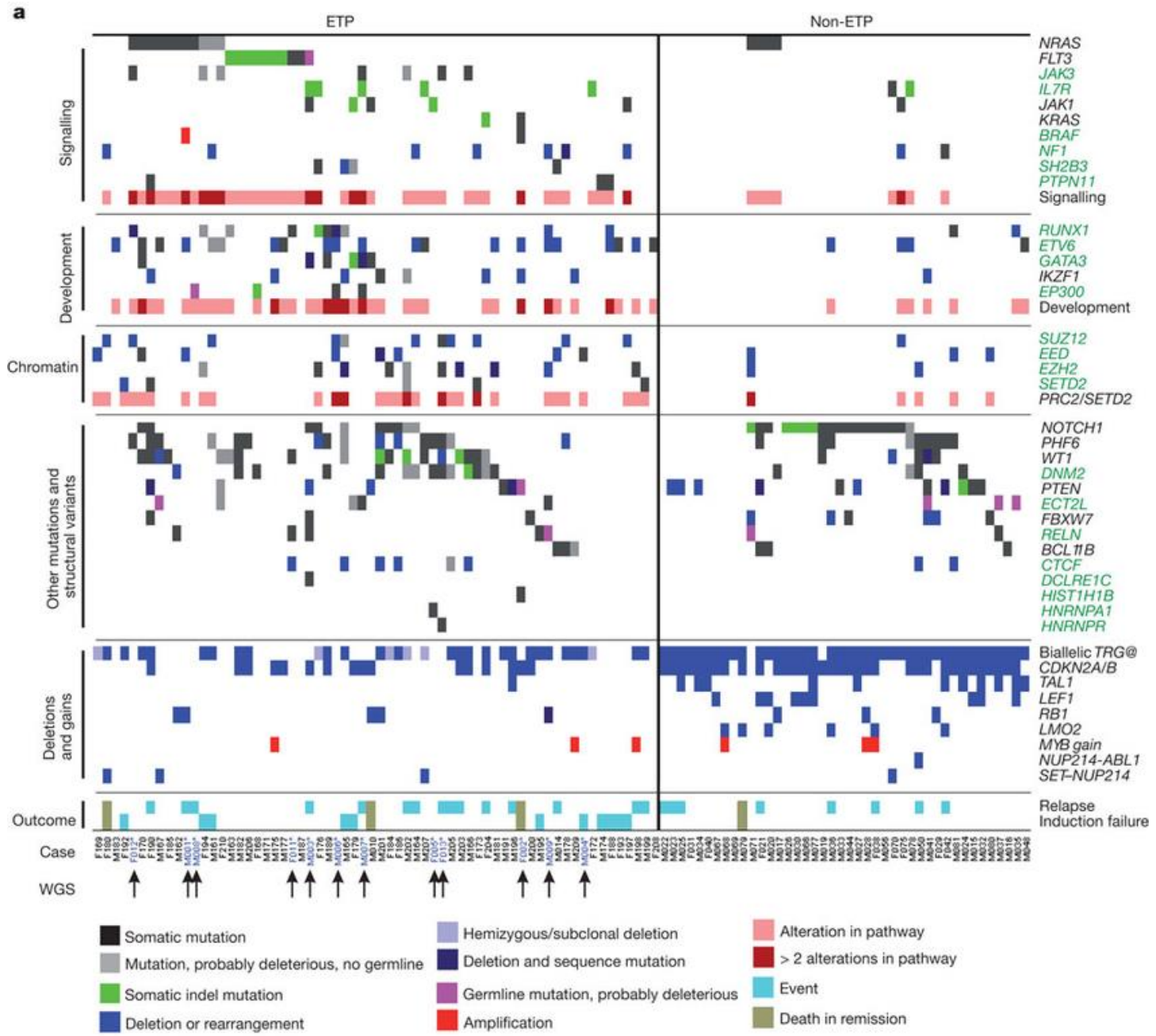
Early T-lineage Progenitor (ETP) leukemias

Early immature



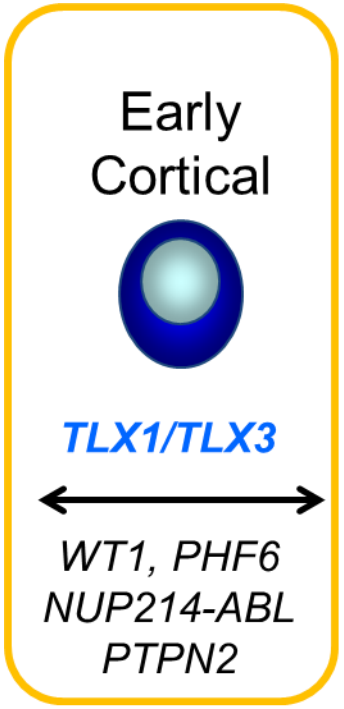
RAS, FLT3
ETV6
EZH2

- Block at the **earliest stages of differentiation**
- **Lower prevalence** of typical T-ALL alterations, including NOTCH1 and CDKN2A deletions
- Associated with genes encoding **signaling factors** (RAS/FLT3) and **epigenetic regulators** (IDH1/2 , EZH2, DNMT3A)
- Associated to loss of function mutations in **master transcription factors** (RUNX1, GATA3, ETV6)
- Historically have **worse prognosis**



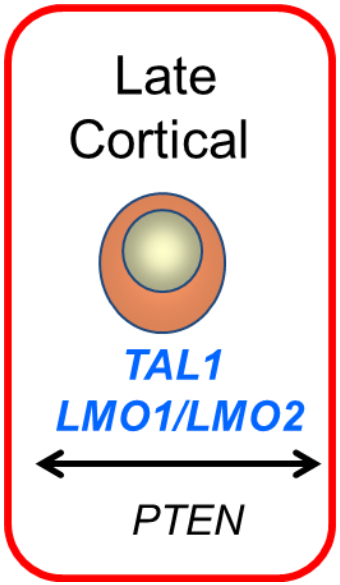
Zhang *et al*., Nature 2012

Early cortical T-ALL: the role of homeobox genes



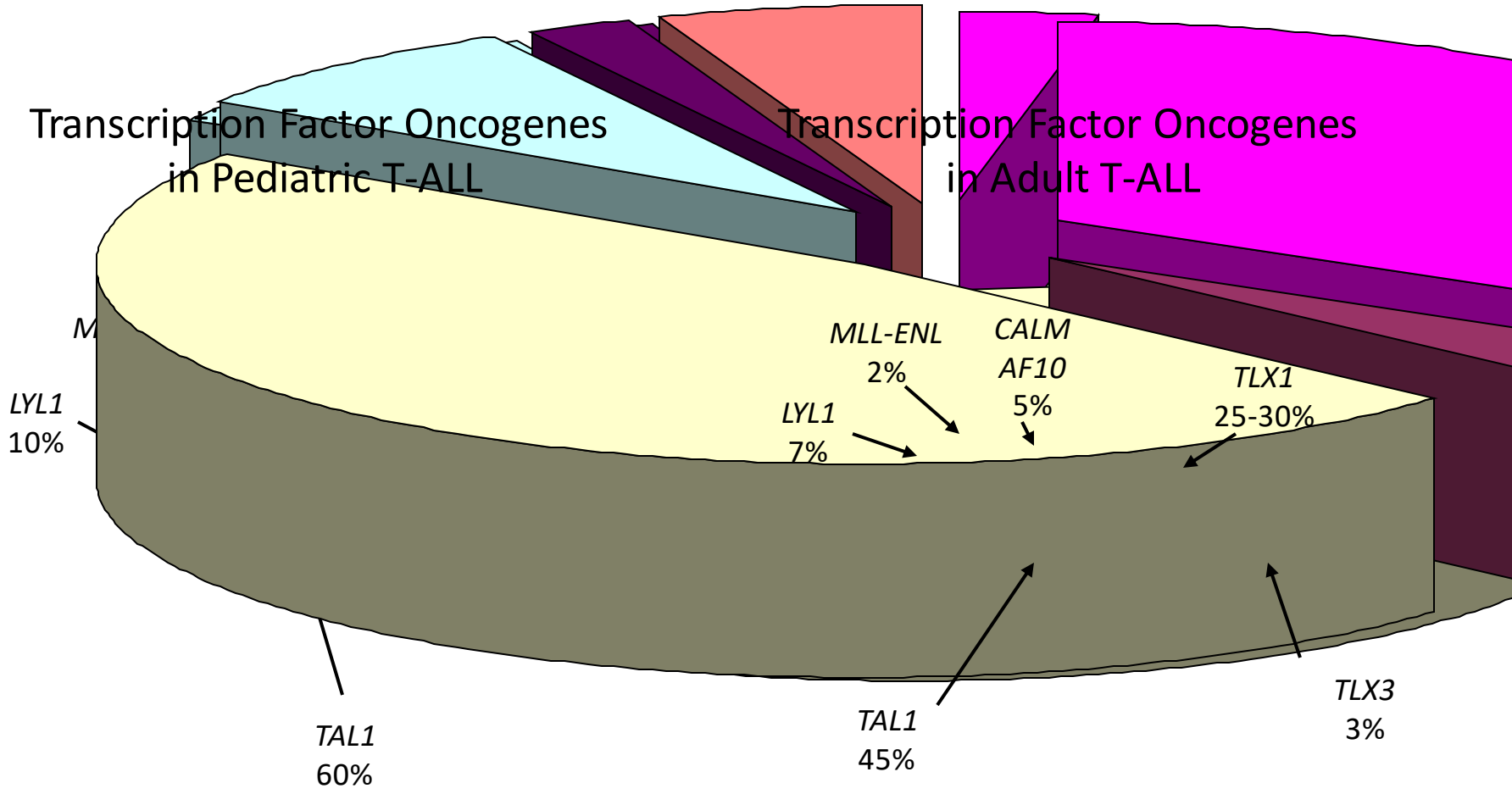
- Early cortical phenotype: $CD1a^{+}$ $CD4^{+}$ $CD8^{+}$
- **Highest prevalence** of NOTCH1 mutations and CDKN2A deletions
- Associated with activation of homeobox genes, most characteristic are TLX1/TLX3 orphan homeobox genes that are activated through recurrent translocations (30% T-ALL)
- **TLX1** t(10;14) translocation; Transgenic mouse for TLX1 develops T-ALL and identified role for Tlx1 in promoting aneuploidy and genomic instability
- **TLX3** t(5;14) translocation
- High overlapping transcriptional signatures
- **Favorable** prognosis

Late cortical T-ALL: TAL1 and LMO factors

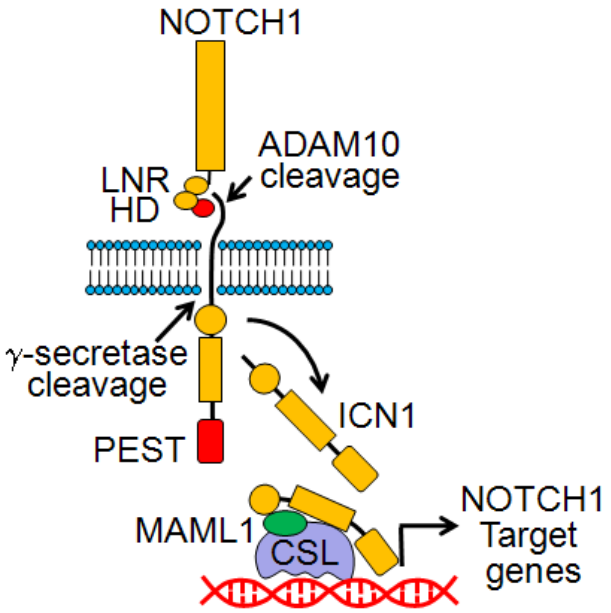


- late cortical phenotype: $CD3^+$ $CD4^+$ $CD8^+$
- **TAL1**: 60% of T-ALL have aberrant expression of class II bHLH (TAL1, TAL2, LYL1 or BHLHB1)**.** Expression can be driven by **translocations**, **intrachromosomal deletion** and **somatic mutations** affecting upstream regulatory regions
- **LMO1/2**: 10% of T-ALL; driven by chromosomal translocations
- LMO proteins and TAL1 form transcriptional complexes, that also contain class I bHLH proteins (E2A/HEB) and GATA3

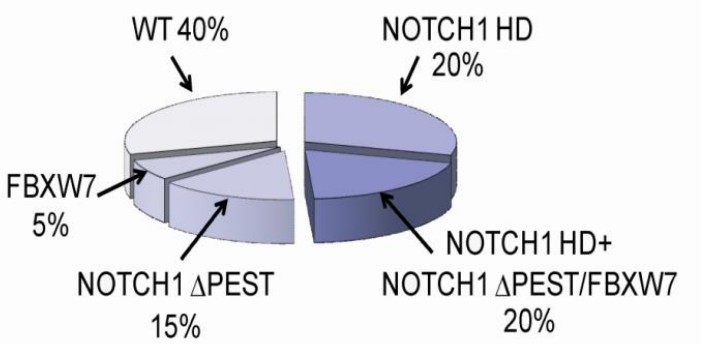
Transcription Factor Oncogenes Translocations



NOTCH1: mastermind of T-ALL



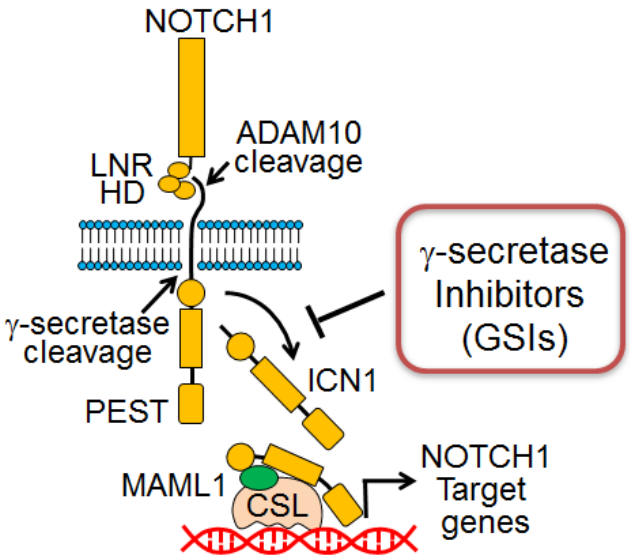
- Activated by mutationsin **65% of T-ALL cases**



- Central role in T-ALL metabolism, growth and survival
- Key driver of oncogenic pathways: **MYC, PI3K**
- **Targetable**: gamma secretase inhibitors (GSIs), NOTCH1 inhibitory antibodies

Weng, Ferrando *et al*., Science. 2004

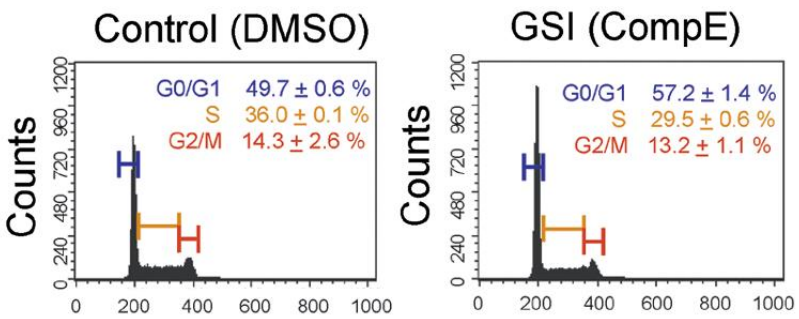
The role of GSI in the treatment of T-ALL



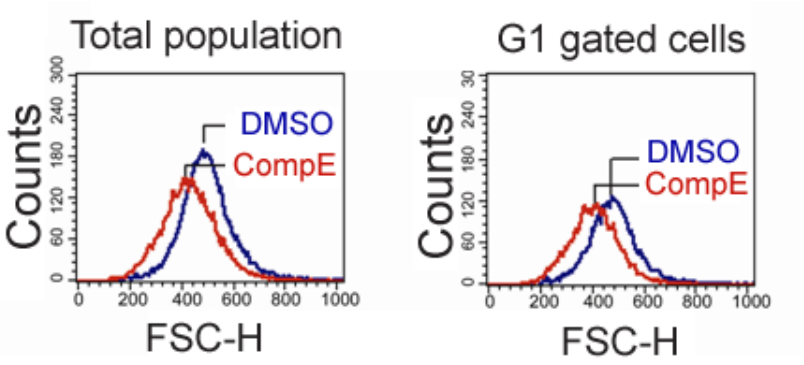
- GSIs are highly active in preclinical models of T-ALL but have shown **limited clinical activity** in early phase trials
- On target **gastrointestinal toxicity**

Mechanism of action of GSI in T-ALL

Cell cycle arrest in G1

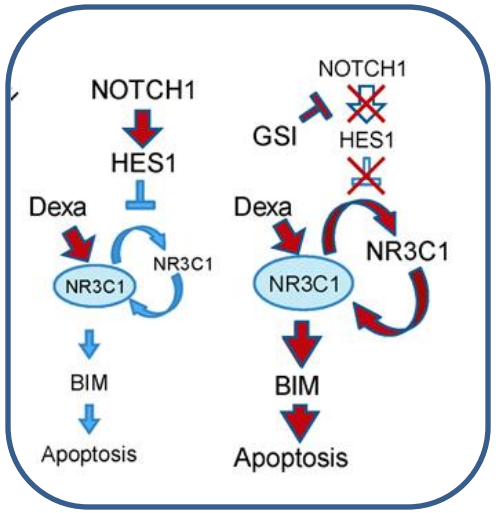


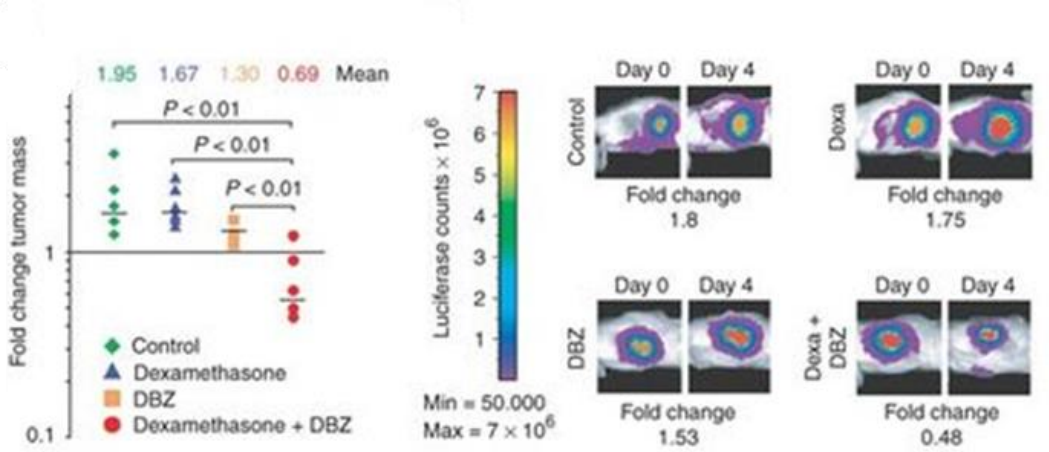
Decreased cell size

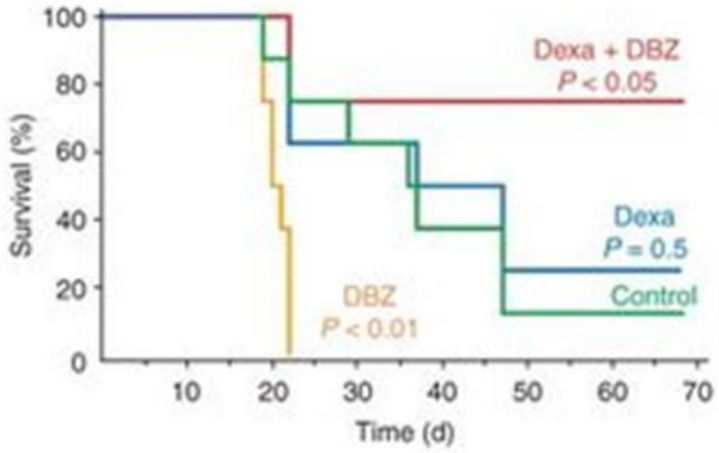


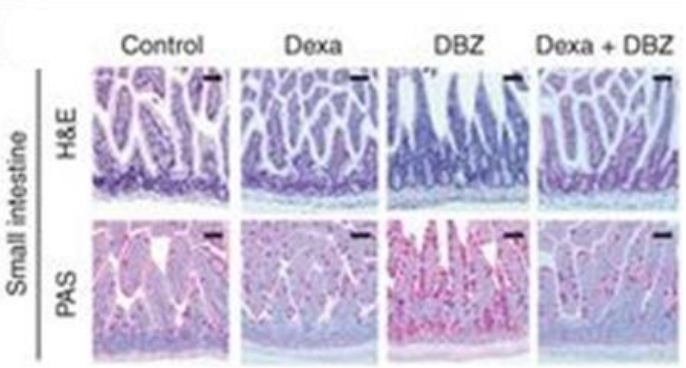
- Delayed phenotype (4-6 days)
- No significant induction of apoptosis
- **Only a fraction** of NOTCH1 mutated T-ALL lines respond
- Limited therapeutic window due to on target **dose limiting gut toxicity**

Synergistic interaction of NOTCH inhibition and glucocorticoids in T-ALL



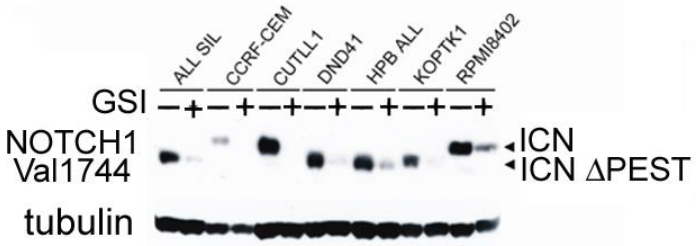


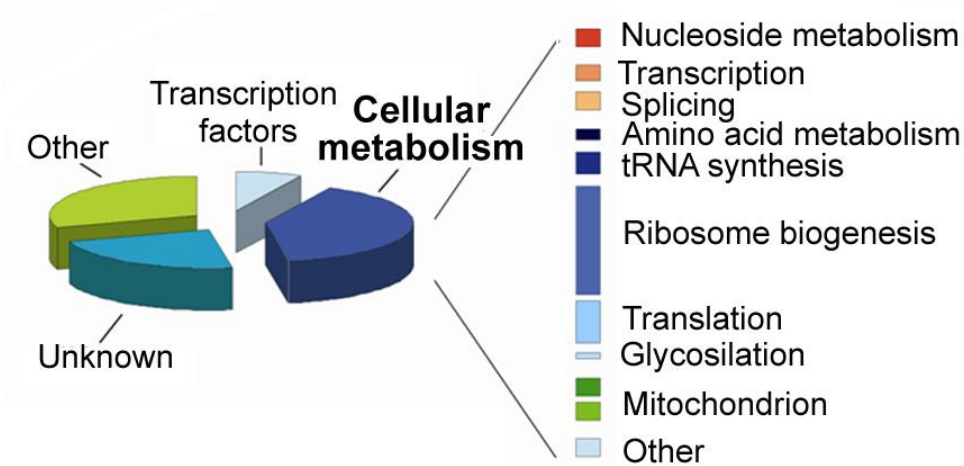


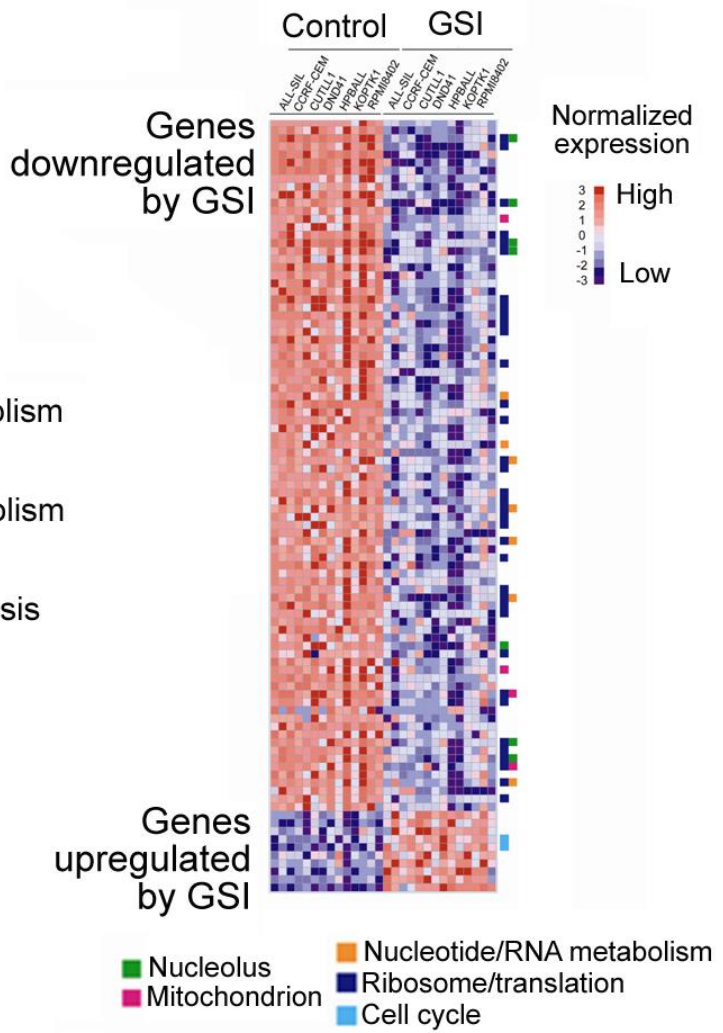


Real *et al*., Nat Med. 2009

NOTCH1 activation controls a cell growth transcriptional program



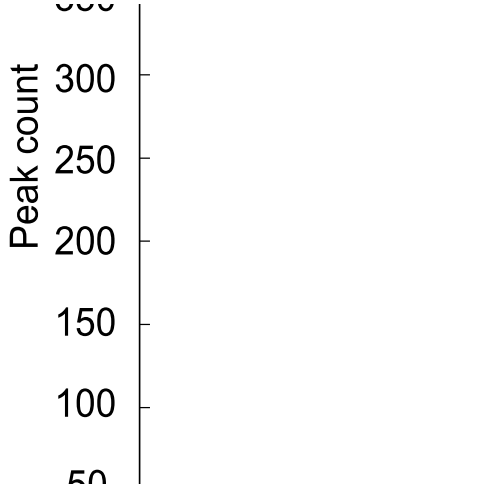


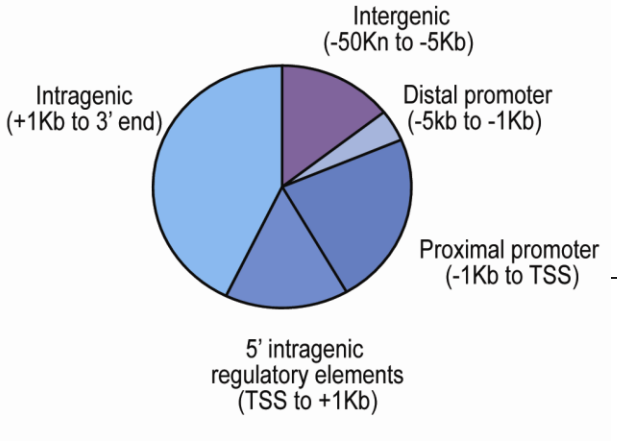


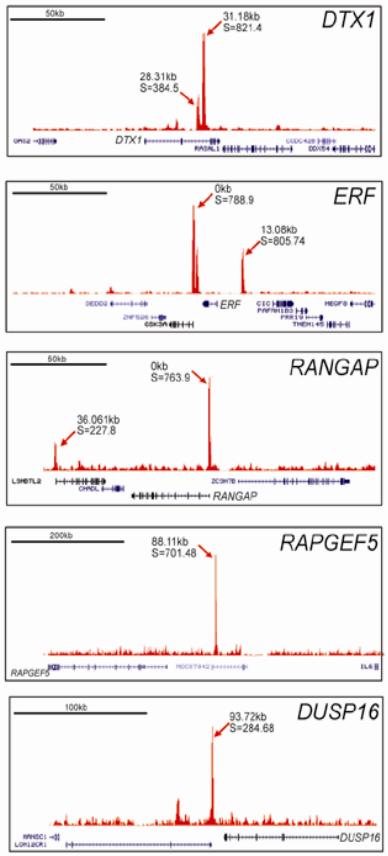
Palomero *et al*. PNAS 2006

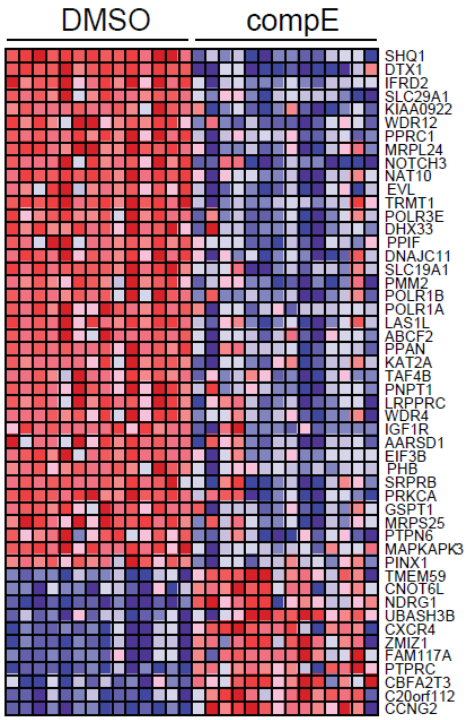
Genome wide mapping of NOTCH1 binding sites in T-ALL

Distribution of NOTCH1 Chip-seq peaks

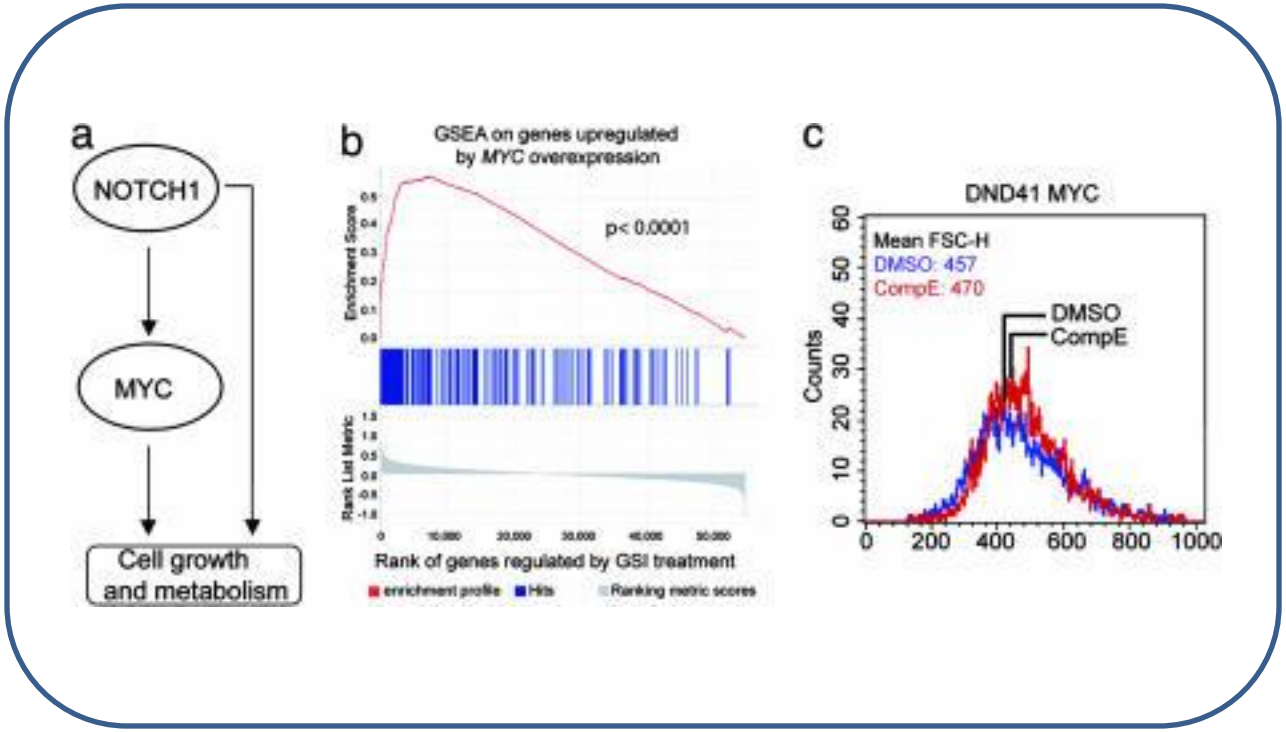








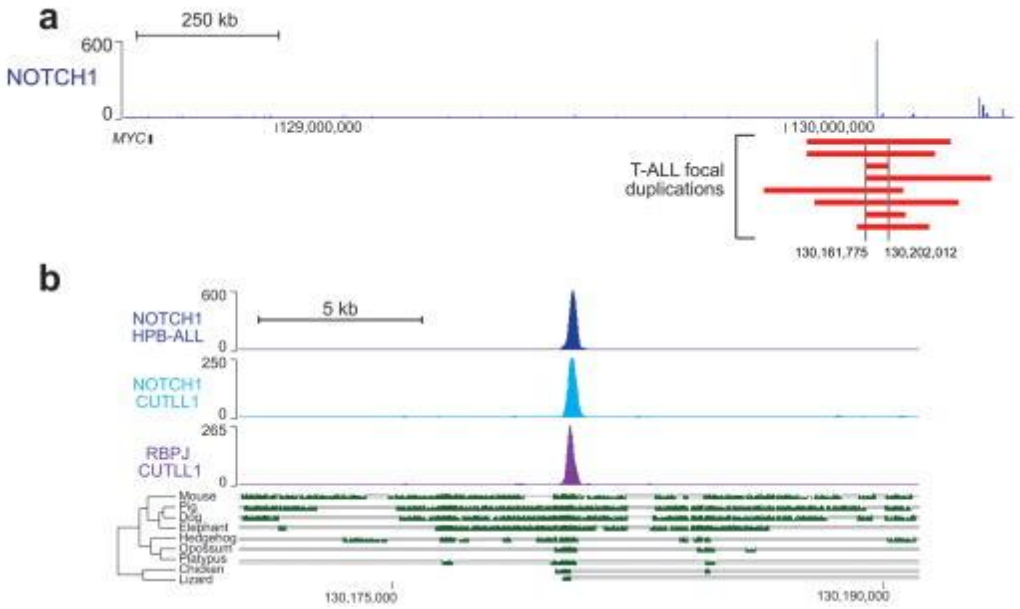
MYC: mediator of NOTCH1 signaling

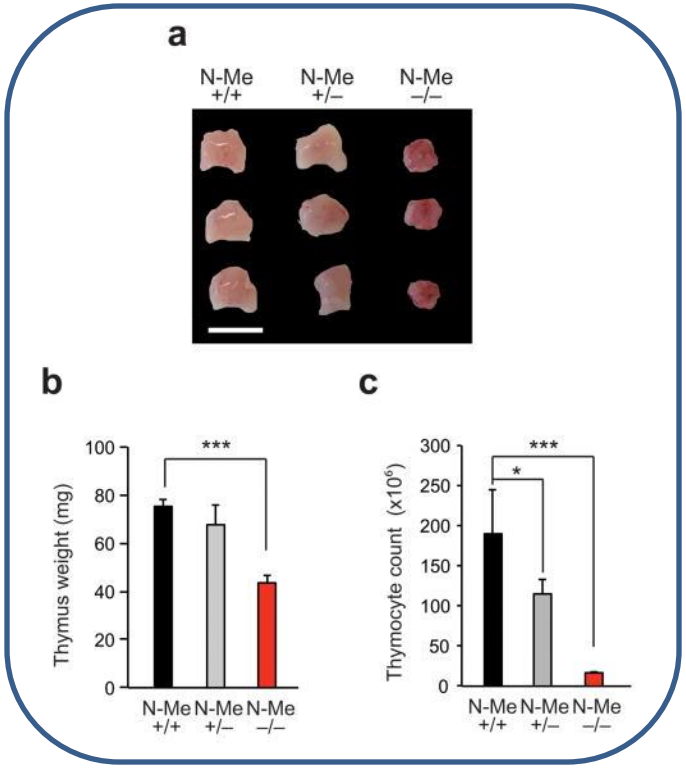


NOTCH1 directly regulates c-MYC and activates a feed-forward-loop transcriptional network promoting leukemic cell growth

Palomero *et al*. PNAS 2006

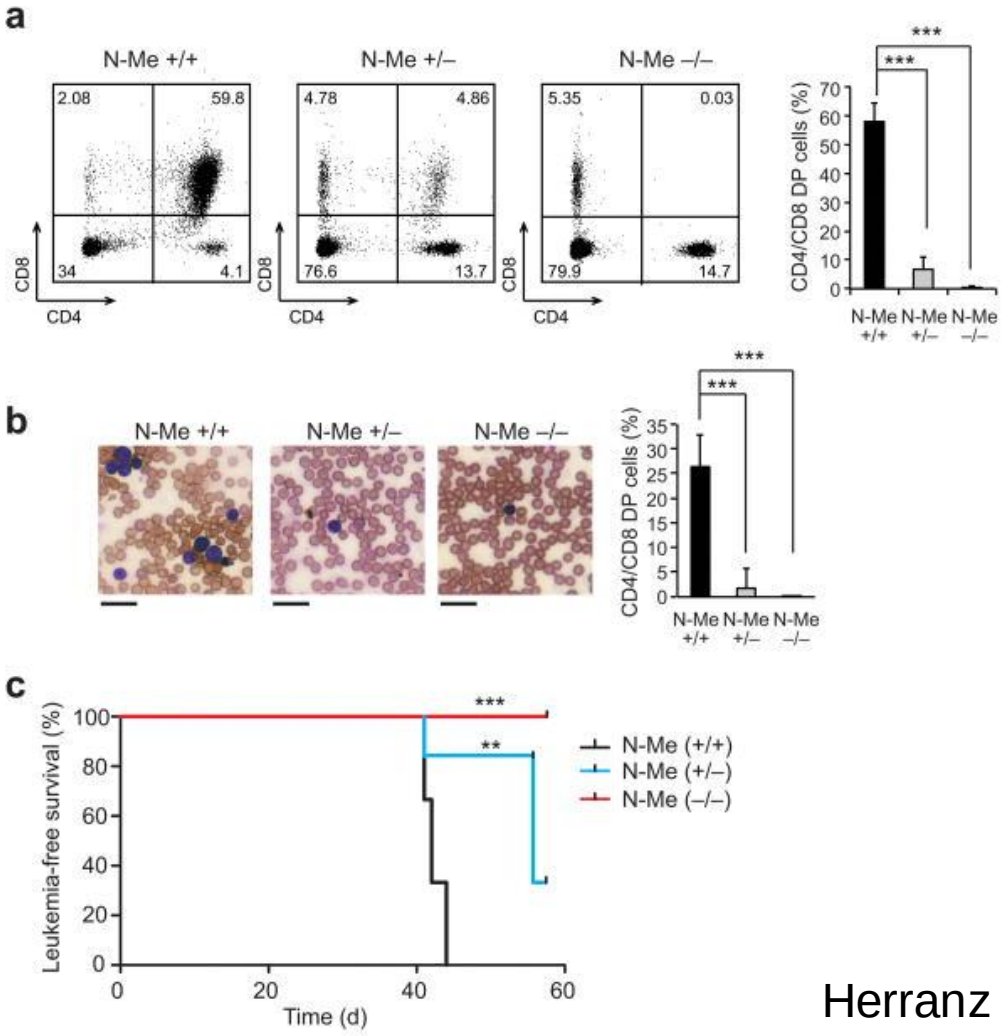
N-Me, a long range MYC enhancer regulated by NOTCH1 in T-ALL





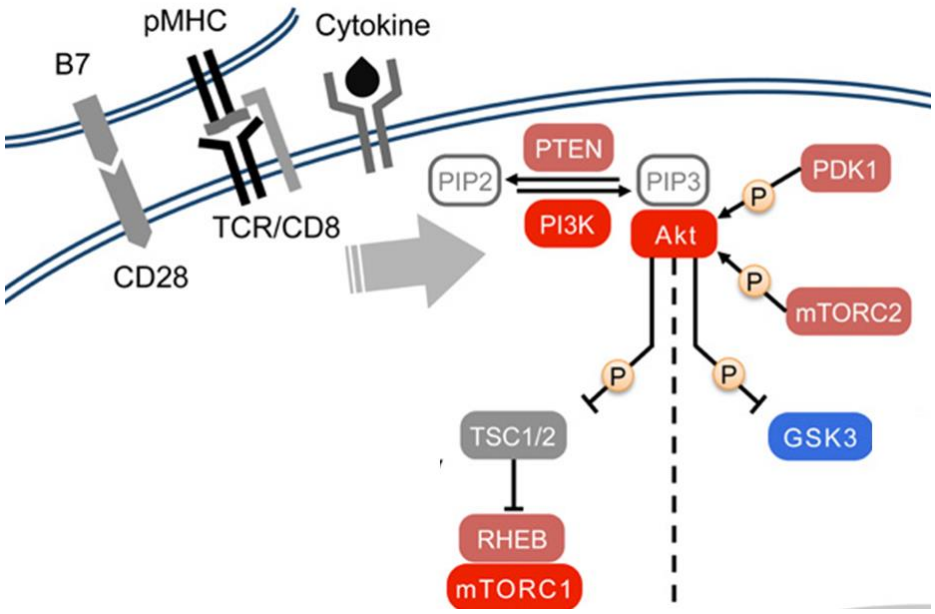
Herranz *et al*., Nat Med 2014

N-Me, a NOTCH1-driven MYC enhancer promotes T-cell development, transformation and acute lymphoblastic leukemia



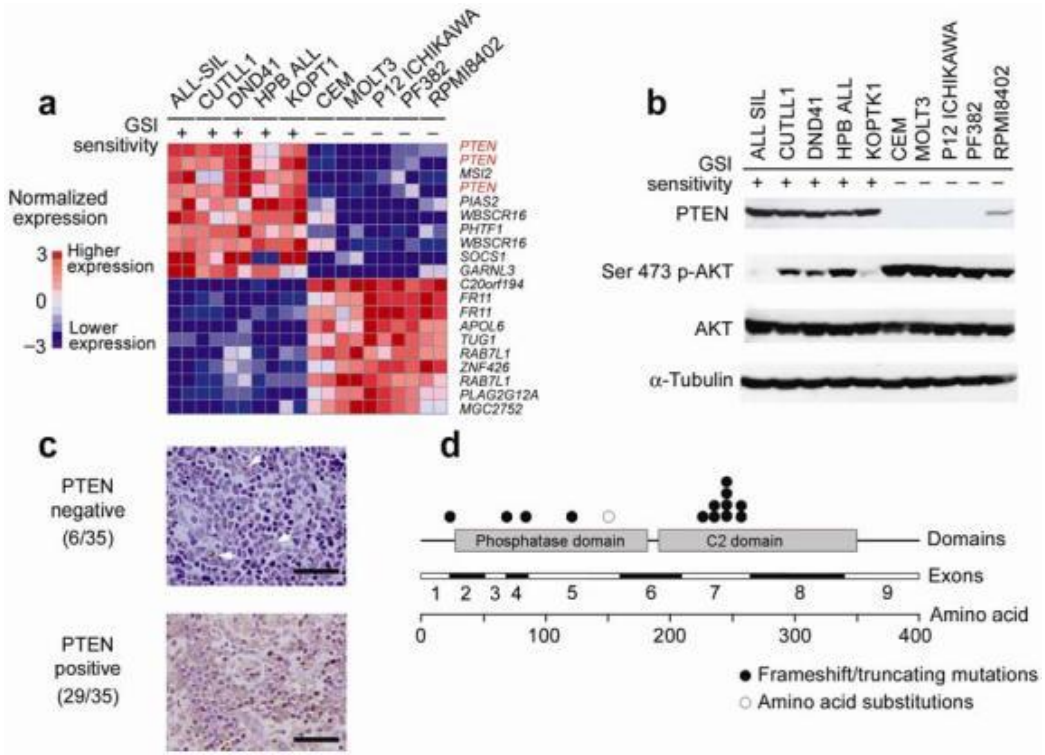
Herranz *et al*., Nat Med 2014

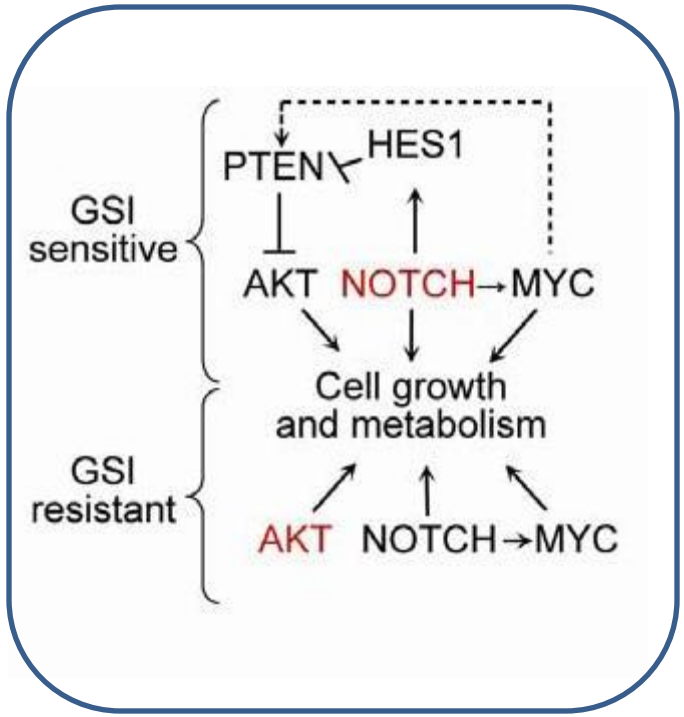
PI3K-AKT pathway in the pathogenesis of T-ALL



- PI3K signaling is a major survival pathway in T-ALL
- PTEN is mutated and deleted in 10% (protein lost in 20%) of T-ALLs
- Associated with **poor prognosis**, impaired glucocorticoid response and response to GSI therapies in preclinical models
- Druggable: inhibition of PI3Kgamma/delta, AKT, mTOR; synergistic with glucocorticoids

Mutations in PTEN induce resistance to gamma-secretase inhibitors in T-ALL

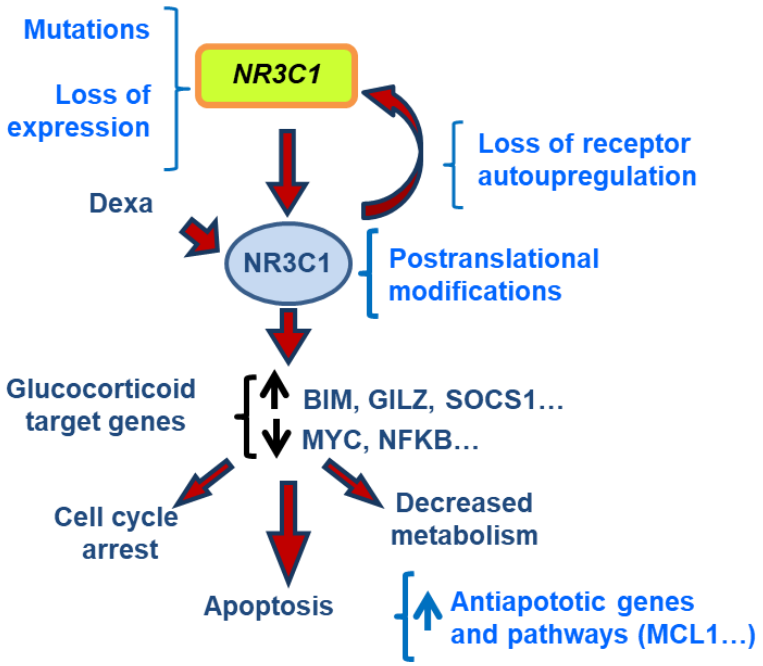




- Mutational loss of PTEN induces an oncogenic metabolic switch and drives in vivo resistance to anti-NOTCH1 therapies in T-ALL
- PTEN mutant T-ALL are highly sensitive to inhibition of AKT

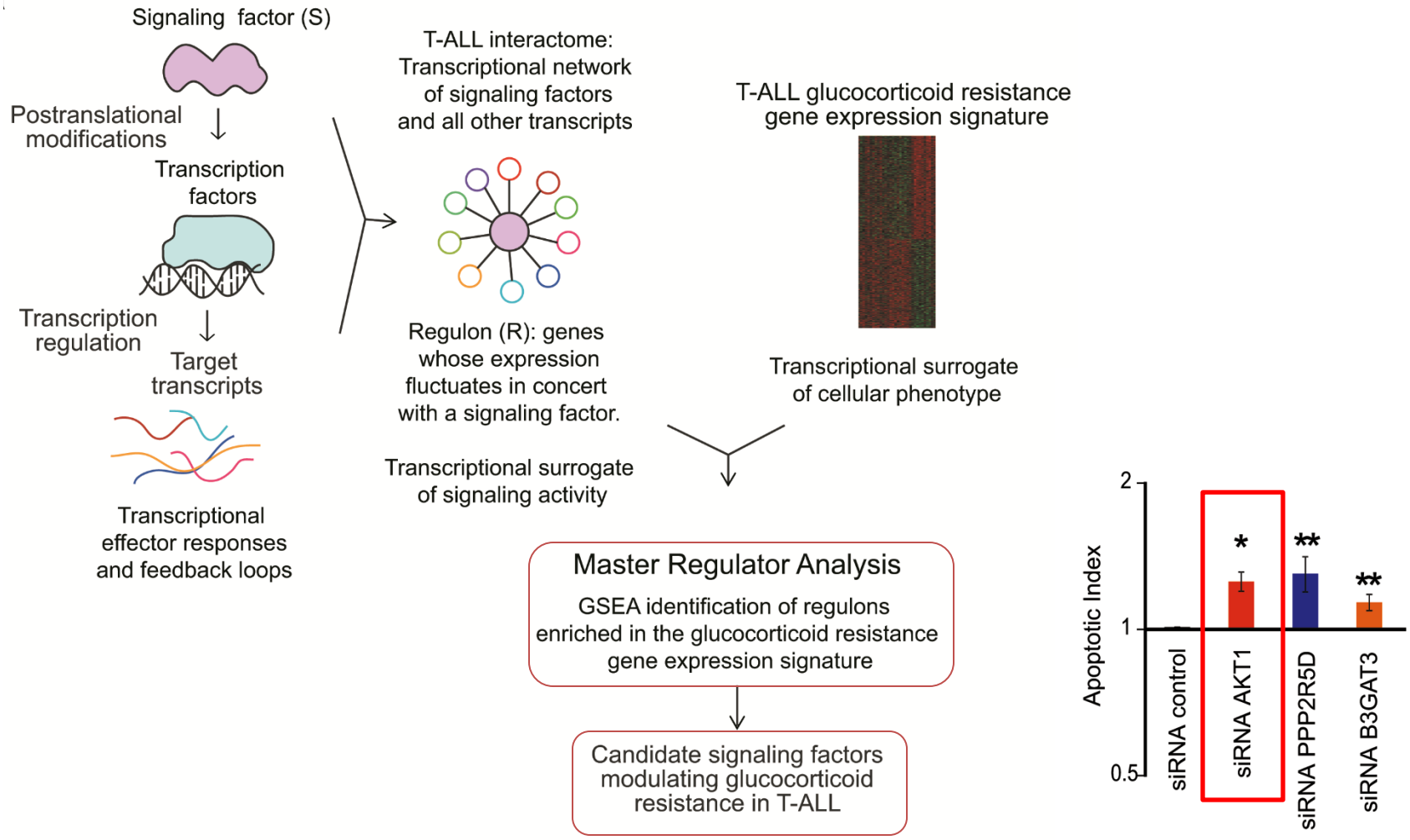
(Palomero et al., Nat Med 2007)

The challenge of glucocorticoid resistance in T-ALL

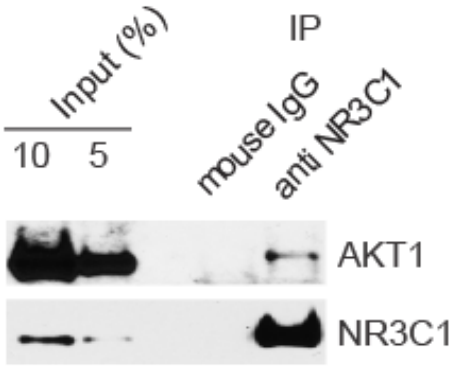


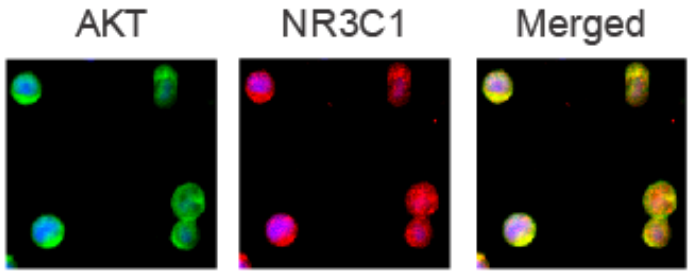
- Glucocorticoids play a fundamental role in the treatment of lymphoid tumors due to their capacity as **inductors of apoptosis**
- **Strong association of primary glucocorticoid resistance with prognosis**
- Prednisone poor response (failure at 7 days) is strongly associated with risk of relapse
- Primary glucocorticoid resistance is **particularly frequent in T-ALL**

Master Regulator analysis of glucocorticoid resistance in T-ALL



AKT interacts with and phosphorylates the NR3C1 glucocorticoid receptor protein



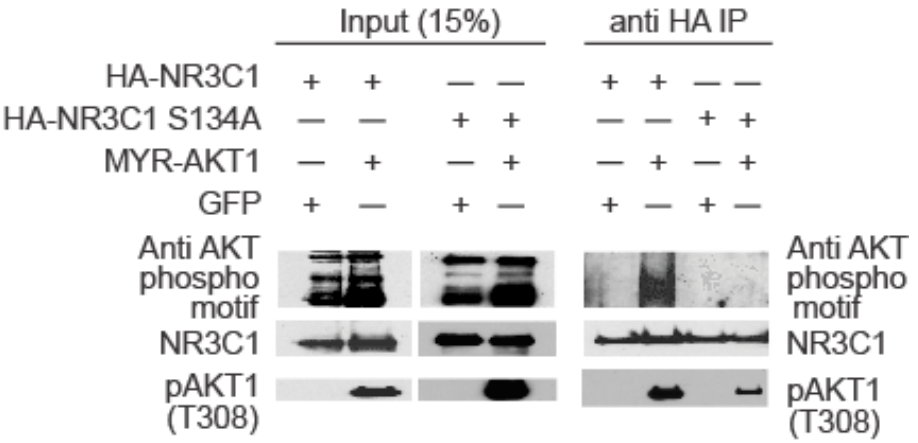


Human NR3C1 ^{129}LNRST**S**VPE^{137}

Mouse NR3C1 ^{146}LNRST**S**RPE^{155}

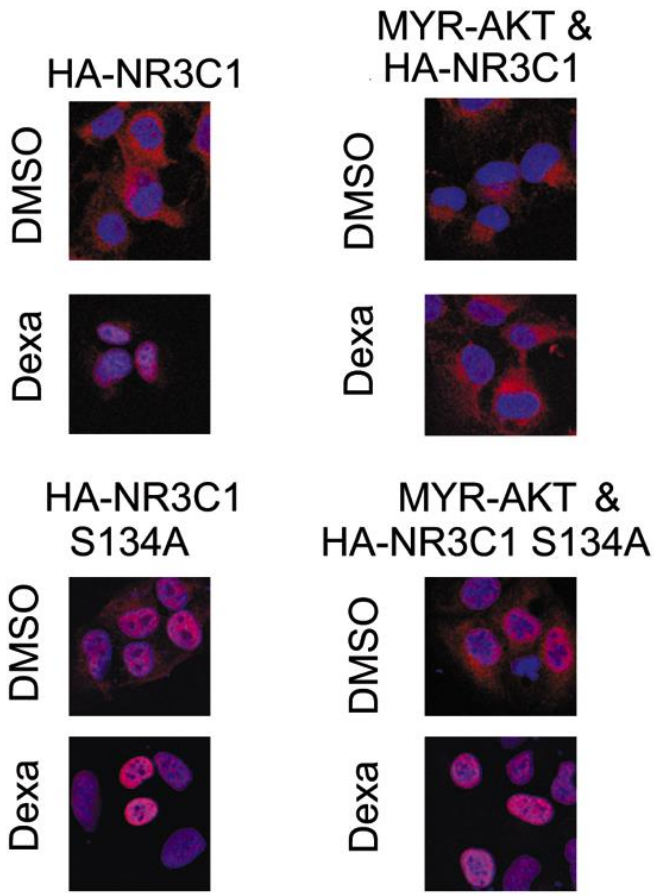
Rat NR3C1 ^{138}LNRST**S**VPE^{146}

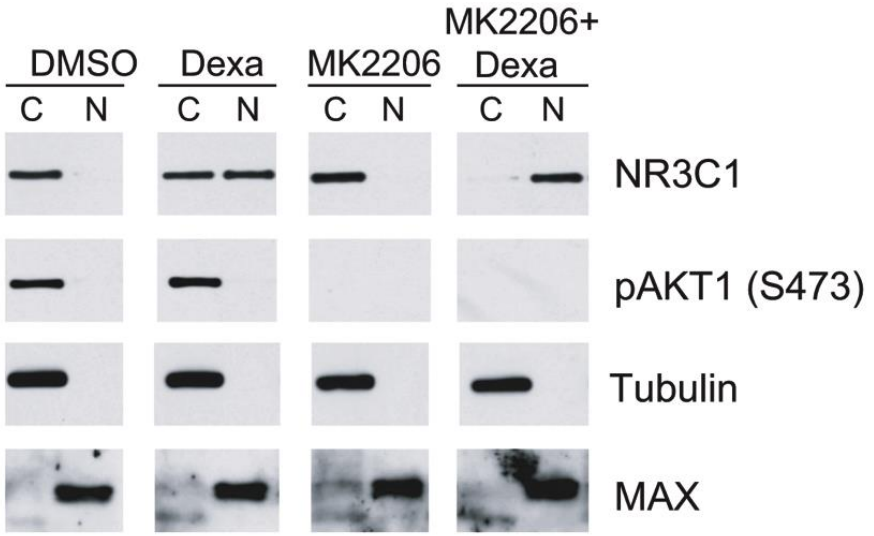
Pig NR3C1 ^{130}LSRST**S**VPE^{138}



Piovan *et al*., Cancer Cell, 2013

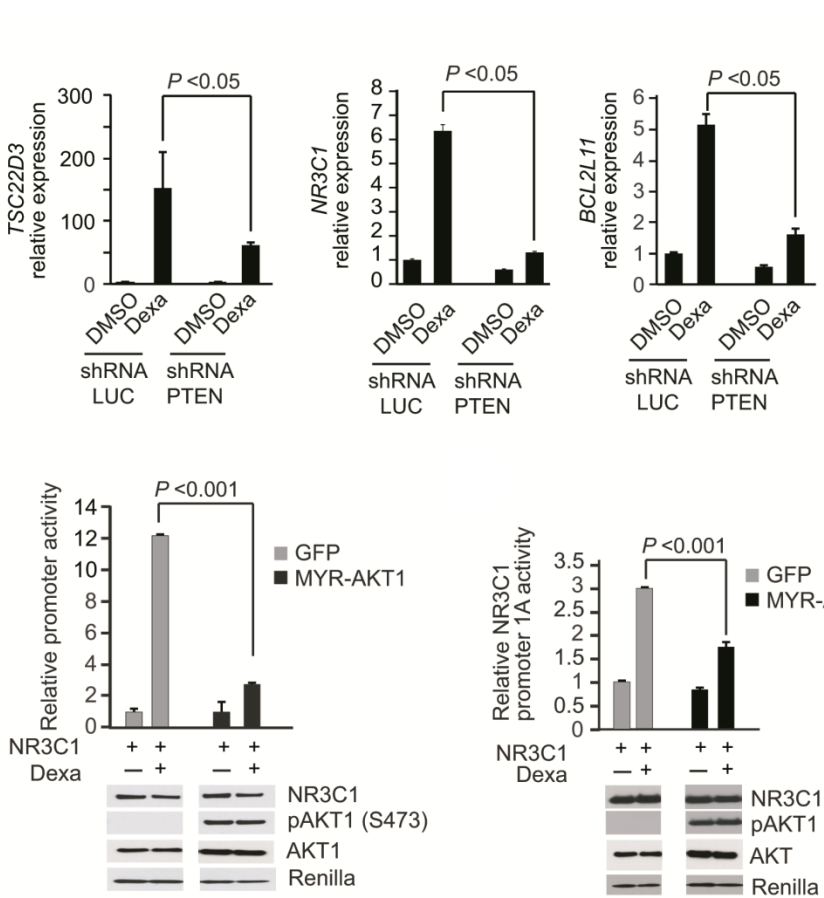
AKT blocks nuclear localization of the glucocorticoid receptor

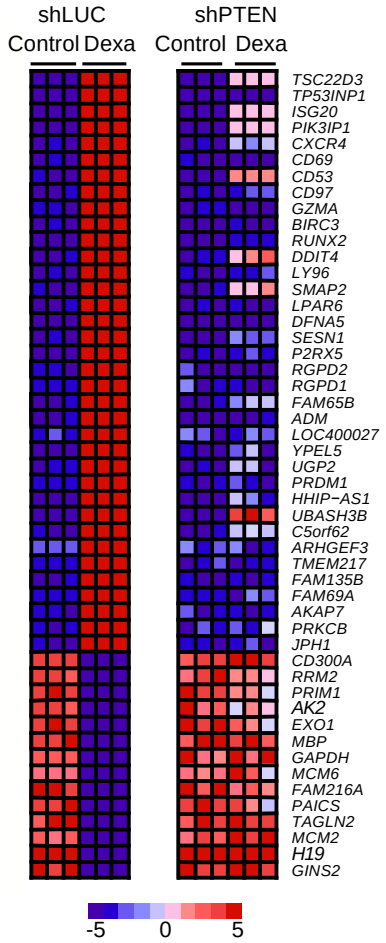


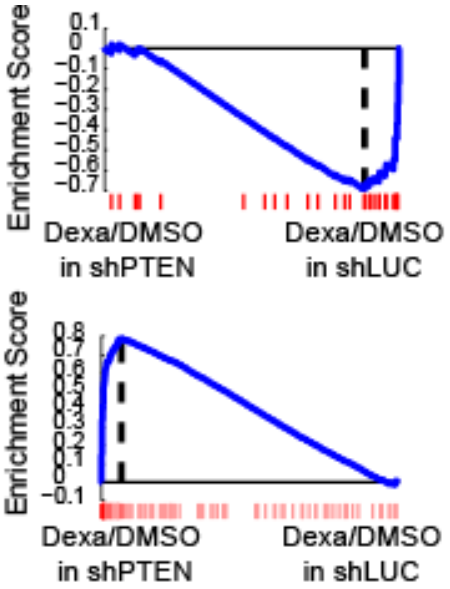


Piovan *et al*., Cancer Cell, 2013

PTEN knockdown and AKT activation blunt glucocorticoid receptor activity

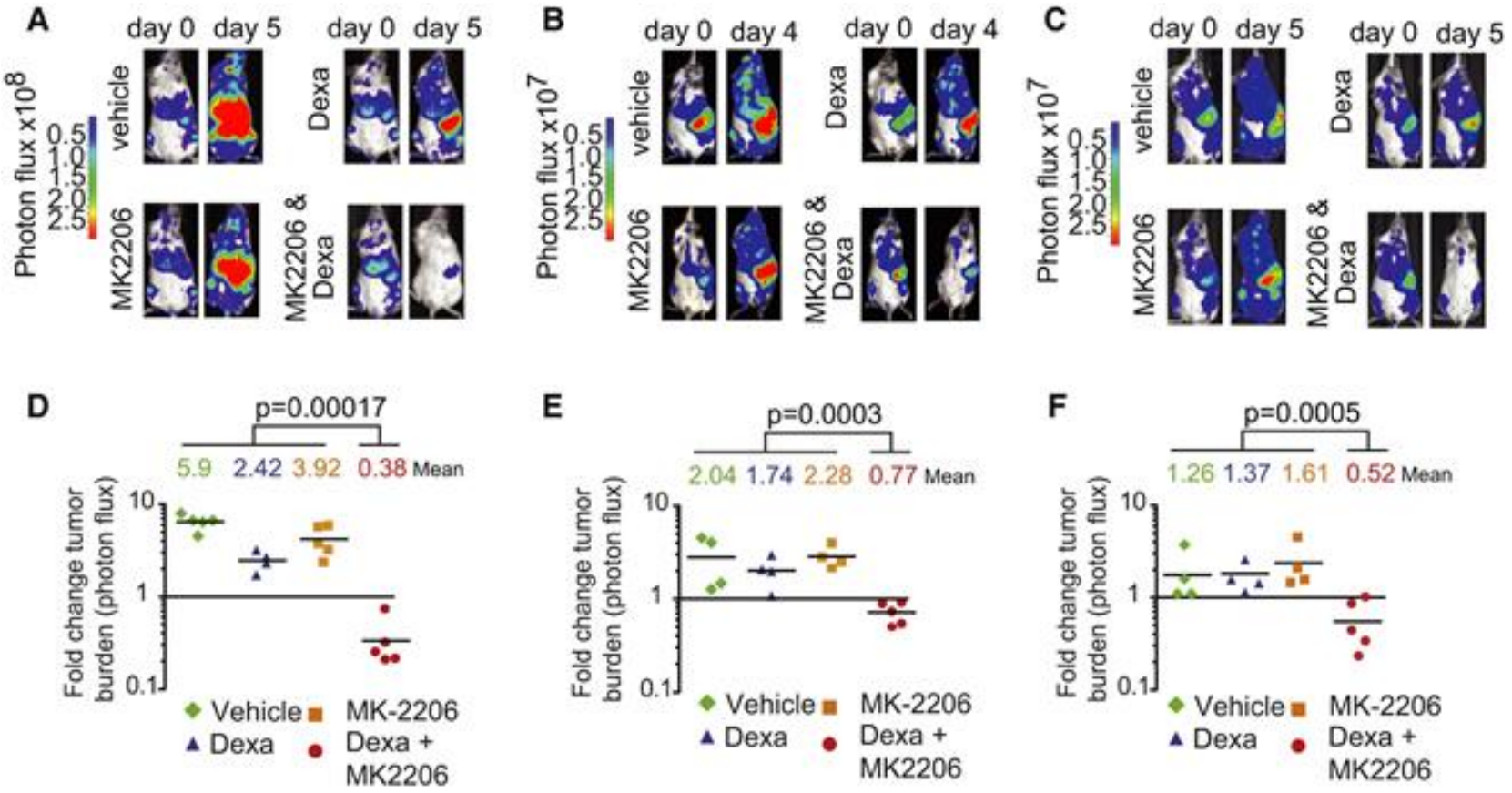






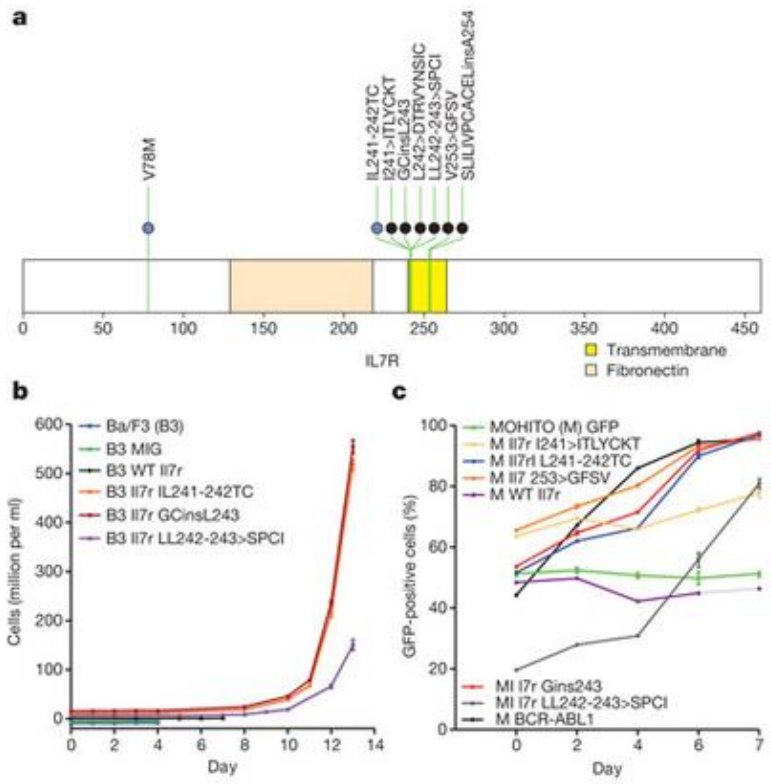
Piovan *et al*., Cancer Cell, 2013

Reversal of glucocorticoid resistance by AKT inhibition in T-ALL



Piovan *et al*., Cancer Cell, 2013

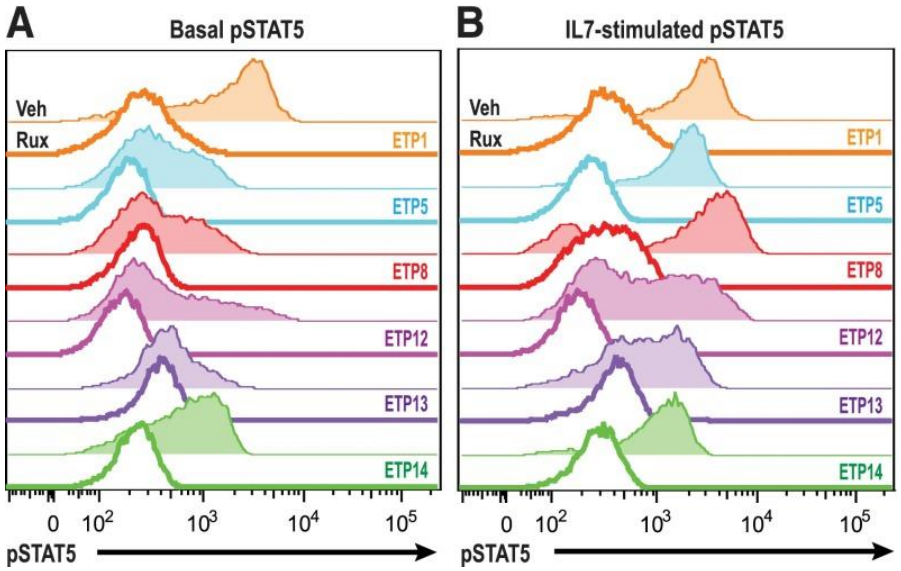
IL7R and JAK/STAT pathway



- Gain of function mutations in the **IL7R in 5-10%** of T-ALLs
- Activating mutations in **JAK1/3** reported in 5-10% of T-ALL
- **STAT5B** activating mutations also identified in 5-10% T-ALLs
- IL7R mutations are **particularly prevalent in ETP T-ALL**, which also show hyperactivation of STAT5 in response to IL7
- Aberrant signaling driven by mutations but also paracrine IL7 signaling may be important in T-ALL
- May contribute to glucocorticoid resistance

Zenatti *et al*., Nat Genet 2011
Zhang *et al*., Nature 2012

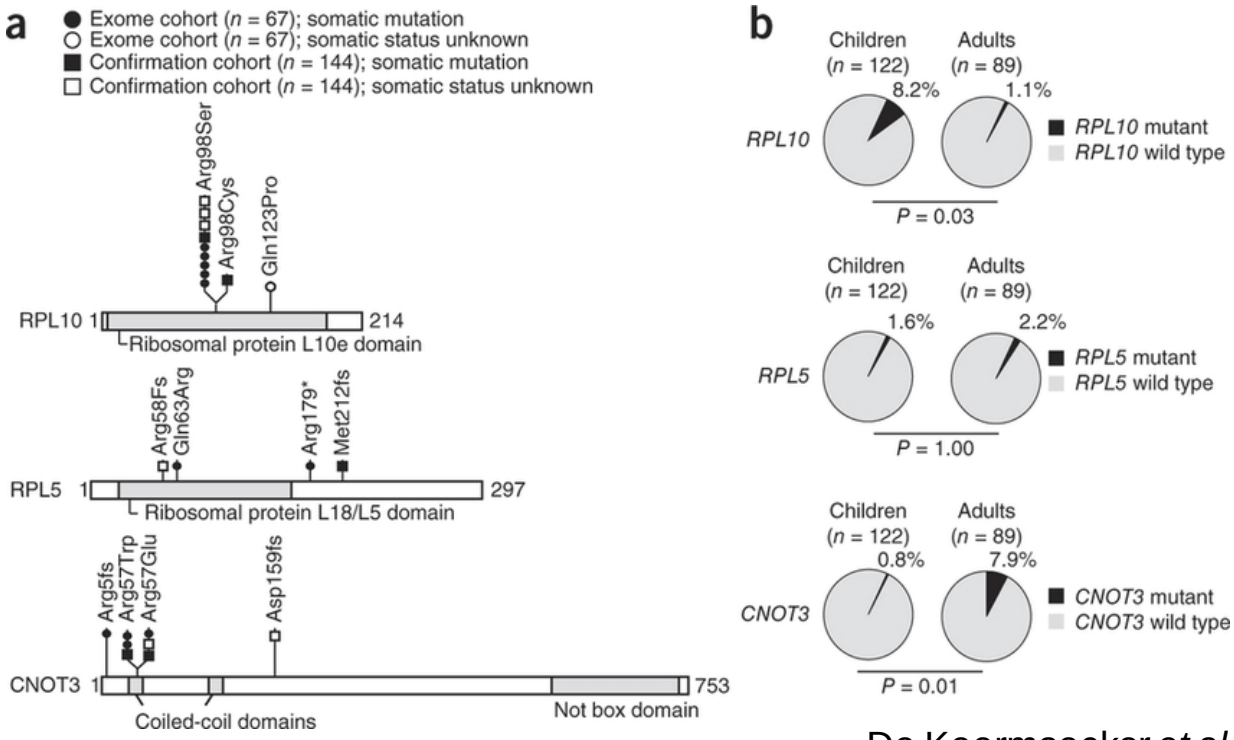
Efficacy of JAK/STAT pathway inhibition in murine xenograft models of ETP-ALL



Ruxolitinib abrogates IL7-induced STAT5 phosphorylation.

Blood. 2015

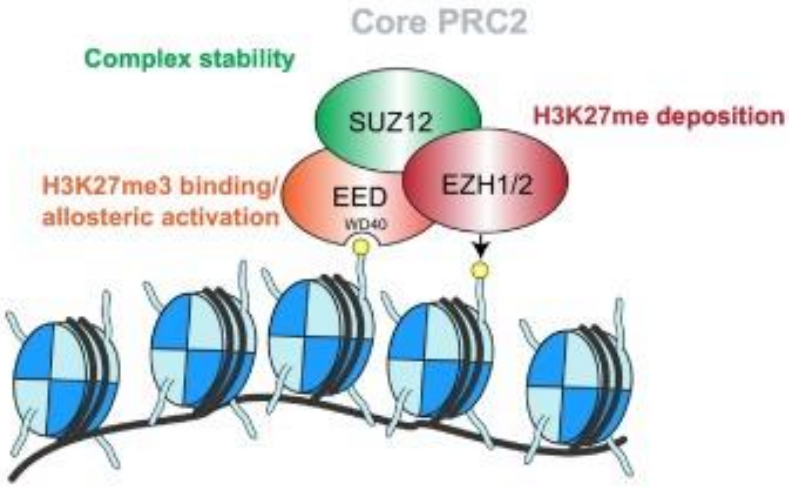
Ribosomal Protein mutations in T-ALL



De Keermaecker *et al*., Nat Genet 2013

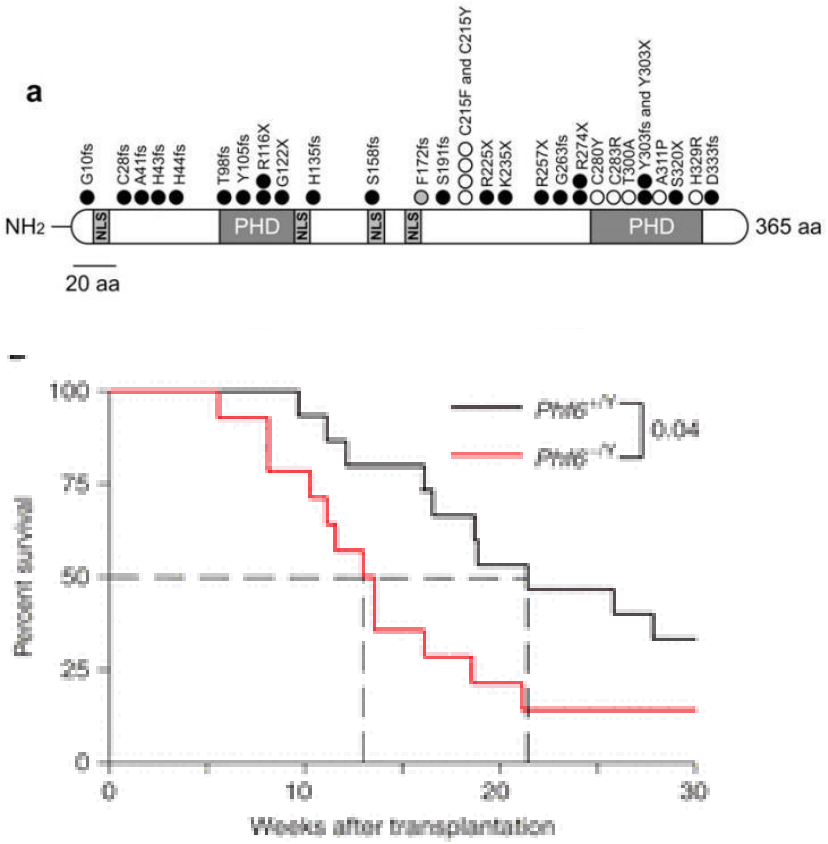
- Recurrent mutations in ribosomal proteins, most frequent in RPL10
- Forced expression of eIF4A translation initiation factor accelerates NOTCH1-induced leukemias
- Resveratrol, a small molecule inhibitor of eIF4A inhibits tumor growth in preclinical models

Epigenetic regulators in T-ALL



- **PRC2** (Polycomb repressive complex 2), responsible for transcriptional repression by adding H3K27me3. EZH2, EED and SUZ12 are are affected by loss of function mutations in 25% T-ALL. Frequently associated with mutations in NOTCH1.
- Loss of PRC2 activity might amplify the oncogenic effect of NOTCH1 by priming the NOTCH1 target genes for transcriptional activation
- **KDM6A** (UTX) targeted by loss of function mutations in 5-15% T-ALL

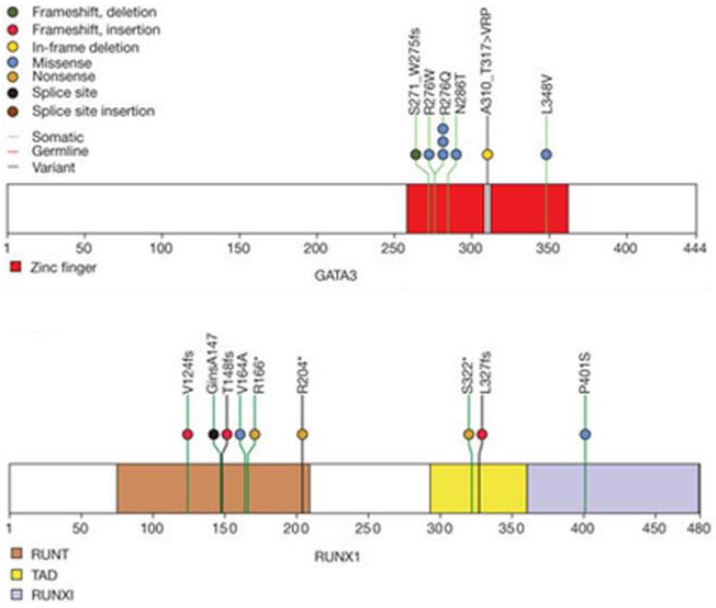
PHF6 epigenetic regulator



- **Loss of function** alterations in 16% pediatric and 38% adult T-ALL (also 3% of AMLs)
- Located in Xq26, mutations are found almost exclusively in males
- **Nucleolar protein i**mplicated in ribosomal biogenesis and splicing, chromatin remodeling and transcriptional regulation
- Phf6 loss enhances **HSC self-renewal** driving tumor initiation and leukemia stem cell activity in T-ALL

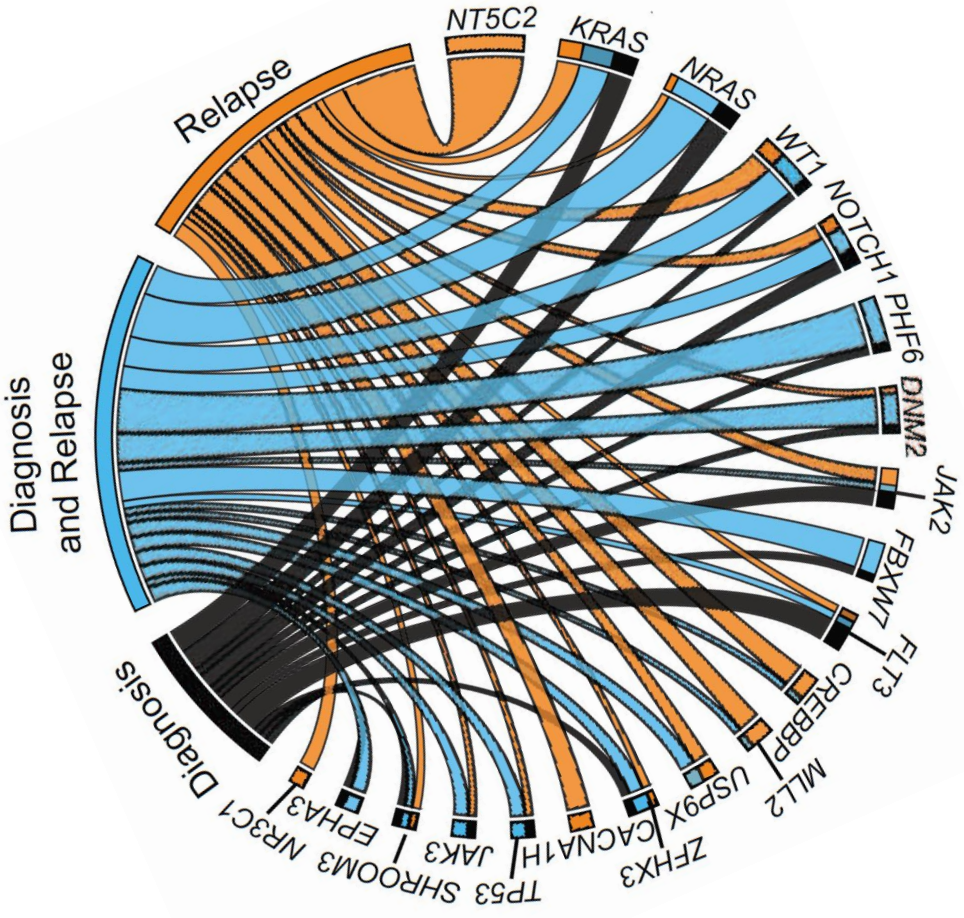
Van Vlierberghe *et al*., Nat Genet 2010
Wendorff *et al*., Cancer Discovery 2019

Transcription factor tumor suppressors in T-ALL



- **ETV6** (ETS transcriptional repressor) 25% of ETP-ALL (DN mutations)
- **RUNX1**, 5% ETP
- **GATA3,** recurrently mutated in ETP
- **BCL11B**, mutated in 10% T-ALL
- **LEF1**, mutated in 10-15% T-ALL; resembles TLX1 tumors
- **WT1**, mutated in 10%T-ALL (frequently associated with TLX1, TLX3 and HOXA oncogenes)

What are the mechanisms driving resistance in T-ALL?



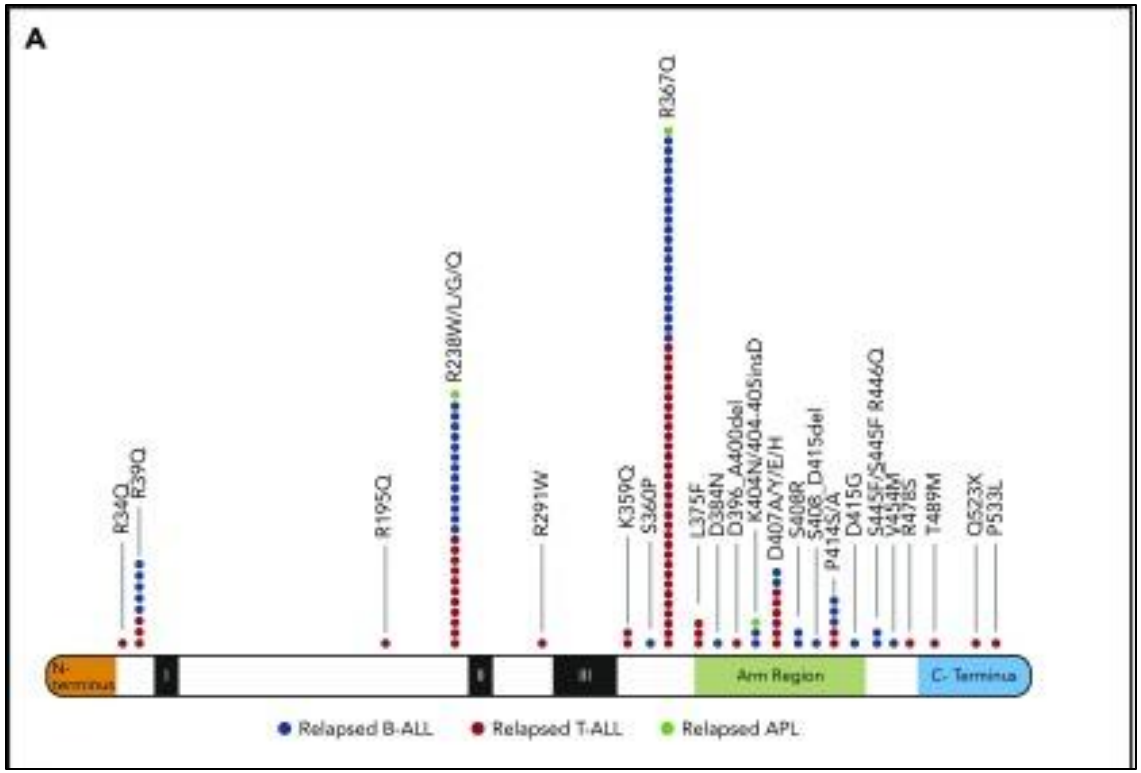
- Leukemia stem cells with intrinsic resistance to chemotherapy and self renewal potential drive relapse.

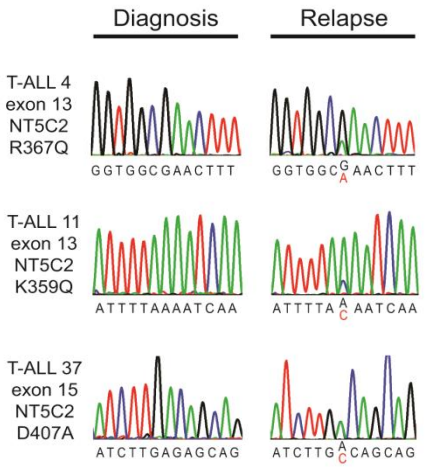
- Safe-heaven niches of stroma and microenvironment protect leukemia cells from chemotherapy

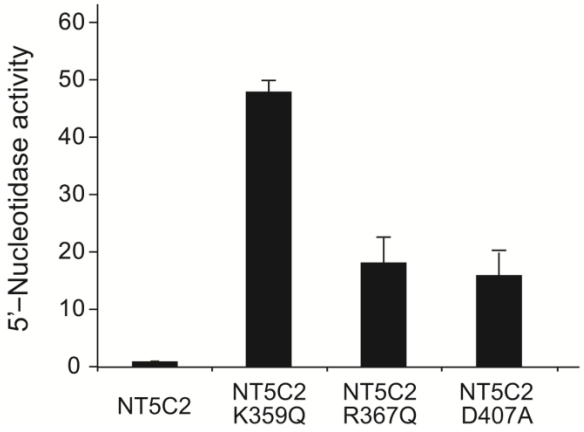
- Darwinian genetic selection driven by chemotherapy select highly resistant subclones responsible for disease progression and relapse

Oshima *et al*., PNAS 2016

Relapse associated NT5C2 mutations are gain of function alleles with increased nucleotidase activity

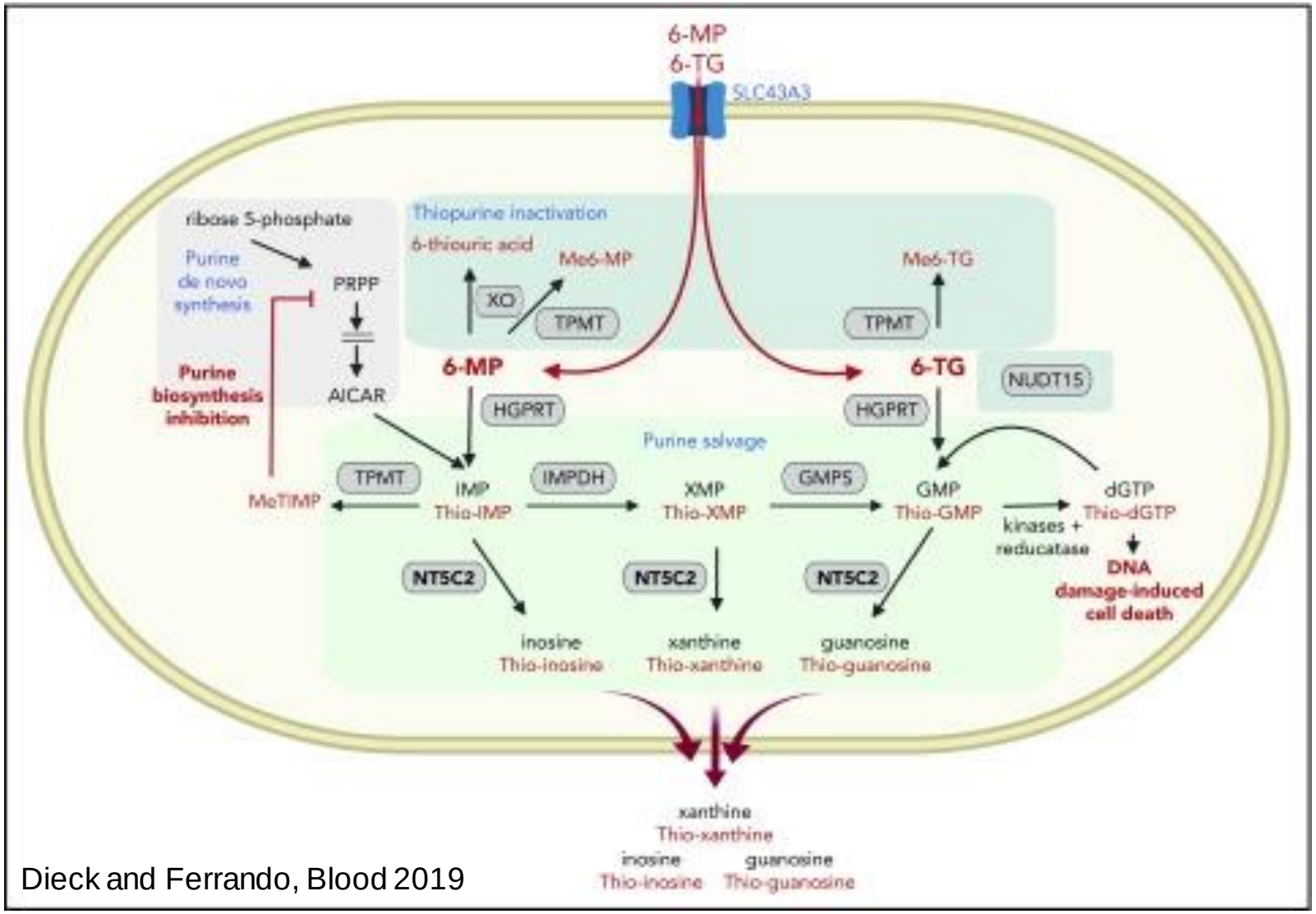






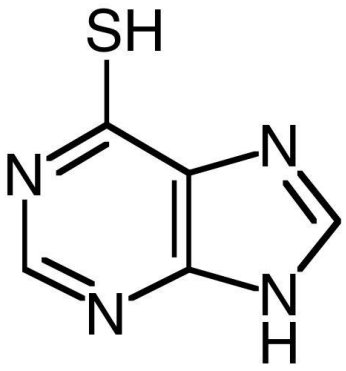
Dieck and Ferrando, Blood 2019

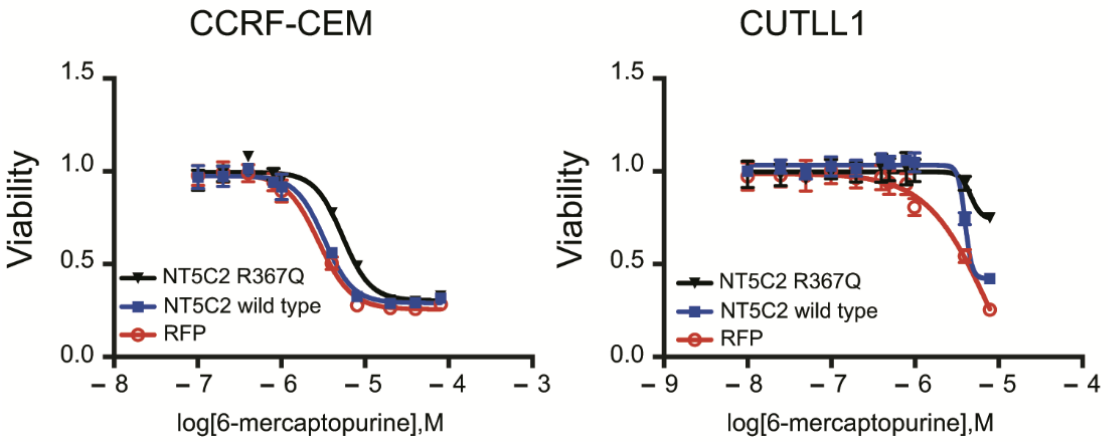
NT5C2 dephosphorylates and inactivates the active metabolites of 6-MP and TG



Dieck and Ferrando, Blood 2019

Relapse associated NT5C2 mutations confer resistance to 6-MP

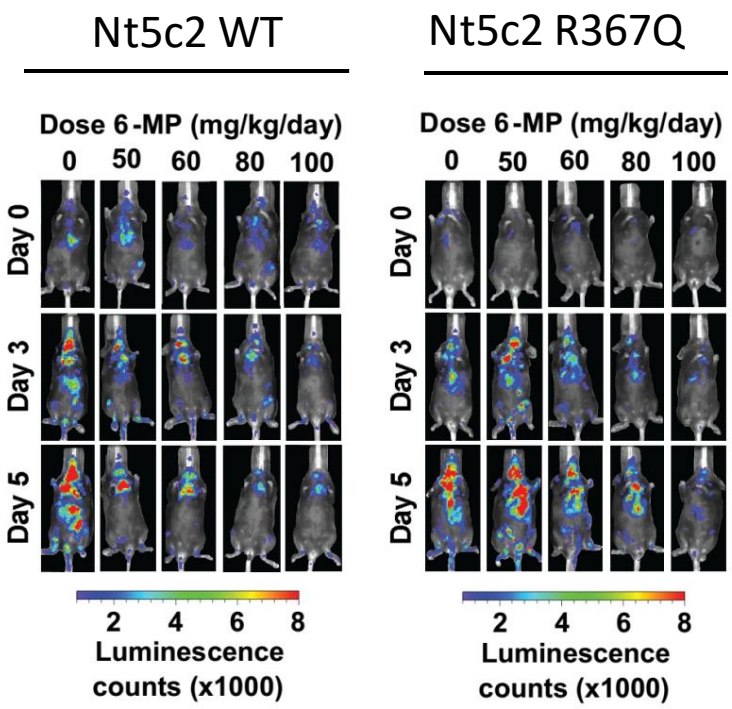


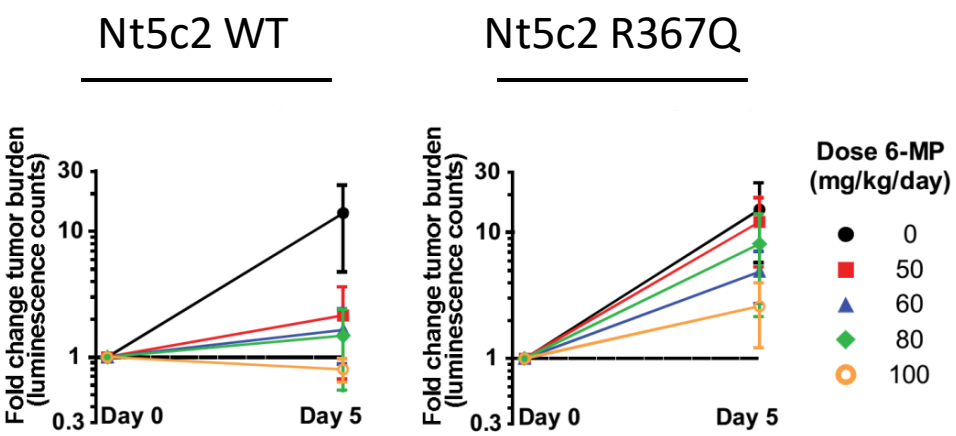


6-mercaptopurine IC50 (μM)

	CCRF-CEM	CUTLL1
RFP	3.07	0.71
NT5C2 WT	3.54	0.41
NT5C2 R367Q	5.57	> 10
NT5C2 R359Q	6.47	> 10
NT5C2 D407A	5.67	> 10

Expression of Nt5c2 R367Q induces resistance to 6-MP in vivo





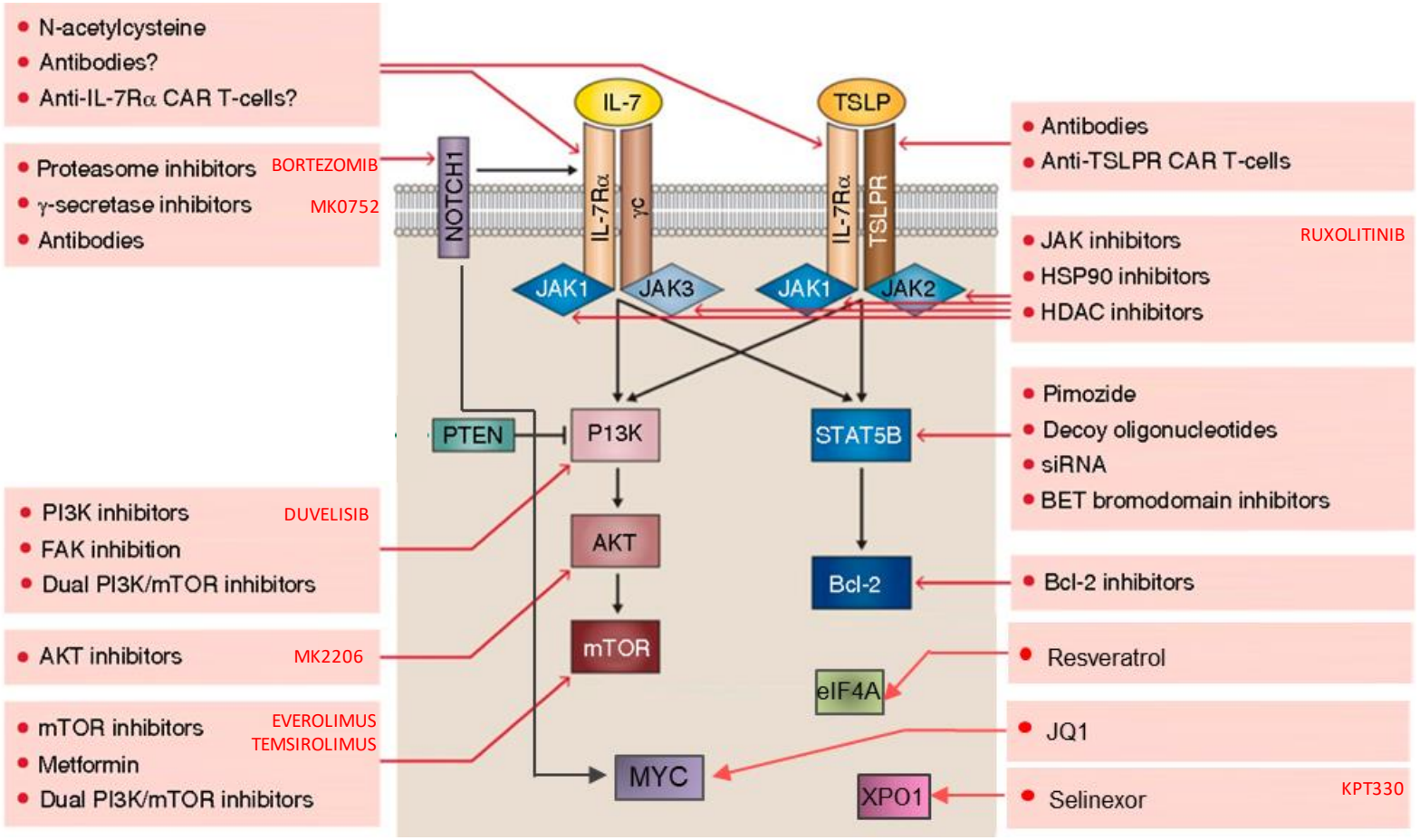
Tzoneva *et al*., Nature 2018
Dieck *et al*., Cancer Cell 2018
Dieck and Ferrando, Blood 2019

Clinical significance of NT5C2 mutations in T-ALL

- Relapse specific and associated with early relapse and progression under therapy
- More frequently associated with T-ALL (22%) than B precursor ALL (3%)
- Highlight the fundamental role of optimal 6-MP dose intensity in maintenance therapy
- May not interfere with nelarabine-based salvage therapies
- Maybe susceptible to small molecule inhibition

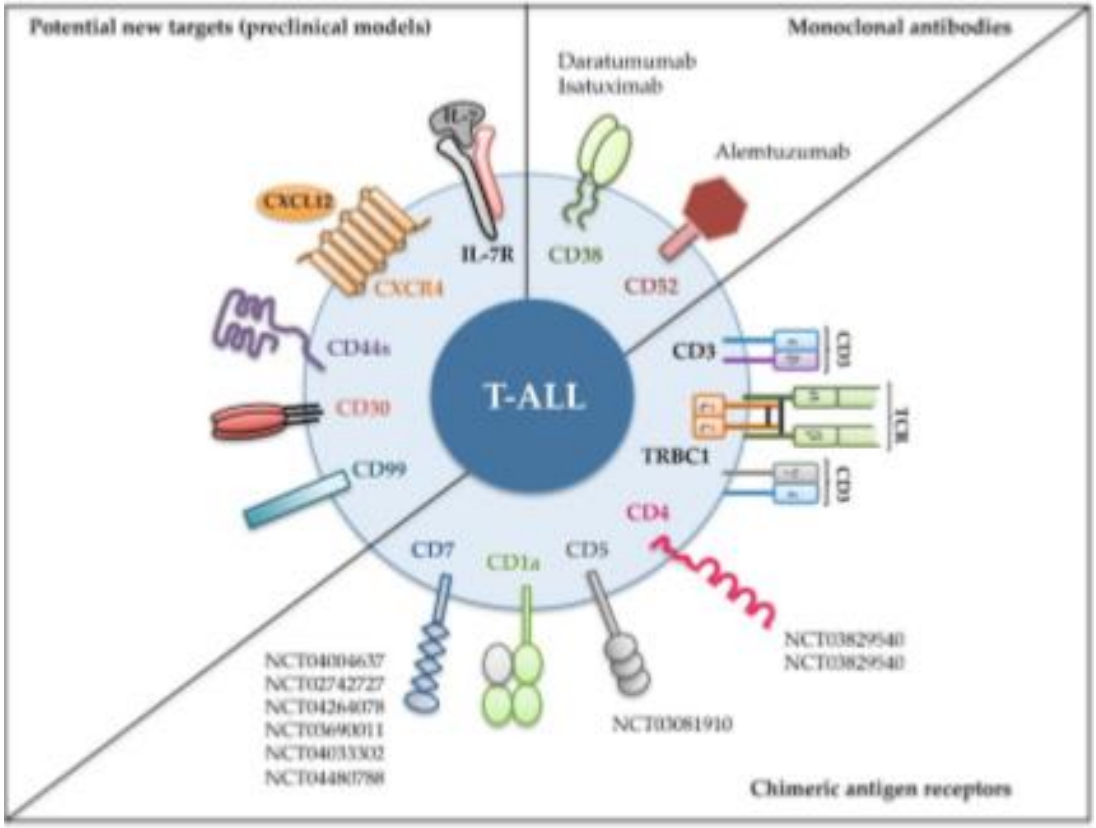
Tzoneva *et al*., Nature 2018
Dieck *et al*., Cancer Cell 2019

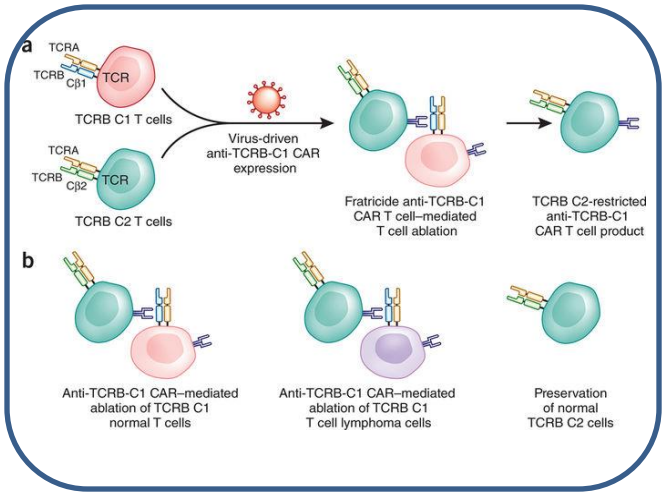
Targeted therapies for T-ALL



(modified from Cramer *et al*., Blood 2016)

Immunotherapy approaches for T-ALL





Targeted TCRB1 directed CAR T immunotherapy for T cell malignancies

Maciocia *et al*., Nat Med. 2017
Palomero and Ferrando, Nat Med. 2017
Bayon-Calderon *et al*., Int. J. Mol. Sci. 2020

Conclusions

- T-ALL are lymphoid malignancies that derive from the transformation of **T-cell precursors**.
- T-ALL is primarily a **NOTCH1 driven disease** in which developmentally important **transcription factor oncogenes** define distinct clinical and biological groups.
- Introduction of **precision medicine** approaches has substantially improved our understanding of the **classification and therapeutic vulnerabilities** in T-ALL.
- **PTEN/PI3K/AKT** pathway play important roles in resistance to glucocorticoids and to gamma secretase inhibitors
- **NT5C2** mutations are relapse specific and associated with early relapse and progression under therapy

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