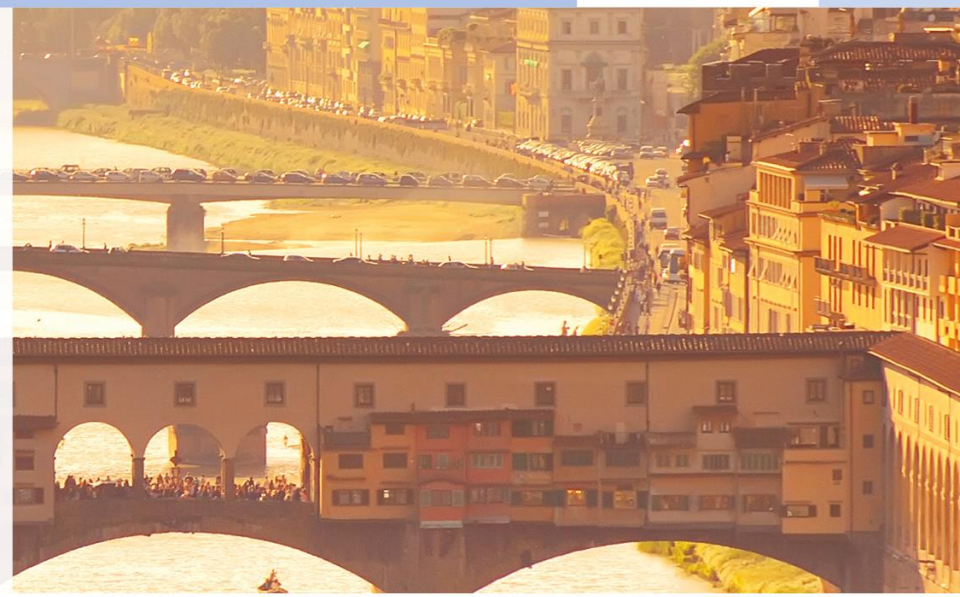
8° WORKSHOP IN EMATOLOGIA TRASLAZIONALE

DELLA SOCIETÀ ITALIANA DI EMATOLOGIA SPERIMENTALE

Firenze - Auditorium CTO - A.O.U. Careggi, 22-23 giugno 2023



Il background genomico delle leucemie acute linfoblastiche a cellule T: identificazione di specifici bersagli terapeutici

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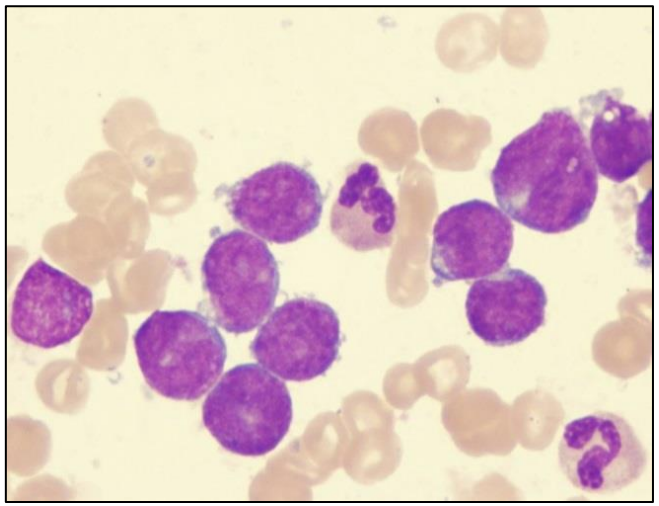
Disclosures of Name Surname

Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other
NOTHING TO DISCLOSE							

T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA (T-ALL)

- **Aggressive neoplasia of thymocytes accounting for 15% of pediatric and 25% of adult ALL cases**
- **In children, intensive chemotherapy results in a high overall survival (90%). Current treatment, however, is frequently complicated by long-lasting side effects**
- **Patients who do not reach remission or experience early relapse (10-15% of children and 40-50% of adults), have a very poor outcome**



Morphology
Flow Cytometry
Pathology
Immunoistochemistry
Genomics….?

→

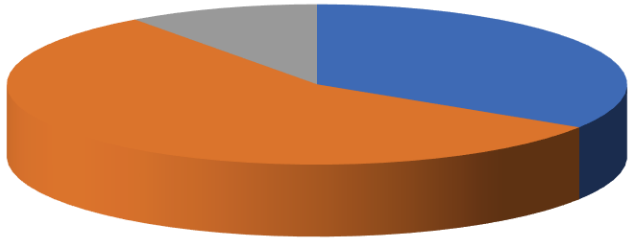
ETP-ALL (myeloid/stem cell markers)
Early (CD5)
Cortical (CD1a)
Mature (sCD3)

T-ALL MULTI-STEP PATHOGENESIS

CELL CYCLE

TP53,RB,p27 *CDKN2A/CDKN2B* *C-MYC* *CCND2*

SELF-RENEWAL CAPACITY

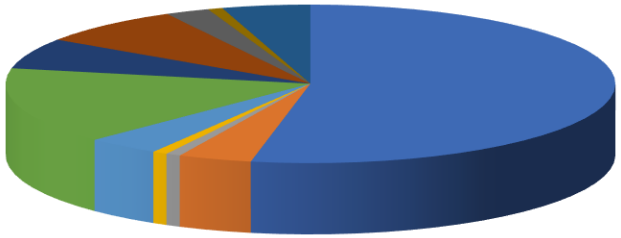


UNKNOWN *NOTCH1* *FBXW7*

MULTI-STEP PATHOGENESIS

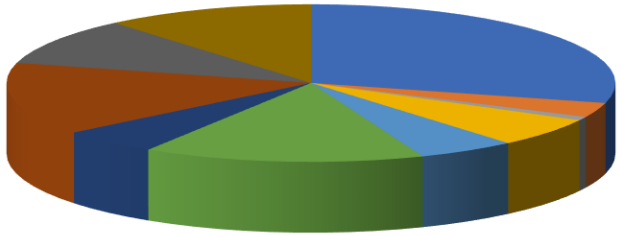
concurrent co-operative events

PROLIFERATION AND SURVIVAL



UNKNOWN *N-RAS* *LCK* *ETV6*
FLT3 *PTEN* *ABL1* *JAK1*
NF1 *LCK* *PTPN2*

DIFFERENTIATION



TAL1+LMO1/2 *MLL* *TAL2*
CALM-AF10 *TLX1* *TLX3*
HOXA *LYL+LMO2* *BCL11B*
LEF1

MULTIPLE GENES AFFECTED

adapted from De Keermaecker K et al, 2005

THE GENOMIC LANDSCAPE OF T-ALL

SUBTYPE-DEFINIG GENE

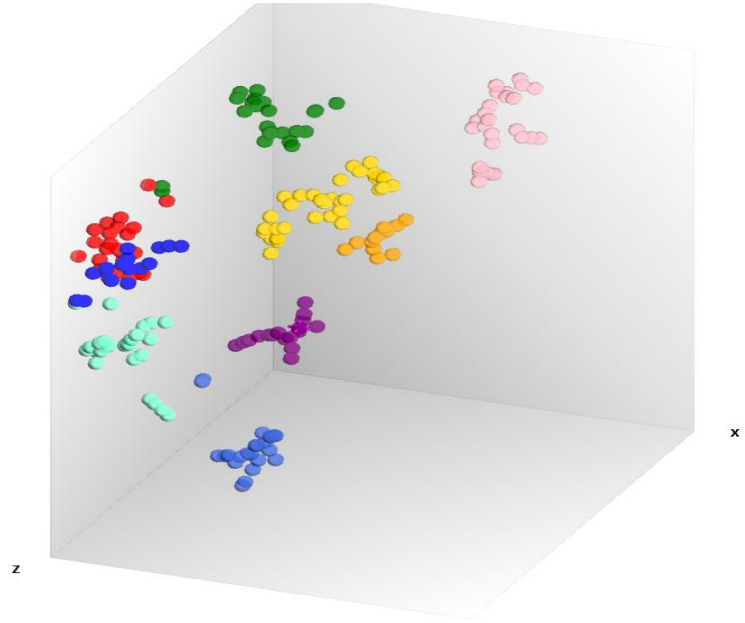
Oncogenes that control

- **thymocyte development**
- **maturation**
- **differentiation**

- **DISTINCT LEUKEMOGENIC PATHWAY**
- **MUTUALLY EXCLUSIVE**
- **SPECIFIC TRANSCRIPTOME PROFILE**

GENETIC SUBGROUPS

- ***HOXA*** [HOXA, *MLLT10*, *NUP214*, NUP98, *KMT2A, ZFP36L2*]
- ***TLX3***
- ***TLX1***
- ***TAL/LMO*** [*TAL1*, *TAL2*, *LMO1*, *LMO2, LMO3*]
- ***NKX2.1***
- ***MEF2C***
- ***BCL11B***
- ***SPI1***



Seki M et al, Nature Genetics 2017; Bardelli V et al, Genes, 2021

THE GENOMIC LANDSCAPE OF T-ALL

ADDITIONAL ALTERATION

Genes regulating/modulating

- **signaling pathways**
- **proliferation**
- **survival**
- **epigenetics**

COOPERATING EVENTS THAT CO-OCCUR WITH BOTH PRIMARY AND OTHER ADDITIONAL ALTERATIONS

***CDKN2A/B* deletion (9p-)**

- >70% of T-ALL
- loss of cell-cycle control

***NOTCH1* mutations**

- >50% of T-ALL
- Thymocyte specification and development

LEF1, CASP8AP2, TP53, RB1, ETV6, TCF7, EED, EZH2, PTEN, MYC, MYB, CDKN1B, CCND2, SUZ12, RUNX1, PHF6, RPL10, RPL22, JAK2……. (1%-10%)

Bardelli V et al, Genes, 2021

T-ALL: PHENOTYPIC and GENETIC SUBTYPES

STAGE OF BLAST DIFFERENTIATION

ETP/near ETP	early	cortical	mature
CD34 and/or CD33,CD13,CD117 CD5-/+	CD5+	CD1a+	sCD3+

SUTYPE-DEFINING GENES

ETP/near ETP	early	cortical	mature
HOXA *MEF2C* *BCL11B*	*HOXA* *TLX3*	*TLX1* *NKX2.1./2.2* *TLX3* *TAL/LMO*	*TAL/LMO*

ADDITIONAL ALTERATIONS

ETP/near ETP	early	cortical	mature
RAS PATHWAY ***(N/KRAS, NF1)*** ***JAK/STAT SIGNALING*** ***(JAK1/2/3, STAT5B)*** ***EPIGENETIC MODULATION*** ***(PHF6,SUZ12,EZH2)***	***NOTCH SIGNALING*** ***TCF7, ABL1***	***NOTCH SIGNALING*** ***BCL11B, RB1, CDKN1B*** ***ABL1***	***PI3K-AKT PATHWAY*** ***(PTEN)*** ***MYC, CASP8AP2, LEF1***

NOTCH1/FBXW7 mutations

CDKN2AB deletions

MAIN CHALLENGES for the GENETIC CLASSIFICATION of T-ALL

Rare leukemia (centralized studies are necessary)

Extremely heterogeneous background (remarkable inter-leukemia diversity)

- Multiple alterations (many concurrent driver events)
- Diverse mechanisms of gene deregulation (comprehensive studies)

Not sufficient evidence to establish genetically defined subtypes with clinical relevance

Alaggio R et al. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Lymphoid Neoplasms. Leukemia 2022

Table 2. WHO Classification of Haematolymphoid Tumours, 5th edition: T-cell and NK-cell lymphoid proliferations and lymphomas.

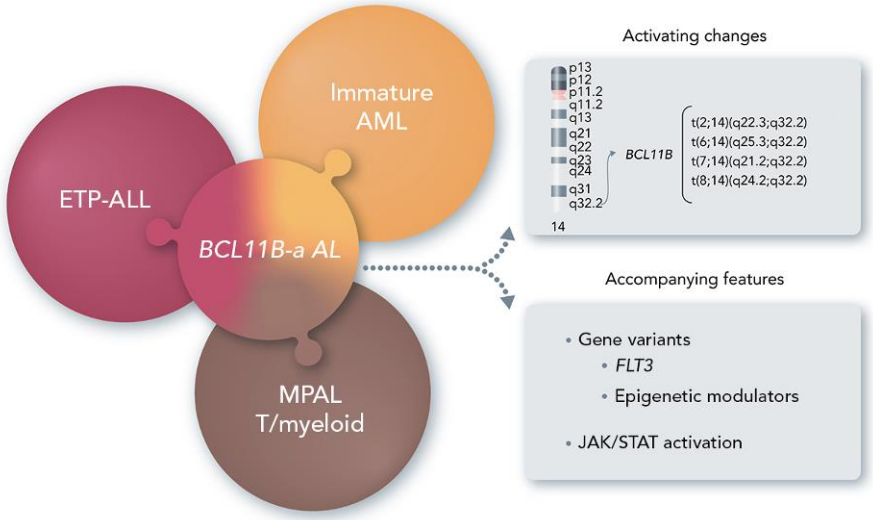
WHO Classification, 5th edition	WHO Classification, revised 4th edition
Precursor T-cell neoplasms	
T-lymphoblastic leukaemia/lymphoma	
T-lymphoblastic leukaemia / lymphoma, NOS	T-lymphoblastic leukaemia/lymphoma
Early T-precursor lymphoblastic leukaemia / lymphoma	Early T-cell precursor lymphoblastic leukaemia

INTERNATIONAL CONSENSUS CLASSIFICATION (ICC 2022)

International Consensus Classification of Myeloid Neoplasms and Acute Leukemia: Integrating Morphological, Clinical, and Genomic Data

T-ALL classification:

- **Early T-cell precursor ALL (ETP) with *BCL11B*-R**
- **Early T-cell precursor ALL, NOS**
- **T-ALL, NOS**



Provisional entities: Suppl Table 7

T-ALL/LL				
Subtype	Frequency	Partner genes/other rearrangements	Immunophenotype	Comment
HOXA dysregulated	15-25%	*HOXA*::TRB/TRG; *KMT2A*-Rearranged; *PICALM*::*MLLT10*; *SET*::*NUP214*	Immature, some ETP	
SPI1 rearrangement	<5%, children	*STMN1*; *TCF7*; *BCL11B*	CD4-, CD8+/-, DR+	Very poor prognosis
TLX1 rearrangement	5-10% children; near 30% adult	TCR	CD4+, CD8+/-,CD1a+, cortical thymocyte	Good prognosis
TLX3 rearrangement	20-25% children <5% adult	TCR; *BCL11B*; *CDK6*	CD4+, CD8+/-, CD1a+, cortical thymocyte, some ETP or near ETP	Good prognosis; BCL11B overexpression different from ETP group
NKX2 rearrangement	<5% children	*NKX2.1/NKX2.2/NKX2.5*::TCR; *BCL11B*; *CDK6*	CD4+, CD8+	Similar GEP to *TLX1*-R
TAL1-2 rearrangement	30-40% (TAL2 rare)	TRA/D; TRB (*TAL2*); 1p32 deletion (*STIL*); intergenic SNV (super enhancer)	CD3+, late cortical	Poor prognosis
LMO1-2 rearrangement	*LMO1*-R -5% *LMO2*-R 10%	TCR; cryptic deletion; enhancer/promoter mutations	Immature but not-ETP	Form LMO complex with bHLH factors. Extremely high LMO expression.
BHLH, other	<2%	*LYL1*::TRB *OLIG2/BHLHB1*::TCR	Immature but not ETP	Extremely high LMO expression *LYL1*-R shows stem cell-like signature

Di Giacomo D et al, Blood 2021; Arber D et al, Blood 2022

MOLECULAR-CYTOGENETIC MARKERS WITH PUTATIVE PROGNOSTIC-PREDICTIVE VALUE

GOOD:

TLX1 (genetic subgroup)

NOTCH1

CDKN2AB

POOR:

HOXA (genetic subgroup)

CASP8AP2

MYC

PTEN

RUNX1

IKZF1

TP53

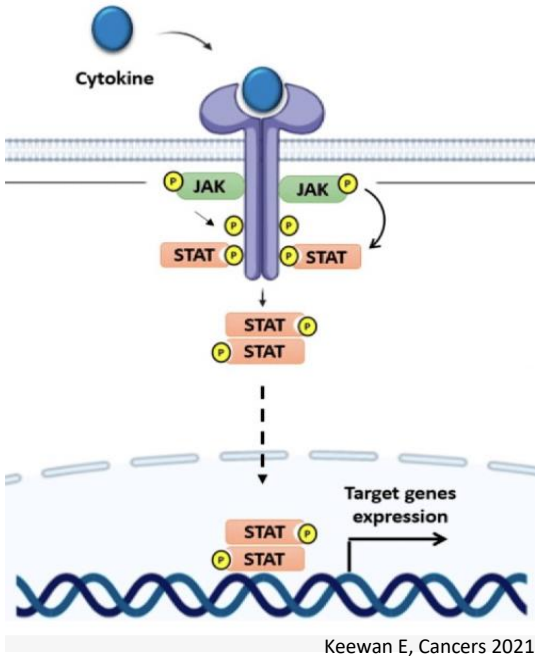
JAK/STAT pathway

RAS pathway

Beldjord K et al, Blood 2014; Gianfelici V et al, Heamatologica 2016; Petit A et al, Blood 2018; Bardelli V et al, Cancer 2021; Simonin M et al, Blood 2021; Simonin M et al Blood 2023

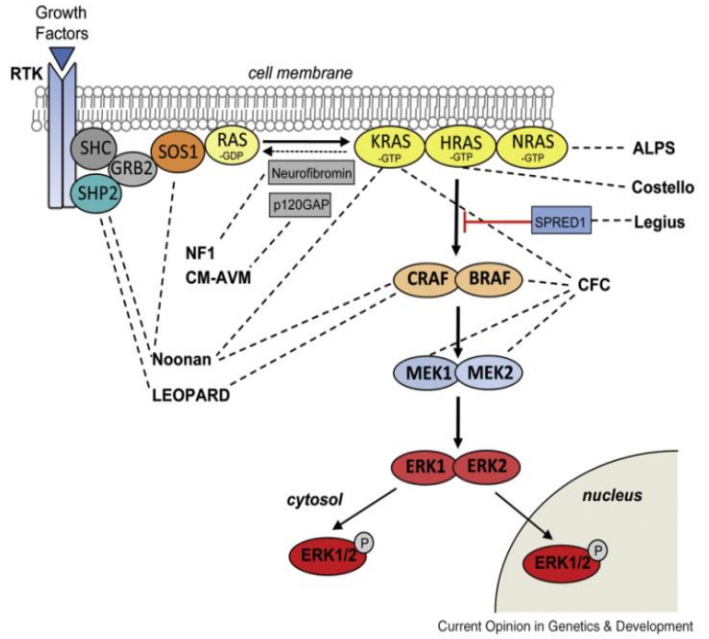
PATHWAYS

JAK/STAT



Keewan E, Cancers 2021

RAS



JAK/STAT ACTIVATION:

- ~25% of T-ALL
- Common in chemorefractory/early relapse
- ETP-ALL
- *TLX1*, *TLX3* and *HOXA* subgroups

RAS ACTIVATION:

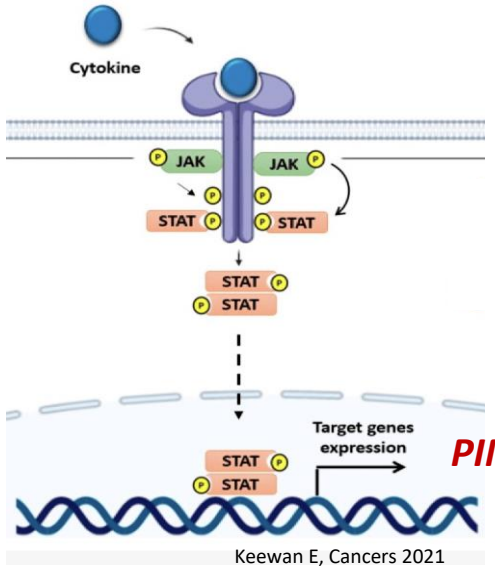
- ~15% of T-ALL
- Common in relapsed disease
- ETP-ALL
- *HOXA* subgroup

Zhang J et al, Nature 2012; Gianfelici V et al, Heamatologica 2016; Liu Y et al, Nature 2017; Bardelli V et al, Genes 2021

JAK/STAT activation in T-ALL

Abnormalities that activates JAK/STAT pathway

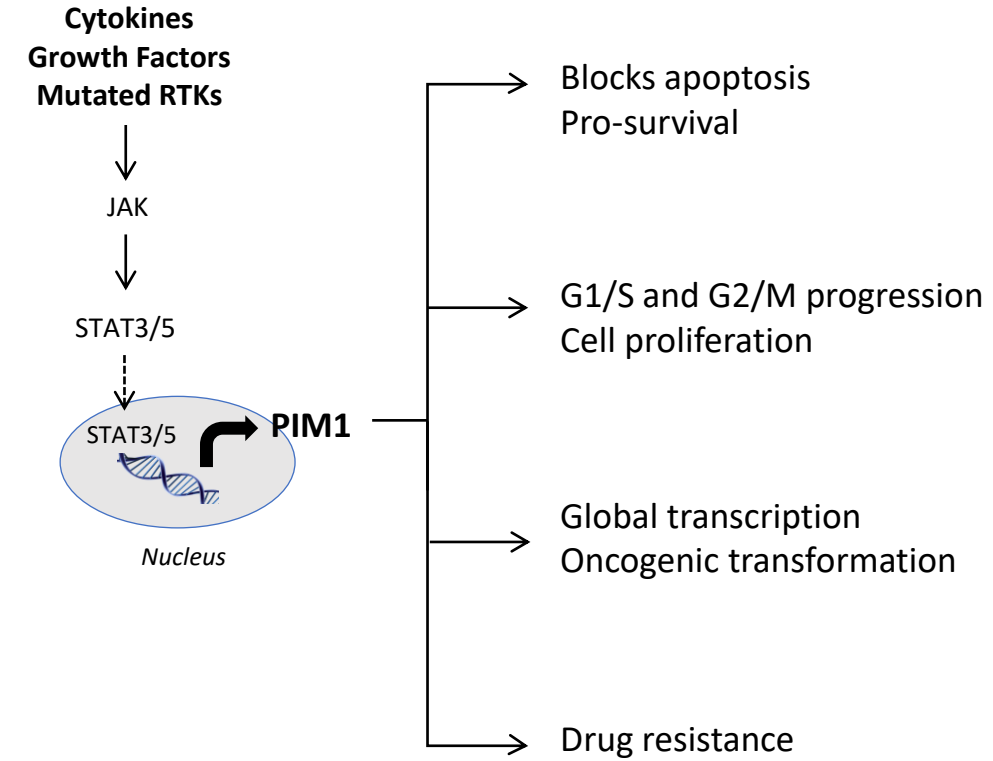
- *IL7R*, *JAK1*, *JAK3* and *STAT5B* gain-of-function mutations
- *PTPN2* loss-of-function mutations
- *PTPN2* deletions
- *DNM2* loss-of-function mutation



Keewan E, Cancers 2021

PIM1 oncogene

- Site of murine T-cell lymphomas retroviral insertion



Zhang J et al, Nature 2012; Gianfelici V et al, Heamatologica 2016; Liu Y et al, Nature 2017; La Starza R et al, Leukemia 2018; Bardelli V et al, Genes 2021

PIM1 is a putative oncogene in T-ALL

COHORT

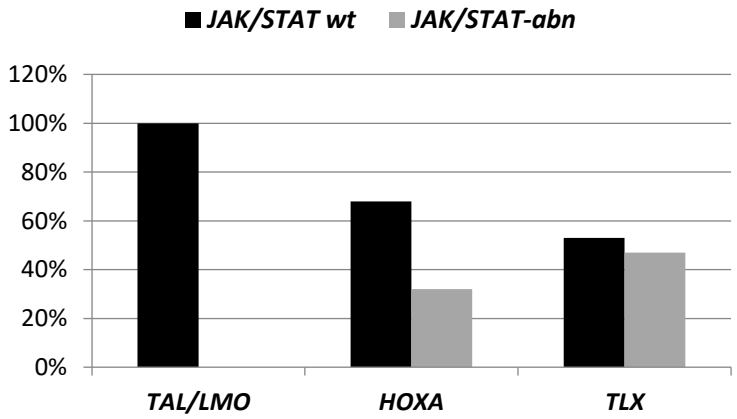
Cases: 96

All ADULTS

Males/Females: 71/25

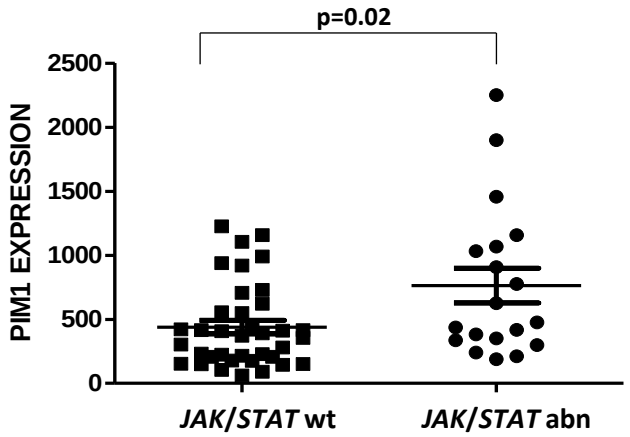
***JAK/STAT* abnormalities: ~30%**

(PTPN2, NUP214-ABL1, JAK2, JAK3, STAT5B, IL7R)



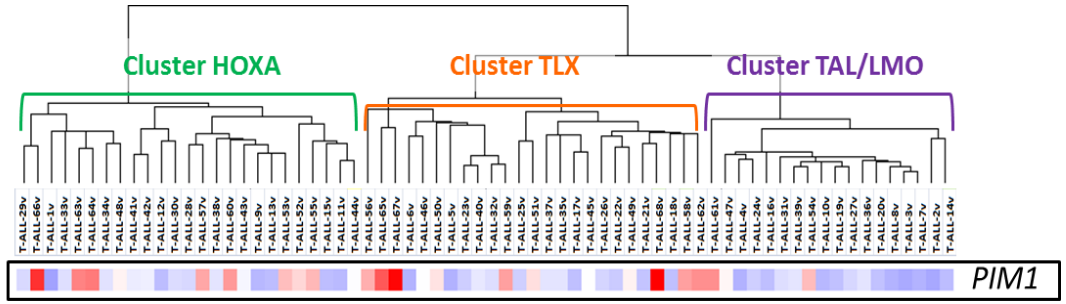
INTEGRATED GENOMIC ANALYSIS:

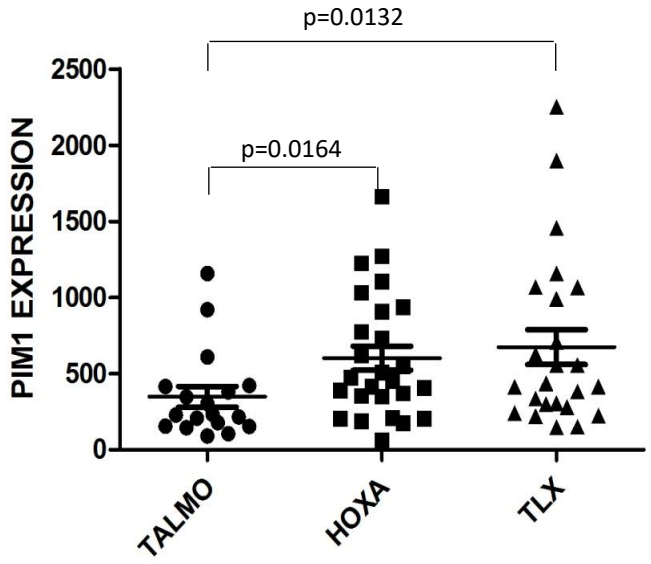
- **CI-FISH** (multiplex genomic clones)
- **SANGER SEQUENCING**
- **WHOLE TRANSCRIPTOME EXPRESSION ARRAY**
  (Human Clariom S)



La Starza R et al, Leukemia 2018

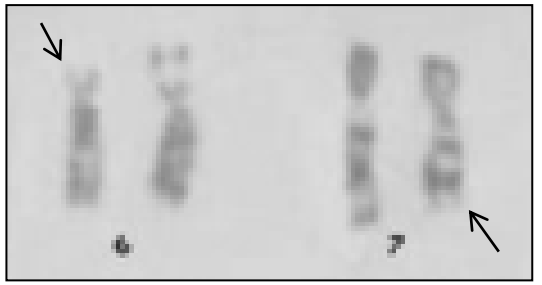
PIM1 and GENETIC GROUPS

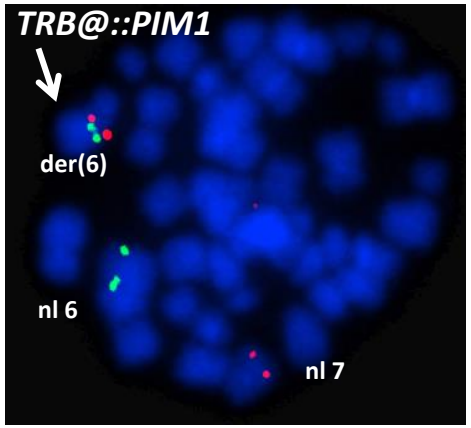


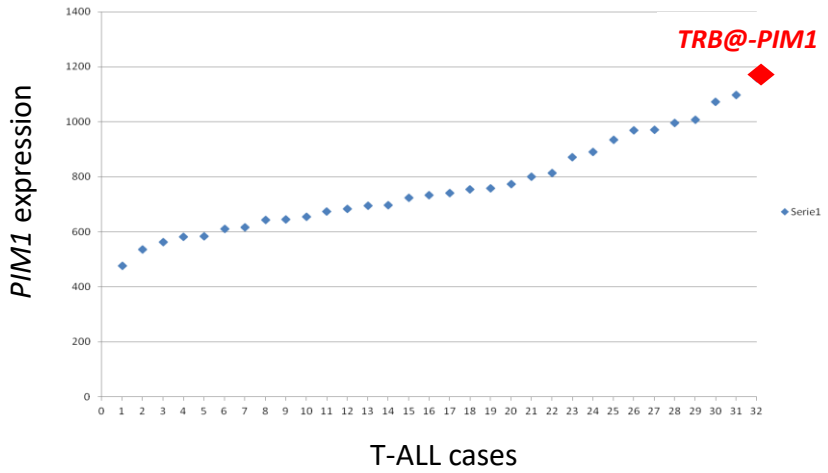


PIM1 REARRANGEMENTS

t(6;7)(p21;q34)/TRB@::PIM1 (<1%)

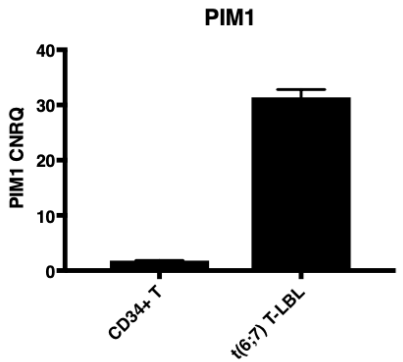






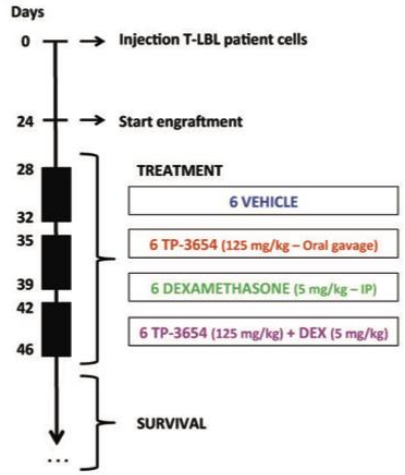
PIM1 as terapeutic target

***TRB@::PIM1* trasnlocation: t(6;7)(p21;q34)**

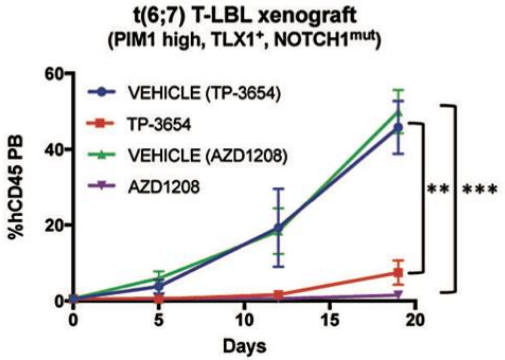


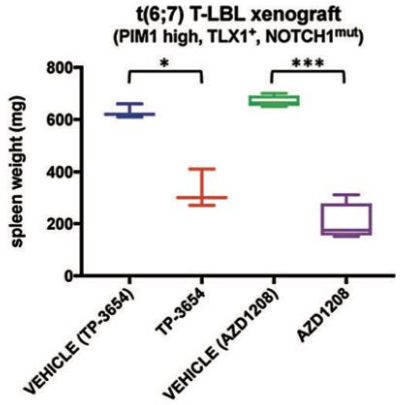
Pediatric T-LBL
TLX1+
NOTCH1+

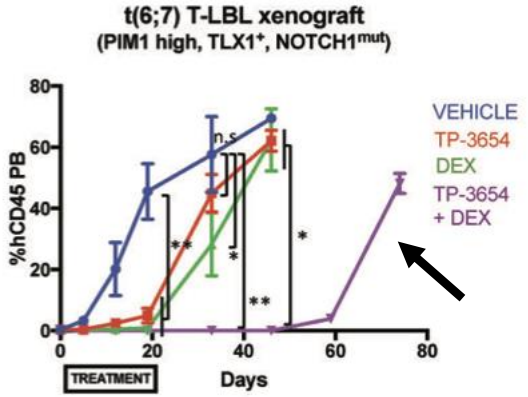
PIM* inhibition and dexamethasone combination therapy *in vivo

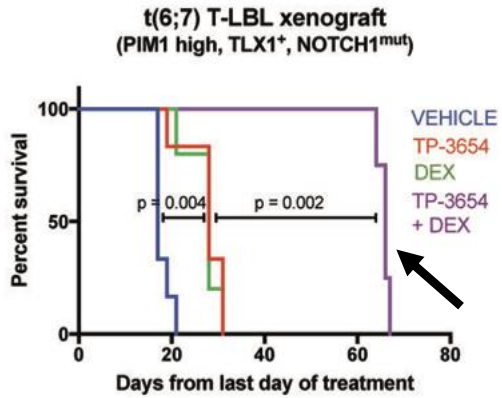


***In vivo* drug evaluation of second-generation pan-PIM inhibitors AZD1208 and TP-3654**









PIM inhibitors caused a significant delay in tumor development

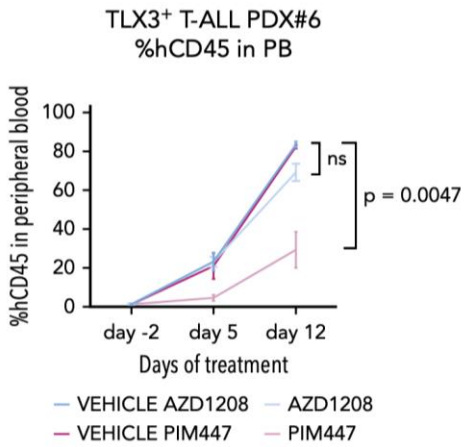
delayed leukemic blast espansion

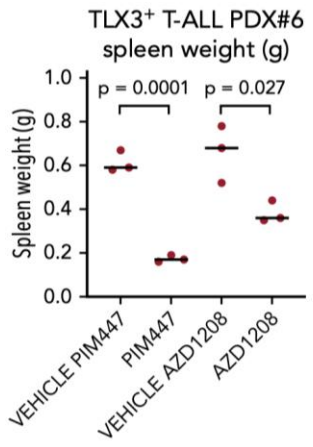
significant increase in survival

De Smedt R et al, Haematologica 2018

PIM1 as terapeutic target

PIM1 inhibition in *IL7R* high expression cases (*IL7R*+)





➡ tumor cells sensitive to in vivo *PIM* inhibition

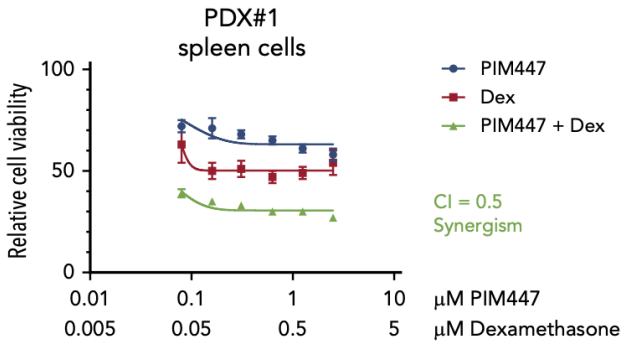
***In 30% T-ALL/T-LBL PIM1 is* actionable**

- ***JAK/STAT* activation**
- **t(6;7)(p21;q34)/*TRB@::PIM1***

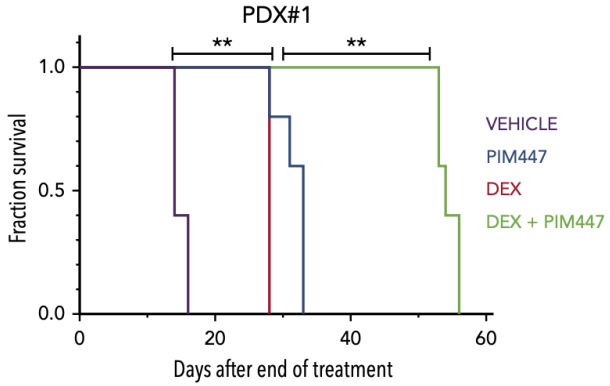
***PIM1* inhibitor TP3654: CLINICAL TRIAL ONGOING IN MIELOFIBROSIS (NCT04176198)**

Combination of PIM447 and dexamethasone in *IL7R*+

Ex vivo



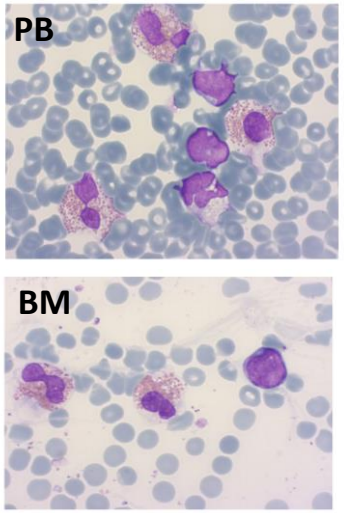
In vivo

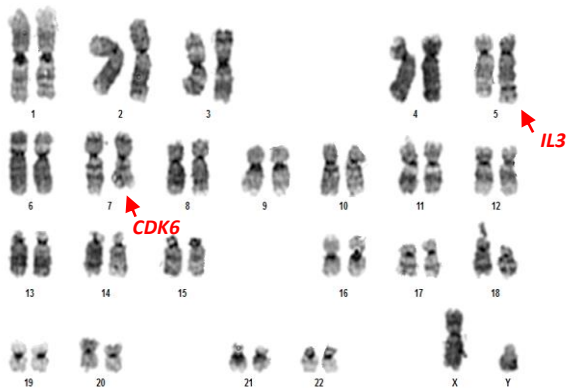


➡ **Improvement of survival in a PDX model of *IL7R*+ T-ALL**

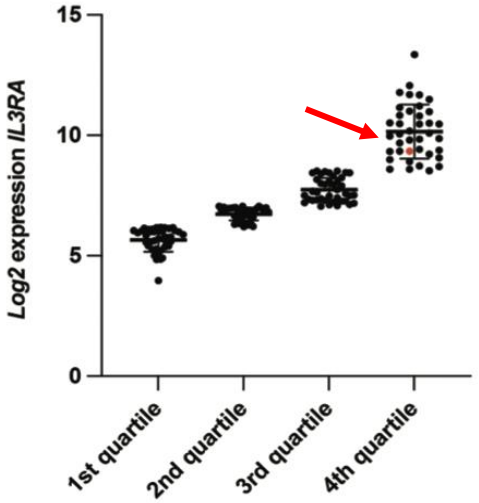
t(5;7)(q31;q21)/*CDK6::IL3*

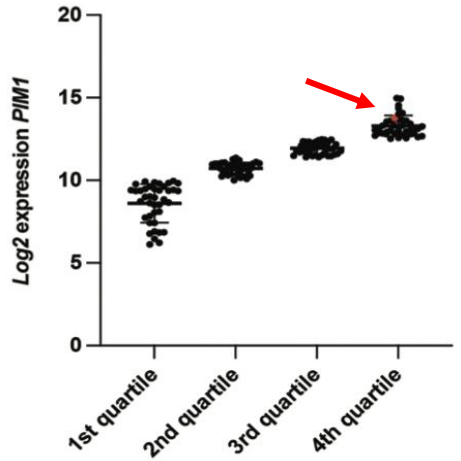
Eosinophilia

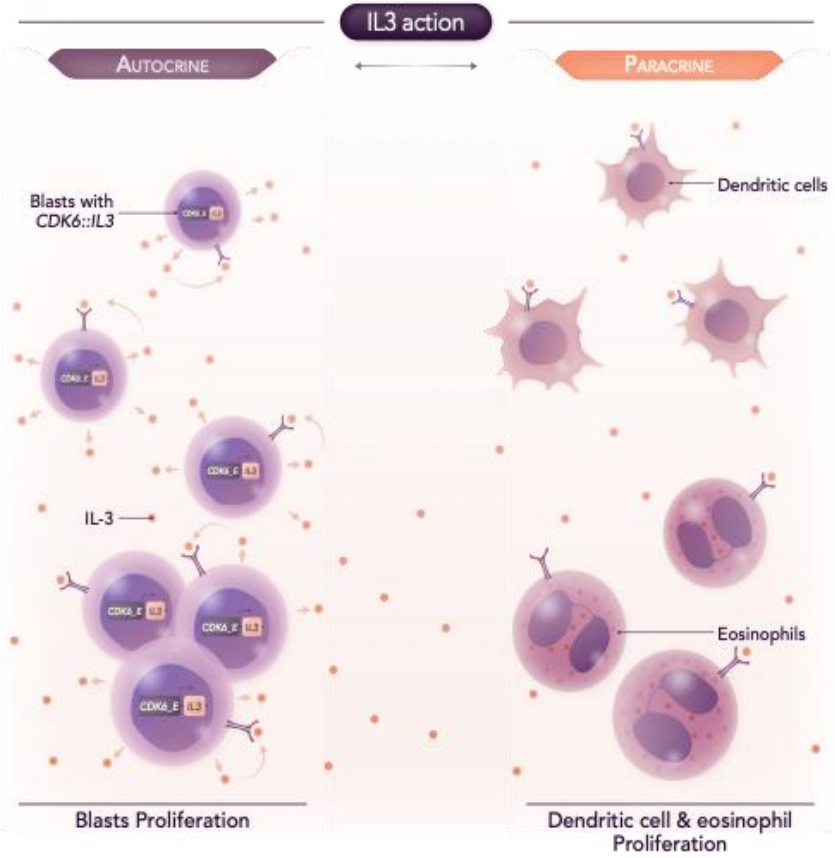




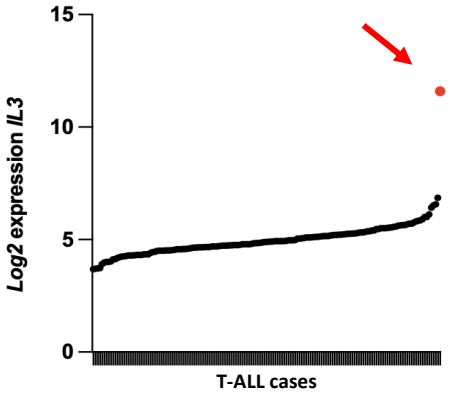
IL3-IL3RA-JAK/STAT signaling

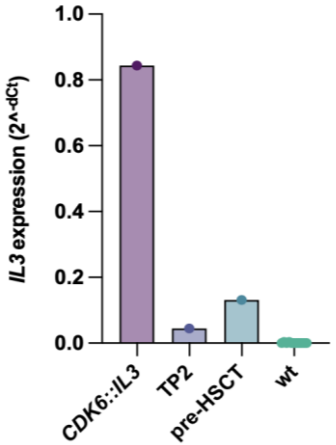






IL3 expression



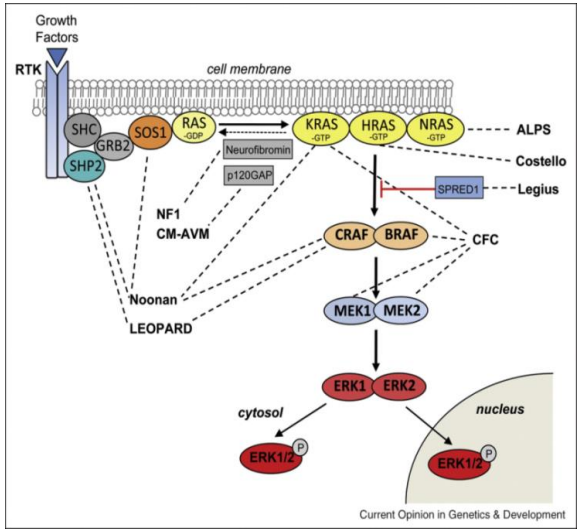


Pierini V et al, Hemasphere, 2022

RAS activation in T-ALL

Abnormalities that activates RAS pathway

- *KRAS/NRAS, BRAF, FLT3* gain-of-function mutations
- *NF1* loss of function mutations
- *PTPN11 deletions*
- *NF1* deletions



OUR STUDY

Patients: 125

Males/Females: 96/29

Adults/Children: 111/14

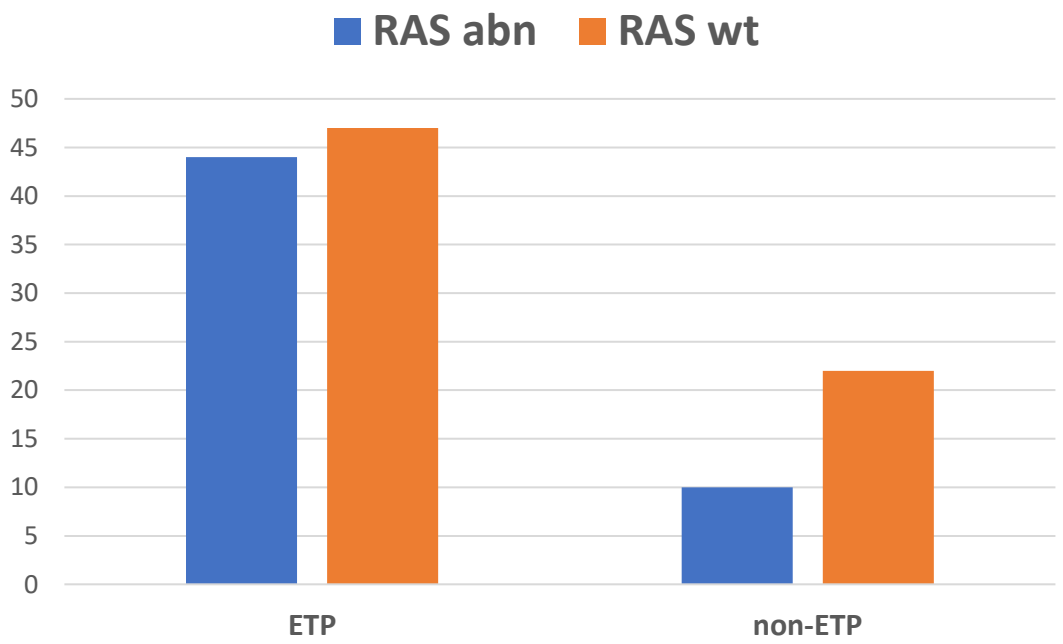
ETP/non-ETP: 91/32

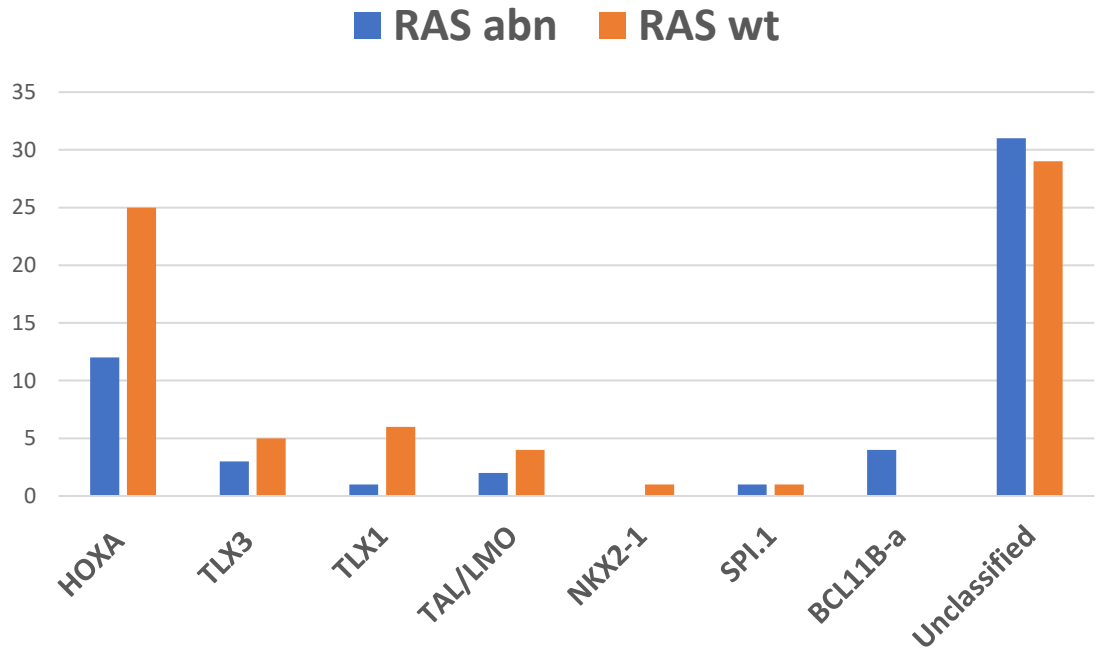
INTEGRATED GENOMIC ANALYSIS:

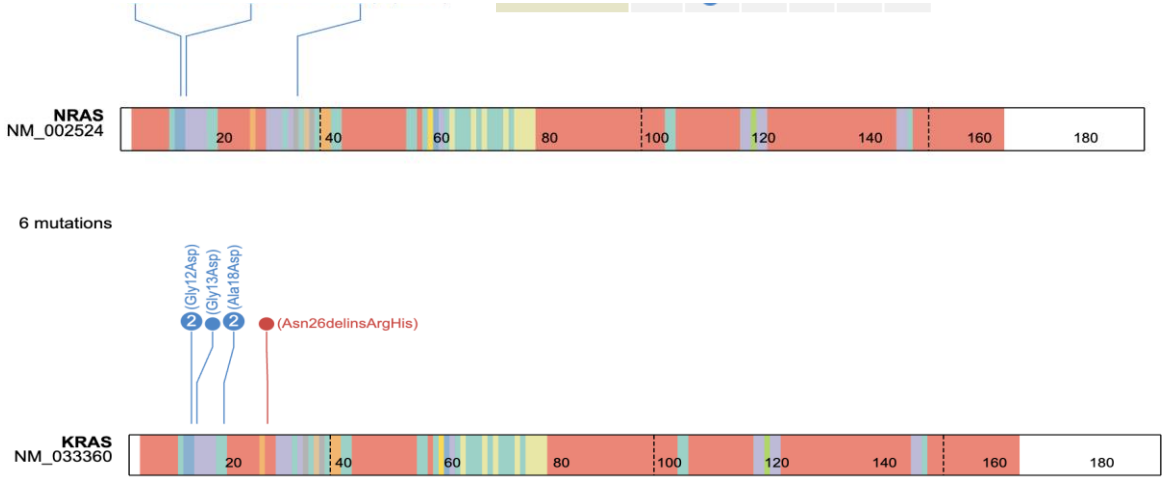
- **CI-FISH** (multiplex genomic clones)
- **SNPa** (CytoScan HD, ONCOSCAN)
- **TARGETED NGS** (SOPHiA Genetics Custom Panel)
- **WHOLE TRANSCRIPTOME EXPRESSION ARRAY** (Human Clariom S)

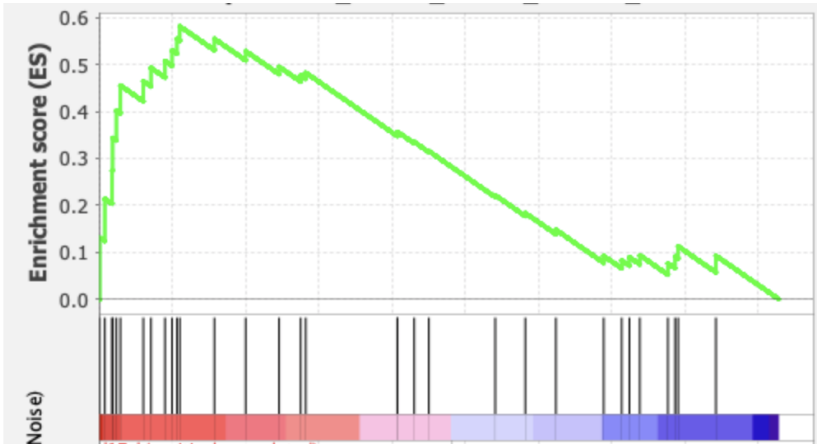
Zhang J et al, Nature 2012; Oshima K et al, PNAS 2016; Liu Y et al, Nature 2017; Bardelli V et al, Genes 2021

RAS PATHWAY ABN: 43%





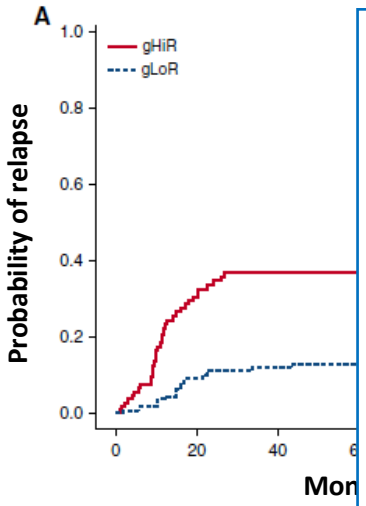




LEUKEMIA-SPECIFIC PROGNOSTIC MARKERS: RISK STRATIFICATION

N/K RAS and/or PTEN: HIGH RISK T-ALL

CHILDREN



Leukemia (2021) 35:3394–3405
https://doi.org/10.1038/s41375-021-01291-5

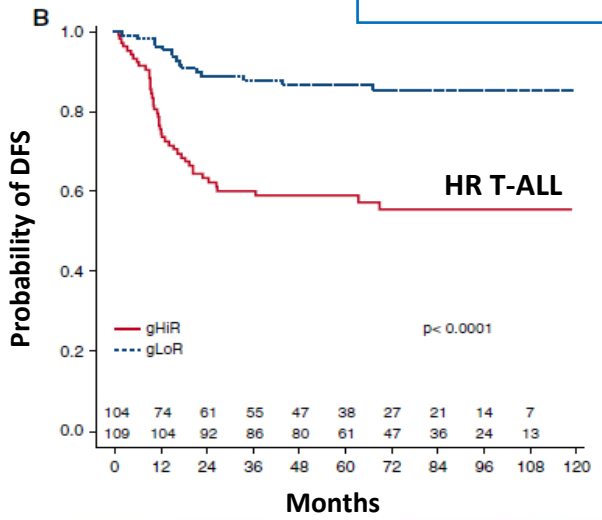
ARTICLE

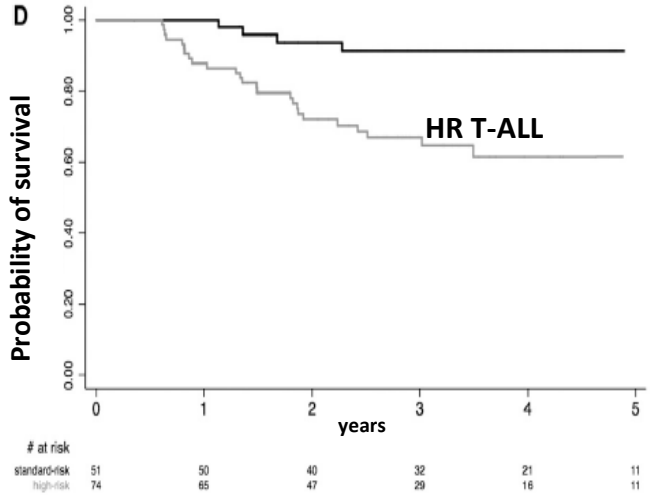
Acute lymphoblastic leukemia

MAPK-ERK is a central pathway in T-cell acute lymphoblastic leukemia that drives steroid resistance

ADULTS

Patients:158
Age range: 15-59
Treatment: GRAALL2003-5





Beldjord K et al, Blood 2014; Petit A et al, Blood 2018; Van der Zwet JCG, Leukemia 2021

CONCLUSION

DIAGNOSTIC WORKFLOW:

Integrated molecolar-cytogenetics and targeted NGS:

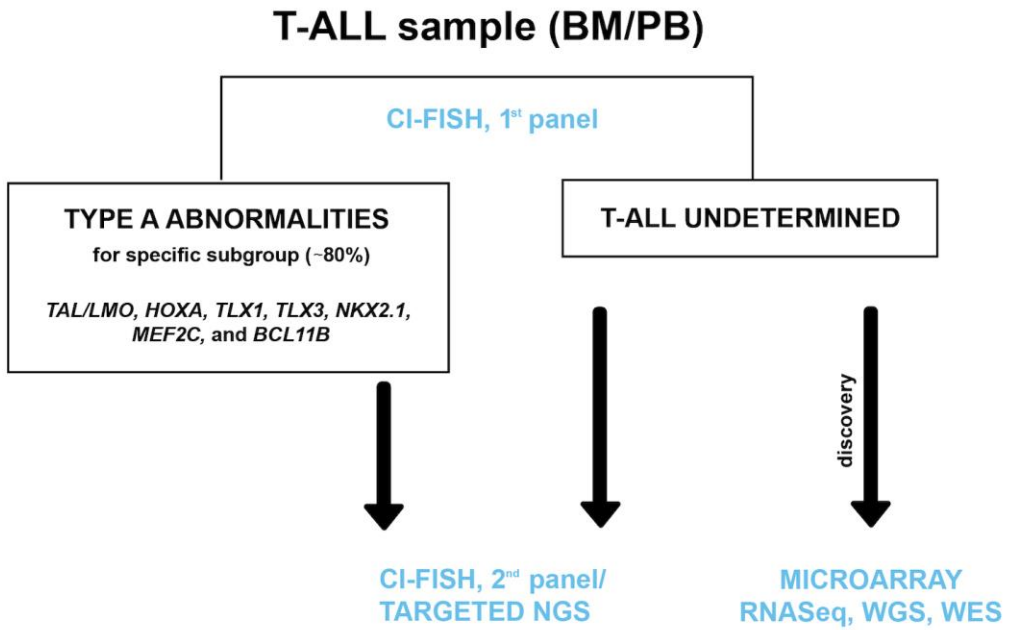
- **define the genetic classification of leukemia**
- **identify cytogenetic markers and gene variants**
- **identify deregulated pathways**

PERSPECTIVE:

Prospective clinical trial to assess:

- **Prognostic value of markers**
- **Predictivity of molecular targets**

WORKFLOW



RELEVANT TYPE B: CNA, REARRANGEMENTS, VARIANTS

(>90%)

- ***NOTCH1, FBXW7*** *(NOTCH signaling)*
- ***PTEN, AKT, PIK3R1*** *(PI3K/AKT/mTOR pathway)*
- ***N/KRAS, BRAF, NF1, PTPN11, FLT3*** *(RAS pathway)*
- ***LEF1, FYN, MYC*** *(Beta-catenin)*
- ***BCL11B. TCF7, WT1, ETV6, IKZF1*** *(T-cell differentiation)*
- ***CDKN2AB, CDKN1B, RB1,TP53*** *(Cell cycle)*
- ***KDM6A, PHF6, SUZ12, EZH2, EED, DNMT3A*** *(Epigenetic modulation)*
- ***IL7R, STAT5B, JAK1/3, PTPN2, PIM1*** *(JAK/STAT signaling)*
- ***ABL1, ABL2, PDGFRB, PSGFRA*** *(ABL-class protein: proliferation, migration, apoptosis)*

Bardelli V et al, Genes 2021

In-vitro/*Ex-vivo* preclinical studies:
Prednisolone + Selumetinib

NGS

SOPHIA Custom Bundle Solution (CHEMA_B_V1, Sophia Genetics)

Genes: AKT1, ATM, BCL11B, BRAF, CCND3, CNOT3, CREBBP, CTCF, DNM2, EED, EP300, ETV6, EZH2, FAT1, FAT3, FBXW7, FLT3, GATA3, GLI1, GLI2, GLI3, IKZF1, IL2RB, IL7R, JAK1, JAK3, KDM6A, KMT2D, KRAS, LEF1, LMO1, (non-coding region), *LMO2* (non-coding region), *MED12, MYB, NF1, NOTCH1, NRAS, NT5C2, PHF6, PIK3CD, PIK3R1, PTCH1, PTEN, RELN, RPL10, RPL22, RPL5, RUNX1, SETD2, SH2B3, SMARCA4, SMO, STAT5B, SUZ12, TAL1* (non-coding region), *TP53, TYK2, USP7, USP9X,* and *WT1*