Les leucémies aiguës lymphoblastiques en 2015 : contribution des facteurs de risque cytogénétiques et moléculaires à une thérapeutique adaptée

Aline Tanguy Schmidt

Thèse de Doctorat

Aline TANGUY SCHMIDT

Mémoire présenté en vue de l'obtention
du **grade de Docteur de l'Université d'Angers**
Université Nantes Angers Le Mans

École doctorale: Biologie de la santé

Discipline: Biologie cellulaire
Unité de recherche: CRCNA INSERM U 892 équipe 7

Soutenue le 14 décembre 2015
Thèse n°108484

Les leucémies aiguës lymphoblastiques en 2015: contribution des facteurs de risque cytogénétiques et moléculaires à une thérapeutique adaptée

JURY

Rapporteurs:	**Jean-Yves CAHN, PU-PH, CHU de Grenoble, Université de Grenoble** **Hervé DOMBRET, PU-PU, AP-HP Hôpital Saint Louis, Université Paris Diderot**
Examinateur:	**Valérie UGO, PU-PH, CHU Angers, Université d'Angers**
Invité:	**Mathilde HUNAULT-BERGER, PU-PH, CHU Angers, Université d'Angers**
Directeur de Thèse:	**Norbert IFRAH, PU-PH, CHU Angers, Université d'Angers**
Co-directeur de Thèse:	**Oliver OTTMANN, Professeur, Institute of Cancer/Genetics University School of Medicine Cardiff**

Université Nantes Angers Le Mans

Ecole doctorale
Biologie de la santé

Doctorat
Biologie cellulaire

Aline TANGUY SCHMIDT

Les leucémies aiguës lymphoblastiques en 2015: contribution des facteurs de risque cytogénétiques et moléculaires à une thérapeutique adaptée

Acute leukemia lymphoblastic in 2015: contribution of the oncogenic and molecular risk factors to an adapted treatment

Thèse dirigée par Norbert IFRAH
Soutenue le 14 décembre 2015 à Angers

ENGAGEMENT DE NON PLAGIAT

Je, soussigné, Aline Tanguy Schmidt déclare être pleinement consciente que le plagiat de documents ou d'une partie d'un document publiés sur toutes formes de support, y compris l'internet, constitue une violation des droits d'auteur ainsi qu'une fraude caractérisée.
En conséquence, je m'engage à citer toutes les sources que nous avons utilisées pour écrire cette thèse de doctorat.

Signature : Aline Tanguy Schmidt

Remerciements

Une thèse n'étant jamais un travail solitaire, ce travail est le fruit d'échanges et de collaborations. Merci à tous ceux et celles qui ont jalonné son parcours.

Aux membres du Jury

A Monsieur le Professeur Jean-Yves Cahn, vous qui me faites l'honneur de présider ce jury de thèse, de juger et de rapporter ce travail.

A Monsieur le Professeur Hervé Dombret, vous me faites l'honneur d'accepter de rapporter ce travail de thèse. Merci de m'avoir permis de travailler avec le GRAALL.

A Madame le Professeur Valérie Ugo, tu as accepté d'être membre du jury de cette thèse. Merci pour tes conseils avisés.

A Monsieur le Professeur Norbert Ifrah
Merci de m'avoir accordé votre confiance depuis toutes ces années.
Sans votre soutien, ce travail n'aurait pas abouti.
Merci pour votre encadrement clinique et scientifique.

A Madame le Professeur Mathilde Hunault-Berger
Merci de m'avoir soutenue et donné l'envie de persévérer.
Merci pour tes encouragements constants.
Merci tout simplement.

A Monsieur le Professeur Oliver Ottmann
Vous m'avez accueillie au sein de votre laboratoire à Frankfort.
Je vous remercie de la confiance que vous m'avez accordée.

A Monsieur le Professeur Vahid Asnafi, à Amélie Trinquant
Merci de m'avoir permis de travailler avec vous.

A Toute l'équipe de l'unité INSERM U 892, à Céline Beauvillain, à Yves Delneste, à Pascale Jeannin et à Isabelle Pellier
Merci m'avoir accueillie au sein de votre équipe, merci pour vos conseils.

A L'ensemble de l'équipe du service des maladies du sang du CHU d'Angers
J'apprécie de travailler avec vous. Merci pour votre aide.

A l'ensemble de l'équipe du laboratoire d'hématologie du CHU de Frankfort
Merci pour votre aide et votre disponibilité.
Annette, Sylvia et Heike, merci de m'avoir guidée pour la réalisation de toutes les techniques. Merci pour la bière et la "curry wurst".

A Tous ceux qui sont toujours là, bienveillants, toujours à l'écoute, sans jugement
Erig, Karine, Joëlle, Jean-Pierre, Hildegard, Patricia, Fabrice, Marie-Pierre, Damien, Séverine, Christine, Séverine, Céline

A Oliver, à Katell et Guilhem
Mon amour, mes amours
Merci pour votre amour, votre présence, votre patience et votre indulgence

Abréviations

ABL gène Abelson
ARN Acide ribonucléique
ATX autotaxine
BCR Break point Cluster Region; gène BCR
CSH cellules souches hématopoïétiques
CSP cellules souches périphériques
ERK Extracellular signal-Regulated Kinase
ETP-ALL Early Thymic Precursor Acute Lymphoblastic Leukemia
Grb2 Growth factor receptor-bound protein 2
GRAALL Group for Research in Adult Acute Lymphoblastic Leukemia
ITK inhibiteur de tyrosine kinase
K-RAS Kristen-RAS
LA leucémie aiguë
LAL leucémie aiguë lymphoblastique
LAL Ph1 leucémie aiguë lymphoblastique à chromosome Philadelphie
LAL T leucémie aiguë lymphoblastique de la lignée T
LAM leucémie aiguë myéloblastique
LMC leucémie myéloïde chronique
LPA lysophosphatidic acid
LPAR LPA recepteur
lysoLPA lysophospholipase D
MAPK Mitogen Activated Protein Kinase
m-BCR minor Breakpoint Cluster
M-BCR major Breakpoint Cluster
MLL Mixed-Lineage Leukemia
MRD maladie résiduelle
mTOR Mammalian Target Of Rapamycine
NFkB Nuclear Factor-kappa B
N-RAS Neuroblastoma-RAS
NOTCH1 Neurogenic locus notch homolog protein 1
PI3K Phosphatidyl Inositol 3-Kinase
PDGFR Platelet Derived Growth Factor Receptor
PTEN phosphatase and tensin homologue deleted on chromosome 10
Ras Rat Sarcoma
RC rémission complète
S1P sphingosine 1-phosphate
SH2 Src homology
TCR récepteur T à l'antigène
TK tyrosine kinase

Sommaire

A - Introduction

I - Généralités

Les leucémies aiguës (LA) de l'adulte représentent un groupe très hétérogène d'hémopathies malignes aiguës dues à la transformation oncogénique de cellules souches hématopoïétiques (CSH).[1] La prolifération maligne qui en résulte est caractérisée par une expansion clonale de précurseurs hématopoïétiques immatures. Selon l'origine du précurseur impliqué, précurseur de la lignée myéloïde ou lymphoïde (lignée B ou T), on distingue les leucémies aiguës myéloblastiques (LAM) et les leucémies aiguës lymphoblastiques (LAL, LAL B ou T). Les principales conséquences de cette prolifération sont l'installation d'un tableau d'insuffisance médullaire pouvant associer une neutropénie fébrile, un syndrome anémique et un syndrome hémorragique et l'apparition d'un syndrome tumoral. Les présentations cliniques sont très variables.

La classification des LA repose sur des critères morphologiques, l'expression de marqueurs membranaires et pour les LAL l'étude des réarrangements des gènes d'immunoglobulines ou du récepteur T. Ces éléments permettent de caractériser le stade de différenciation des cellules leucémiques. Il existe 2 systèmes de classification des LAL: le système de classification franco-américano-britannique (classification FAB) de 1976 qui classe le sous-type de LAL selon la forme et la structure de la cellule et le système de l'OMS (WHO classification). La classification de l'OMS est devenue le système de classification des LAL le plus utilisé, il classe les LAL en sous-type selon le type de lymphocyte (cellule B ou T), le degré de maturité des cellules leucémiques (précurseurs ou matures) et les anomalies cytogénétiques associées (tableau n°1). Le diagnostic des LA repose de ce fait sur un bilan sophistiqué combinant des études cytologiques, immunophénotypiques, cytogénétiques et moléculaires.[2]

Les LA sont rares, elles représentent 1% des cancers. L'incidence globale est faible, en moyenne de 3 pour 100 000 habitants/an dans les pays occidentaux, mais considérablement variable selon le type de LA et l'âge (15 à 20/100 000 après 60 ans pour les LAM).

Chez l'enfant, les LAL sont néanmoins le premier type de cancer (25% des cancers pédiatriques) et les plus fréquentes des LA. En revanche après 15 ans, leur fréquence intrinsèque se réduit ainsi que leur proportion au sein des LA (< 1% des cas de cancers adultes avec 20 % de LAL contre 80% de LAM). Cette incidence des LAL chez l'adulte est de 1 à 2/100 000 habitants et s'accroît à nouveau après 50 ans. La maladie a donc une distribution bimodale avec un premier pic chez les enfants de 2 à 5 ans (80% des LA dont 80% de LAL B) et un second pic chez les adultes autour de 50 ans.[3-5]

La proportion des LAL T est environ deux fois moindre chez les enfants (10-15%) que chez les adultes (20-30%).[5,6]
Comme dans la grande majorité des cancers, les LAL ne relèvent pas d'une cause unique mais d'une série d'altérations successives combinatoires. Moins de 5 % d'entre elles sont liées à des facteurs génétiques associés à des processus d'instabilité génomique tel que le syndrome de Down, la neurofibromatose, le syndrome de Shwachman, l'ataxie télangiectasie, le syndrome de Wiskott-Aldrich et le syndrome de Klinefelter. De nombreux agents (notamment les rayons X avec exposition prénatale, le benzène et l'oxyde d'éthylène) sont également incriminés dans leur génèse, mais leur responsabilité est régulièrement contestée.[7,8] Seules les études épidémiologiques impliquant les rayonnements ionisants chez les survivants des bombes atomiques de 1945 ne semblent pas contestables.[9]

Tableau n°1: Classification OMS des LA

WHO classification of acute leukemia

Acute myeloid leukemia and related neoplasms
- Acute myeloid leukemia with recurrent genetic abnormalities
  - AML with t(8;21)(q22;q22); *RUNX1-RUNX1T1*
  - AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*
  - APL with t(15;17)(q22;q12); *PML-RARA*
  - AML with t(9;11)(p22;q23); *MLLT3-MLL*
  - AML with t(6;9)(p23;q34); *DEK-NUP214*
  - AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); *RPN1-EVI1*
  - AML (megakaryoblastic) with t(1;22)(p13;q13); *RBM15-MKL1*
  - *Provisional entity: AML with mutated NPM1*
  - *Provisional entity: AML with mutated CEBPA*
- Acute myeloid leukemia with myelodysplasia-related changes
- Therapy-related myeloid neoplasms
- Acute myeloid leukemia, not otherwise specified
  - AML with minimal differentiation
  - AML without maturation
  - AML with maturation
  - Acute myelomonocytic leukemia
  - Acute monoblastic/monocytic leukemia
  - Acute erythroid leukemia
    - Pure erythroid leukemia
    - Erythroleukemia, erythroid/myeloid
  - Acute megakaryoblastic leukemia
  - Acute basophilic leukemia
  - Acute panmyelosis with myelofibrosis
- Myeloid sarcoma
- Myeloid proliferations related to Down syndrome
  - Transient abnormal myelopoiesis
  - Myeloid leukemia associated with Down syndrome
- Blastic plasmacytoid dendritic cell neoplasm

Acute leukemias of ambiguous lineage
- Acute undifferentiated leukemia
- Mixed phenotype acute leukemia with t(9;22)(q34;q11.2); *BCR-ABL1*
- Mixed phenotype acute leukemia with t(v;11q23); *MLL* rearranged
- Mixed phenotype acute leukemia, B-myeloic, NOS
- Mixed phenotype acute leukemia, T-myeloid, NOS
- *Provisional entity: natural killer (NK) cell lymphoblastic leukemia/lymphoma*

B lymphoblastic leukemia/lymphoma
- B lymphoblastic leukemia/lymphoma, NOS
- B lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities
  - B lymphoblastic leukemia/lymphoma with t(9;22)(q34;q11.2); *BCR-ABL1*
  - B lymphoblastic leukemia/lymphoma with t(v;11q23); *MLL* rearranged
  - B lymphoblastic leukemia/lymphoma with t(12;21)(p13;q22) *TEL-AML1 (ETV6-RUNX1)*
  - B lymphoblastic leukemia/lymphoma with hyperdiploidy
  - B lymphoblastic leukemia/lymphoma with hypodiploidy
  - B lymphoblastic leukemia/lymphoma with t(5;14)(q31;q32) *IL3-IGH*
  - B lymphoblastic leukemia/lymphoma with t(1;19)(q23;p13.3); *TCF3-PBX1*

T lymphoblastic leukemia/lymphoma

Vardiman JW et al. Chem Biol Interact 2010

II - Physiopathologie

L'origine des LAL réside dans la dérégulation de l'homéostasie hématopoïétique conduisant à un blocage de la maturation des précurseurs hématopoïétiques avec expansion clonale.[10] Ce dysfonctionnement peut être dû à une expression inadaptée ou à des altérations structurales de gènes suite à des mutations ponctuelles ou à des anomalies de structure chromosomique. L'expression anormale de gènes normaux, l'expression de gènes anormaux (oncogènes ou proto-oncogènes) soit par mutation soit par fusion entre deux gènes, ou la disparition de gènes (gènes suppresseurs de tumeur) contrôlant le processus mutagène représentent les trois mécanismes principaux.[11,12] Ces anomalies génétiques acquises ont été au départ identifiées à partir d'anomalies chromosomiques décelables au microscope. Elles donnent souvent lieu à un avantage prolifératif et concernent en général des gènes intervenant dans la survie et/ou la croissance cellulaire et/ou l'apoptose.[5,13,14]

Les tableaux n°2 et n°3 récapitulent les sous-types et les altérations génétiques clefs actuels des LAL avec les différences de répartition selon l'âge résumées dans la figure n°1.[14,15]

Tableau n°2: Les sous-types de LAL

Table 1. Key subtypes of ALL

Subtype	Prevalence (%)*	Comment
B-cell precursor ALL		
Hyperdiploidy with >50 chromosomes	20-30	Excellent prognosis
Hypodiploidy with <44 chromosomes	2-3	Poor prognosis; high frequency of Ras pathway and Ikaros gene family mutations
t(12;21)(p13;q22) translocation encoding *ETV6-RUNX1*	15-25	Excellent prognosis
t(1;19)(q23;p13) translocation encoding *TCF3-PBX1*	2-6	Increased incidence in African-Americans; generally excellent prognosis; association with CNS relapse
t(9;22)(q34;q11.2) translocation encoding *BCR-ABL1*	2-4	Historically poor outcome; improved with addition of imatinib and/or dasatinib to intensive chemotherapy
Ph-like ALL	10-15	Multiple kinase-activating lesions; associated with older age, elevated white blood cell count, and *IKZF1* alteration; potentially amenable to TKI therapy
t(4;11)(q21;q23) translocation encoding *MLL-AF4* fusion	1-2	Common in infant ALL (especially age <6 mo); poor prognosis
t(8;14)(q24;q32), t(2;8)(q12;q24), t(2;8)(q12;q24) encoding; *MYC* rearrangement	2	Favorable prognosis with short-term high-dose chemotherapy
CRLF2 rearrangement (*IGH-CRLF2*; *P2RY8-CRLF2*)	5-7	Common in Down syndrome–associated and Ph-like ALL (~50% each); associated with *IKZF1* deletion and/or mutation and *JAK1/2* mutation and poor prognosis in non-Down syndrome–associated ALL
ERG-dysregulated ALL	~7	Distinct gene expression profile; the majority have focal ERG deletions and favorable outcome despite *IKZF1* alterations
PAX5 rearrangement	~2	Multiple partners, commonly from dic(7;9), dic(9;12), and dic(9;20)
iAMP21	~2	Complex structural alterations of chromosome 21; rarely associated with a constitutional Robertsonian translocation rob(15;21)(q10;q10)c; poor prognosis
T-lineage ALL		
t(1;7)(p32;q35) and t(1;14)(p32;q11) translocations and interstitial 1p32 deletion; *TAL1* dysregulation	15-18	Generally favorable outcome
t(11;14)(p15;q11) translocation and 5′ *LMO2* deletion; LMO2 dysregulation	10	Generally favorable outcome
t(10;14)(q24;q11) and t(7;10)(q35;q24) translocations; *TLX1 [HOX11]* dysregulation	7	Good prognosis
t(5;14)(q35;q32) translocation; *TLX3* dysregulation	20	Commonly fused to *BCL11B*, also a target of deletion and/or mutation; poor prognosis
t(10;11)(p13;q14) translocation; *PICALM-MLLT10 [CALM-AF10]*	10	May have poor outcome
MLL-MLLT1 [MLL-ENL]	2-3	Superior prognosis to other types of *MLL*-rearranged leukemia
9q34 amplification encoding *NUP214-ABL1*	6	Potentially amenable to TKIs, also identified in high-risk B-ALL; other kinase fusions identified in T-ALL include *EML1-ABL1*, *ETV6-JAK2*, and *ETV6-ABL1*
t(7;9)(q34;q34) translocation	<1	Rearrangement of *NOTCH1*; also sequence mutations in >50% T-ALL
Early T-cell precursor ALL	10-15	Immature immunophenotype; expression of myeloid and/or stem cell markers; historically poor outcome, although improved in recent studies; genetically heterogeneous with mutations in hematopoietic regulators, cytokine, and Ras signaling, and epigenetic modifiers

CNS, central nervous system.
*Frequencies refer to childhood ALL.

Hunger SP et al. Blood 2015

Tableau n°3: Les altérations génétiques clefs actuels des LAL

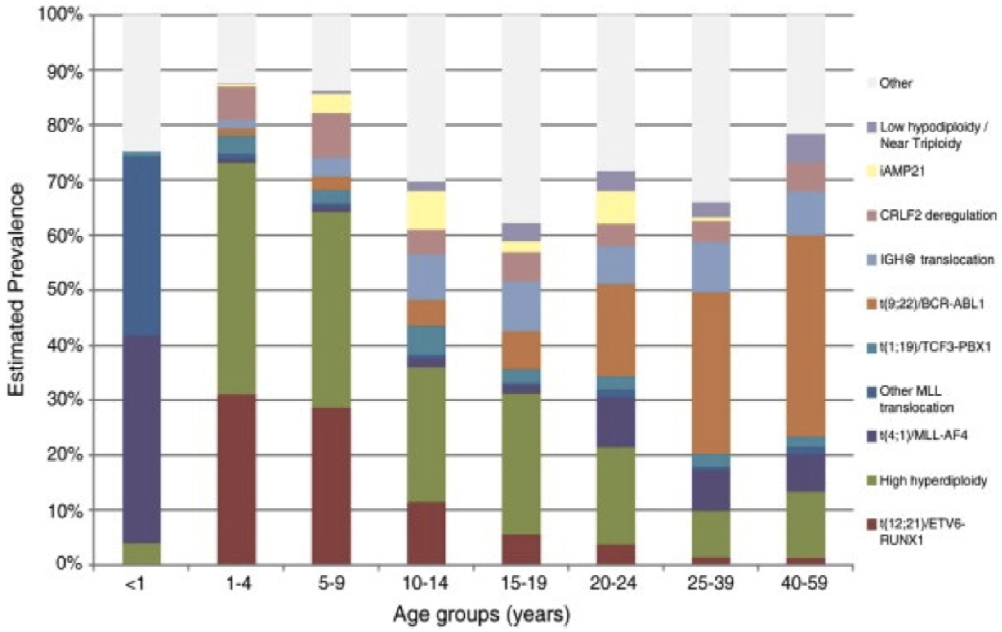
Table 2. Key genetic alterations in ALL

Gene	Alteration	Frequency	Pathway and consequences of alteration	Clinical relevance	Reference
PAX5	Focal deletions, translocations, and sequence mutations	30% of B-ALL	Transcription factor required for B-lymphoid development; mutations impair DNA binding and transcriptional activation	Important in leukemogenesis, but not associated with adverse outcome	3-6
IKZF1	Focal deletions or sequence mutations	15% of all pediatric B-ALL cases, including 70%-80% of *BCR-ABL1*-positive ALL and one-third of high-risk *BCR-ABL1*-negative B-ALL	Transcription factor required for development of HSC to lymphoid precursor; deletions and mutations result in loss of function or dominant-negative isoforms	Associated with poor outcome	4, 5, 7, 51, 102
JAK1/2	Pseudokinase and kinase domain mutations	18%-35% of DS ALL and 10.7% high-risk *BCR-ABL1*-negative ALL	Constitutive JAK-STAT activation	Potential for targeting with TKIs that inhibit JAK1/2	8, 39, 43
CRLF2	Rearrangement as *IGH-CRLF2* or *P2RY8-CRLF2* resulting in overexpression	5%-16% of pediatric and adult B-ALL and >50% DS ALL; 50% of Ph-like ALL	Associated with mutant JAK in up to 50% of cases; CRLF2 mutations and JAK mutations are cotransforming in cell lines/Ba/F3 cells and result in constitutive STAT activation; in high-risk B-ALL, associated with *IKZF1* alterations, JAK mutations, and poor outcome	Detected by flow cytometry or molecular assays; potential for targeting with JAK inhibitors	6, 29, 41, 42, 44
Kinase-activating alterations in Ph-like ALL	Rearrangements of 13 cytokine receptors and tyrosine kinases; sequence mutations of *IL7R* and *FLT*; deletions of *SH2B3*	10% childhood B-ALL, up to 30% ALL in adult ALL; associated with Ph-like gene expression profile	Activation of kinase signaling pathways and amenable to TKI therapy	Associated with high-risk features and increased risk of relapse; anecdotal reports of response to TKI therapy	14, 22, 30, 54
NOTCH1	Mutations, rarely deletions	>60% T-ALL	Activation of NOTCH1 signaling; mutations in FBXW7 and PTEN also influence NOTCH1 signaling and prognosis	Key pathogenic lesion in T-ALL; direct targeting limited by toxicity; variable associations with outcome	62
CREBBP	Focal deletion and sequence mutations	19% of relapsed ALL; also mutated in non-Hodgkin lymphoma	Mutations result in impaired histone acetylation and transcriptional regulation	Mutations selected for at relapse and associated with glucocorticoid resistance	11, 35
NT5C2	Focal mutations	Up to 20% relapsed mutation	Mutations in gain of function	Mutations selected at relapse and associated with resistance to thiopurines	17, 84
TP53	Deletions and focal mutations	~90% of LH ALL, otherwise uncommon in predominant clone at diagnosis	Frequently germline in LH ALL; confer resistance to therapy	Hallmark of LH ALL at diagnosis; associated with disease relapse	15, 83

DS, Down syndrome; HSC, hematopoietic stem cell; NR, none reported.

Hunger SP et al. Blood 2015

Figure n°1: Répartition des sous-types de LAL en fonction de l'âge



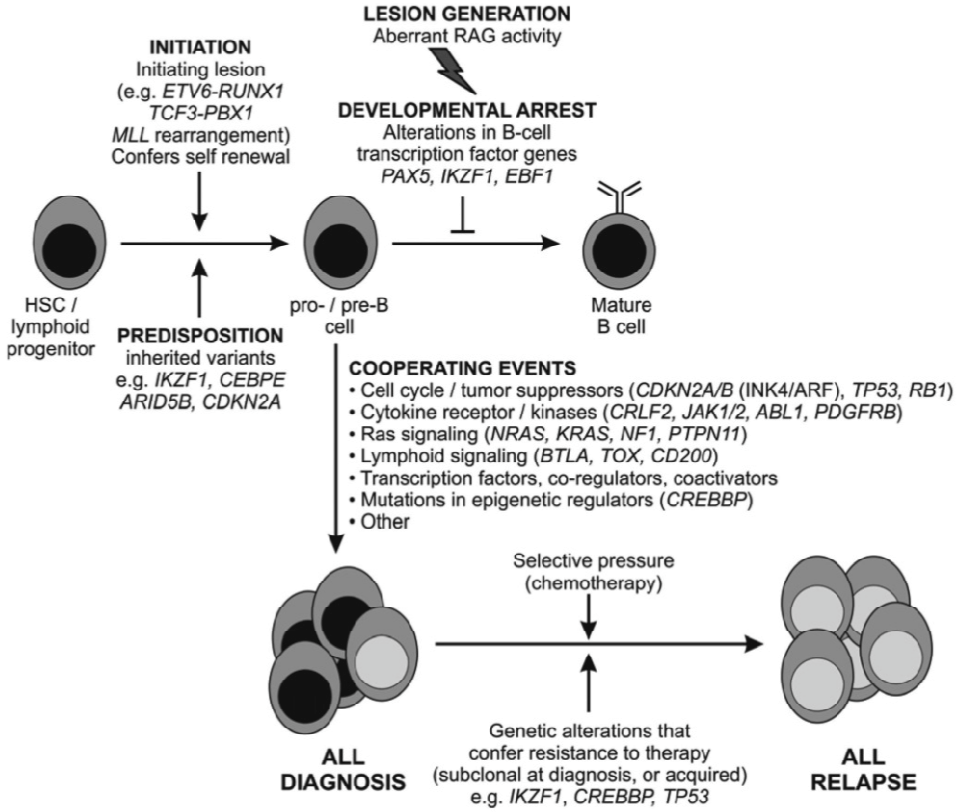
Moorman AV et al. Blood Rev 2012

1 - Les LAL B

Plus d'une centaine d'anomalies génétiques codant des protéines aux fonctions très diverses y sont décrites.[14-16] Un grand nombre de ces anomalies impliquent des facteurs de transcription tel que PAX5, des coactivateurs ou corépresseurs transcriptionnels comme ETV6, des tyrosines kinases comme Kit ou PDGFR, des protéines intervenant dans le contrôle du cycle cellulaire ou des protéines anti-apoptotiques. La liste de ces remaniements peut être consultée dans l'« Atlas of genetics and cytogenetics in oncology and haematology ».[17]
Dans les modèles expérimentaux, les réarrangements chromosomiques peuvent aboutir à des activations oncogéniques, des activations constitutives de tyrosines kinases ou des perturbations de gènes impliqués dans la lymphopoïèse (par exemple : ETV6-RUNX1). Cependant ces perturbations géniques n'entraînent pas à elles seules la transformation leucémique. La leucémogénèse est induite par l'association aux mutations génétiques primaires, d'altérations additionnelles de nature et de fréquence très diverses. Par exemple, les LAL avec un réarrangement MLL (Mixed-Lineage Leukemia) portent en moyenne une seule mutation additionnelle alors que les LAL avec une translocation ETV6-RUNX1 présentent 6 à 8 altérations additionnelles.[5,15,16]
Des réarrangements chromosomiques sont probablement acquis très tôt dans la leucémogénèse et conduisent à une dérégulation transcriptionnelle et épigénétique et à un renouvellement cellulaire anormal. Des lésions et/ou des changements génétiques secondaires perturbent le développement lymphoïde aboutissant à un blocage de la maturation. L'ensemble de ces événements aboutit

à l'établissement et à la prolifération d'un clone leucémique. Des changements génétiques dans des clones mineurs présents au diagnostic peuvent conférer la résistance à la chimiothérapie et être responsable des rechutes.[14,16]
Le schéma ci-dessus résume le rôle des anomalies génétiques dans la leucémogénèse des LAL B (figure 2).[13]

Figure n°2: Rôle des anomalies génétiques dans la leucémogénèse des LAL B



Mullighan CG. Hematology 2012

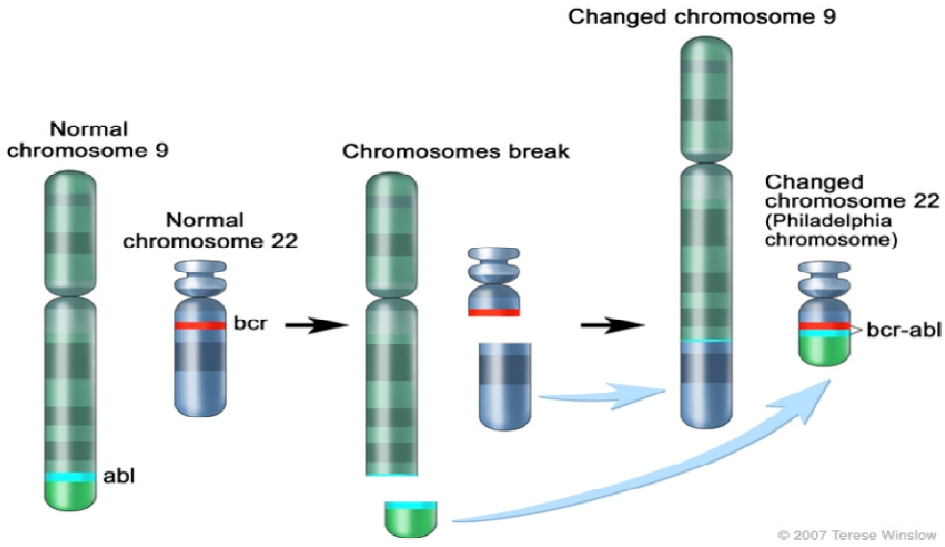
La compréhension de la leucémogénèse est essentielle pour la prise en charge thérapeutique et l'adaptation des traitements aux différents groupes pronostiques.

2 - Les LAL à Ph1

La fréquence des LAL à chromosome Philadelphie (LAL à Ph1) varie avec l'âge (figure n°2). Ces LAL représentent de 2 à 3 % des LAL de l'enfant et en moyenne 20 à 30 % des LAL de l'adulte ainsi reparties : de 10 à 15 % chez l'adulte jeune, de 50 % environ à 50 ans et de 70 % à 70 ans. Leur incidence est en augmentation depuis 10 ans sans cause évidente.[18,19]

Le chromosome Philadelphie est issu de la translocation réciproque entre les chromosomes n° 9 et n° 22, respectivement en position 9q34 au niveau du gène Abelson (ABL) codant la protéine Abl et en position 22q11 au niveau du gène BCR (pour Break point Cluster Region) codant la protéine Bcr (figure n°3). Il a été décrit pour la première fois en 1960 par P. Nowell et D. Hungerford et est présent dans la leucémie myéloïde chronique (LMC), hémopathie maligne appartenant aux syndromes myéloprolifératifs.[20,21]

Figure n°3: Le chromosome Philadelphie



Kurzrock R et al. Ann Intern Med 2003

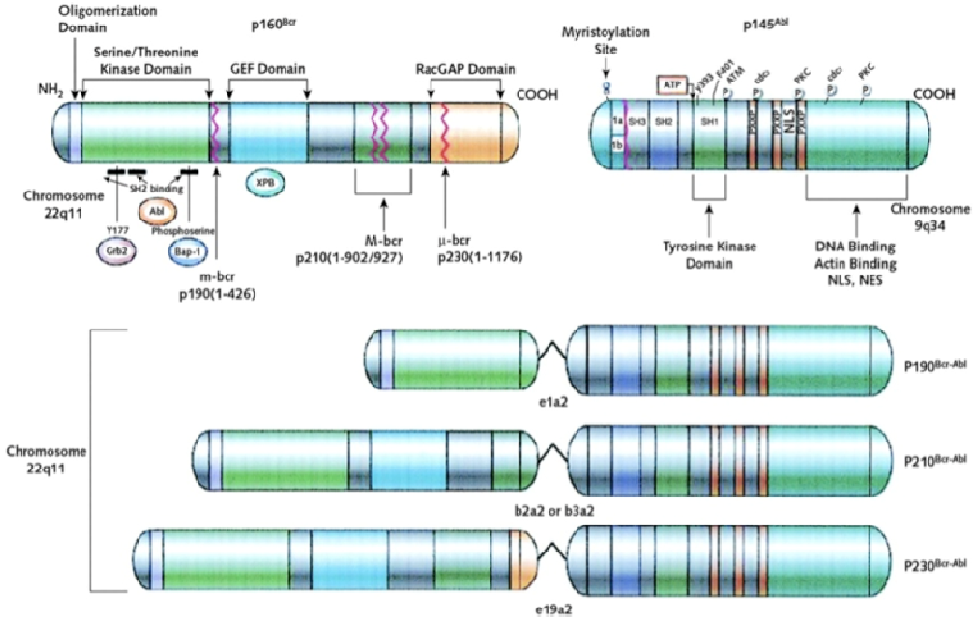
La protéine Abl est une protéine à activité tyrosine kinase (TK) ayant un rôle essentiel dans le fonctionnement cellulaire avec des fonctions à la fois nucléaires et cytoplasmiques. Sa structure comprend trois régions SH (SH1, 2 et 3) dans la partie NH2 terminale, une région riche en proline dans la partie centrale et des séquences de localisation nucléaire dans la partie COOH-terminale. L'activité TK est assurée par le domaine SH1 où sont localisés le site de liaison à l'ATP, site majeur d'autophosphorylation, et le domaine catalytique phosphotransférase.[20,21]

La protéine Bcr est d'expression ubiquitaire au niveau des cellules hématopoïétiques et possède deux domaines fonctionnels essentiels, un domaine d'oligomérisation et un domaine présentant une activité sérine-thréonine kinase. Le domaine d'oligomérisation est responsable de l'homotétramérisation de la molécule et est indispensable à son activité. Le domaine à activité sérine-thréonine kinase contient un résidu tyrosine essentiel (Y177) qui assure la liaison avec l'adaptateur moléculaire GRB2 conduisant à l'activation de la voie de signalisation intracellulaire Ras jouant un rôle important dans la régulation de la prolifération, de la survie, de la différenciation, de la migration cellulaire, et de l'angiogenèse.[20,21]

a - Le transcrit BCR-ABL

Sur le plan cytogénétique la translocation t(9;22) met en continuité la partie centromérique 5' du BCR avec la partie C terminale du protooncogène ABL (figure n°4). Sur le chromosome 9, les points de cassure du gène ABL surviennent pratiquement toujours entre les exons 2 et 1a. Sur le chromosome 22, les points de cassure sont moins stéréotypés survenant en M-BCR (pour Major Breakpoint Cluster) codant une protéine de 210 kilodaltons ($Bcr\text{-}Abl^{p210}$) ou en m-BCR (pour minor Breakpoint Cluster) codant une protéine de 190 kilodaltons ($Bcr\text{-}Abl^{p190}$) ou en μ-BCR codant une protéine de 230 kilodaltons ($Bcr\text{-}Abl^{p230}$).[20,22,23] Des transcrits rares sont également décrits. Typiquement la protéine p190 est associée à la LAL à Ph1 (70 % des LAL à Ph1), la protéine p210 à la LMC et la protéine p230 à certaines leucémies à neutrophiles, généralement classées comme formes variantes de LMC.[23,24] Cependant, dans les LAL à Ph1 la protéine p210 peut être exprimée avec ou sans co-expression de la protéine p190. La protéine p210 est observée dans 25 à 50 % des LAL à Ph1 de l'adulte.[25] Cependant, les LAL à Ph1 représentent un groupe nettement plus hétérogène du fait de l'existence d'épissages alternatifs ou atypiques, de l'incorporation d'introns ou de mutations ponctuelles dans le transcrit BCR ou l'insertion d'introns ou d'exons dans le transcrit ABL avec des altérations différentes du domaine kinase de la protéine.[20,23,26]

Figure n° 4: Les différentes protéines Bcr-Abl



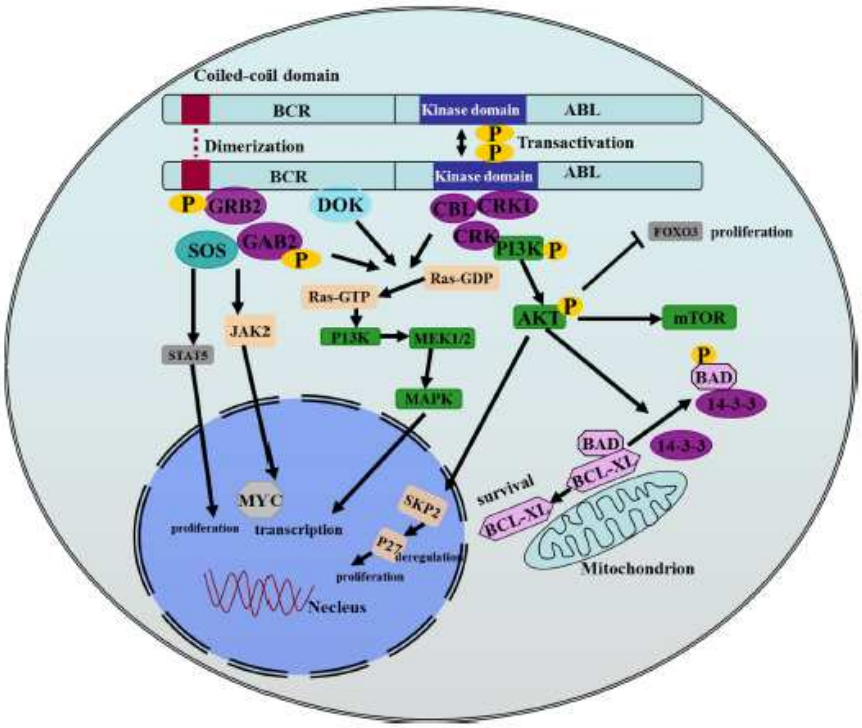
Advani AS et al. Leuk Res 2002

Au niveau moléculaire, la translocation t(9; 22) induit la formation d'un gène de fusion BCR-ABL codant une protéine chimérique Bcr-Abl avec trois isoformes (Bcr-Abl^{p190}, Bcr-Abl^{p210} et Bcr-Abl^{p230}).[20,21,27]

La protéine chimérique Bcr-Abl, membranaire, possède un domaine cytoplasmique. La dimérisation de Bcr-Abl entraîne l'activation constitutive du site kinase de Bcr-Abl. L'activité kinase est de ce fait activée en permanence.[20,22,28,29]

Les substrats phosphorylés par Bcr-Abl sont nombreux: GRB-2, SHC, STAT5, DOK, CRK (figure n°5).[29]

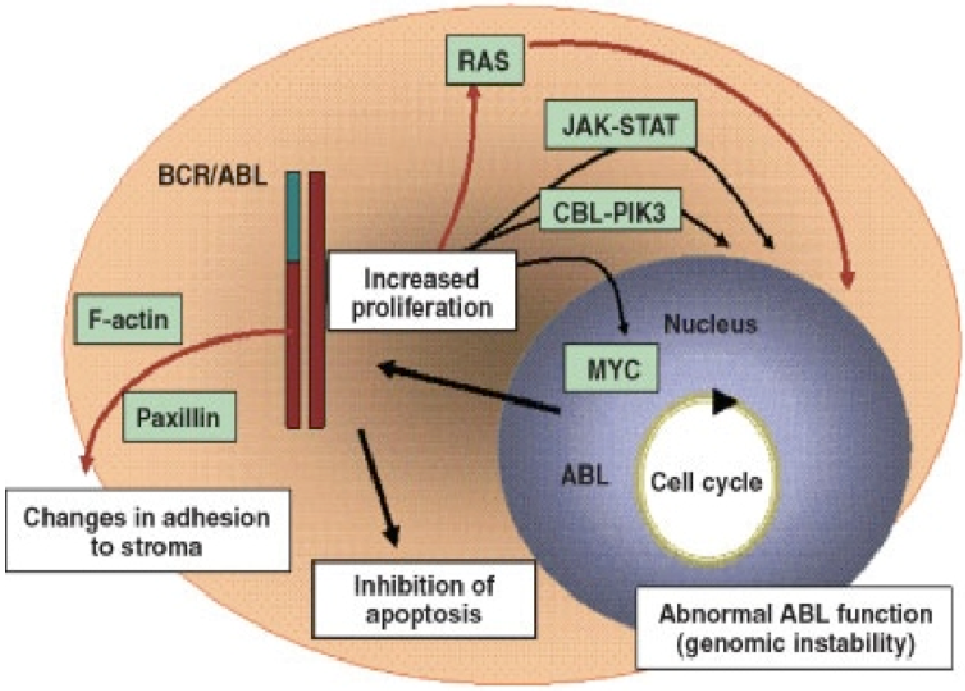
Figure n°5: Les substrats phosphorylés par Bcr-Abl



Yang K et al. Critical Reviews in Oncology/Hematology. 2015

Au niveau cellulaire, cette phosphorylation, conséquence de l'activité TK entraîne l'activation de nombreuses voies de signalisation cellulaire: les voies des Ras-MAP kinases, les voies Stat qui induisent une augmentation de la production des facteurs de croissance myéloïdes tel que IL3, GM-CSF, G-CSF, la voie PI3 Kinase (PI3K), la voie MYC, la voies des Src kinase et la voie NF-KB. La signalisation Ras conduit à l'activation de la cascade des MAPK avec une voie de signalisation RAF/MEK/ERK et une voie de signalisation JNK avec, comme conséquence, une activation de la transcription (figure n°6). L'activation des voies Stat, essentiellement de STAT 5 et à un moindre degré, de STAT 1 et 6 et de la voie PI3K/AKT est responsable de l'activité anti-apoptotique. L'activation de ces différentes voies provoque une prolifération excessive, une inhibition de l'apoptose, des modifications de l'adhésion cellulaire et une instabilité génétique.[20-22,27]

Figure n° 6: Les voies de signalisation du Bcr-Abl



Pane F et al. Oncogene 2002

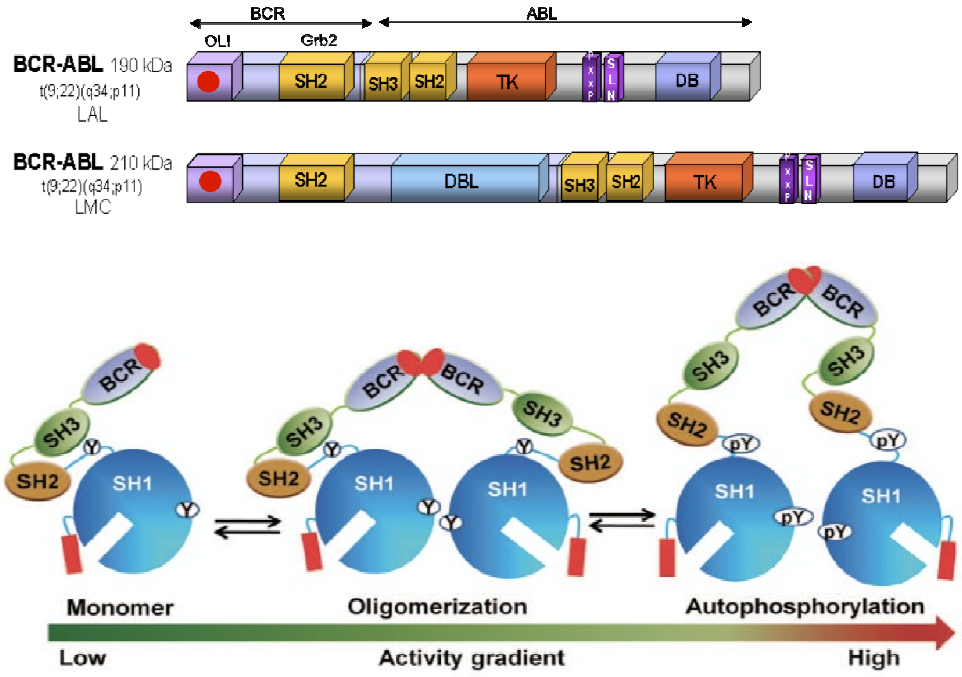
Outre la compréhension de la physiopathologie, la description de toutes ces voies est une porte ouverte aux thérapeutiques ciblées en complément du traitement majeur des LAL à Ph1, les inhibiteurs de tyrosine kinase (ITK), traitement que nous aborderons ultérieurement.

b - Conséquences protéiques du transcrit BCR-ABL

Dans les LAL à Ph1, la protéine de fusion Bcr-Abl^{p190} a des caractéristiques communes aux 3 isoformes (Bcr-Abl^{p190}, Bcr-Abl^{p210} et Bcr-Abl^{p230}).[20,22]
La partie Abl des différentes isoformes de la protéine Bcr-Abl comporte différents domaines: le domaine tyrosine kinase qui possède des domaines d'homologies SH semblables à ceux de l'oncogène Src, responsable de l'activation de voies de signalisation, des domaines régulateurs de l'activité tyrosine (domaine SH2 régulateur positif, domaine SH3 régulateur négatif), le domaine NLS pour la localisation intracellulaire du signal et le domaine DBL, segment de fixation de l'ADN au cytosquelette.
La partie Bcr de la protéine Bcr-Abl comporte un domaine OLI pour la dimérisation nécessaire à l'activité de la protéine, un domaine GrB2, domaine de fixation de protéines adaptatrices qui interagit avec le domaine SH2 et deux sites de liaison aux domaines SH2 qui permettront la liaison de Bcr avec le domaine SH2 d'ABL (figure n°7). La protéine Bcr normale peut former des

hétérodimères avec les protéines de fusion Bcr-Abl, avec comme conséquence une délocalisation possible de la protéine Bcr normale.[20,23,27]

Figure n° 7: Dimérisation de la protéine Bcr-Abl



Cilloni D et al. Clin Cancer Res 2012

c - Particularités de l'isoforme BCR-ABL^{p190}

La protéine Bcr-Abl^{p190} possède des caractéristiques propres.
Le gène BCR est plus court, sans domaine DBL, domaine d'échange de GTP par rapport à BCR-ABL^{p210} mais suffisant à la structure active. La transcription puis la traduction de ce gène chimère conduit à la génération d'une protéine de fusion Bcr-Abl^{p190}, possédant une importante majoration de son activité TK par rapport à Bcr-Abl^{p210}. La protéine Bcr-Abl^{p190} a un très haut niveau de phosphorylation, une perte d'autoinhibition et une activité kinase, supérieurs à Bcr-Abl^{p210}. *In vitro*, Bcr-Abl^{p190} stimule davantage la croissance des lignées lymphoïdes et est un agent oncogène plus puissant.[20-22,30]

d - Autres mécanismes impliqués dans la physiopathologie des LAL à Ph1

D'autres spécificités interviendraient dans le lignage lymphoïde. Le transcrit réciproque ABL-BCR^{p96}, mutant de BCR participerait à ce lignage en n'effectuant pas de régulation négative de l'oncogénèse lymphoïde contrairement à la protéine Bcr.[31,32] D'autres éléments seraient des éléments clés de

l'oncogénèse des LAL à Ph1, notamment Gads un régulateur de GrB2, PECAM-1 immunorécepteur présent à la surface des cellules lymphoïdes et vav3 facteur d'échange de guanine de la voie ROS.[33-35]

D'autres voies de signalisation sont spécifiques des LAL à Ph1. La voie STAT5 serait nécessaire au maintien de la prolifération lymphoïde. La voie STAT6 interviendrait dans la différenciation lymphoïde.[36] La protéine chaperon HSP90 stabiliserait le transcrit BCR-ABL et la voie Src serait activée.[37,38] Certaines études montrent une dérégulation de la méthylation avec une altération des microARN et une hyperméthylation donc l'inhibition d'antioncogènes intervenant normalement dans la régulation de l'oncogénèse lymphoïde.

Un autre fait marquant dans les LAL à Ph1 est l'existence de nombreuses anomalies cytogénétiques et génétiques associées, reflet probable de l'instabilité génétique.[13,14,39,40]

Ces caractéristiques propres aux LAL à Ph1 permettent de comprendre pourquoi l'inhibition de la voie du BCR-ABL n'est pas suffisante pour interrompre la croissance des cellules leucémiques contrairement à la LMC, autre hémopathie maligne caractérisée par la présence du chromosome Ph1 avec l'isoforme BCR-ABL^{p210}. Différentes questions restent en suspens. Le gène BCR-ABL est-il le premier ou un deuxième événement dans l'oncogénèse des LAL à Ph1? Existe–t-il plusieurs clones ou des sous-clones? Certains modèles de LAL à Ph1 retrouvent le gène BCR-ABL uniquement dans la lignée lymphoïde, d'autres dans les précurseurs myéloïdes.[13,16,21,30,37,39-41]

3 - Les LAL T

Les LAL T représentent environ 15 % de toutes les leucémies avec des variations de fréquence en fonction de l'âge et une prédominance chez les adolescents.[42] De 10 à 15% des LAL de l'enfant, leur fréquence s'élève à 20 à 30 % chez l'adulte avec un sexe ratio homme/femme de 4:1.[4-6] Cette incidence chez l'enfant est aussi plus élevée chez le garçon avec un sex ratio de 3:1.[42]
Les LAL T sont dues à la transformation oncogénique de précurseurs hématopoïétiques de la lignée lymphoïde T. Elles correspondent à des proliférations clonales malignes développées à partir des précurseurs thymiques. L'oncogénèse des LAL T et le développement physiologique lymphoïde T sont liés, en particulier en ce qui concerne les mécanismes de régulation des recombinaisons somatiques, à l'origine de la mise en place d'un récepteur T à l'antigène (TCR). De nombreux signaux de prolifération et/ou d'apoptose physiologiques sont de ce fait impliqués dans la leucémogénèse des LAL T.[43-46]
La classification des LAL T repose sur des critères morphologiques, sur l'expression d'antigènes de différenciation spécifiques de la lignée lymphoïde T et sur l'étude des réarrangements des TCR. L'expression du CD3 cytoplasmique ou membranaire est nécessaire et suffisante pour affirmer l'appartenance des blastes à la lignée lymphoïde T. Le groupe européen de caractérisation immunologique des leucémies (EGIL) a établi une classification immunologique en fonction des stades de différenciation thymique, laquelle n'est toutefois pas tout-à-fait précise pour définir le stade d'arrêt de maturation et a peu d'impact thérapeutique.[47]

Les stades de blocage de maturation des LAL T reproduisent les différentes étapes de maturation thymique humaine.[48] A partir de l'analyse des réarrangements des loci des TCRs, l'expression de la chaîne du TCRβ en intra-cytoplasmique et l'expression du TCRγδ ou αβ à la surface des cellules blastiques, les LAL T se scindent en trois groupes : les LAL T immatures, les LAL T pré-αβ et les LAL T matures.[43,48,49]
Les LAL-T immatures présentent les caractéristiques des précurseurs thymiques immatures non-T restreints. Elles comportent 4 sous groupes, correspondant à l'ordre physiologique des réarrangement successifs des TCRs: les IM0, les IMδ, les IMγ et les IMβ.[49] Parmi elles, un autre sous-groupe est décrit: les ETP-ALL (Early Thymic Precursor Acute Lymphoblastic Leukemia). Il s'agit d'un sous groupe qui présente des marqueurs d'expression antigénique myéloides ou de cellules souches.[50,51]
Les LAL T préαβ ont effectué des réarrangements complets du TCRδ et du TCRβ mais n'ont pas commencé à réarranger le locus du TCRα. Les blastes expriment de ce fait un pré-TCR.

Les LAL T matures ont réarrangé le TCRδ, le TCRγ et le TCRβ de façon incomplète et expriment le complexe CD3/TCRγδ ou CD3/TCRαβ.
Les LAL T de l'adulte présentent souvent un blocage de maturation plus immature que celles de l'enfant.[44,45,48]

Ces maladies constituent un modèle de pathologie dite «multihits». La transformation blastique d'un lymphocyte T est un processus de multi-étapes dans lequel différentes lésions géniques s'accumulent et modifient la prolifération, la survie, le cycle cellulaire et la différenciation de cellules T. De nombreuses altérations telles que des translocations réciproques ou non, des mutations, des délétions et des amplifications doivent être acquises par la cellule lymphoïde avant d'aboutir à cette transformation maligne. Les oncogènes impliqués dans une translocation avec un TCR sont très nombreux (figure n°8).[44,46]
La liste de ces remaniements peut être consultée dans l'« Atlas of genetics and cytogenetics in oncology and haematology ».[17]

Figure n°8: Les lésions géniques les plus fréquentes dans les LAL T

TABLE 2. Recent Genetic Determinants in ALL by Lineage

ALL Lineage	Cytogenetic Aberration	Involved Genes	Protein	Comments
B-cell	BCR/ABL+ (Ph+)	IKZF1	Ikaros	Poor outcome; 80% of Ph+ cases
		CRLF2 + Ig heavy chain locus or interstitial PAR1 deletion	CRLF2	5%-10% of cases with no molecular rearrangement; poor outcome; 50% of children with Down syndrome
	BCR/ABL-like	IKZF1 deletions; rearrangements/ mutations in CRLF2, IGH-CRLF2, NUP214-ABL1; in-frame fusions of EBF1-PDGFRB, BCR-JAK2, or STRN3-JAK2; cryptic IGH-EPOR rearrangements		15% of cases; potential use of TKIs and/ or mTOR and JAK2 inhibitors
	Near-hypodiploid	NRAS, KRAS, FLT3, NF1		70% of cases
	Low-hypodiploid	IKZF2 and, by TP53 disruptions, CDKN2A/B locus deletion		91% of cases
	Hyperdiploid	CREBBP		
		NT5C2 mutations	NT5C2	
		TP53 mutations		6% of cases
T-cell		PICALM-MLLT10, NUP214-ABL1 fusion, EML-ABL1, SET-NUP214 fusion, MLL, NOTCH1, FBW7, BCL11B, JAK1, PTPN2, IL-7R, PHF6, RAS/PTEN		NOTCH1 (>60%) and/or FBW7 (~20%) mutations associated with a favorable outcome; RAS/PTEN and JAK1 usually associated with a poor outcome

Abbreviations: ABL1, Abelson murine leukemia viral oncogene homolog; ALL, acute lymphoblastic leukemia; BCL11B, B-cell lymphoma/leukemia 11B; BCR, breakpoint cluster region; CDKN, cyclin-dependent kinase inhibitor; CREBBP, CREB-binding protein; CRLF2, cytokine receptor-like factor 2; EBF1, early B-cell factor 1; EML, Echinoderm microtubule-associated protein-like; EPOR, erythropoietin receptor; FBW7, F-box/WD repeat-containing protein 7; FLT3, fms-related tyrosine kinase 3; Ig, immunoglobulin; IGH, immunoglobulin heavy; IKZF, IKAROS family zinc finger; IL-7R, interleukin 7 receptor; JAK, Janus kinase; MLL, mixed-lineage leukemia; mTOR, mammalian target of rapamycin; NF1, neurofibromin 1; NT5C2, 5′-nucleotidase cytosolic II; NUP214, nucleoporin 214; PAR1, pseudo-autosomal region 1; PDGFRB, platelet-derived growth factor receptor β; Ph, Philadelphia chromosome; PHF6, PHD finger protein 6; PICALM, phosphatidylinositol binding clathrin assembly protein; PTEN, phosphatase and tensin homolog; PTPN2, protein tyrosine phosphatase non-receptor type 2; STRN3, striatin 3; TKI, tyrosine kinase inhibitor.

Jabbour E et al. Cancer 2015

Une classification simplifiée de ces nombreuses altérations a été proposée, laquelle s'inspire du mécanisme cellulaire qu'elles affectent tel que la différenciation, les étapes de contrôle au niveau du cycle cellulaire, la

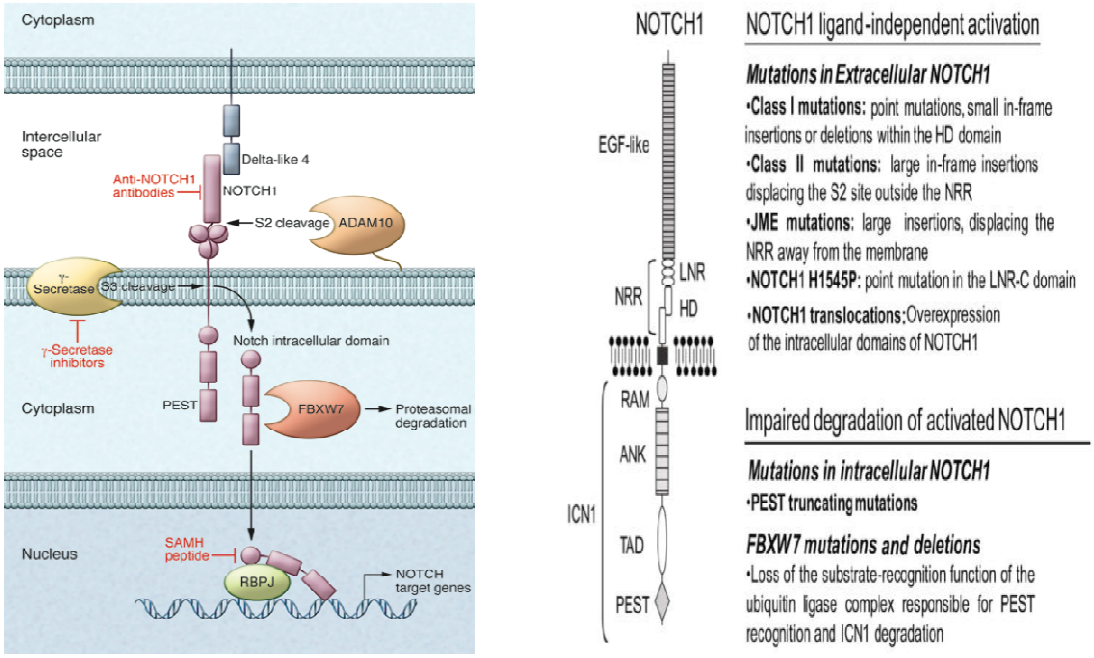
prolifération ou la survie. Deux grands types d'anomalies sont distinguées, selon qu'elles touchent des oncogènes de type 1 ou de type 2.[52-55]

Les anomalies de type 1 sont corrélées à un stade d'arrêt de maturation. Elles seraient responsables du blocage de maturation à des stades spécifiques du développement des lymphocytes T.
Elles sont mutuellement exclusives et probablement fondatrices.[52-55]
Différents sous-groupes cytogénétiques et moléculaires, impliquant TAL/LMO, c-MYC, HOXA, TLX1, TLX3, sont distingués en tenant compte également du profil d'expression transcriptionnel associé.[52,53,56,57] La surexpression de ces oncogènes peut être due à différents mécanismes propres à chaque oncogène. Il peut par exemple s'agir d'une surexpresion induite par une translocation avec les loci du TCRβ et TCRα/δ ou par une délétion mettant l'oncogène sous le contrôle d'un promoteur ou par une expression ectopique du fait de l'existence de transcrits de fusion.
Dans le sous-groupe des gènes HOXA, HOXA peut être dérégulé par la présence des transcrits de fusion impliquant MLL, CALM-AF10 ou SET-NUP214, ou par une translocation avec les loci du TCR.[58,59]

Les anomalies de type 2 ne sont pas corrélées à un stade précis de maturation. Plus de 30 d'entres sont actuellement décrites.
Redondantes entre elles et avec les anomalies de type 1, elles correspondent souvent à des lésions impliquées dans la régulation du cycle cellulaire, dans l'auto-renouvellement, dans la voie de signalisation du TCR dans la différenciation T. Elles peuvent induire l'activation des tyrosines kinases avec en conséquence une prolifération et/ou une survie excessive. Ces anomalies agissent en synergie dans le processus de transformation. La perte de CDKN2A/B et les anomalies de la voie de signalisation de Notch1 constituent les lésions oncogènes les plus prédominantes.[13,40,41,52,54,58,59]
Dans ces LAL T, L'inactivation génomique homozygote ou hétérozygote des loci CDKN2A et CDKN2B est l'anomalie la plus fréquente avec une incidence d'environ 90 %.[60] Elle entraine des anomalies du cycle cellulaire telles qu'une entrée incontrôlée en cycle, la désactivation des points de contrôles et une apoptose.
La dérégulation de la voie Notch1 a été découverte par l'étude de la translocation t(7; 9), laquelle implique le récepteur Notch1 et le TCRβ.[61] Des mutations de NOTCH1 sont désormais identifiées dans 50 à 60 % des LAL T soit, et essentiellement, une mutation touchant le domaine d'hétérodimérisation, soit une mutation affectant le domaine PEST. Des mutations plus rares peuvent affecter le domaine juxta-membranaire ou le domaine de trans-activation.[62-64]
Les mutations de NOTCH1 sont couplées avec les mutations du gène FBXW7 qui appartient à la famille de E3 ligase et permet la polyubiquitination de

NOTCH1 ainsi que sa dégradation par le protéasome. Les mutations de FBXW7 sont retrouvées dans environ 10 % des LAL T.[65,66]
Les mutations de NOTCH1 et/ou FBXW7 portent la dérégulation de cette voie dans environ 70 % des LALT (figure n°9). [43,66,67]

Figure n°9: La voie de signalisation Notch1 et les anomalies de NOTCH1



Paganin M. et al. Blood Reviews 2011

De nombreuses autres anomalies sont décrites, parmi lesquelles des altérations de facteurs de transcription tel que RUNX1 ou ZEB2, des mutations de médiateurs de la voie Ras tels que IL7R, JAK3, JAK1, KRAS, and NRAS, des mutations de PTEN impliquées dans la régulation du cycle cellulaire et de mutations de régulateurs épigénétiques. Ces lésions ont une fréquence variable selon le stade auquel la maturation est bloquée.
L'implication de micros ARN et de large ARN non-codant intergéniques, ARN non codants est aussi démontrée. Les micros ARN coopérent notamment avec les mutations de Notch, FBXW7 et PTEN.[45,63,68,69]
La compréhension de l'oncogénèse des LAL T est essentielle pour l'identification de groupes pronostiques et une prise en charge thérapeutique adaptée, nous y reviendrons.

III - pronostic et traitement des LAL de l'adulte

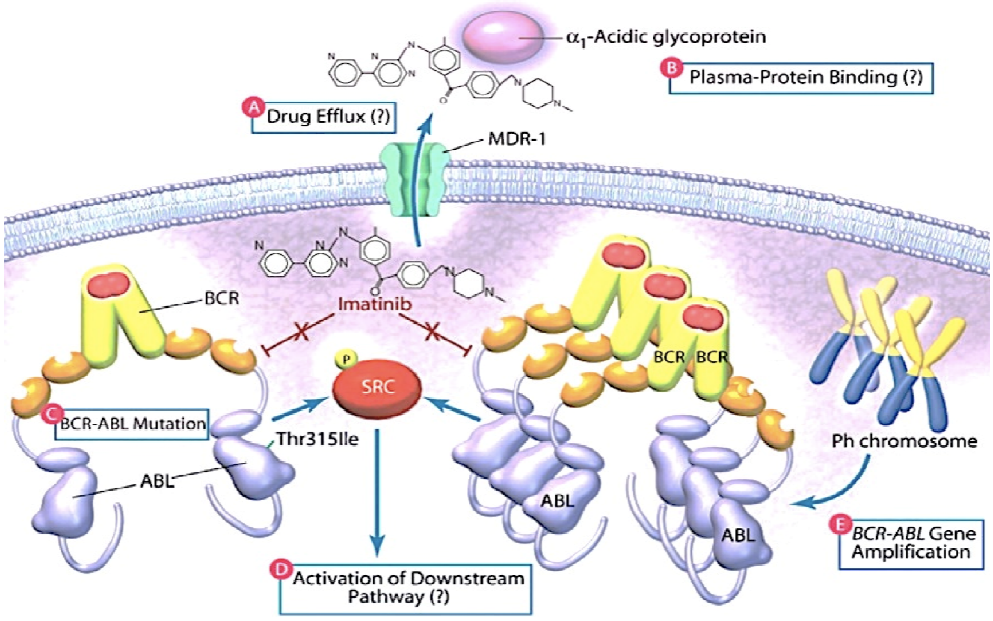
Le traitement moderne des LAL des sujets jeunes repose sur une chimiothérapie intensive inspirée des protocoles pédiatriques, incluant une préphase de corticoïdes, une induction favorisant des médicaments non myélotoxiques dont la L-asparaginase, une consolidation comprenant plusieurs blocs de chimiothérapie et une intensification adaptée aux facteurs pronostiques (intensification retardée ou allogreffe de CSH selon la situation pronostique). L'intensité de ce traitement est diminuée chez les sujets âgés.[4-6,18,70,71]
Ce traitement permet d'obtenir des taux de rémission complète (RC) de l'ordre de 80 à 90 %. Mais la survie à long terme n'est pas comparable au taux de survie de 90% obtenue chez les enfants. La survie globale à long terme n'est que de 40 à 50 %, du fait des rechutes et de la toxicité des traitements, notamment de la toxicité de l'allogreffe.[18,72,73] Cette survie à long terme est de environ de 50% pour les LAL B, de 50 à 60 % pour les LAL à Ph1 et de 50 à 60 % pour les LAL T.[5,18,72,74-81] L'impact de l'âge demeure fort: 50% à 60% des moins de 60 ans survivent à long terme, chiffre qui s'abaisse à 15% des patients entre 60 et 70 ans et moins de 10% des patients de plus de 70 ans. Ces résultats sont en partie liés à la fréquence importante des formes de mauvais pronostic et à la tolérance médiocre des sujets âgés aux chimiothérapies lourdes.[6,18,70,78]

Ainsi le taux de survie à long terme dépend-il de l'âge et des caractéristiques de la LAL avec des facteurs d'évolution péjoratifs tels que l'âge avancé, les leucocytes élevés au diagnostic, le caractère non T, la présence d'un chromosome Ph1, la mauvaise réponse initiale au traitement (corticorésistance, chimiorésistance, nécessité d'un rattrapage pour l'obtention de la RC) et la lenteur de réponse au traitement (persistance d'une maladie résiduelle précoce détectable).
Les facteurs pronostiques ont évolué et différents groupes pronostiques basés sur la cytogénétique et les analyses moléculaires se dégagent actuellement. Les thérapeutiques sont adaptées à ces groupes.[14,15,82] Les survies longues sont de l'ordre de 20 à 30 % pour les leucémies à haut risque contre 60 à 70 % pour les leucémies à risque standard.[14] L'enjeu de l'identification de ces différents groupes est de diminuer l'intensité du traitement et donc de limiter sa toxicité dans les groupes de pronostic favorable et de renforcer ou d'utiliser d'autres thérapeutiques dans les groupes de pronostic défavorable. Affiner le pronostic permet une meilleure prise en charge thérapeutique, adaptée au mieux au profil de la maladie.
Pour les LAL B, sur le plan cytogénétique et moléculaire, les facteurs de mauvais pronostic actuellement reconnus conduisant à réalisation d'une intensification par allogreffe de CSP comprennent la MRD, la présence d'une translocation t(4;11) ou transcrit MLL-AF4, ou d'une délétion IKZF1.[14,70,71,73,75,78,83-88]

1 - Traitement des LAL à Ph1

Le pronostic péjoratif conféré par la présence d'une t(9;22) a été modifié par l'utilisation des inhibiteurs de tyrosines kinases de type PDGFR (ITK). Les ITK dont le chef de fil est l´imatinib ou STI 571 (inhibiteur de première génération) sont spécifiques des TK Bcr-Abl.[89,90] Leur action est intracellulaire. Ils inactivent les TK cytoplasmiques par liaison compétitive au niveau du site ATP du domaine kinase Abl et stabilisent la forme inactive. Les conséquences sont une inhibition de la croissance tumorale et une mort des cellules leucémiques par apoptose. Les ITK ont bouleversé le devenir des patients atteints de LMC avec des taux de RC proche de 90% et de survie à long terme proche de 80%.[89-92] Dans la LAL à Ph1, cet inhibiteur a amélioré le taux de réponse initiale au traitement, permettant d'obtenir des taux de RC de 90 à 95%. Cependant la réponse n'est pas durable, du fait du développement précoce, voire préexistant de mécanismes de résistance aux ITK. Plusieurs mécanismes de résistances existent (figure n°10). Il peut s'agir de mutations ponctuelles de BCR-ABL, d'une amplification du gène BCR-ABL, de la surexpression protéique de Bcr-Abl, de la perte d'activité de Bcr-Abl ou de la résistance multidrogue par surexpression de glycoprotéine P. Ces mécanismes peuvent être additionnels. A la rechute environ 80% des patients développent une résistance secondaire aux ITK, principalement par mutations du domaine kinase. En revanche, les résistances primaires sont multifactorielles et non totalement élucidées.[89-95]

Figure n°10: Les mécanismes de résistances aux ITK



Yang K et al. Crit Rev Oncol Hematol 2015

A ce jour, une chimiothérapie conventionnelle associée aux ITK suivie d'une intensification thérapeutique par allogreffe de CSP demeure de ce fait le standard thérapeutique quand ce traitement est réalisable. L'objectif des protocoles basés sur les ITK est d'obtenir la meilleure réduction tumorale possible au moment de l'allogreffe. L´intensification thérapeutique par allogreffe de CSP améliore la survie mais celle-ci reste médiocre à long terme proche de 50%, de par la mortalité liée à la toxicité du traitement, à la maladie du greffon contre l'hôte (GvHD, Graft versus Host Disease) et aux rechutes par un effet GvL (Graft versus leukemia) insuffisant.[76,77,96]
Il convient de développer des stratégies thérapeutiques nouvelles afin d´améliorer la survie de ces patients, incluant l'autogreffe de CSP et les ITK. Identifier des facteurs pronostiques au sein de ce groupe permettrait d'adapter le traitement. La compréhension des mécanismes de résistance aux inhibiteurs, des mécanismes d´apoptose et de migration lymphocytaire peut être à la base de développement de nouvelles cibles thérapeutiques.[18,19,76,97]

2 - Traitement des LAL T

L'utilisation d'une chimiothérapie inspirée des protocoles pédiatriques a notablement amélioré le pronostic des LAL T. Leur taux de RC atteint 90 à 95 %. Cependant leur taux de survie à long terme n'est que de 60 % du fait de la toxicité du traitement et des rechutes survenant dans environ 40 à 50 % des cas. Différents études ont permis d'affiner les facteurs pronostiques et de leur donner un sens biologique. L’impact pronostique favorable des mutations de NOTCH1 ou de FBXW7 a été démontré.[67] L'absence de mutation de NOTCH1 ou de FBXW7 et la lenteur de réponse au traitement (persistance d'une maladie résiduelle précoce positive) sont des facteurs péjoratifs. Dans ce sous-groupe de patients à haut risque de rechute, l'intensification par allogreffe de CSP, malgré son cortège de morbidité et mortalité, reste nécessaire pour espérer une survie prolongée.[75,79] La stratification des groupes de risques demeure perfectible. En effet dans le sous-groupe LAL T NOTCH1 muté, un tiers des patients rechutent. Il faudrait pouvoir repérer ce sous-groupe pour renforcer le traitement.[98,99]

B - objectifs généraux

Les taux de survie des patients adultes atteints de LAL continuent d'être inférieurs à ceux obtenus chez l'enfant (survie de 90% chez l'enfant): un démembrement plus fin des groupes pronostiques pourrait autoriser des traitements adaptés et parfois réellement nouveaux.[5,18,73]

Mon travail de thèse s'inscrit dans cette identification de nouveaux groupes pronostiques. A la lumière des résultats des protocoles thérapeutiques actuels, nous avons travaillé sur l'oncogenèse des LAL T et des LAL à Ph1 avec un intérêt particulier pour les voies de signalisation Notch1 et PI3K/Akt/mTor et les mécanismes de résistance aux ITK dans les LAL à Ph1.

Dans la première partie de ce travail, notre objectif a été d'identifier les patients atteints de LAL T NOTCH1 muté, sous-groupe considéré de bon pronostic, qui rechutent. Nous nous sommes intéressés aux voies de signalisation Ras/RAF/MEK/ERK et PI3K/PTEN/Akt/mTOR, voies de signalisation impliquées dans la signalisation du pré-TCR.

Dans la seconde partie de ce travail portant sur les LAL à Ph1, un premier objectif a été d'envisager la possibilité de réaliser une autogreffe en alternative à l'allogreffe et de définir quels patients pourraient être concernés par cette option thérapeutique, ces interrogations se posant avec l'avènement des ITK et leur efficacité sur la survie à court terme. Cependant, devant la résistance aux ITK de patients atteints de LAL à Ph1, un deuxième objectif a été d'identifier des mécanismes de résistance non élucidés afin de pouvoir proposer des alternatives thérapeutiques, indépendantes de l'activation de Bcr-Abl.

C - Résultats

I - Les LAL T
Nouvelle classification pronostique des LAL T basée sur NOTCH1/FBXW7/RAS/PTEN

Article n°1
Tanguy-Schmidt A*, Trinquand A* et al. Toward a NOTCH1/FBXW7/RAS/PTEN-based oncogenetic risk classification of adult T-cell acute lymphoblastic leukemia: a Group for Research in Adult Acute Lymphoblastic Leukemia study. J Clin Oncol 2013;31:4333-4342
***Co-first authors**

Les LAL T, caractérisées par un processus oncogénique multi-étape, représentent 30 % des LAL de l'adulte et sont considérées de bon pronostic depuis l'utilisation d'un schéma de chimiothérapie intensive d'inspiration pédiatrique. Cependant malgré un taux de RC de 90%, la survie à long terme n'est que de 50 à 60% du fait de la toxicité des traitements et des rechutes. Ce groupe est très hétérogène. De nombreuses altérations géniques sont décrites.
La dérégulation de la voie de signalisation de Notch1 (voie de signalisation juxtacrine entre deux cellules au contact l'une de l'autre) constitue l'une des lésions oncogènes prédominantes.
La dérégulation de la voie Notch1 peut être due à des mutations de NOTCH 1 ou à des mutations de FBXW7, membre de la famille de E3 ligase, impliqué dans la dégradation de Notch1, en permettant sa polyubiquitination et sa dégradation par le protéasome.
Les mutations de NOTCH1 sont identifiées dans 50 à 60 % des LAL T. Elles peuvent essentiellement affecter le domaine d'hétérodimérisation, ou le domaine PEST, qui est le propre régulateur négatif de NOTCH1. Elles peuvent être couplées à des mutations de FBXW7, lesquelles sont observées dans environ 10 % des LAL T et aboutissent à une activation de la voie Notch1.
Le groupe du GRAALL a précédemment montré l'impact pronostiquefavorable, au sein des LAL T, d'une mutation de NOTCH1et/ou FBXW7. Au sein de ce groupe coopérateur, la stratification pronostique des LAL T repose sur l'absence de mutations NOTCH1 et/ou FBXW7 et sur la persistance d'une maladie résiduelle (MRD). Un sous-groupe pronostique à haut risque de rechute se dégage ainsi, seul à être réellement justiciable d'une l'intensification thérapeutique par allogreffe de CSP.
Cependant au sein même de la fraction NOTCH1/FBXW7 muté, sous-groupe pourtant considéré de bon pronostic, un tiers des patients rechutent : ceci suggère l'existence d'anomalies associées de mauvais pronostic encore cryptiques.

La dérégulation de deux voies de signalisation, la voie Ras/RAF/MEK/ERK et la voie PI3K/PTEN/Akt/mTOR, apporte un avantage prolifératif. Cette dérégulation décrite en pédiatrie a été peu étudiée chez l'adulte. RAS est un oncogène impliqué dans la vie cellulaire normale, qui favorise la prolifération tumorale en cas de mutation. PTEN est un gène suppresseur de tumeur, régulant la prolifération des cellules. Une mutation génétique l'empêchant de s'exprimer engendre une prolifération cellulaire et une résistance à la chimiothérapie.
Dans le cadre du groupe du GRAALL, nous nous sommes intéressé à ces deux voies de signalisation.
Nous avons comparé le devenir de 212 adultes atteints de LAL T avec ou sans mutation de NOTCH1 et/ou FBXW7. Comme le groupe du GRAALL l'avait déjà montré, c'est l'absence de mutation de NOTCH1et/ou FBWX7 qui est de mauvais pronostique, puisqu'elle s'accompagne de la réduction de la survie (OS) à 5 ans qui passe de 75 % à 42%. Cependant un tiers du groupe muté présente une rechute (EFS à 5 ans de 69 %), essentiellement pendant les 2 premières années.
Nous avons montré que les mutations de RAS (K-RAS et N-RAS) sont fréquentes (20 sur 191 patients étudiés) comme les anomalies PTEN (21 sur 175 patients étudiés) et que ces deux anomalies sont exclusives.
La présence de mutations de RAS ou d'anomalie de PTEN est de mauvais pronostic, réduisant l'OS à 5 ans de 69 % à 42 %.
En combinant ces différentes anomalies, nous avons identifié des sous-groupes au sein de la fraction mutée pour NOTCH1 et proposé une nouvelle classification pronostique reposant sur le statut de NOTCH1/FBXW7/RAS/PTEN. Les patients à haut risque sont ceux ne présentant pas de mutation pour NOTCH1 et/ou FBXW7 ou présentant une mutation pour RAS ou une anomalie de PTEN (OS à 5 ans de 44 %). Ainsi, seuls les patients avec une mutation de NOTCH1et/ou FBXW7 sans mutation de RAS ni d'anomalie de PTEN sont de bon pronostic (OS à 5 ans de 82 %).

Cette classification pronostique pourrait permettre l'adaptation des thérapeutiques dès le diagnostic par la connaissance du profil de mutation.

VOLUME 31 · NUMBER 34 · DECEMBER 1 2013

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Toward a *NOTCH1/FBXW7/RAS/PTEN*–Based Oncogenetic Risk Classification of Adult T-Cell Acute Lymphoblastic Leukemia: A Group for Research in Adult Acute Lymphoblastic Leukemia Study

Amélie Trinquand, Aline Tanguy-Schmidt, Raouf Ben Abdelali, Jérôme Lambert, Khetra Beldjord, Etienne Lengliné, Noémie De Gunzburg, Dominique Payet-Bornet, Ludovic Lhermitte, Hussein Mossafa, Véronique Lhéritier, Jonathan Bond, Françoise Huguet, Agnès Buzyn, Thibaud Leguay, Jean-Yves Cahn, Xavier Thomas, Yves Chalandon, André Delannoy, Caroline Bonmatt, Sébastien Maury, Bertrand Nadel, Elizabeth Macintyre, Norbert Ifrah, Hervé Dombret, and Vahid Asnafi

Author affiliations appear at the end of this article.

Published online ahead of print at www.jco.org on October 28, 2013.

Support information appears at the end of this article.

Written on behalf of the Group for Research in Adult Acute Lymphoblastic Leukemia (GRAALL).

A.T. and A.T.-S. are co-first authors.

H.D. and V.A. are co-director authors.

Presented in part at the 54th Annual Meeting of the American Society of Hematology, Atlanta, GA, December 8-11, 2012; at the 2013 Congress of the Société Française d'Hématologie, Paris, France, March 27-29, 2013; and at the Second European School of Haematology-European Hematology Association Scientific Workshop on T-Cell Acute Lymphoblastic Leukemia, Lisbon, Portugal, March 22-24, 2013.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

Clinical trial information: NCT00327678, NCT00222027.

Corresponding author: Vahid Asnafi, MD, Hôpital Necker-Enfants Malades, Laboratoire d'Hématologie, 149 rue de Sèvres, 75015 Paris, France; e-mail: vahid.asnafi@nck.aphp.fr.

0732-183X/13/3134w-4333$20.00

DOI: 10.1200/JCO.2012.48.5292

ABSTRACT

Purpose
The Group for Research in Adult Acute Lymphoblastic Leukemia (GRAALL) recently reported a significantly better outcome in T-cell acute lymphoblastic leukemia (T-ALL) harboring *NOTCH1* and/or *FBXW7* (*N/F*) mutations compared with unmutated T-ALL. Despite this, one third of patients with *N/F*–mutated T-ALL experienced relapse.

Patients and Methods
In a series of 212 adult T-ALLs included in the multicenter randomized GRAALL-2003 and -2005 trials, we searched for additional *N/K-RAS* mutations and *PTEN* defects (mutations and gene deletion).

Results
N/F mutations were identified in 143 (67%) of 212 patients, and lack of *N/F* mutation was confirmed to be associated with a poor prognosis. *K-RAS*, *N-RAS*, and *PTEN* mutations/deletions were identified in three (1.6%) of 191, 17 (8.9%) of 191, and 21 (12%) of 175 patients, respectively. The favorable prognostic significance of *N/F* mutations was restricted to patients without *RAS/PTEN* abnormalities. These observations led us to propose a new T-ALL oncogenetic classifier defining low-risk patients as those with *N/F* mutation but no *RAS/PTEN* mutation (97 of 189 patients; 51%) and all other patients (49%; including 13% with *N/F* and *RAS/PTEN* mutations) as high-risk patients. In multivariable analysis, this oncogenetic classifier remained the only significant prognostic covariate (event-free survival: hazard ratio [HR], 3.2; 95% CI, 1.9 to 5.15; $P < .001$; and overall survival: HR, 3.2; 95% CI, 1.9 to 5.6; $P < .001$).

Conclusion
These data demonstrate that the presence of *N/F* mutations in the absence of *RAS* or *PTEN* abnormalities predicts good outcome in almost 50% of adult T-ALL. Conversely, the absence of *N/F* or presence of *RAS/PTEN* alterations identifies the remaining cohort of patients with poor prognosis.

J Clin Oncol 31:4333-4342. © 2013 by American Society of Clinical Oncology

INTRODUCTION

T-cell acute lymphoblastic leukemia (T-ALL) corresponds to a heterogeneous group that accounts for 30% of adult *BCR-ABL*–negative acute lymphoblastic leukemias (ALLs).[1] Recognized T-ALL oncogenic pathways include proto-oncogene activation, tumor suppressor gene deletion, and activation of the Notch1 pathway by *NOTCH1/FBXW7* (*N/F*) mutations,[2,3] leading to various combinations of gene alterations and complex oncogenic networks.[4-8] *N/F* mutations involve either the heterodimerization domain, probably facilitating cleavage of the NOTCH receptor, and/or the negative regulatory PEST domain,[9] increasing the half-life of intracellular NOTCH. An alternative mechanism of constitutive Notch1 pathway activation involves loss-of-function mutations of *FBXW7*, leading to the inhibition of ubiquitin-mediated degradation of activated NOTCH1.[10]

Even if the complete remission (CR) rate in adults with *BCR-ABL*–negative ALLs reaches 90%,

long-term outcome remains unsatisfactory, with a 5-year overall survival (OS) rate of approximately 45%.[1] Historical prognostic factors used for therapeutic stratification are predominantly initial clinical features, including age, WBC count, immunophenotype, and CNS involvement.[11] Minimal residual disease (MRD) quantification is a strong prognostic factor[12] but requires stringent standardization and is obviously not available at baseline. The Group for Research in Adult Acute Lymphoblastic Leukemia (GRAALL) reported a significant improvement in the outcome of adults with *BCR-ABL*–negative ALL using a pediatric-inspired intensified treatment protocol,[13] which in T-ALL was particularly beneficial for patients harboring *N/F* mutations, compared with unmutated patients.[2] Despite this, approximately one third of patients with *N/F*-mutated T-ALL experience relapse, suggesting that other factors may dampen the positive effect of *N/F* and making the identification of a subgroup with a favorable outcome a desirable goal.

The two pro-proliferative Ras/Raf/MEK/ERK and PI3K/PTEN/Akt/mTOR pathways have also been reported to be deregulated in limited series of pediatric T-ALL,[14,15] but corresponding data for adult T-ALL are scanty. More specifically, *RAS*, a regulator of the Ras/Raf/MEK/ERK pathways, and *PTEN*, the main negative regulator of the PI3K/PTEN/Akt/mTOR pathways, both play roles in cell proliferation and resistance to chemotherapy.

Here, we identified *PTEN* loss-of-function deletions/mutations or *K-RAS/N-RAS* activating mutations as two virtually mutually exclusive genetic abnormalities found in 23% of adult T-ALLs treated on GRAALL trials. Importantly, the absence of *N/F* or presence of *RAS/PTEN* alterations identifies the 50% of patients who are most likely to benefit from alternative therapies that target either the PI3K/PTEN/Akt/mTOR or the Ras/Raf/MEK/ERK pathways.

PATIENTS AND METHODS

The GRAALL-2003 protocol was a multicenter phase II trial that enrolled 76 adults with T-ALL between November 2003 and November 2005,[13] of whom 57 had material available for the present study and have been previously reported.[3] The multicenter randomized GRAALL-2005 trial was the following phase III trial and was similar to the GRAALL-2003 trial, with the addition of the randomized evaluation of an intensified sequence of hyperfractionated cyclophosphamide during induction and late intensification. Between May 2006 and May 2010, 189 adults with T-ALL were randomly assigned in the GRAALL-2005 study. The present study concerns 155 of these patients, for whom diagnostic DNA and/or cDNA was available. As for the GRAALL-2003 trial, these 155 patients were representative of the total GRAALL-2005 T-ALL population, with a 3-year OS of 67% (95% CI, 60% to 74%). The GRAALL-2003 and GRAALL-2005 protocols are briefly described in the Data Supplement. Informed consent was obtained from all patients at trial entry. Both trials were conducted in accordance with the Declaration of Helsinki and approved by local and multicenter research ethical committees. In these trials, allogeneic (allo) stem-cell transplantation (SCT) was offered in first CR in patients who had a matched sibling or 10/10 fully matched unrelated donor and at least one of the following criteria: CNS involvement at diagnosis; early resistance to

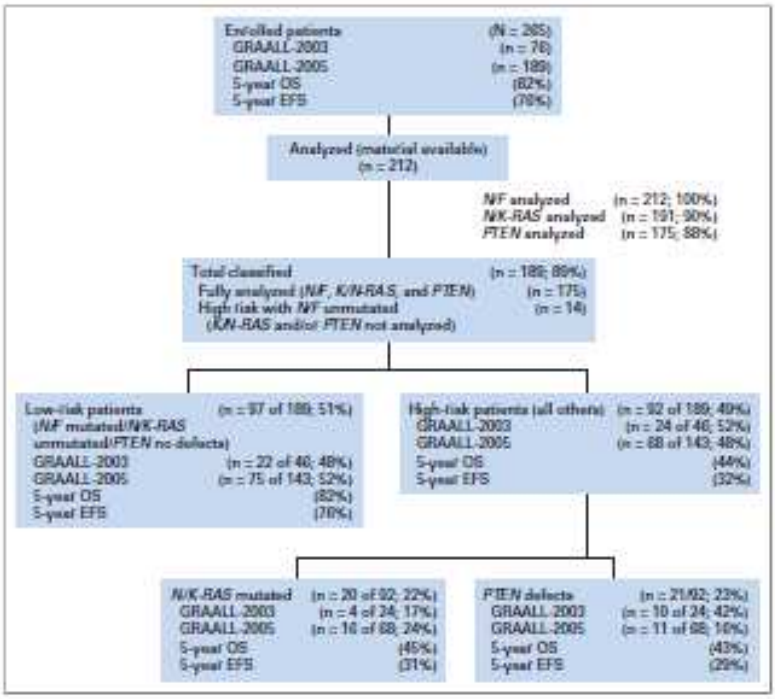


Fig 1. Patient flow diagram. EFS, event-free survival; GRAALL, Group for Research in Adult Acute Lymphoblastic Leukemia; *N/F*, *NOTCH1/FBXW7*; OS, overall survival.

corticosteroid after the first 1-week prephase; early resistance to chemotherapy after 1 additional week of treatment; and CR not achieved after first induction.

Among the 212 consecutive adults with T-ALL included in the present study (57 GRAALL-2003 and 155 GRAALL-2005 patients), 133 were eligible for allo-SCT and 67 actually received transplantation in first CR (16 GRAALL-2003 and 51 GRAALL-2005 patients). With a point date on December 31, 2011, the median follow-up time was 4.2 years (6.0 and 3.3 years for GRAALL-2003 and GRAALL-2005 patients, respectively). Complete methods are available in the Data Supplement.

Patient characteristics and CR rates were compared using either the Fisher's exact test or the Mann-Whitney *U* test. Median comparisons were performed using the Mann-Whitney *U* test. OS and event-free survival (EFS) were calculated from the date of prophase initiation. Events accounting for EFS were induction failure, first hematologic relapse, and death from any cause in first CR. Cumulative incidence of relapse (CIR) and relapse-free survival (RFS) were calculated from the date of CR achievement. For the analysis of survival outcomes, SCT was not considered to be a censoring event in patients who received allo-SCT in first CR. OS and EFS were estimated using the Kaplan-Meier method and then compared using the log-rank test.[28] Multivariable regressions were performed with the Cox model.[27] CIR was estimated taking into account death in first CR for competing risk and then compared using cause-specific hazard Cox models. Specific hazards of relapse (SHRs) and hazard ratios (HRs) were given with 95% CIs. Interactions were assessed by introducing an interaction term in the Cox model. Prognostic discriminatory powers were evaluated by concordance probability estimates[29] and then

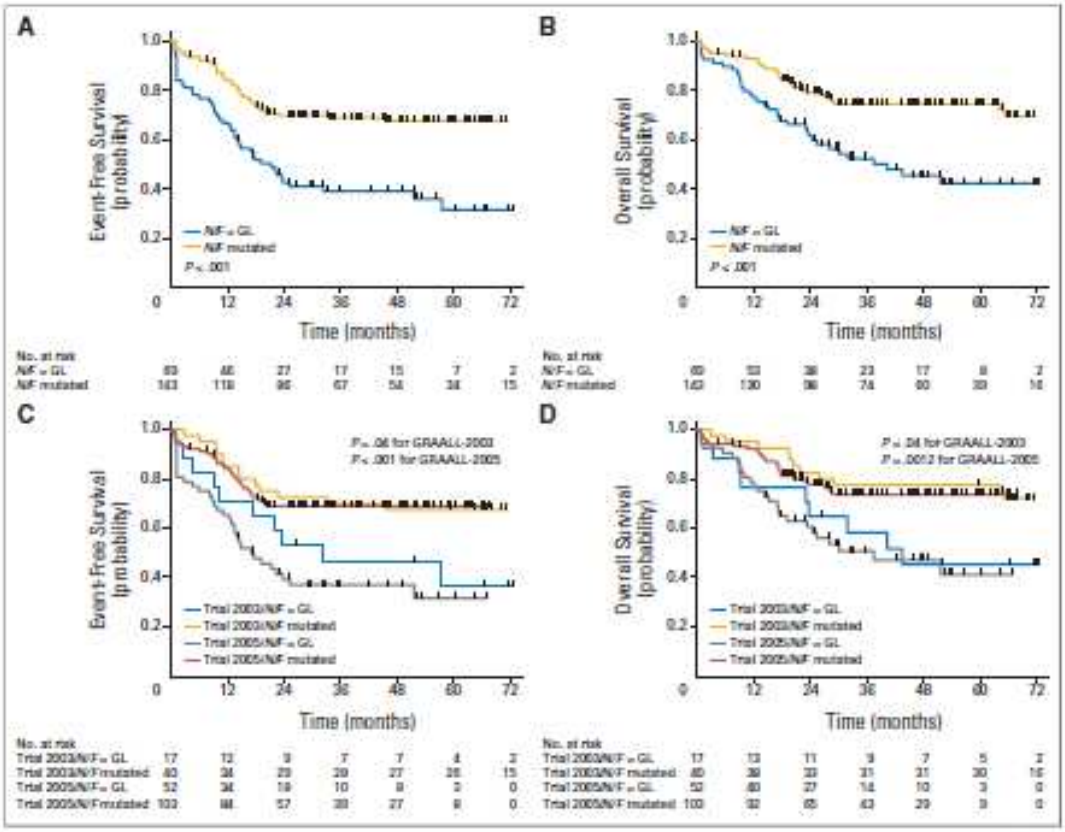


Fig 2. Event-free survival (EFS) and overall survival (OS) by *NOTCH1/FBXW7* (*N/F*) status and trial. (A) EFS by *N/F* status. At 5 years, EFS was estimated at 32% (95% CI, 19% to 45%) in patients with unmutated *N/F*, compared with 69% (95% CI, 60% to 76%) in those with *N/F* mutation. The hazard ratio (HR) for shorter EFS in the former group was 2.6 (95% CI, 1.7 to 3.9; $P < .001$). (B) OS by *N/F* status. At 5 years, OS was estimated at 42% (95% CI, 29% to 55%) in patients with unmutated *N/F*, compared with 75% (95% CI, 66% to 81%) in those with *N/F* mutation. The HR for shorter OS in the former group was 2.45 (95% CI, 1.5 to 3.9; $P < .001$). (C) EFS by *N/F* status in the Group for Research in Adult Acute Lymphoblastic Leukemia (GRAALL)–2003 and GRAALL-2005 trials. For GRAALL-2003 patients, 5-year EFS was estimated at 27% (95% CI, 14% to 61%) in patients with unmutated *N/F*, compared with 67.5% (95% CI, 51% to 80%) in those with *N/F* mutation. The HR for shorter EFS in the former group was 2.3 (95% CI, 1.01 to 5.2; $P = .04$). For GRAALL-2005 patients, 5-year EFS was estimated at 32% (95% CI, 18% to 47%) in patients with unmutated *N/F*, compared with 69% (95% CI, 59% to 77%) in those with *N/F* mutation. The HR for shorter EFS in the former group was 2.65 (95% CI, 1.6 to 4.3; $P < .001$). (D) OS by *N/F* status in the GRAALL-2003 and GRAALL-2005 trials. For GRAALL-2003 patients, 5-year OS was estimated at 45% (95% CI, 21% to 67%) in patients with unmutated *N/F*, compared with 77.5% (95% CI, 61% to 88%) in those with *N/F* mutation. The HR for shorter OS in the former group was 2.45 (95% CI, 1.01 to 5.9; $P = .04$). For GRAALL-2005 patients, 5-year OS was estimated at 41% (95% CI, 24% to 57%) in patients with unmutated *N/F*, compared with 74% (95% CI, 63% to 81%) in those with *N/F* mutation. The HR for shorter OS in the former group was 2.4 (95% CI, 1.4 to 4.2; $P = .0012$). GL, germline.

compared using the bootstrap method. STATA/SE 10.1 software (STATA, College Station, TX) was used. All tests were two-sided, with a type I error at 5%.

RESULTS

Lack of N/F Mutation Identifies a Poor Prognostic Subset of Adult T-ALL

N/F mutations were identified in 143 (67%; 95% CI, 61% to 74%) of the 212 analyzed patients with T-ALL (Fig 1). The mutation rate of *N/F* was similar in the GRAALL-2003 (70%; 95% CI, 57% to 82%) and GRAALL-2005 (67%; 95% CI, 58% to 74%) cohorts. In keeping with our previous report,[1,2] EFS and OS were significantly ($P < .001$ and $P < .001$, respectively) better in T-ALLs harboring *N/F* mutations, compared with unmutated T-ALL (Figs 2A and 2B, respectively). Furthermore, as shown in Figures 2C and 2D, the favorable impact of *N/F* mutation was also observed when GRAALL-2003 and GRAALL-2005 patients were analyzed separately.

Despite this, one third of patients with *N/F* mutations experienced an EFS event, mostly within the first 2 years of follow-up (Fig 2A). To identify a genetic surrogate for relapsing T-ALLs, we studied Ras/Raf/MEK/ERK and PI3K/PTEN/Akt/mTOR pathway activation by *N/K-RAS* and *PTEN* alteration, respectively.

N/K-RAS Mutations Are Frequent Events in Adult T-ALL

Among the 212 patients with T-ALL tested for *N/F* mutations, 191 were explored for activating *RAS* mutations. *K-RAS* and *N-RAS* mutations were identified in three (2%; 95% CI, 0.3% to 5%) of 191 and 17 (9%; 95% CI, 5% to 14%) of 191 patients, respectively. Overall, 20 (11%; 95% CI, 7% to 16%) of 191 GRAALL T-ALLs harbored activating *RAS* mutations. Clinical, immunophenotypic and oncogenic features of the patients were analyzed according to the absence or presence of *RAS* mutations (Table 1), and full details of individual patients with *RAS* abnormalities are reported in the Data Supplement.

Patients with *RAS* mutations did not differ significantly from patients without mutations with respect to age, sex, or WBC counts greater than 100×10^9/L at diagnosis (Table 1). CNS involvement was found in 25% of patients with *RAS* mutations versus 6% of patients without mutations ($P = .02$). *RAS* mutations were also more frequently observed in T-ALL with no classical oncogenic markers compared with T-ALLs harboring *TLX1/3*, *SIL-TAL1*, or *CALM-AF10* abnormalities (78% *v* 50%, respectively; $P = .03$). No significant correlation was found with European Group for the Immunological Classification of Leukemias class or *N/F* status or early sensitivity to corticosteroids and chemotherapy, but *RAS* mutations were notably absent in T-cell receptor (TCR)–positive T-ALLs.

PTEN Genomic Deletions and Mutations Lead to PTEN Loss in 12% of Adult T-ALLs

PTEN mutations were identified in 17 (10%; 95% CI, 6% to 15%) of 175 patients with available material (all of whom had been tested for *RAS* mutations). All mutations were nonsense or, more frequently, frameshift insertions or insertions/deletions as reported in the Data Supplement. We then analyzed the whole *PTEN* locus by high-resolution comparative genomic hybridization (CGH) array for 100 patients already screened for *PTEN* exon7 mutations. Overall, *PTEN* deletions were detected in five (5%; 95% CI, 2% to 11%) of 100 patients. The deletions were mainly large, ranging from 60 to 7,464 kb, but were focal and intragenic in two patients (Fig 3A) and biallelic in one patient. Because the breakpoints were relatively heterogeneous, a common deleted region, including exon 2, was identified (Fig 3A, right panel).

To validate the CGH array findings, *PTEN* (introns 2 and 8) genomic allele quantification by quantitative polymerase chain reaction was performed. As shown in Figure 3A, all patients with *PTEN* deletions identified by CGH array demonstrated a low *PTEN/ALBUMIN* gene dosage ratio (range, 0.05 to 0.59) compared with 39 patients without deletions (range, 0.72 to 1.4). This genomic quantitative polymerase chain reaction system was then used to identify

Table 1. Characteristics of Patients With T-ALL According to Their *RAS* Status

Characteristic	All Patients		*N/K-RAS* Exon 1 Mutation		*N/K-RAS* Exon 1 Wild Type		*P**
	No.	%	No.	%	No.	%	
Total patients	191		20	10	171	90	
TCR subsets analyzed	172						
Immature	44	26	7	41	37	24	.14
Pre-αβ	92	53	10	59	82	53	9
TCR positive	36	21	0	0	36	23	.025†
EGIL	180						
I,II	70	39	9	50	61	36	.32
III	89	49	9	50	80	49	1.0
IV	21	12	0	0	21	13	.14
Genotype subsets analyzed	183						
CALM-AF10	9	5	0	0	9	5	.6
SIL-TAL1	16	9	0	0	16	10	.37
TLX1	37	20	2	11	35	21	.54
TLX3	25	14	2	11	23	14	1.0
None of above	96	52	14	78	82	50	.031†
N/F mutation	132	69	16	80	116	68	.32
Clinical subsets analyzed							
Male	143	75	14	70	129	76	.59
Age, years							
Median	31		34		31		.23
> 35	77	41	9	45	68	40	.64
WBC count, × 10⁹/L							
Median	36.4		47.1		36.4		.99
> 100	53	28	5	25	48	28	1.0
CNS involvement	16	8	5	25	11	6	.021†
CR	176	92	19	95	157	92	.56
Cs	105	55	11	55	94	55	1.0
CHs	108	57	12	60	96	56	.92

Abbreviations: CHs, chemosensitivity; CR, complete remission; Cs, corticosteroid sensitivity; EGIL, European Group for the Immunological Classification of Leukemias; *N/F*, *NOTCH1* and/or *FBXW7*; T-ALL, T-cell acute lymphoblastic leukemia; TCR, T-cell receptor.

*Determined using *t* test or Fisher's exact test when appropriate.

†Significant value.

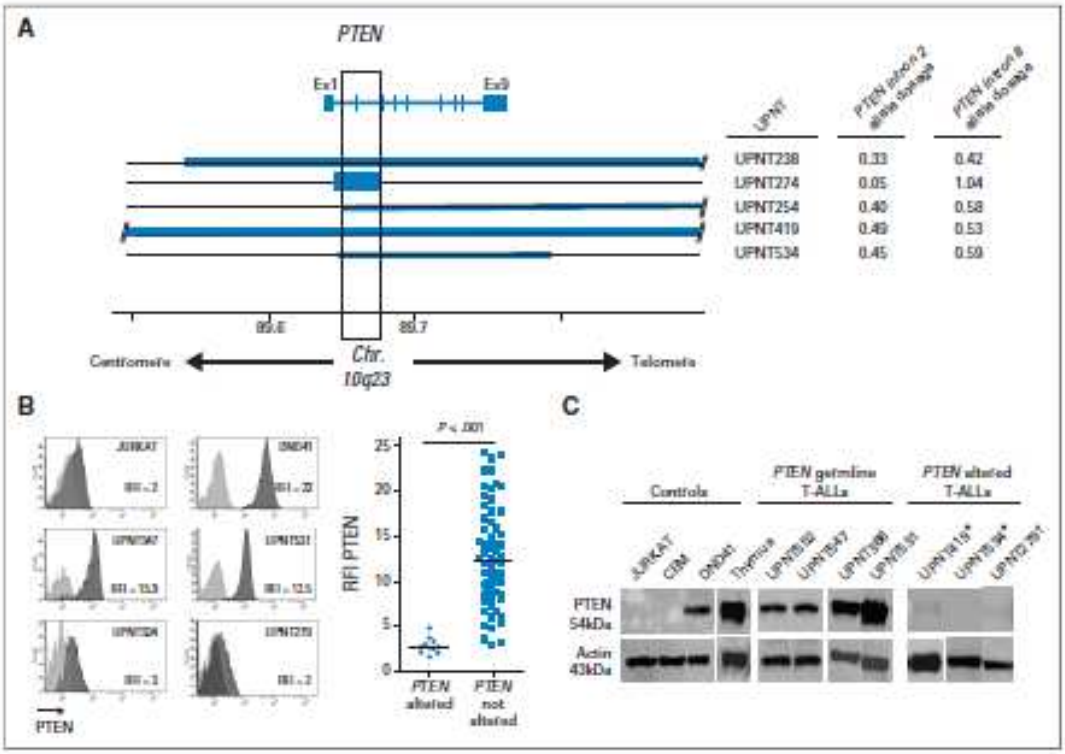


Fig 3. *PTEN* analysis. (A) Schematic representation of *PTEN* deletions (left) and *PTEN* intron 2/*ALB* and *PTEN* intron 8/*ALB* genomic quantitative polymerase chain reaction (qPCR) ratios (right) in five T-cell acute lymphoblastic leukemias (T-ALLs). Patient UPNT238 harbors a monoallelic deletion of *PTEN* concordant with gene dosage results (*PTEN* intron 2/*ALB* and *PTEN* intron 8/*ALB* qPCR ratios, 0.33 and 0.42, respectively). Patient UPNT274 harbors a biallelic deletion of the exon 2 and intron 2 of *PTEN* concordant with gene dosage results (*PTEN* intron 2/*ALB* qPCR ratio of 0.05 and *PTEN* intron 8/*ALB* qPCR ratio of 1.04). (B) Flow cytometry analysis of PTEN expression in T-ALL cell lines and primary T-ALL samples (left) and representation of PTEN ratio of fluorescence intensity (RFI) according to *PTEN* status (right). Lighter gray histograms represent the isotypic control, and the darker gray histograms represent PTEN levels. JURKAT is a *PTEN*-null cell line. DND41 harbors *PTEN* levels similar to germline *PTEN* primary T-ALLs. The two *PTEN*-altered primary T-ALLs show low PTEN protein. RFI was less than 5 in all *PTEN*-altered T-ALLs, whereas RFI ranged from 2.0 and 44.2 (median, 12.6) in *PTEN*-nonaltered T-ALLs. (C) PTEN Western blot analysis in T-ALL cell lines, normal human thymus, and primary T-ALL samples. Tested T-ALLs with germline *PTEN* status harbored higher level of PTEN protein compared with T-ALLs with genomic *PTEN* alterations. Actin was used as a loading control. Western blot data are in concordance with flow cytometric results. (*) Deletion; (†) Mutation.

PTEN deletion in the remaining 75 patients tested for *PTEN* mutations but not by CGH array. This allowed identification of one additional patient with *PTEN* deletion (*PTEN/ALBUMIN* ratio, 0.53). Overall, *PTEN* genomic deletions occurred in six (3%; 95% CI, 0.9% to 6%) of 175 patients. Two patients with heterozygous *PTEN* deletions also harbored *PTEN* mutations (Data Supplement). Altogether, *PTEN* genomic abnormalities by deletion and/or mutation were identified in 21 (12%; 95% CI, 7.8% to 18%) of 175 patients.

To determine whether the observed *PTEN* genomic abnormalities led to inactivation of PTEN expression and function, we then analyzed protein expression by immunophenotyping and Western blot in 82 and 57 T-ALLs, respectively, with available material. All tested patients harboring *PTEN* genomic alteration (four deletions and seven mutations) demonstrated loss of or low-level PTEN protein expression as measured by Western blot or flow analysis (Figs 3B and 3C).

PTEN *Genomic Abnormalities Occur Frequently in Unmutated N/F- and SIL-TAL1–Positive Adult T-ALLs but Are Mutually Exclusive With N/K-RAS Mutations*

Clinical, immunophenotypic, and oncogenic features of patients were analyzed as a function of *PTEN* status (Table 2). Full clinical, immunophenotypic, oncogenic, and karyotypic data of individual patients with *PTEN* abnormalities are reported in the Data Supplement. *PTEN* abnormalities were more frequent in unmutated *N/FT*-ALLs; only eight (38%; 95% CI, 18% to 62%) of 21 T-ALLs with *PTEN* mutations/deletions harbored *N/F* mutations compared with 112 (73%; 95% CI, 65% to 80%) of 154 germline *PTEN* T-ALLs ($P = .002$). With respect to recurrent oncogenic subtypes, *SIL-TAL1*–positive patients demonstrated the highest rate of *PTEN* abnormalities; seven (33%; 95% CI, 15% to 57%) of 21 T-ALLs with *PTEN* mutations/deletions harbored *SIL-TAL1* fusion compared with only nine (6%; 95% CI, 2.8% to 11.2%) of 149 *PTEN* wild-type T-ALLs ($P = .001$).

Table 2. Characteristics of Patients With T-ALL According to Their *PTEN* Status (*PTEN* CGH array, *PTEN*/*ALB* allelic ratios, and *PTEN* exon 7 mutation)

Characteristic	All Patients		PTEN				
			Altered		Not Altered		
	No.	%	No.	%	No.	%	*P*
Total patients	175		21	12	154	88	
TCR subsets analyzed	160						
Immature	41	26	1	5	40	28	.048†
Pre-αβ	85	53	9	47	76	54	.6
TCR positive	34	21	9	47	25	18	.006†
EGIL	167						
I-II	65		5	25	60	41	.22
III	82		10	50	72	49	1.0
IV	20		5	25	15	10	.07
Genotype subsets analyzed	170						
CALM-AF10	9	5	1	5	7	5	1.0
SIL-TAL1	16	9	7	33	9	6	< .001†
TLX1	31	18	1	5	30	20	.13
TLX3	24	14	2	10	22	15	.74
None of above	91	54	10	48	81	54	.64
N/F mutated	120	69	8	38	112	73	.002†
Clinical subsets analyzed							
Male	131	75	18	86	113	73	.29
Age, years							
Median	30.8		24.9		31.4		.001†
> 35	69	39	3	14	66	43	.021
WBC, × 10⁹/L							
Median	36.8		110		29.9		.001†
> 100	49	28	13	62	36	23	< .001†
CNS involvement	18	9	4	19	12	8	.11
CR	160	96	20	95	140	96	1.0
Cs	94	54	8	38	86	56	.16
CHs	98	56	15	71	83	54	.16

Abbreviations: CGH, comparative genomic hybridization; CHs, chemosensitivity; CR, complete remission; Cs, corticosteroid sensitivity; EGIL, European Group for the Immunological Classification of Leukemias; *N/F*, *NOTCH1* and/or *FBXW7*; T-ALL, T-cell acute lymphoblastic leukemia; TCR, T-cell receptor.

*Determined using *t* test or Fisher's exact test when appropriate.

†Significant value.

PTEN-altered patients did not significantly differ from wild-type patients with respect to sex, CNS involvement, or early sensitivity to corticosteroids or chemotherapy (Table 2), but WBC counts greater than 100×10^9/L at diagnosis were found in 62% of *PTEN*-altered patients compared with 23% of unmutated patients ($P < .001$). *PTEN*-altered status was also more frequently observed in patients younger than 35 years of age ($P = .02$) and in mature T-ALLs expressing surface TCR (47% *v* 18% not expressing surface TCR; $P = .006$). Overall, *PTEN* alteration was more frequent in younger, mature, TCR-positive, *SIL-TAL1*–positive, *N/F* unmutated patients with high leukemic bulk tumors. Interestingly, only one patients with *RAS* mutation was also mutated for *PTEN* but only within a subpopulation of leukemic cells (Data Supplement), suggesting that these two oncogenic alterations affecting two different interlinked pro-proliferative pathways may be virtually mutually exclusive in adult T-ALL.

N/K-RAS *Mutations and* PTEN *Genomic Abnormalities Predict Similar Poor Outcome*

Figures 4A, 4B, and 4C show that both *N/K-RAS* mutations and *PTEN* genomic abnormalities were associated with marked trends to shorter CIR, RFS, and OS (see the Data Supplement for *PTEN* abnormalities alone and within *N/F* subgroups). Because of their biologic pro-proliferative function, mutual exclusion, and similar poor prognostic significance, we regrouped all patients with *N/K-RAS* mutations or *PTEN* genomic abnormalities in one unique *RAS/PTEN* alteration subgroup. Figures 4D, 4E, and 4F illustrate the significant prognostic impact of these oncogenetic alterations on CIR, RFS, and OS, respectively.

RAS, PTEN, *and N/F Mutational Status Identifies a Strong Classifier in Adult T-ALL*

We then analyzed how the presence of these virtually exclusive *N/K-RAS* mutations and *PTEN* genomic abnormalities may modulate the good prognosis associated with *N/F* mutations and whether prognostic interactions may exist between these two genomic pathways. For this purpose, we performed a multivariable Cox model for CIR, RFS, and OS, entering the two *N/F* and *RAS/PTEN* covariates, as well as their interaction term. As illustrated in Figures 5A, 5B, and 5C, this analysis indicated that the prognostic significance of *N/F* mutations was still observed but with significant interactions between *N/F* and *RAS/PTEN* mutations, indicating that the favorable impact of *N/F* mutation was only observed in patients without *RAS/PTEN* mutation (Figs 5A to 5C). Importantly, sensitivity analyses of patients treated as part of the GRAALL-2003 trial or during the GRAALL-2005 trial demonstrated that statistical significance of the classifier was consistent in both groups (Data Supplement).

These observations led us to propose a new T-ALL oncogenetic classifier defining low-risk patients as those with *N/F* mutation but no *RAS/PTEN* mutation (here, 97 of 189 studied patients; 51%) and all other patients (49%) as high-risk patients. Figures 5D, 5E, and 5F show CIR, RFS, and OS according to this new strong oncogenetic classifier. As a whole, 23 patients who would have been classified as low risk based on their *N/F* status joined the high-risk subgroup based on their *RAS/PTEN* status. Importantly, these patients did not differ from their *N/F*-mutated, *RAS/PTEN*-unaltered counterparts (Data Supplement). Comparing the oncogenetic risk classification based only on the *N/F* mutational status to this refined oncogenetic classifier, HRs for high-risk patients increased from 2.6 (95% CI, 1.7 to 4.0) to 3.25 (95% CI, 2.0 to 5.3) for EFS and from 2.5 (95% CI, 1.5 to 4.0) to 3.3 (95% CI, 1.9 to 5.8) for OS. Concordance probability estimates of the old *N/F* versus the new *N/F-RAS-PTEN* classifier were 0.603 (95% CI, 0.561 to 0.645) versus 0.633 (95% CI, 0.589 to 0.677) for EFS and 0.600 (95% CI, 0.552 to 0.647) versus 0.636 (95% CI, 0.587 to 0.684) for OS, respectively.

When adjusting the effect of the *N/F-RAS-PTEN* classifier to age (using the 35-year cutoff) and WBC count (using the 100×10^9/L cutoff), the oncogenetic classifier remained the only significant prognostic covariate (EFS: HR, 3.2; 95% CI, 1.9 to 5.15; $P < .001$; and OS: HR, 3.2; 95% CI, 1.9 to 5.6; $P < .001$).

A limited subset of 89 patients (46 new low-risk and 43 new high-risk patients, according to this *N/F-RAS-PTEN* classifier) were evaluated for genomic immunoglobulin/TCR MRD level at time of CR achievement after the first induction course. Using the 10^{-4} MRD cutoff, there was only a nonstatistically significant trend toward a

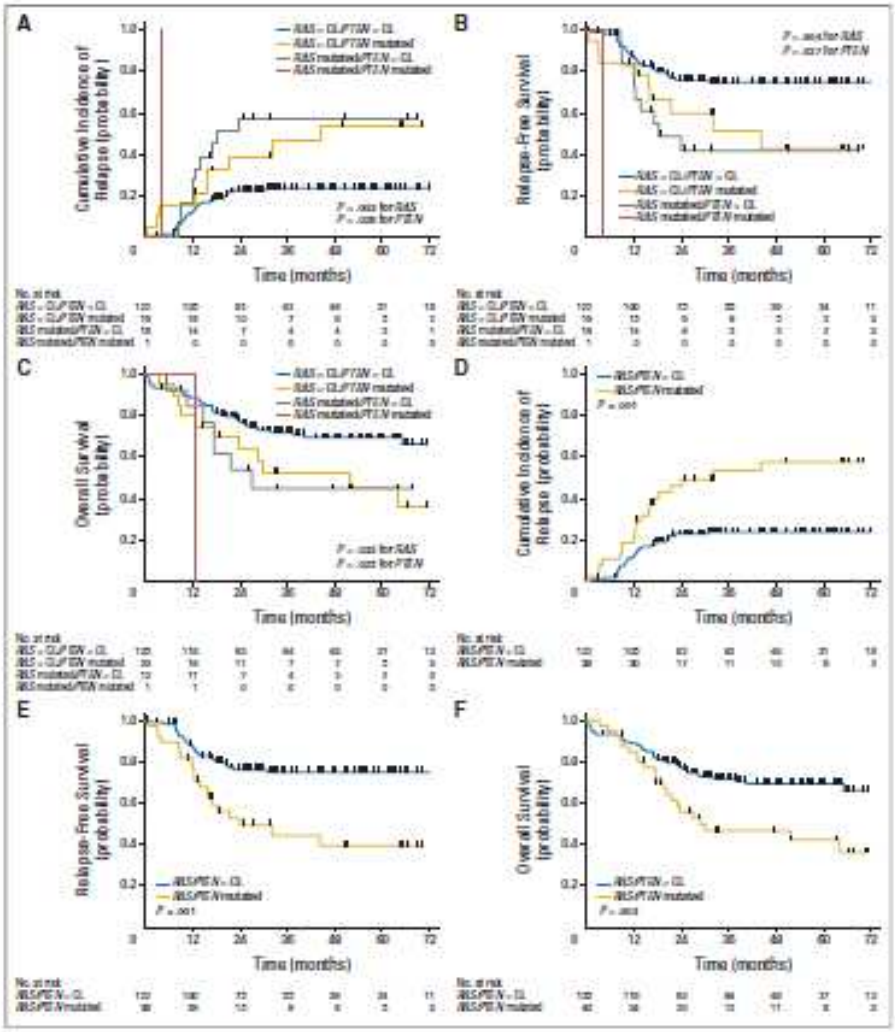


Fig 4. Cumulative incidence of relapse (CIR), relapse-free survival (RFS), and overall survival (OS) by *NIK-RAS* mutation or *PTEN* genomic abnormality. (A) CIR according to the presence of *NIK-RAS* mutation alone, *PTEN* genomic abnormality alone, or both (one single patient). At 5 years, CIR was estimated at 24% (95% CI, 17% to 33%) in patients with no *NIK-RAS* mutation or *PTEN* genomic abnormality, compared with 57% (95% CI, 36% to 80%) in those with *NIK-RAS* mutation and 54% (95% CI, 32% to 79%) in those with *PTEN* genomic abnormality. In the latter subgroups, the specific hazards of relapse (SHRs) were 2.6 (95% CI, 1.4 to 5.1; $P = .003$) and 2.1 (95% CI, 1.1 to 4.3; $P = .028$), respectively. (B) RFS according to the presence of *NIK-RAS* mutation alone, *PTEN* genomic abnormality alone, or both (one single patient). At 5 years, RFS was estimated at 75% (95% CI, 66% to 82%) in patients with no *NIK-RAS* mutation or *PTEN* genomic abnormality, compared with 42% (95% CI, 19% to 64%) in those with *NIK-RAS* mutation and 43% (95% CI, 18% to 66%) in those with *PTEN* genomic abnormality. In the latter groups, hazard ratios (HRs) for shorter RFS were 2.6 (95% CI, 1.3 to 5.0; $P = .004$) and 2.2 (95% CI, 1.1 to 4.3; $P = .027$), respectively. (C) OS according to the presence of *NIK-RAS* mutation alone, *PTEN* genomic abnormality alone, or both (one single patient). At 5 years, OS was estimated at 69% (95% CI, 60% to 77%) in patients with no *NIK-RAS* mutation or *PTEN* genomic abnormality, compared with 45% (95% CI, 19% to 69%) in those with *NIK-RAS* mutation and 43% (95% CI, 20% to 64%) in those with *PTEN* genomic abnormality. In the latter groups, HRs for shorter OS were 2.0 (95% CI, 1.04 to 3.8; $P = .033$) and 2.0 (95% CI, 1.06 to 3.8; $P = .028$), respectively. (D) CIR according to the presence of *NIK-RAS* mutation and/or *PTEN* genomic abnormality. At 5 years, CIR was estimated at 24% (95% CI, 17% to 33%) in patients with no *NIK-RAS* mutation or *PTEN* genomic abnormality, compared with 58% (95% CI, 41% to 75%) in those with *NIK-RAS* mutation and/or *PTEN* genomic abnormality. The SHR was 2.9 (95% CI, 1.5 to 4.9) in the latter group ($P < .001$). (E) RFS according to the presence of *NIK-RAS* mutation and/or *PTEN* genomic abnormality. At 5 years, RFS was estimated at 75% (95% CI, 66% to 82%) in patients with no *NIK-RAS* mutation or *PTEN* genomic abnormality, compared with 40% (95% CI, 22% to 57%) in those with *NIK-RAS* mutation and/or *PTEN* genomic abnormality. The HR for shorter RFS in the latter group was 2.7 (95% CI, 1.5 to 4.9; $P < .001$). (F) OS according to the presence of *NIK-RAS* mutation and/or *PTEN* genomic abnormality. At 5 years, OS was estimated at 69.5% (95% CI, 60% to 77%) in patients with no *NIK-RAS* mutation or *PTEN* genomic abnormality, compared with 42% (95% CI, 28% to 56%) in those with *NIK-RAS* mutation and/or *PTEN* genomic abnormality. The HR for shorter OS in the latter group was 2.1 (95% CI, 1.3 to 3.6; $P = .003$). GL, germline.

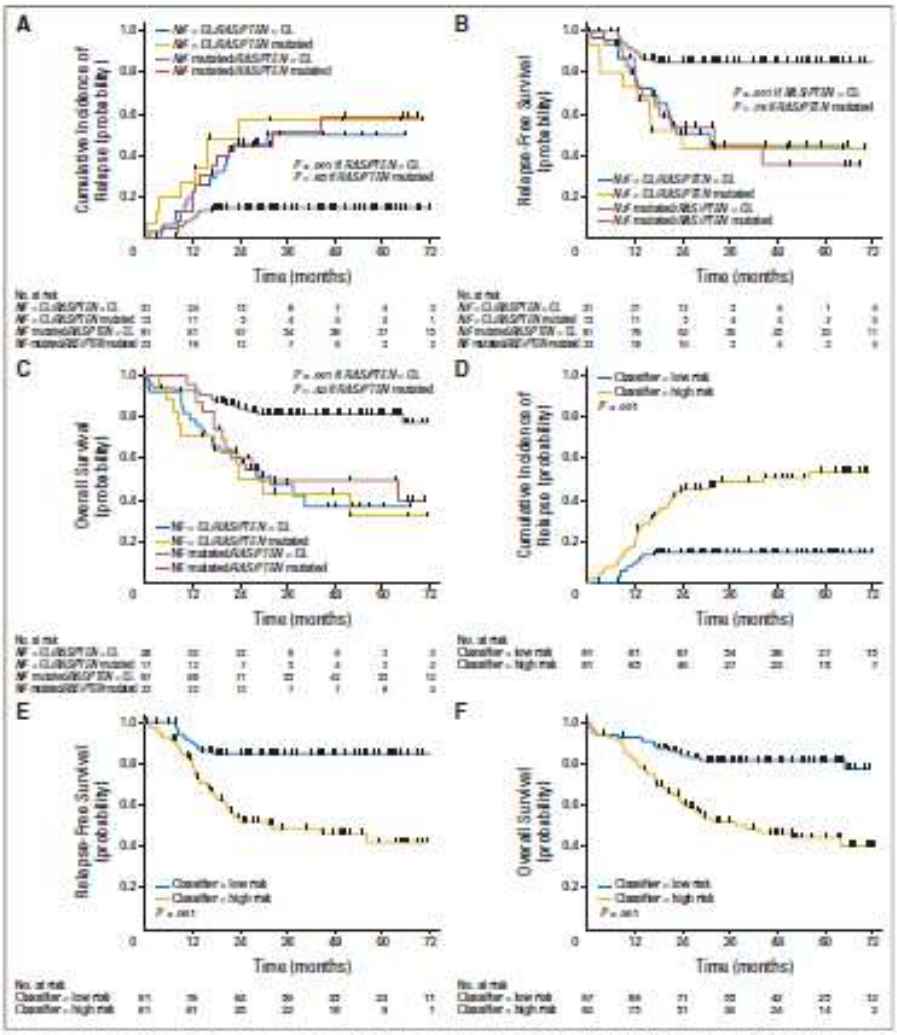


Fig 5. Cumulative incidence of relapse (CIR), relapse-free survival (RFS), and overall survival (OS) by *NOTCH1/FBXW7* (*NF*) and *RAS/PTEN* mutational status. (A) CIR according to the presence of *NF* and/or *RAS/PTEN* mutations. In patients with no *NK-RAS* mutation or *PTEN* genomic abnormality, 5-year CIR was estimated at 15% (95% CI, 9% to 24%) in patients with *NF* mutation, compared with 50% (95% CI, 34% to 64%) in those without *NF* mutation. The specific hazard of relapse (SHR) was 3.3 (95% CI, 2.0 to 10.0) in the latter group (*P* < .001). Conversely, in those with *NK-RAS* mutation and/or *PTEN* genomic abnormality, 5-year CIR was similarly poor in patients with *NF* mutation and in those without *NF* mutation (58%; 95% CI, 37% to 80% *v* 57%; 95% CI, 33% to 83%, respectively). The SHR was 1.25 (95% CI, 0.5 to 3.3) in the latter group (*P* = .65). (B) RFS according to the presence of *NF* and/or *RAS/PTEN* mutations. In patients with no *NK-RAS* mutation or *PTEN* genomic abnormality, 5-year RFS was estimated at 85% (95% CI, 76% to 91%) in patients with *NF* mutation, compared with 46% (95% CI, 25% to 63%) in those without *NF* mutation. The hazard ratio (HR) for shorter RFS in the latter group was 4.0 (95% CI, 2.0 to 10.0; *P* < .001). Conversely, in those with *NK-RAS* mutation and/or *PTEN* genomic abnormality, 5-year RFS was similarly poor in patients with *NF* mutation and in those without *NF* mutation (38%; 95% CI, 13% to 59% *v* 43%; 95% CI, 17% to 67%, respectively). The HR for shorter RFS in the latter group was 1.1 (95% CI, 0.45 to 2.5; *P* = .79). (C) OS according to the presence of *NF* and/or *RAS/PTEN* mutations. In patients with no *NK-RAS* mutation or *PTEN* genomic abnormality, 5-year OS was estimated at 82% (95% CI, 72% to 88%) in patients with *NF* mutation, compared with 37% (95% CI, 19% to 55%) in those without *NF* mutation. The HR for shorter OS in the latter group was 3.3 (95% CI, 2.0 to 7.1; *P* < .001). Conversely, in those with *NK-RAS* mutation and/or *PTEN* genomic abnormality, 5-year OS was similarly poor in patients with *NF* mutation and in those without *NF* mutation (49%; 95% CI, 27% to 68% *v* 32%; 95% CI, 10% to 57%, respectively). The HR for longer OS in the former group was 0.7 (95% CI, 0.3 to 1.7; *P* = .43). (D) CIR according to the new *NF*, *NK-RAS*, and *PTEN* oncogenetic classifier. At 5 years, CIR was estimated at 15% (95% CI, 9% to 24%) in low-risk patients, compared with 54% (95% CI, 42% to 66%) in high-risk patients. The SHR was 4.1 (95% CI, 2.2 to 7.7) in the latter group (*P* < .001). (E) RFS according to the new *NF*, *NK-RAS*, and *PTEN* oncogenetic classifier. At 5 years, RFS was estimated at 85% (95% CI, 76% to 91%) in low-risk patients, compared with 42% (95% CI, 29% to 55%) in high-risk patients. The HR for shorter RFS in the latter group was 4.2 (95% CI, 2.3 to 8.0; *P* < .001). (F) OS according to the new *NF*, *NK-RAS*, and *PTEN* oncogenetic classifier. At 5 years, OS was estimated at 82% (95% CI, 72% to 89%) in low-risk patients, compared with 44% (95% CI, 33% to 55%) in high-risk patients. The HR for shorter OS in the latter group was 3.3 (95% CI, 1.9 to 5.8; *P* < .001). GL, germline.

higher MRD response rate in low-risk compared with high-risk patients (74% *v* 60%, respectively; $P = .18$). When adjusting the effect of the *N/F-RAS-PTEN* classifier to age (using the 35-year cutoff), WBC count (using the 100×10^9/L cutoff), and MRD response (using the 10^{-4} cutoff) in these 89 patients, the oncogenetic classifier remained the only significant prognostic factor for OS (HR, 4.8; 95% CI, 1.6 to 14.8; $P = .006$).

Taken together, these data demonstrate that the detection of *RAS* and *PTEN* mutations adds significant prognostic value to assessment of the *N/F* status in isolation and allows identification of a significant proportion (48%) of good prognosis adult T-ALLs with *N/F* mutations but no *RAS/PTEN* abnormalities that cannot be identified by classical parameters.

DISCUSSION

Much progress has been made recently toward the identification of molecular-genetic abnormalities in T-ALL.[7] A number of these genetic events, sometimes defined as type A mutations,[20] act mainly to block T-cell differentiation at a specific developmental stage and delineate T-ALL subgroups displaying specific gene expression profiles.[5,6] In contrast, type B mutations act by gain-of-function alterations affecting cell cycle, self-renewal, pre-TCR signaling, or constitutive tyrosine kinase activation. *RAS* and *PTEN* defects belong to this category and are involved in pre-TCR complex signaling (reviewed in Van Vlierberghe et al[20]), which leads to the downstream activation of both the RAS/MAPK and PI3K/AKT pathways.[21] There is also increasing recognition of the role played by tumor suppressor gene inactivation in T-ALL.[7] PTEN is a lipid and protein phosphatase that negatively regulates the PI3K/AKT/mTOR pathway through dephosphorylation of the PIP3 lipid second messenger.[22] PTEN plays critical roles in cell growth, survival, and migration.[23] The *PTEN* expression level can be regulated by multiple mechanisms.[23] In leukemia, *PTEN* loss promotes self-renewable leukemia stem-cell formation and leukemogenesis.[24] Whether *PTEN* abnormalities are of prognostic value remains debated in childhood T-ALLs.[15,25,26] In general, *PTEN* genomic deletions are of poor prognosis, but *PTEN* mutations were reported to be without significant prognostic impact,[15] albeit in a small series of pediatric T-ALL. We now show that *PTEN* modification is disproportionately associated with TCR-positive, high WBC, younger adult T-ALLs that demonstrate a relatively low incidence of *N/F* mutation and poor prognosis.

Several studies have also highlighted the oncogenic role of *RAS* in leukemogenesis.[27,28] Oncogenic *K-RAS* and *N-RAS* mutations are described in only 2% of pediatric T-ALLs without clinical impact,[29] *RAS*-mutated adult T-ALLs represent 10% and tend to have more frequently an immature immunophenotype. This association has been recently suggested[30] and, because immature phenotypes are more frequent in adult compared with pediatric T-ALLs,[31] might explain the higher incidence of *RAS* mutation in our series. As such, *RAS*- and *PTEN*-mutated patients have distinct features, in keeping with their virtually mutually exclusive occurrence.

Taken together, we have identified a significant subgroup (40 of 175 patients; 23%) of adult patients with poor prognosis T-ALL with genetic anomalies of either the PI3K/PTEN/Akt/mTOR or the Ras/Raf/MEK/ERK pathway. The intricate links in cell signaling between these pathways and the rationale for targeting both to prevent chemotherapeutic drug resistance and re-emergence of cancer-initiating cells have led to the development of specific inhibitors of these two pathways. Therefore, it was logical to regroup *RAS/PTEN*-modified T-ALLs and to develop an oncogenetic classifier of T-ALL as an extension of our previous *N/F*-based classification. Adults with *N/F*-mutated, *RAS/PTEN* germline T-ALL compose approximately 50% of patients and have an excellent prognosis. It is important to note that these new risk factors are independent from the two most important classical prognostic factors (ie, WBC count $> 100 \times 10^9$/L and European Group for the Immunological Classification of Leukemias class).[32,33] The added value of MRD assessment in these oncogenetically defined subgroups remains to be determined.

At a practical level, increasing availability of high-throughput sequencing strategies will facilitate rapid genotyping (including allelic mutation or deletion of *PTEN*) of diagnostic samples, thus allowing therapeutic stratification at an earlier stage that is possible with MRD-based stratification. These considerations are currently impacting the design of the next GRAALL T-ALL study.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) and/or an author's immediate family member(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

Employment or Leadership Position: None **Consultant or Advisory Role:** Françoise Huguet, Amgen (C), ARIAD Pharmaceuticals (C), Bristol-Myers Squibb (C), Novartis (C), Pfizer (C) **Stock Ownership:** None **Honoraria:** Françoise Huguet, ARIAD Pharmaceuticals, Bristol-Myers Squibb, Novartis **Research Funding:** None **Expert Testimony:** None **Patents:** None **Other Remuneration:** None

AUTHOR CONTRIBUTIONS

Conception and design: Hervé Dombret, Vahid Asnafi
Provision of study materials or patients: All authors
Collection and assembly of data: All authors
Data analysis and interpretation: Amélie Trinquand, Vahid Asnafi
Manuscript writing: All authors
Final approval of manuscript: All authors

REFERENCES

1. Pui CH, Evans WE: Treatment of acute lymphoblastic leukemia. N Engl J Med 354:166-178, 2006
2. Aifantis I, Raetz E, Buonamici S: Molecular pathogenesis of T-cell leukaemia and lymphoma. Nat Rev Immunol 8:380-390, 2008
3. Ben Abdelali R, Asnafi V, Leguay T, et al: Pediatric-inspired intensified therapy of adult T-ALL reveals the favorable outcome of NOTCH1/FBXW7 mutations, but not of low ERG/BAALC expression: A GRAALL study. Blood 118:5099-5107, 2011
4. De Keersmaecker K, Atak ZK, Li N, et al: Exome sequencing identifies mutation in CNOT3 and ribosomal genes RPL5 and RPL10 in T-cell acute lymphoblastic leukemia. Nat Genet 45:186-190, 2013
5. Fernando AA, Neuberg DS, Staunton J, et al: Gene expression signatures define novel oncogenic pathways in T cell acute lymphoblastic leukemia.

Cancer Cell 1:75-87, 2002
6. Soulier J, Clappier E, Cayuela JM, et al: HOXA genes are included in genetic and biologic networks defining human acute T-cell leukemia (T-ALL). Blood 106:274-286, 2005
7. Van Vlierberghe P, Ferrando A: The molecular basis of T cell acute lymphoblastic leukemia. J Clin Invest 122:3398-3406, 2012
8. Zhang J, Ding L, Holmfeldt L, et al: The genetic basis of early T-cell precursor acute lymphoblastic leukaemia. Nature 481:157-163, 2012
9. Weng AP, Ferrando AA, Lee W, et al: Activating mutations of NOTCH1 in human T cell acute lymphoblastic leukemia. Science 306:269-271, 2004
10. O'Neil J, Grim J, Strack P, et al: FBW7 mutations in leukemic cells mediate NOTCH pathway activation and resistance to gamma-secretase inhibitors. J Exp Med 204:1813-1824, 2007
11. Rowe JM, Buck G, Burnett AK, et al: Induction therapy for adults with acute lymphoblastic leukemia: Results of more than 1500 patients from the international ALL trial: MRC UKALL XII/ECOG E2993. Blood 106:3760-3767, 2005
12. Campana D: Minimal residual disease in acute lymphoblastic leukemia. Hematology Am Soc Hematol Educ Program 2010:7-12, 2010
13. Huguet F, Leguay T, Raffoux E, et al: Pediatric-inspired therapy in adults with Philadelphia chromosome-negative acute lymphoblastic leukemia: The GRAALL-2003 study. J Clin Oncol 27:911-918, 2009
14. Bonnet M, Lacombe M, Montpellier B, et al: Posttranscriptional deregulation of MYC via PTEN constitutes a major alternative pathway of MYC activation in T-cell acute lymphoblastic leukemia. Blood 117:6650-6659, 2011
15. Gutierrez A, Sanda T, Grebliunaite R, et al: High frequency of PTEN, PI3K, and AKT abnormalities in T-cell acute lymphoblastic leukemia. Blood 114:647-650, 2009
16. Kaplan EL, Meier P: Nonparametric estimation from incomplete observations. J Am Stat Assoc 53:457-481, 1958
17. Cox D: Regression models and life tables. J R Stat Soc B 34:187-220, 1972
18. Gönen M, Heller G: Concordance probability and discriminative power of proportional hazards regression. Biometrika 92:965-970, 2005
19. Asnafi V, Buzyn A, Le Noir S, et al: NOTCH1/FBXW7 mutation identifies a large subgroup with favorable outcome in adult T-cell acute lymphoblastic leukemia (T-ALL): A Group for Research on Adult Acute Lymphoblastic Leukemia (GRAALL) study. Blood 113:3918-3924, 2009
20. Van Vlierberghe P, Pieters R, Beverloo HB, et al: Molecular-genetic insights in paediatric T-cell acute lymphoblastic leukaemia. Br J Haematol 143:153-168, 2008
21. Germain RN, Stefanová I: The dynamics of T cell receptor signaling: Complex orchestration and the key roles of tempo and cooperation. Annu Rev Immunol 17:467-522, 1999
22. Leslie NR, Downes CP: PTEN: The down side of PI 3-kinase signalling. Cell Signal 14:285-295, 2002
23. Song MS, Salmena L, Pandolfi PP: The functions and regulation of the PTEN tumour suppressor. Nat Rev Mol Cell Biol 13:283-296, 2012
24. Guo W, Lasky JL, Chang CJ, et al: Multi-genetic events collaboratively contribute to Pten-null leukaemia stem-cell formation. Nature 453:529-533, 2008
25. Bandapalli OR, Zimmermann M, Kox C, et al: NOTCH1 activation clinically antagonizes the unfavorable effect of PTEN inactivation in BFM-treated children with precursor T-cell acute lymphoblastic leukemia. Haematologica 98:928-936, 2013
26. Zuurbier L, Petricoin EF 3rd, Vuerhard MJ, et al: The significance of PTEN and AKT aberrations in pediatric T-cell acute lymphoblastic leukemia. Haematologica 97:1405-1413, 2012
27. Parikh C, Subrahmanyam R, Ren R: Oncogenic NRAS rapidly and efficiently induces CMML- and AML-like diseases in mice. Blood 108:2349-2357, 2006
28. Zhang J, Wang J, Liu Y, et al: Oncogenic Kras-induced leukemogenesis: Hematopoietic stem cells as the initial target and lineage-specific progenitors as the potential targets for final leukemic transformation. Blood 113:1304-1314, 2009
29. Perentesis JP, Bhatia S, Boyle E, et al: RAS oncogene mutations and outcome of therapy for childhood acute lymphoblastic leukemia. Leukemia 18:685-692, 2004
30. Van Vlierberghe P, Ambesi-Impombato A, Perez-Garcia A, et al: ETV6 mutations in early immature human T cell leukemias. J Exp Med 208:2571-2579, 2011
31. Asnafi V, Beldjord K, Libura M, et al: Age-related phenotypic and oncogenic differences in T-cell acute lymphoblastic leukemias may reflect thymic atrophy. Blood 104:4170-4180, 2004
32. Digel W, Schultze J, Kunzmann R, et al: Poor prognosis of prethymic phenotype acute lymphoblastic leukemia (pre-T-ALL). Leukemia 8:1406-1409, 1994
33. Thiel E, Kranz BR, Raghavachar A, et al: Prethymic phenotype and genotype of pre-T (CD7+/ER1-) cell leukemia and its clinical significance within adult acute lymphoblastic leukemia. Blood 73:1247-1258, 1989

Affiliations

Amélie Trinquand, Raouf Ben Abdelali, Etienne Lengliné, Noëmie De Gurunburg, Ludovic Lhermitte, Jonathan Bond, Agnès Buzyn, Elizabeth Macintyre, and Vahid Asnafi, University Paris Descartes, Centre National de la Recherche Scientifique (CNRS) Unité Mixte de Recherche (UMR)-8147, and Assistance Publique–Hôpitaux de Paris (AP-HP), Hôpital Necker-Enfants Malades; Jérôme Lambert, UMR-S-717, Hôpital Saint-Louis, AP-HP; Kheira Beldjord, Etienne Lengliné, and Hervé Dombret, University Paris 7, Hôpital Saint-Louis, AP-HP, and Institut Universitaire d'Hématologie, EA3518, Paris; Aline Tanguy-Schmidt and Norbert Ifrah, Pôle de Recherche et d'Enseignement Supérieur L'Université Nantes Angers Le Mans, Centre Hospitalier Universitaire Angers Service des Maladies du Sang et L'Institut National de la Santé et de la Recherche Médicale (INSERM) U892, Angers; Dominique Payet-Bornet and Bertrand Nadel, Center of Immunology of Marseille Luminy, Aix-Marseille University, INSERM U1104 and Centre National de la Recherche Scientifique (CNRS) UMR-7280, Marseille; Hossein Mossafa, Laboratoire Cerba, Cergy-Pontoise; Véronique Lhéritier and Xavier Thomas, Centre Hospitalier Lyon Sud, Lyon; Françoise Huguet, Hôpital Purpan, Toulouse; Thibaud Leguay, Centre Hospitalier du Haut Lévêque, Pessac; Jean-Yves Cahn, UMR-5525 CNRS–Université Joseph Fourier, Grenoble; Caroline Bonnati, Centre Hospitalier Régional Hôpital de Brabois, Vandoeuvre Les Nancy; Sebastien Maury, Hôpital Henry Mondor, Creteil, France; Yves Chalandon, University Hospital of Geneva, Geneva, Switzerland; and André Delannoy, Hopital de Jolimont, La Louviere, Belgium.

Support

Supported by grants to Necker Laboratory from the Association Laurette Fugain, the Comité Départemental de la Ligue Contre le Cancer, and the Institut National du Cancer. The Group for Research in Adult Acute Lymphoblastic Leukemia (GRAALL) was supported by Grants No. P020070I and P030425/AOM03081 from Le Programme Hospitalier de Recherche Clinique, Ministère de l'Emploi et de la Solidarité, France, and the Swiss Federal Government in Switzerland. Samples were collected and processed by the Assistance Publique–Hôpitaux de Paris Direction de Recherche Clinique Tumor Bank at Necker-Enfants Malades. AT was supported by Soutien pour la formation à la recherche translationnelle en cancérologie dans le cadre du Plan cancer 2009-2013 and Fondation pour la Recherche Médicale. I.B. was supported by a Kay Kendall Leukaemia Fund Intermediate Research Fellowship.

II - Les LAL à Ph1

1 - Survie à long terme améliorée par les ITK

Article n°2
Tanguy-Schmidt A et al. Long-term follow-up of the imatinib GRAAPH-2003 study in newly diagnosed patients with de novo Philadelphia chromosome-positive acute lymphoblastic leukemia: a GRAALL study. Biol Blood Marrow Transplant 2013;19:150-155

Dans le cadre du GRAALL, nous avons rapporté les résultats à long terme du GRAAPH-2003, avec un suivi médian de 46 mois. Plusieurs études qui ont suggéré le bénéfice des ITK pour les patients atteints de LAL à Ph1 souffraient d'un suivi médian n'excédant pas 2 ans.
Nous avons étudié le devenir à long terme de 45 patients atteints de LAL à Ph1 inclus dans le protocole GRAAPH-2003 de janvier 2003 à Octobre 2005. Dans ce protocole reposant sur l'association d'une chimiothérapie et d'un ITK, l'imatinib, aucune maintenance par ITK n'était prévue. Nous avons comparé leur devenir à long terme au devenir à long terme du protocole LALA 94, protocole français de pure chimiothérapie utilisé avant l'ère des ITK.
L'association d'imatinib à la chimiothérapie a permis non seulement un taux de RC de 96 %, mais surtout d'améliorer la survie à long terme, passée depuis l'époque du LALA94 de 20 % à 52 % à 4 ans. Ces résultats montrent l'impact à long terme des ITK dans l'amélioration de la survie.
Dans le cadre de leur traitement, 24 patients ont reçu une allogreffe de CSH, 10 patients ont reçu une autogreffe de CSP du fait de leur âge ou de l'absence de donneur et 9 patients n'ont pas pu recevoir d'intensification thérapeutique. Parmi les 10 patients autogreffés, 7 avaient une MRD indétectable.
L'OS à 4 ans de patients sans intensification n'est que de 33 %. Elle est de 50 % pour les patients allogreffés et de 80% pour les patients autogreffés, sans différence significative entre les patients allogreffés et autogreffés compte tenu des effectifs.
Ces résultats montrent que l'intensification thérapeutique dans les LAL à Ph1 garde toute sa place même à l'ère des ITK, les patients rechutant en l'absence d'intensification thérapeutique.
Le taux de rechute est de 27 % à 4 ans pour les patients allogreffés, de 50 % pour les patients autogreffés et de 39 % en l'absence d'intensification. Les 3 patients autogreffés avec une maladie moléculaire élevée avant intensification ont rechuté, tandis que seulement 2 des 7 patients autogreffés avec une MRD basse avant l'intensification ont rechuté. Les taux de mortalité lié au traitement sont de 40 % pour les patients allogreffés, de 0 % pour les patients autogreffés et de 28 % en l'absence d'intensification. Le risque global de rechute dans

l'autogreffe - dont il faut retenir la modestie si la maladie résiduelle est indétectable - est compensé par la faible mortalité de cette procédure.

L'intensification thérapeutique dans les LAL à Ph1 reste d'actualité même à l'ère des ITK, les patients rechutant en l'absence d'intensification thérapeutique.
L'autogreffe de CSP pourrait trouver sa place chez les patients avec une MRD basse ou mieux indétectable, ne pouvant pas recevoir d'allogreffe mais éligibles à l'autogreffe. Cette possibilité reste à confirmer compte tenu de l'effectif de l'étude et des études récentes en faveur d'une maintenance par ITK.

Biol Blood Marrow Transplant 19 (2013) 150–155

Long-Term Follow-Up of the Imatinib GRAAPH-2003 Study in Newly Diagnosed Patients with De Novo Philadelphia Chromosome-Positive Acute Lymphoblastic Leukemia: A GRAALL Study

Aline Tanguy-Schmidt [1], Philippe Rousselot [2], Yves Chalandon [3], Jean-Michel Cayuela [4], Sandrine Hayette [5], Marie-Christiane Vekemans [6], Martine Escoffre [7], Françoise Huguet [8], Delphine Réa [9], André Delannoy [10], Jean-Yves Cahn [11], Jean-Paul Vernant [12], Norbert Ifrah [1], Hervé Dombret [9], Xavier Thomas [13,*]

[1] Department of Hematology, Service des Maladies du sang INSERM UMR3, PRES UNAM, University Hospital, Angers, France
[2] Department of Hematology, Hôpital André Mignot, Versailles, France
[3] Department of Hematology, University Hospital, Geneva, Switzerland
[4] Molecular Biology Department, Hôpital Saint Louis, AP-HP, University Paris 7, Paris, France
[5] Molecular Biology Department, University Hospital Lyon Sud, Pierre-Bénite, France
[6] Department of Hematology, Cliniques Universitaires Saint-Luc, Brussels, Belgium
[7] Department of Hematology, University Hospital, Rennes, France
[8] Department of Hematology, Hôpital Purpan, Toulouse, France
[9] Department of Hematology, Hôpital Saint-Louis, AP-HP, University Paris 7, UMR 3518, Paris, France
[10] Department of Hematology, Hôpital de Jolimont, La Louvière, Belgium
[11] Department of Hematology, University Hospital, Grenoble, France
[12] Department of Hematology, Hôpital Pitié-Salpêtrière, Paris, France
[13] Department of Hematology, Hôpital Lyon-Sud, Lyon, France

Article history:
Received 16 April 2012
Accepted 29 August 2012

Key Words:
Acute lymphoblastic leukemia
Philadelphia chromosome
Imatinib
Stem cell transplantation

ABSTRACT

We report here the results of the GRAAPH-2003 trial with long-term follow-up in 45 patients with de novo Philadelphia chromosome-positive (Ph+) acute lymphoblastic leukemia (ALL). Imatinib-based strategy improved the 4-year overall survival (OS) up to 52% versus 20% in the pre-imatinib LALA-94 trial ($P = .0001$). Despite the selection in patients who actually underwent transplantation, these results suggest that allogeneic or autologous stem cell transplants (SCTs) still have a place in overcoming the poor prognosis of Ph+ ALL in the era of imatinib therapy. OS was 50% after allogeneic SCT (24 patients), 33% in patients without a transplantation (9 patients), and 80% after autologous SCT (10 patients without allogeneic donor or >55 years, including 7 patients in complete molecular response).

INTRODUCTION

Philadelphia chromosome positive (Ph+) is the most frequent recurrent cytogenetic abnormality observed in adult patients diagnosed with acute lymphoblastic leukemia (ALL). Before the tyrosine kinase inhibitor (TKI) era, the outcome of patients with Ph+ ALL was very poor, with long-term survival rates, at best, reaching 20% in most studies [1,2]. Allogeneic stem cell transplant (SCT) has been considered as the consolidation treatment of choice once achieving a first complete remission (CR); as it provides the best outcome in this setting [3]. In recent years, the most significant advance in term of treatment has been the introduction of TKIs into Ph+ ALL treatment protocols. Several groups have shown that the combination of concurrent or alternating use of imatinib with high-dose chemotherapy has significantly improved the outcome of adults and children with newly diagnosed Ph+ ALL, with higher CR rates (nearly 95%) and 12-month overall survival (OS) reaching approximately 75% [4-9]. However, to date, there is no evidence that TKIs may impact on the long-term outcome of patients with Ph+ ALL, because the follow-up of published studies did not exceed 2 years [4-10]. In a first analysis of the GRAAPH-2003 study, CR rate, 18-month disease-free survival (DFS), and OS were 96%, 51%, and 65%, respectively [11]. In the present analysis, results of the GRAAPH-2003 study have been actualized with a longer median follow-up reaching 3.86 years.

PATIENTS AND METHODS

From January 2004 to October 2005, 45 patients with newly diagnosed de novo Ph+ ALL (median age, 45 years; range, 16–59 years) were included in the GRAAPH-2003 study. The Ph+ was detected by standard karyotype and/or fluorescence in situ hybridization analysis and/or BCR-ABL fusion transcript detection by reverse transcription-PCR. Patients with previous chronic myeloid leukemia or myeloproliferative disorders were excluded. All patients gave their written informed consent before the study. The study was approved in March 2003 by the Institutional review board of Hôpital Purpan, Toulouse, France, and conducted in accordance with the Declaration of Helsinki. Details regarding this study have been published elsewhere [11].

The protocol schedule is summarized in Figure 1. Following a 7-day prednisone pre-phase, corticosteroid sensitivity (CS) was assessed by peripheral blood examination and was defined as $<1.0 \times 10^9$/L residual circulating blasts. Chemotherapy sensitivity (CtS) was assessed by peripheral blood and bone marrow (BM) examination 8 days after the start of an induction chemotherapy including daunorubicin, cyclophosphamide,

Financial disclosure: See Acknowledgments on page 155.

* Correspondence and reprint requests: Xavier Thomas, MD, Hématologie, Hôpital Lyon-Sud, Chemin du Grand Revoyet, 69495 Pierre Benite, France.
E-mail address: xavier.thomas@chu-lyon.fr (X. Thomas).

1083-8791/$ – see front matter © 2013 American Society for Blood and Marrow Transplantation.
http://dx.doi.org/10.1016/j.bbmt.2012.08.021

GRAPH-2003: induction and consolidation therapy

	Dose	Time, d
Prephase		
Prednisone	60 mg/m²/d PO	Between −7 and −1
Methotrexate	15 mg IT	Between −7 and −1
Standard induction, wks 1-2		
Daunorubicin	50 mg/m²/d IV	1 to 3
Cyclophosphamide	750 mg/m²/d IV	1
Vincristine	2 mg IV	1, 8
Prednisone	60 mg/m²/d PO	1 to 14
L-asparaginase	6000 IU/m²/d IV	8, 10, 12
Triple IT	—‡	1, 8
Standard induction, wks 3-4		
Daunorubicin	30 mg/m²/d IV	15 to 16
Cyclophosphamide	750 mg/m²/d IV	15
Vincristine	2 mg IV	15, 22
L-asparaginase	6000 IU/m²/d IV	20, 22, 24, 26, 28
G-CSF lenograstim	150 μg/m²/d SC or IV	From 17
DIV combination*		
Vincristine	2 mg IV	1, 8, 15, 22
Dexamethasone	40 mg PO or IV	1-2, 8-9, 15-16, 22-23
Imatinib	800 mg/d PO	1 until SCT
Triple IT	—‡	1, 8, 15, 22
HAMI combination†		
Mitoxantrone	10 mg/m²/d, IV	1 to 3
Cytarabine	2000 mg/m²/12h, IV	1 to 4
Imatinib	800 mg/d PO	1 until SCT
Triple IT	—‡	8, 15
G-CSF filgrastim	5 μg/kg/d SC or IV	From 9

PO indicates per os (orally); IT, intrathecally; IV, intravenously; SC, subcutaneously.

*Administered at day 15 of the standard induction course in patients with corticoresistant and/or chemoresistant ALL.

†Administered after hematologic CR achievement as consolidation in patients with corticosensitive and chemosensitive ALL.

‡Consisted of 15 mg methotrexate, 40 mg cytarabine, and 40 mg dexamethasone, all administered intrathecally.

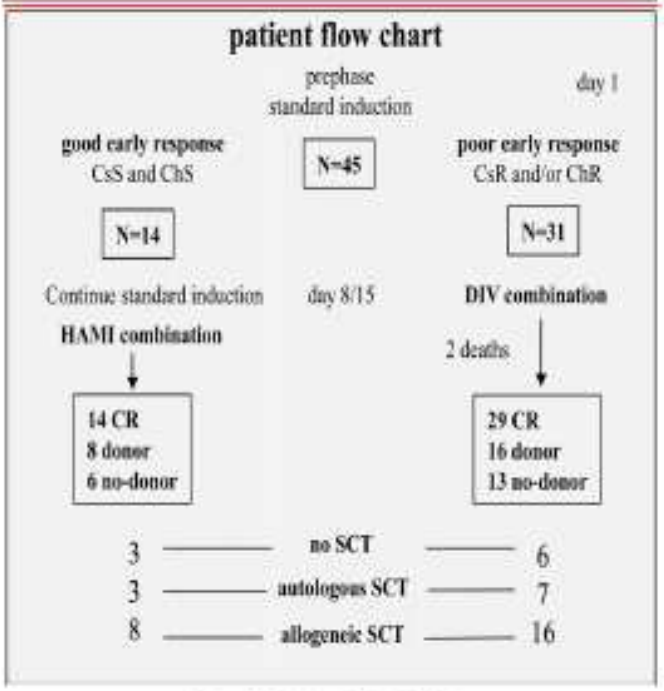


Figure 1 Treatment and patient flow chart.

vincristine, prednisone, and L-asparaginase. ChS was defined as the absence of circulating blasts and <5% of marrow blasts. Treatment strategy was stratified according to CS and ChS. Patients with a disease defined as corticosensitive and chemo-sensitive (good early responders) received a standard induction. Subsequently, patients achieving hematological CR received imatinib (600 mg per day) combined with consolidation chemotherapy including mitoxantrone and cytarabine. Imatinib was given from day 1 of the consolidation course to SCT. Patients with corticosteroid-resistant or chemo-resistant disease (poor early responders) received, either by day 8 (if cortico-resistant) or day 15 (if chemo-resistant), high-dose imatinib (800 mg per day) combined with vincristine and dexamethasone as the second part of the induction regimen (DIV combination), and subsequently imatinib alone until SCT.

All patients in hematological CR, age <55 years, with either an HLA-identical sibling donor or an HLA-matched unrelated donor (matched at 9 or 10 of 10 HLA antigens) were eligible for allogeneic SCT. For SCT, the preparative regimen included high-dose cyclophosphamide (120 mg/kg total dose, 60 mg/kg/day iv. for 2 consecutive days) and total body irradiation (12 Gy total dose in 6 fractions in 3 days). Graft-versus-host disease prophylaxis associated methotrexate (15 mg/m^2 at day +1, 10 mg/m^2 at day +3, day +6, and day +11) and cyclosporine (3 mg/kg per day by continuous i.v. infusion started at day −1, then switched orally, with adaptations according to blood concentration and tapered by day +60). No imatinib maintenance therapy was planned after transplantation, either autologous or allogeneic.

Marrow minimal residual disease (MRD) was monitored using reverse transcription-PCR, centralized in predefined reference laboratories. According to a minimum sensitivity level of 10^{-5}, low *BCR-ABL* level was defined as no *BCR-ABL* transcript detection or as a *BCR-ABL/ABL* ratio between 10^{-5} and 10^{-4}.

Patients with a BM MRD ratio $< 10^{-4}$, but without any donor or >55 years, were eligible for autologous SCT. Patients who failed to achieve this BM MRD level were further treated with imatinib combined with various chemotherapy regimens.

Results of the GRAAPH-2003 study were compared to those obtained by the pre-imatinib French trial, LALA-94 [2], which included 154 patients with de novo Ph+ ALL between 1994 and 2000, and used standard induction chemotherapy combining prednisone, vincristine, and cyclophosphamide with an anthracycline (daunorubicin or idarubicin) followed by a consolidation course combining mitoxantrone with intermediate-dose cytarabine (HAM). All of the 103 patients in hematological CR after these 2 chemotherapy courses underwent allogeneic SCT if a matched donor was identified, or autologous SCT if not.

Statistical Analysis

An update of the database was performed by May 18, 2012. At this time point, the median follow-up was 2.46 years for the overall group of patients (range, 0.05–4.98 years) and 3.86 years for those alive at the last follow-up evaluation (range, 0.9–4.98 years). All analyses were conducted according to the intention-to-treat principle. DFS was measured from the time of first CR to the date of relapse or death, whatever the cause. Relapse was defined as leukemia recurrence after first CR. OS was defined as the time interval from randomization until death, whatever the cause. The probabilities of DFS and OS were estimated according to the Kaplan-Meier product limit method and compared using either the 2-tailed log-rank test or Cox proportional hazard regression analysis. Departure from the proportional hazards assumption was assessed using methods based on partial residuals and a graphical approach. Competing risks analysis and the Gray test were used to calculate and compare the cumulative incidence of treatment-related death and relapse between groups. The Fine and Gray model was used to estimate relative risks (RRs) and 95% confidence intervals (CIs) for competing risks analyses. According to group size, chi-square analysis or Fisher exact test was used to compare categorical covariates. The Mann-Whitney *U* test was used to compare medians. All statistical analyses were conducted with S-Plus 8.0 for Windows Enterprise Developer (Insightful Corporation, Seattle, WA).

RESULTS

As previously reported [11], the CR rate after the induction phase was 96%. First CR was achieved in 14 patients (31%) after mitoxantrone, intermediate dose cytarabine, and imatinib, and 29 patients (64%) after DIV (Figure 1). Two patients died during DIV chemotherapy. Twenty-four of the 43 patients in CR received allogeneic SCT from either an HLA-identical sibling donor (n = 15) or an HLA-matched unrelated donor (n = 8), or from an unrelated cord blood donor (n = 1).

Nineteen patients in CR did not receive allogeneic SCT because they were >55 years (n = 4) or had no available or identified donor (n = 15). Among them, 10 received intensification therapy followed by autologous SCT. Seven patients achieved at least a low MRD level before undergoing the transplantation procedure, whereas 3 patients had positive pretransplantation MRD. Although no maintenance therapy

Table 1
Patient Characteristics According to the Type of Consolidation Therapy

Covariate	Allogeneic SCT Sibling Donor N = 15	Allogeneic SCT Unrelated Donor N = 9	Autologous SCT N = 10	Chemotherapy N = 9
Age at diagnosis				
Median (years)	40	40	44	50
Range (years)	20-55	16-56	27-59	42-55
Gender				
Female	5	5	6	4
Male	10	4	4	5
WBC at diagnosis				
Median, range (G/L)	20	22	8	12
Range (G/L)	2-143	1-159	2-151	7-78
WBC ≥ 50 G/L	4	2	1	1
Cytogenetics				
Complex karyotype	4	3	1	1
Other karyotypes	8	6	9	8
Missing/failure	3	0	0	0
Early response				
CS and ChS	9	7	7	6
Other profiles	6	2	3	3
MRD1 level				
Median	0.00003	0.00002	0.000045	0.00005
Range	0-0.026	0-0.05	0-0.02	0-0.001
High	6	2	3	4
Low	4	4	4	3
Negative	5	3	3	2
MRD2 level				
Median	0.0017	0.00002	0.00053	0.00005
Range	0-0.026	0-0.05	0-0.02	0-0.001

SCT indicates stem cell transplant; CS, corticosteroid sensitivity; ChS, chemotherapy sensitivity; MRD, minimal residual disease.

Table 2
Four-Year OS and DFS of the GRAAPH-2003 and LALA-94 Trials

	OS: 4-Years Estimated, % (95% CI)	DFS: 4-Years Estimated, % (95% CI)	CIRS (95% CI)	TRM of Patients in CR %
LALA-94 (103 patients in CR)	*20 (14-26)	*20 (14-28)	42 (34-50)	10
GRAAPH-2003 (43 patients in CR)	52 (36-66) *P = .0001	43 (27-58) *P = .002	24 (14-40)	11

OS indicates overall survival; DFS, disease-free survival; CI, confidence interval; CIR, cumulative incidence of relapse; CR, complete remission; TRM, treatment-related mortality; *, P.

was planned after SCT, 4 of these 10 patients received imatinib. Furthermore, 9 patients received an "off protocol" maintenance chemotherapy with imatinib because they failed to achieve a low MRD level (n = 4) or because investigators waited for the identification of an HLA-matched unrelated donor before to resolve to perform autologous SCT (Table 1).

For the entire cohort, the 4-year OS and DFS were 52% (95% CI, 39%-70%) and 44% (95% CI, 31%-62%), respectively (Figure S1A and B, online only). A highly significant improvement of the outcome was observed when compared to the LALA-94 trial (Table 2). The 4-year cumulative incidence rates of relapse and treatment-related mortality (TRM) were 36% (95% CI, 21%-51%) and 21% (95% CI, 8%-34%), respectively. In univariate analysis, WBC count at the time of diagnosis was the only covariate affecting significantly relapse incidence and DFS (Figure S1C and D, online only) (when considering the logarithm of the WBC count at diagnosis, i.e., leukocytosis as a continuous covariate: RR for the 4-year DFS, 1.45; 95% CI, 1.03-2.05; $P = .034$; RR for the 4-year relapse incidence, 1.61; 95% CI, 1.06-2.45; $P = .027$).

Table 3 summarizes the 43 remitters, the 4-year cumulative incidence of relapse, the 4-year TRM rate, the 4-year OS, and the 4-year DFS (Figure S2A and B, online only) according to the type of consolidation treatment (allogeneic SCT versus autologous SCT versus chemotherapy alone). The only significant difference was observed in terms of OS between patients who received autologous SCT and those who did not undergo transplantation.

Regarding the outcome of the 24 patients who received allogeneic SCT, a significant difference was observed according to the type of donor (HLA-identical sibling versus unrelated donor) in terms of OS, DFS, and TRM (Table 3 - Figure 2). The best results were observed in patients who underwent transplantation from an HLA-identical sibling donor, whereas those who underwent transplantation from an unrelated donor presented a higher TRM rate (1-year TRM, 22%; 95% CI, 0%-51% versus 7%; 95% CI, 0%-20%), without any gain in terms of relapse incidence. Patients undergoing autologous SCT had a similar 4-year OS than those receiving allogeneic SCT from an HLA-identical sibling donor. The higher relapse incidence observed in patients who received autologous SCT was balanced by a lower TRM rate.

Table 3 also shows outcome according to the MRD level achieved after the induction phase. There were no statistically significant differences in terms of survival between patients with high, low, or negative MRD levels after the induction phase (Figure S3, online only). Similar results were observed when considering the MRD level as a continuous variable. For the entire cohort, relapse incidence decreased concomitantly to MRD level (Figure 3). TRM was inversely correlated to the relapse incidence, and higher TRM rates were correlated to negative MRD levels (Figure 3). Similar results were observed when only considering patients who received allogeneic SCT (data not shown).

Table 3
Outcome of the Patients According to the Type of Consolidation Therapy and the MRD Level after the Induction Phase

Covariates	4-Year OS	4-Year DFS	4-Year RIS	4-Year TRM
Allogeneic SCT sibling (1)	70%	71%	27%	7%
95% CI	50%-100%	51%-100%	4%-50%	0%-20%
Allogeneic SCT URD (2)	11%	11%	44%	44%
95% CI	2%-70%	2%-70%	8%-80%	0%-82%
Autologous SCT (3)	80%	50%	50%	0%
95% CI	50%-100%	27%-93%	17%-83%	–
No SCT (4)	33%	33%	59%	28%
95% CI	11%-68%	11%-68%	0%-81%	0%-60%
High MRD level	51%	29%	71%	0%
95% CI	29%-68%	12%-71%	41%-100%	–
Low MRD level	51%	47%	40%	13%
95% CI	31%-65%	27%-65%	14%-65%	0%-31%
Negative MRD level	61%	54%	8%	38%
95% CI	39%-95%	33%-69%	0%-23%	10%-65%
P value				
(1) versus (2)	.003	.009	.40	.042
(1) versus (3)	.44	.79	.23	.41
(1) versus (4)	.17	.026	.54	.24
(2) versus (3)	.09	.0007	.88	.024
(2) versus (4)	.45	.52	.85	.52
(3) versus (4)	.47	.024	.74	.09
High versus low MRD level	0.52	0.89	0.34	0.15
High versus negative MRD level	0.98	0.26	0.002	0.016
Low versus negative MRD level	0.58	0.38	0.041	0.19

MRD indicates minimal residual disease; OS, overall survival; DFS, disease-free survival; RIS, relapse; TRM, treatment-related mortality; SCT, stem cell transplant; CI, confidence interval; URD, unrelated donor.

DISCUSSION

This updated analysis of the GRAAPH-2003 trial confirms and extends the previously published results [11]. Results are in agreement with those from other reports suggesting that, in patients with Ph+ ALL, the addition of imatinib to first-line chemotherapy is associated with an improved long-term outcome [12-14].

Our results suggest that even when including imatinib during the induction phase, the prognosis of patients can again be improved by intensifying the post-remission phase, through either autologous SCT (especially if a low or negative MRD level is achieved after the induction phase) or allogeneic SCT, especially from an HLA-identical sibling. In both "optimized situations," the 4-year OS was above 75% (4-year OS, 76%; 95% CI, 56%-100% with allogeneic SCT from an HLA-identical sibling (4-year OS, 86%; 95% CI, 63%-100% with autologous SCT and MRD low or negative), and the 4-year DFS above 70% (4-year DFS, 71%; 95% CI, 51%-100% with allogeneic SCT from an HLA-identical sibling (4-year DFS, 78%; 95% CI, 55%-100% with autologous SCT and MRD low or negative). Results of autologous SCT were encouraging with no TRM and a relapse incidence not statistically different from that observed after allogeneic SCT. These results might be explained by the fact that imatinib administration was stratified according to the MRD level assessed after the

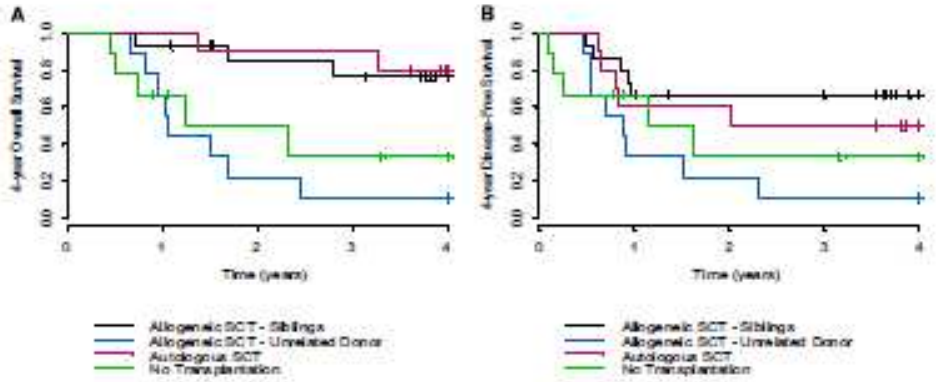


Figure 2. Four-year overall survival (OS) and disease-free survival (DFS) of the 43 patients who achieved complete remission (CR) in the GRAAPH-2003 study according to the consolidation treatment. (A) The 4-year OS was 78% (95% confidence interval [CI], 58%-100%) for those who received allogeneic stem cell transplant (SCT) from an HLA-identical sibling donor (black curve), 11% (95% CI, 2%-70%) for those who received allogeneic SCT from an unrelated donor (blue curve), 80% (95% CI, 59%-100%) for those who received autologous SCT (red curve), and 33% (95% CI, 11%-98%) for those who did not undergo transplantation (green curve) (*P* value [log-rank test] < .0003). (B) The 4-year DFS was 71% (95% CI, 51%-100%) for those who received allogeneic SCT from an HLA-identical sibling donor (black curve), 11% (95% CI, 2%-70%) for those who received allogeneic SCT from an unrelated donor (blue curve), 50% (95% CI, 27%-93%) for those who received autologous SCT (red curve), and 33% (95% CI, 11%-98%) for those who did not undergo transplantation (green curve) (*P* value [log-rank test] = .07).

induction phase, in contrast with the methods of imatinib administration in previous studies [13,14]. Therefore, most of the patients who underwent autologous SCT were good early responders. The 3 patients who received autologous SCT, despite a positive pretransplantation MRD evaluation, relapsed (at 1, 4, and 18 months posttransplantation, respectively). Although not planned in the protocol, 4 patients received imatinib as maintenance therapy after autologous SCT, which could have influenced the outcome of this group of patients. Among them, 3 patients were still alive in first CR at 37, 41, and 46 months posttransplantation, and 1 patient who has relapsed after 1 month remained alive at 48 months posttransplantation. Finally, although not statistically significant, lower WBC counts at diagnosis were observed in patients who received autologous SCT comparatively to those observed in patients who received an allogeneic SCT group from an HLA-identical sibling donor (median leukocytosis: 8 G/L versus 26 G/L, respectively). This covariate was the only one associated with 2 of the main endpoints for the entire cohort: the 4-year DFS and the relapse incidence. Only 1 patient with a WBC count above 50 G/L received autologous SCT. He rapidly relapsed despite the achievement of a low MRD level after the induction phase.

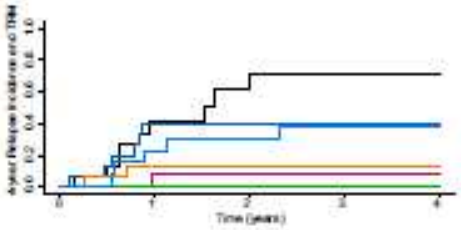


Figure 3. Four-year relapse incidence and treatment-related mortality (TRM) of the 43 patients who achieved complete remission (CR) in the GRAAPH-2003 study according to the minimal residual disease (MRD) level after the induction phase. The 4-year relapse incidence was 8% (95% confidence interval [CI], 0%-23%) for those who had a negative MRD evaluation (red curve), 40% (95% CI, 14%-66%) for those who had a low MRD level evaluation (dark blue curve), and 71% (95% CI, 43%-100%) for those who had a high MRD level evaluation (black curve) (*P* value [Gray test] < .014). The 4-year TRM was 38% (95% CI, 16%-60%) for those who had a negative MRD evaluation (light blue curve), 0% (95% CI, 0%-31%) for those who had a low MRD level evaluation (orange curve), and 0% for those who had a high MRD level evaluation (green curve) (*P* value [Gray test] = .038).

Our results, although based on a limited number of patients, suggest that the autologous SCT option should be reconsidered in the imatinib era for Ph+ ALL patients with a low or negative MRD level after the induction phase. In our previous LALA-94 trial [2], we reported a favorable prognostic value when molecular remission was obtained after 2 chemotherapy courses independently of the presence of a donor. Molecular response was therefore considered as a good surrogate endpoint of the disease, and autologous SCT was avoided in patients with persistent MRD. The potential role of imatinib, as maintenance therapy after SCT in order to further reduce relapse incidence, remains an important issue that should be addressed prospectively. Radich et al. [15], in a retrospective series of 36 patients with Ph+ ALL who underwent transplantation at the Fred Hutchinson Cancer Research Center, and subsequently Stiewalt et al. [16], analyzing the outcome of 90 patients with Ph+ ALL who underwent transplantation at the same center, showed that the persistent expression of *BCR-ABL* during the first 100 days posttransplantation was associated with a higher incidence of relapse and a lower DFS. Both studies argue in favor of a maintenance therapy with imatinib after transplantation in patients with a positive MRD evaluation, especially when no chronic graft-versus-host immune reaction can be expected.

Unexpectedly, early MRD evaluation did not significantly influence patient outcome, both in terms of OS and DFS. The benefit of a low or negative MRD level, which translated into a significant lower incidence of relapse, was almost completely abrogated by the fact that these patients

experienced the highest TRM rate. This was observed mainly for patients who received allogeneic SCT, and especially for those who underwent transplantation with an unrelated donor. In this group, a particularly poor outcome was observed with a 4-year TRM greater than 40% and a 4-year OS and DFS of only 11%. Combined with the relatively advanced age of patients with Ph+ ALL and the high probability of graft-versus-leukemia effect in this disease [17], these results tend to argue in favor of allogeneic SCT after reduced-intensity conditioning for adult patients with Ph+ ALL when no HLA-identical sibling donor is available. Whether autologous SCT with a low or negative MRD level should be performed instead of allogeneic SCT using an HLA-matched unrelated donor in patients with Ph+ ALL is a critical issue that should further be addressed through a prospective controlled study.

ACKNOWLEDGMENTS

This work was supported by grants P020070l and P030425/AOM03081 from Le Programme Hospitalier de Recherche Clinique, Ministère de l'Emploi et de la Solidarité, France.

We thank Philippe Guardiola for assistance with the statistical analyses.

Financial disclosure statement: The authors declare no conflict of interest.

SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.bbmt.2012.08.021.

REFERENCES

1. Fadeel S, O'Brien S, Pui CH, et al. Adult acute lymphoblastic leukemia: concepts and strategies. *Cancer*. 2010;116:1165–1176.
2. Dombret H, Gabert J, Boiron JM, et al. Outcome of treatment in adults with Philadelphia chromosome-positive acute lymphoblastic leukemia—results of the prospective multicenter LALA-94 trial. *Blood*. 2002; 100:2357–2366.
3. Fielding AK, Goldstone AH. Allogeneic haematopoietic stem cell transplant in Philadelphia-positive acute lymphoblastic leukaemia. *Bone Marrow Transplant*. 2008;41:447–453.
4. Lee KW, Lee JH, Choi SJ, et al. Clinical effect of imatinib added to intensive combination chemotherapy for newly diagnosed Philadelphia chromosome-positive acute lymphoblastic leukemia. *Leukemia*. 2005;19:1509–1516.
5. Lee S, Kim YJ, Min CK, et al. The effect of first-line imatinib interim therapy on the outcome of allogeneic stem cell transplantation in adults with newly diagnosed Philadelphia chromosome-positive acute lymphoblastic leukemia. *Blood*. 2005;105:3449–3457.
6. Thomas DA, Faderl S, Cortes J, et al. Treatment of Philadelphia chromosome-positive acute lymphocytic leukemia with hyper-CVAD and imatinib mesylate. *Blood*. 2004;103:4396–4407.
7. Towatari M, Yanada M, Usui N, et al. Combination of intensive chemotherapy and imatinib can rapidly induce high-quality complete remission for a majority of patients with newly diagnosed BCR-ABL-positive acute lymphoblastic leukemia. *Blood*. 2004;104:2507–2512.
8. Vignetti M, Fazi P, Cimino G, et al. Imatinib plus steroids induces complete remissions and prolonged survival in elderly Philadelphia chromosome-positive patients with acute lymphoblastic leukemia without additional chemotherapy: results of the Gruppo Italiano Malattie Ematologiche dell'Adulto (GIMEMA) LAL0201-B protocol. *Blood*. 2007;109:3676–3678.
9. Yanada M, Takeuchi J, Sugiura I, et al. High complete remission rate and promising outcome by combination of imatinib and chemotherapy for newly diagnosed BCR-ABL-positive acute lymphoblastic leukemia: a phase II study by the Japan Adult Leukemia Study Group. *J Clin Oncol*. 2006;24:460–466.
10. Ottmann OG, Pfeifer H. First-line treatment of Philadelphia chromosome-positive acute lymphoblastic leukaemia in adults. *Curr Opin Oncol*. 2009;21(Suppl 1):S43–S46.
11. de Labarthe A, Rousselot P, Huguet-Rigal F, et al. Imatinib combined with induction or consolidation chemotherapy in patients with de novo Philadelphia chromosome-positive acute lymphoblastic leukemia: results of the GRAAPH-2003 study. *Blood*. 2007;109: 1408–1413.
12. Milone JH, Estrov A. Treatment of Philadelphia chromosome-positive acute lymphoblastic leukemia. *Anal Lymphoma*. 2009;50(Suppl 2):9–15.
13. Ribera JM, Oriol A, Gonzalez M, et al. Concurrent intensive chemotherapy and imatinib before and after stem cell transplantation in newly diagnosed Philadelphia chromosome-positive acute lymphoblastic leukemia. Final results of the CSTIBES02 trial. *Haematologica*. 2011;96:587–95.
14. Bassan R, Rossi G, Pogliani EM, et al. Chemotherapy-phased imatinib pulse improves long-term outcome of adult patients with Philadelphia chromosome-positive acute lymphoblastic leukemia: Northern Italy Leukemia Group protocol 09/00. *J Clin Oncol*. 2010;28:3644–3652.
15. Radich J, Gehly G, Lee A, et al. Detection of bcr-abl transcripts in Philadelphia chromosome-positive acute lymphoblastic leukemia after marrow transplantation. *Blood*. 1997;89:2602–2609.
16. Sobrevals DL, Gutiérrez KA, Beggio L, et al. Predictors of relapse and overall survival in Philadelphia chromosome-positive acute lymphoblastic leukemia after transplantation. *Bol Blood Marrow Transplant*. 2003;9:206–212.
17. Laport CC, Alvarnas JC, Palmer JM, et al. Long-term remission of Philadelphia chromosome-positive acute lymphoblastic leukemia after allogeneic hematopoietic cell transplantation from matched sibling donors: a 20-year experience with the fractionated total body irradiation-etoposide regimen. *Blood*. 2008;112:905–909.

II - Les LAL à Ph1

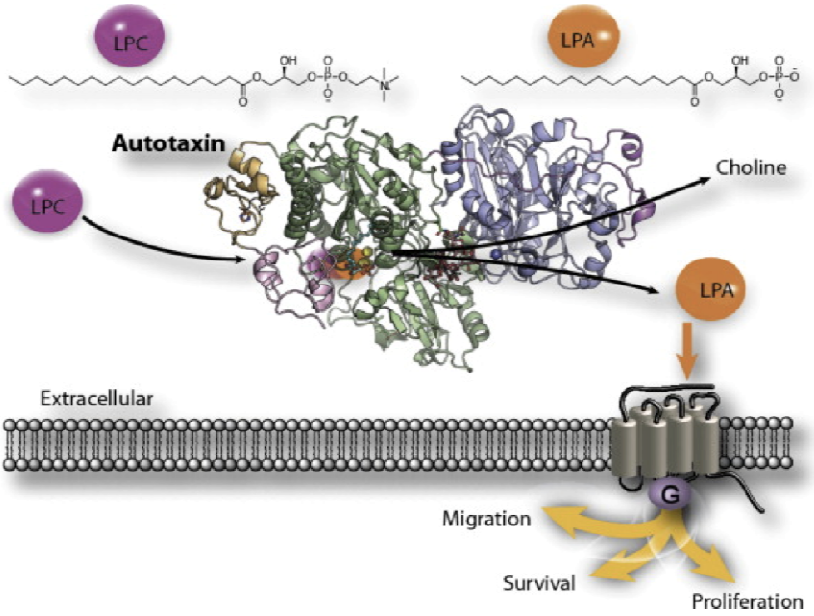
2 - Implication d'ATX dans la résistance aux ITK des LAL à Ph1

Article n° 3
Schmidt-Tanguy A*, Romanski A* et al. Upregulation of autotaxin in tyrosine kinase inhibitor resistant human Philadelphia chromosome acute lymphoblastic leukemia is associated with altered migration and resistance to imatinib. *Co-first authors

L'association des ITK à la chimiothérapie est devenue le standard dans le traitement des LAL à Ph1. Cependant de nombreux patients développent une résistance secondaire à ces ITK, principalement par mutations du domaine kinase. De plus, un certain nombre de patients présentent des résistances primaires, multifactorielles et non totalement élucidées.
Nous nous sommes intéressés aux mécanismes de résistance aux ITK. Comme rapporté dans un travail précédent, nous avons mis au point un modèle de lignée cellulaire humaine résistante aux ITK SupB15RT, obtenue par exposition croissante progressive à l'imatinib de la lignée cellulaire humaine SupB15W (LAL pro-B BCR-ABL positif sensible à l'imatinib). La lignée cellulaire SupB15RT présente une résistance croisée aux ITK de seconde génération sans implication de mécanismes de résistance connus (absence de mutation ponctuelle de BCR-ABL, absence d'amplification du gène BCR-ABL, absence de surexpression protéique de Bcr-Abl, absence de perte d'activité de Bcr-Abl et absence de surexpression de glycoprotéine P).
Nous avons étudié la voie de signalisation de Bcr-Abl et montré que notre modèle de résistance non mutationnelle était indépendant de la voie d'activation de Bcr-Abl avec une activation constitutive de AKT.

Nous avons démontré par étude comparative de profil génique entre SupB15W et SupB15RT que l'expression de l'autotaxine (ATX) était augmentée dans SupB15RT. La protéine ATX est une protéine extracellulaire à activité enzymatique. Grâce à son activité lysophospholipase D (lysoPLD), ATX synthétise deux lipides biologiques, l'acide lysophosphatidique (LPA) et la sphingosine 1-phosphate (S1P).[100-102] La sécrétion d´ATX est augmentée dans différentes pathologies tumorales.[102-104] La protéine ATX a une activité prométastatique et proangiogénique.[105-109] Son implication est évoquée dans la régulation du système immunitaire [action sur les lymphocytes, cellules dendritiques (DC)], la croissance tumorale et la métastatique par action sur la mobilité tissulaire, la prolifération tissulaire, l´apoptose. Cependant, son rôle exact dans la progression des cancers est mal connu (figure n° 11).[104,110-112]

Figure n°11: Axe ATX/LPA/LPAR



Perrakis A et al. J Lip Res 2015

En utilisant des modèles murins de LAL à Ph1, nous avons surexprimé ATX et montré qu'ATX augmentait la prolifération et la migration des cellules leucémiques et diminuait la réponse à l'imatinib.

Dans ce modèle cellulaire SupB15W, le LPA augmentait la prolifération cellulaire. De plus, il existait une différence d'expression de récepteurs au LPA entre SupB15W et SupB15RT avec absence d'expression de LPAR1 dans SupB15RT.

Ainsi, nous avons démontré l´implication d'ATX et de l´axe ATX/LPA/LPA récepteurs dans la résistance primaire non mutationnelle aux ITK. Cette voie pourrait être à la base de nouvelles cibles thérapeutiques.
Ce travail vient d'être soumis.

Title

Upregulation of autotaxin in tyrosine kinase inhibitor resistant human Philadelphia chromosome acute lymphoblastic leukemia is associated with altered migration and resistance to imatinib

Authors

Aline Schmidt-Tanguy#[1,2], Annette Romanski#[1], Patricia Neh Gwanmesia Mambou[1], Tanja Rossmanith[1], Heike Pfeifer[1], Susanne Badura[1], Wolf-Karsten Hofmann[3], Mathilde Hunault-Berger[2], Martin Ruthardt[1] and Oliver G. Ottmann[1].

[1]Department of Medicine, Hematology/Oncology, J. W. Goethe University Frankfurt, Germany, [2]Department of Hematology, service des maladies du sang, INSERM U892, PRES UNAM, University Hospital, Angers, France and [3]Department of Medicine, Hematology/Oncology, Medicine University, Mannheim, Germany.

# AS-T and AR contributed equally to the manuscript.

Correspondence: O.G.Ottmann

Address: ZIM, Medizinische Klinik II,Abteilung für Hämatologie, Onkologie

Klinikum der JWG Universität, Theodor Stern Kai 7, D-60590 Frankfurt

Tel.:0049 (0) 69 6301 3947 - FAX:0049 (0) 69 6301 7463

E-mail: ottmann@em.uni-frankfurt.de

Abstract

Background

The prognosis of Philadelphia Chromosome-positive acute lymphoblastic leukemia (Ph+ ALL) remains poor due to the inactivation of ABL-tyrosine kinase inhibitors (TKI). Cellular resistance to ABL-TKIs at ensuing relapse is the predominant cause of treatment failure of Ph+ ALL. Mechanisms other than TKD mutations clearly conduct to resistance but remain poorly characterized.

Methods

We previously determined, by comparative gene expression profiling of the human Ph+ ALL cell line SupB15-RT exhibiting non-mutational resistance against currently available Abl-TKIs with the TKI-sensitive parental line SupB15-WT, Autotaxin (ATX) is profoundly upregulated in SupB15-RT cells. We investigated whether ATX, a tumor cell mobility factor, antagonizes responsiveness of Ph+ ALL to the TKI.

Results

BCR-ABL transduced Ba/F3 cells (Ba/F3^{p185}) genetically modified to additional express the 2 major isoforms of ATX (ATXα or ATXβ; Ba/F3$^{p185/ATX\alpha}$, Ba/F3$^{p185/ATX\beta}$) were impervious to imatinib treatment. Since ATX mediates its biological activity by lysophosphatidic acid (LPA), we examined the effect of LPA on leukemic growth and responsiveness to imatinib. Adding of LPA mitigated the inhibitory effect of imatinib on the parental sensitive SupB15-WT cells. In addition we showed that cell migration of SupB15-RT cells was enhanced compared to SupB15-WT cells. ATX mediates at least partial resistance to TKI and has a pro-migratory effect on Ph+ ALL cells.

Conclusions

This is the first study to report a role of ATX in primary mutation-independent TKI resistance through the ATX-LPA axis. Inhibiting this axis in combination with TKI could provide a beneficial effect to treatment of Ph+ ALL patients.

Keywords

acute lymphoblastic leukemia

Philadelphia chromosome

tyrosine kinase inhibitor resistance

autotaxin

Background

The BCR-ABL translocation, corresponding molecular aberration of the Philadelphia chromosome (Ph+), is a constitutively active tyrosine kinase (TK) that is associated to chronic myeloid leukemia (CML) and a subset of acute lymphoblastic leukemia (ALL). Ph+ ALL represent in approximately 25 % of adult patients with ALL and are historically associated with a poor prognosis [1], [2]. Current front-line treatment regimens, including high dose of chemotherapy and imatinib, the first ABL TK inhibitor (TKI), induce complete remission in more than 90 % of patients with Ph+ ALL. However, the majority of patients not undergoing allogeneic stem cell transplantation (SCT) relapse [3], [4], [5]. Resistance to TKI is the major reason for the failure of therapy in these patients. More potent second generation TKIs (dasatinib, nilotinib) are also highly effective as initial therapy but do not appear to abrogate the development of resistance.

At relapse approximately 80 % of the patients with ALL display mutations in the TK domain (TKD) of BCR-ABL, with predominance of mutations known to confer high-level resistance to available TKI [6], [7]. However, several lines of evidence strongly implicate additional resistance mechanisms that are unrelated to BCR-ABL mutations: i) TKD mutations are not detected in approximately 20 % of patients at the time of relapse; ii) patients with a TKD mutation at relapse often show no clinical response to second generation TKI even if the mutation is considered to be sensitive to this TKI; iii) patients with pre-existing low-level TKI mutations at diagnosis may achieve a prolonged hematologic remission in response to TKI-based therapy, but subsequently relapse with outgrowth of this mutated leukemic clone while still on therapy, indicating a change of biological behavior resulting from acquisition of non-mutational resistance mechanisms [8].

Numerous mechanisms of resistance other than TKD mutations have been proposed to play a role in patients with primary or acquired resistance to TKI, particularly in CML [9]. These include genomic amplification or transcriptional up-regulation of BCR-ABL, sequestration of TKI by binding to plasma proteins, increased drug efflux by up-regulation of multidrug resistance gene 1 (MDR1), low activity of drug import pumps, and presence of additional genetic or epigenetic aberrations resulting in the activation of signaling pathways that are able to bypass BCR-ABL kinase inhibition [10]. However, the clinical relevance of these altered for Ph+ALL remains unclear.

Microarray gene expression profiling has been employed as a methodology for identifying mechanisms of TKI resistance in CML. We previously reported that clinical responsiveness to imatinib

in patients with advanced Ph+ ALL was associated with a predictive gene expression signature determined by microarray gene expression profiling [11]. Subsequently functional analysis linking differentially expressed genes with a mechanism of TKI resistance have met with only limited success, possibly due to the large degree of interpatient heterogeneity. This limitation might be overcome by directly comparing cells with and without a TKI resistance phenotype that we derived from same genetic background.

Here, we used a previously developed model of cellular resistance to ABL-directed TKI derived from the human BCR-ABL positive B-precursor cell line SupB15 [12]. Ph+ ALL cells highly resistant to TKI (SupB15-RT) were generated by exposing "wild-type" SupB15 cells (SupB15-WT) to increasing concentrations of imatinib. These cells were found to be cross-resistant to the more potent second generation TKI, and do not harbor BCR-ABL kinase domain mutations. This previously described model is relevant because it is unique and reproduced the mutation-independent TKI resistance which is present at 20 % of the patients without clarified mechanisms.

Using microarray gene expression analysis to compare SupB15 cells resistant or sensitive to TKI we examined differentially expressed genes as possible contributors to TKI resistance. Among the differentially expressed genes that to date have not been associated with resistance to TKI the most highly up-regulated was Autotaxin (ATX). ATX was originally identified as a motility factor in melanoma cells [13]. ATX is identical to lysophospholipase D, which catalyzes lysophosphatidylcholine (LPC) to the bioactive lysophosphatidic acid (LPA) [14],[15]. LPA acts as a ligand for three of the endothelial differentiation gene (EDG) family G-protein coupled receptors (GPCR), LPA1, LPA2, and LPA3, or the non-EDG GPCRs, LPA4, LPA5, LPA6 [16]. The mechanism by which ATX induced motility remained elusive until it was identified that its phosphodiesterase catalytic site was required to enhance cell migration [17]. ATX and LPA promote chemotaxis, migration, invasion, angiogenesis and tumorigenesis of multiple types of cancer [15], [18], [19]. Four alternative splicing isoforms of ATX have been described in humans. These isoforms are ATXα (925aa, teratocarcinoma-derived), ATXβ (863aa, melanoma-derived), ATXγ (888aa, brain specific) and ATXδ (859 aa, expressed in various human tissues), the role of which is not clearly established [14], [23].

Here, we describe functional studies to determine whether ATX is involved in TKI resistance of Ph+ALL, and whether the functional effects of ATX on Ph+ ALL cells are mediated by modulation of the synthesis of LPA.

Methods

Cells lines and reagents

The human leukemic cell line SupB15 (Ph+ B precursor ALL, p185), and the murine IL-3-dependent pre-B cell line Ba/F3 were purchased from the German Collection of Microorganisms and Cell Cultures (*DSMZ, Braunschweig, Germany*). The SupB15 cells are sensitive to imatinib and are subsequently referred to as SupB15-WT (wild type). Cells were cultured at 37°C in 5 % CO_2 in humidified atmosphere. The ecotropic packaging cells Phoenix (293T) were obtained as described [20]. SupB15-WT cells were maintained in RPMI 1640 medium supplemented with 15% FCS, 1 % Glutamine and 1 % Penicillin/Streptomycin (all from *Invitrogen, Karslruhe, Germany*). Ba/F3 were cultured in RPMI 1640 medium supplemented with 10 % FCS containing 10 ng/ml mIL-3 (*Peprotech, Hamburg, Germany*), 1 % Glutamine and 1 % Penicillin/Streptomycin and Ba/F3^{p185} cells were maintained in the same medium without mIL-3. To select the TKI-resistant phenotype, SupB15-WT were cultured with gradually increasing concentrations of imatinib (*Novartis, Basel, Switzerland*), dissolved in 0.1 N HCl and stocked at 10 µM solution at 4°C for 6 months. Starting concentration was 0.05 µM imatiniband increased every 10 to 14 days by 0.05 µM. The treated cells, designated SupB15-RT, were able to grow in up to 10 µM imatinib and were then maintained in the presence of 1 µM imatinib [12]. Parental SupB15-WT cells were maintained without imatinib. L-α-lysophosphatidic acid (LPA) using in proliferation assay was purchased from *Sigma-Aldrich (Steinheim, Germany)*. Dasatinib and nilotinib were provided by *Bristol Myer Squib (Munich, Germany)* and from *Novartis (Basel, Switzerland)*.

Western blot analysis

WB was essentially performed as previously described [21]. The following primary antibodies were used: polyclonal rabbit antibodies included anti-phospho-c-Abl (Tyr245)-antibody (α-p-c-Abl), anti-phospho-Akt (Tyr326)-antibody (α-p-Akt), anti-Akt-antibody (α-Akt) and monoclonal mouse anti-phospho-Stat5 (Tyr694) (clone 14H2)-antibody (α-p-Stat5) (all from *Cell Signaling Technologies, Frankfurt, Germany*). Monoclonal mouse anti-c-Abl (clone 24-11)-antibody (α-Abl) was purchased from *SantaCruz (Heidelberg, Germany)*. Monoclonal mouse anti-Stat5 (clone 89)-antibody (α-Stat5) was from *Becton Dickinson (Heidelberg, Germany)*. Secondary polyclonal goat-anti-rabbit-antibody and polyclonal goat-anti-mouse-antibody were purchased from *Dianova GmbH, (Hamburg, Germany)*. ATX was detected with an anti-peptide antibody generated by *Peptide Specialty Laboratories GmbH (Heidelberg, Germany)*.

Comparative gene expression analysis

Gene expression profiling was done using Affymetrix (Santa Clara, Ca, USA) GeneChip Human Genome U133A Plus 2.0 microarrays as previously described [22]. Ten µg of total RNA was transcribed in cRNA and doublestrand-cDNA which was then used for *in vitro* transcription for the synthesis of biotinylated cRNA. 15 µg of fragmented and biotinylated cRNA was used for hybridization of the array (*Affymetrix One-Cycle cDNA synthesis Kit*). After hybridization, the array was washed,

colored in "Affymetrix fluidics station", and scanned. The fluorescence intensity was normalized with the average fluorescence of the whole microarray.

Results were analyzed by Affymetrix Microarray Suite Software 5.0, and individual arrays were analyzed with the MAS 5.0 algorithm for single-array analysis. Data analysis was performed with the GeneSpring software version 4.2 (*Silicon Genetics, Redwood City, CA*). For differential gene expression, a minimum of 3-fold change was required. Hierarchical clustering analysis with Spearman's confidence correlation was used to identify gene clusters.

Analysis of ATX by quantitative RT-PCR

Total RNA and first strand DNA were prepared as described previously [7]. The qPCR was conducted following standard protocols [7]. ATX was amplified with the following oligonucleotide-primers: ATX-global-fwd (5'-CCC TAC ATG AGG CCG GTG TAC-3'), ATX-global-rev (5'-GAT CGA ATT CTT CCT AGA GTT CCA GGC AC-3'). The Sequence Detector Software SDS 2.0 (*Applied Biosystems, Carlsbad, USA*) was used for data analysis. CT values were exported into an Excel worksheet for calculation of fold changes using the comparative CT method. Internal reference gene was glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The expression data were evaluated using the ΔΔCT method.

RT-PCR analysis of ATXα and ATXβ were performed using standard protocols and the following oligonucleotide-primer: ATXα/β_fwd (CCCTACATGAGGCCGGTGTAC), ATXα_rev (CCTCTTA-GGGGAAGCTTTCCTCT) and ATXβ_rev (GGATTTGTCATCTCAGGGCC).Actin was used as internal reference: Actin_fwd (GTG GGG CGC CCC AGG CAC CA) and Actin_rev (CTC CTT AAT GTC ACG CAC GAT TTC).

Analysis of LPAR

Total RNA and first strand DNA were prepared as described previously [7]. PCR was carried following standard protocols. LPAR1, LPAR2 and LPAR3 were amplified with the following primers: LPAR1-fwd (5'-CTG CCA TCT CTA CTT CCA TC-3'), LPAR1-rev (5'-AGA TTG CCA CCA TGA CCA ATA G-3'), LPAR2-fwd (5'-TTG TCT TCC TGC TCA TGG TG-3'), LPAR2-rev (5'-CAG GAC TCA CAG CCT AAA CC-3'), LPAR3-fwd (5'-TTG CCT CTG CAA CAT CTC GG-3'), LPAR3-rev (5'-CAT GAC GGA GTT GAG CAG TG-3') with these conditions 40 cycles: 30s 94℃, 30s 60℃, 30s 72℃ for LPAR1 and LPAR2; 40 cycles: 30s 94℃, 30s 50℃, 30s 72℃ for LPAR3.

Retroviral infection: plasmids, retroviral vectors, retroviral transfection and transduction

The cDNA encoding ATXαwas cloned from SupB15-RT by RT-PCR and confirmed by sequencing. ATXα encoding sequence was generated by PCR on cDNA as template with the following oligonucleotides: ATX-BamHI-fwd (5'-GGA TCC ATG GCA AGG AGG AGC TC-3'), ATX1022-HindIII-rev (5'-CCT CTT AGG GGA AGC TTT CCT CT-3'), ATX1022-HindIII-fwd (5'-AGA GGA AAG CTT CCC CTA AGA GG-3'), ATX-XhoI-rev (5'-CTC GAG TTA AAT CTC GCT CTC AT-3'). Correction of

the ATXα-cDNA was performed using the QuickChange® II Site-Directed Mutagenesis Kit (*Stratagene; La Jolla, CA*) according to the manufacturer's instructions. The following primers were used: ATX-mut-fwd (5'-GAC CTA AGA GGA AAG TTG CCC CTA AGA GGA G-3') and ATX-mut-rev (5'-GTC TCC TCT TAG GGG CAA CTT TCC TCT TAG G-3'). PCR products were controlled by sequencing, and cloned into the BamHI and XhoI sites of pENTR1A. cDNA encoding ATXβ cloned into pDONR was derived from Genecopoeia (*Rockville, MD, USA*).

All retroviral expression vectors used in this study were based on the bi-cistronic vector Pinco as described before [20]. The Pinco variant Paulo (PIDE harbouring the functionally inactive "low affinity growth factor receptor" {ΔLNGFR} as a reporter) was created through conventional cloning techniques. These vectors were converted into Gateway®-destination vectors by the introduction of a Gateway® cassette according to the manufacturer's instructions (*Invitrogen, Darmstadt, Germany*). All related inserts were cloned into the Gateway® entry-vector (pENTR1A) or pDONR for recombination into the destination vectors by using the "LR-clonase" enzyme kit (*Invitrogen, Darmstadt, Germany*).

Ecotropic Phoenix packaging cells were transiently transfected with the indicated retroviral vectors as described before [23],[24]. Retroviral supernatant was collected at days 2 and 3 after transfection, shock-frozen in liquid nitrogen and stored at -80℃. For the infection, the retroviral supernatant was thawed on ice. Target cells were plated onto retronectin-coated (Takara-Shuzo, Shiga, Japan) non tissue culture treated 24-well plates and exposed to the retroviral supernatant for 3 h at 37℃ in the presence of 4 µg/mL polybrene (Sigma-Aldrich, Steinheim, Germany). Cells were centrifuged at 2200 rpm for 45 min. Infection was repeated three times and infection efficiency was measured after 48 h by determining the percentage of GFP- or ΔNGFR-positive cells by FACS analysis. The protein expression was controlled by WB.

Proliferation and apoptosis assays and cell-surface marker analysis

Viability of cells was determined by the trypan blue dye exclusion. For the analysis of apoptosis, cells were stained using the Annexin V kit according to the instructions of the manufacturer (*Roche, Mannheim, Germany*) by FACS analysis. For cell-surface marker expression analysis (ΔNGFR), cells were stained with antibodies or an isotype-matched nonspecific control antibody, and analyzed using FACScan *(all from BD Bioscience, Heidelberg, Germany)*.

Migration assay

Migration of leukemic cells was examined using transwells with a pore size of 3 µm (*BD Falcon, Heidelberg, Germany*) according to the manufacturer's instructions. The wells were coated with fibronectin. Fibronectin(5 µg/cm^2; *BD Biosciences, Heidelberg, Germany)* diluted in PBS was adsorbed to wells 1 h by room temperature. SupB15-WT and SupB15-RT (pre-incubated with or without 1 µM imatinib over night) were washed once in PBS and seeded in the upper chamber at 2 x10^4 cells in the adequate medium. Cells were allowed to migrate through the pores of the membrane for 24 h at 37℃. After incubation, filters were wa shed with PBS by gentle agitation and migrated cells attached to the lower surface of the membrane were fixed with 3.7 % formaldehyde and stained with

Coomassie staining solution (2% Coomassie Brilliant Blue; 45 % methanol; 10 % acetic acid). Stained cells were documented by photographs of three independent fields and counted.

FS-3 ATX-activity assay

FS-3 was obtained from Echelon Bioscience (Salt Lake City, UT, USA) and maintained as a 1mM stock solution in Tris buffered saline. Concentrated conditioned medium and cellular lysates were diluted in 50 mM Tris, 140 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, pH 8.0 and FS-3 at a concentration of 0.5 µM in a 96 well plate and incubated in the dark overnight at 37°C. The increase in fluorescence was measured in a Tecan plate reader (485/535 nm) (Tecan, Crailsheim, Germany).

Statistical analysis

Data are expressed as mean of triplicates ± SD, unless stated otherwise. All experiments were done 3 times. Data were compared by a students t-test, a two-tailed Bonferroni and Anova tests; p values < 0.05were considered to be significant (SPSS 16.0, inc.)

Results

Cell model of mutation-independent TKI resistance

We have established a unique *in vitro* model for the analysis of non-mutational TKI-resistance in Ph+ ALL by generating a TKI-resistant subclone of the B-precursor cells SupB15, denoted SupB15-RT, as described previously [12].

SupB15-RT and SupB15-WT were comparable at the level of the cytogenetics: cytogenetic analysis performed by the MLL Leukämielabor GmbH (Munich, Germany), revealed a largely identical karyotype of these two cell lines.

Expansion of SupB15-WT but not SupB15-RT cells was inhibited in a dose-dependent manner by imatinib. Proliferation of SupB15-WT was inhibited by 82 % compared to control by 72 h exposure to 0.5 µM imatinib, representing clinically achievable concentration. In contrast, exposure of SupB15-RT cells to 0.5 - 5 µM imatinib did not inhibit proliferation (Figure 1A). Moreover, SupB15-RT cells display cross-resistance to the 2nd generation TKI dasatinib, nilotinib and ponatinib (data not shown).

Apoptosis was analyzed in parallel by Annexin V staining. A 72 h imatinib exposure at concentrations as low as 0.5 µM induced 70 % apoptosis in SupB15-WT cells, compared to 20 % in untreated cells, whereas there was no specific induction of apoptosis at concentrations up to 5 µM imatinib in SupB15-RT cells (Figure 1B).

Western blot (WB) analysis of PARP confirmed Annexin V staining. SupB15-WT cells induced PARP cleavage 72 h after imatinib treatment with 0.5 - 5 µM imatinib, but no induction of PARP cleavage was detected in SupB15-RT cells at up to 5 µM imatinib (data not shown).

In an extension of these reported results, we demonstrate pronounced cross-resistance of SupB15-RT cells to all clinically approved ABL-directed TKI (imatinib, dasatinib and nilotinib). Thus, exposure of SupB15-RT cells to nilotinib or dasatinib failed to induce apoptosis (17 % for 1 µM nilotinib, 24 % for 300 nM dasatinib, 18 % for 10 µM imatinib, 35 % for untreated control), whereas nilotinib and dasatinib potently induced apoptosis in SupB15-WT (53 % at 1 µM nilotinib and 73 % at 300 nM dasatinib), respectively (Figure 1C).

No mutations of the TKD of BCR-ABL could be identified. BCR-ABL protein expression was not increased in SupB15-RT vs. SupB15-WT cells as determined by WB analysis. Genomic amplification of BCR-ABL was ruled out by FISH analysis (Harald Rieder, Department of Genetics, University Marburg, Germany). The Calcein-assay, a functional transporter assay showed no differences

between SupB15-RT and SupB15-WT. Similarly, treatment with verapamil, a potent inhibitor of the multi-drug resistance protein did not abrogate the proliferative advantage of SupB15-RT over SupB15-WT cells in the presence of imatinib (data not shown). Thus, the commonly implicated mechanisms of TKI resistance did not play a role in conferring this TKI-resistance.

In summary, our model reproduced a mutation-independent TKI resistance, concerning 20 % of the patients without clarified mechanisms.

Akt kinase is constitutively activated in TKI-resistant SupB15-RT cells

To investigate this mutation-independent TKI-resistance phenotype, we first analyzed the signaling pathway of BCR-ABL.

Both SupB15-RT and SupB15-WT were treated with imatinib (0 to 5 µM) and analyzed for differences of AKT and STAT5 protein levels by WB analysis.

Imatinib reduced BCR-ABL phosphorylation in both SupB15-RT and SupB15-WT cells. Despite its pronounced effect on BCR-ABL phosphorylation, up to 5 µM imatinib did not decrease levels of phospho-STAT5 in the TKI-resistant SupB15-RT cells, in contrast to TKI-sensitive SupB15-WT. Moreover, phosphorylation of phospho-Tyr-AKT was inhibited by 0.5 µM imatinib in SupB15-WT cells, whereas imatinib in concentrations up to 5 µM had no impact on the level of phospho-AKT in SupB15-RT cells (Figure 1D), indicating constitutive activation of AKT during imatinib treatment.

In summary, this non-mutational TKI-resistance phenotype was independent of BCR-ABL pathway.

ATX is up-regulated in TKI-resistant SupB15-RT cells

As reported previously microarray gene expression analysis comparing TKI-resistant (SupB15-RT) and TKI-sensitive (SupB15-WT) cells we identified 29 genes that were differentially regulated based on a cut off of at least 3-fold.

Interestingly, the most profoundly up-regulated gene in TKI-resistant SupB15-RT cells was ATX (12-fold) (Figure 2A).

Up-regulation of ATX mRNA in SupB15-RT was confirmed by quantitative Real-time PCR which showed a 190-fold higher level in SupB15-RT than in SupB15-WT (Figure 2B).

As the ATXα and ATXβ isoform are known to play a role in migration, invasion and tumorigenesis [19], [29], [30], we examined whether these isoforms are overexpressed in a different manner. Both, ATXα

and ATXβ were upregulated in SupB15-RT vs. SupB15-WT cells. By RT-PCR, ATXα transcript was detected exclusively in SupB15-RT, whereas ATXβ was highly expressed in SupB15-RT cells but was also detected at a low level in SupB15-WT cells (Figure 2C).

In conclusion, the expression of ATXα and ATXβ in the TKI-resistant SupB15-RT cells was increased.

Since ATX is synthesized as a pre-proenzyme and is secreted into the extracellular space following two N-terminal cleavages [14],[15] we were unable to detect this protein in cell lysates of SupB15-WT and SupB15-RT cells. We analyzed the amount of ATX of SupB15-WT and SupB15-RT in conditioned medium (supernatant) that was concentrated. As shown in Figure 2D, ATX was detected only in concentrated supernatant of SupB15-RT cells but not of SupB15-WT. As published by Houben et al. we examined the expression of ATX under reducing and non-reducing Western blot conditions. When analyzed by reducing conditions ATX was detected to a high amount as cleavage product (~40 kDa and ~70kDa), respectively. With non-reducing conditions we detected ATX as a 120 kDa in supernatant of SupB15-RT.

Impact of ATX on proliferation of Ba/F3^{p185} cells

ATX is involved in signalling pathways related to cellular proliferation in several non-hematological cell types [14], [19]. To examine the effect of ATX isoforms (ATXα and ATXβ) on leukemic cell proliferation, we used Ba/F3 cells expressing BCR-ABL (Ba/F3^{p185}) as a murine Ph+ ALL model as described previously [31]. This Ph+ ALL model allowed us to selectively examine the impact of ATX on sensitivity of BCR-ABL expressing cells to TKI.

Ba/F3 cells infected with Paulo-mock (Ba/F3^{Mock}) or Paulo-p185$^{(BCR-ABL)}$ (Ba/F3^{p185}) were co-infected with Pinco-mock (Ba/F3$^{Mock/Mock}$, Ba/F3$^{p185/Mock}$), Pinco-ATXα (Ba/F3$^{Mock/ATXα}$, Ba/F3$^{p185/ATXα}$) or Pinco-ATXβ (Ba/F3$^{Mock/ATXβ}$, Ba/F3$^{p185/ATXβ}$). Infection efficiency was analyzed by two reporter genes (GFP for Pinco and ΔNGFR for Paulo). After cell sorting by FACS more than 80 % of the cells were double-infected and could be used for further experiments.

ATXβ was detected by WB analysis in the cell extracts of Ba/F3$^{p185/ATXβ}$ and Ba/F3$^{Mock/ATXβ}$ cells, but not in the concentrated conditioned medium (supernatant). Conversely, ATXα was present in the supernatant of Ba/F3$^{p185/ATXα}$ and Ba/F3$^{Mock/ATXα}$ but was not detectable in cellular extracts as shown in Figure 3A. The amount of ATX protein in cellular extracts and supernatant of ATX infected Ba/F3 cells was not affected by imatinib treatment (Figure 3A).

$Ba/F3^{p185/ATX\beta}$ and $Ba/F3^{Mock/ATX\beta}$ showed enhanced proliferation (1.5-fold) compared to mock-infected Ba/F3 cells ($Ba/F3^{p185/Mock}$ and $Ba/F3^{Mock/Mock}$). In contrast, proliferation of ATXα expressing cells ($Ba/F3^{p185/ATX\alpha}$ and $Ba/F3^{Mock/ATX\alpha}$) was not significantly different from proliferation of mock-infected Ba/F3 cells (Figure 3B). This may indicate different roles of ATXα and ATXβ in cellular signalling or reflect differences in cellular localization of the different isoforms.

ATX impairs responsiveness of $Ba/F3^{p185}$ cells to imatinib

Based on the role of ATX as a drug-resistance gene in ovarian cancer [32], and our demonstration that ATX is upregulated in TKI resistant cells (SupB15-RT), we investigated whether ATX modulates the sensitivity of BCR-ABL expressing leukemic cells to imatinib.

As expected, imatinib (1 µM) almost entirely abrogated cell growth of mock-infected $Ba/F3^{p185/Mock}$ cells. In contrast, additional expression of either ATXα ($Ba/F3^{p185/ATX\alpha}$) or ATXβ ($Ba/F3^{p185/ATX\beta}$) almost completely overturned the inhibitory effect of imatinib ($p<0.05$) (Figure 3B). This indicates that both ATX (α and β isoform) are implied in the TKI resistance by inhibiting the proliferation.

Effect of ATX activity in migratory capacity of SupB15

As ATX is involved in migration of non-hematological cells [14], [19] we examined in transwell experiments whether the SupB15-RT cells migrate to a greater extend than SupB15-WT. The proportion of SupB15-RT cells that migrated through the filter was 2-fold higher than that of SupB15-WT (Figure 4A), suggesting that ATXα or ATXβ may enhance the migratory capacity of leukemic cells. Migration was not affected by imatinib ($p<0.05$).

Different enzymatic activity of ATXα and ATXβ

Enzymatic activity of ATX is determined by quantifying hydrolysis of its substrate provided as a fluorescent activity-based probe (FS-3). Using this assay we determined the enzyme activity present in concentrated conditioned medium derived from cultures of SupB15-WT and SupB15-RT cells and from $Ba/F3^{p185/Mock}$, $Ba/F3^{p185/ATX\alpha}$ and $Ba/F3^{p185/ATX\beta}$ cells, respectively.

As shown in Figure 4B, supernatant of SupB15-RT cells displayed significantly higher ATX enzymatic activity than the supernatant of SupB15-WT cells ($p=0.04$) at the same µg protein level.

To examine whether the ATX isoforms ATXα and ATXβ differ in their enzymatic activity, we compared the ATX enzymatic activity of supernatants of Ba/F3 cells infected either with ATXα (Ba/F3$^{p185/ATX\alpha}$) or ATXβ (Ba/F3$^{p185/ATX\beta}$). Supernatant of Ba/F3$^{p185/ATX\alpha}$ showed high enzymatic activity, whereas supernatants derived from Ba/F3$^{p185/ATX\beta}$ and mock controls (Ba/F3$^{p185/Mock}$) showed low and not significantly different activity (Figure 4C). ATX-activity measured in lysates of Ba/F3$^{p185/Mock}$, Ba/F3$^{p185/ATX\beta}$ and Ba/F3$^{p185/ATX\alpha}$ cells was uniformly high (Figure 4D), i.e. high endogenous level already in mock control.

Results of the activity assays indicate that enzymatic activity of ATX in leukemic cells can be attributed primarily to ATXα, the longest of the known isoforms of this protein.

LPA increases proliferation in SupB15-WT

Because the conversion of LPC to LPA is the central mechanism by which ATX mediates its biological activity [15], [17], [19], we hypothesized that ATX may contribute to TKI resistance due to modulation of this signaling molecule.

To investigate whether LPA is able to confer TKI resistance, we examined the effect of LPA on proliferation of SupB15 cells in the presence or absence of 1 µM imatinib.

Proliferation of SupB15-WT cells increased in response to LPA in a dose dependent manner, with an up to 2.6-fold enhancement at 5 µM (Figure 5). Concentrations above 5 µM did not further enhance proliferation.

To determine whether LPA is able to counteract the inhibitory effect of TKIs, we examined the proliferation of SupB15-WT cells after combined treatment with LPA and 1 µM imatinib. As shown in Figure 5, we observed an increase (3.2 to 3.9-fold) of proliferation of imatinib-treated (1 µM) cells in a dose dependent manner in response to 1 - 10 µM LPA.

This indicates that the signal molecule LPA not only promotes proliferation of the leukemic cells but also mitigates the inhibitory effect of imatinib.

Downstream signaling of LPA is mediated via LPA receptors (LPAR) of which 6 different types have been described to date (LPAR1-LPAR6) [27]. We focused on the analysis of LPAR1, LPAR2 and LPAR3 expression by RT-PCR. LPAR1 was expressed in SupB15-WT cells but not in SupB15-RT cells (Figure 5B), whereas LPAR2 and LPAR3 were expressed in both SupB15-WT and SupB15-RT cells.

Discussion

Development of clinical resistance to ABL-TKI is a clinically relevant problem observed most frequently in patients with Ph+ ALL or advanced stage CML [6], [10]. Despite the well established role of TKD mutations [7], [8], [33], it is clear that additional mechanisms exist which enable leukemic cells to evade the inhibitory action of TKI.

The TKI resistant Ph+ ALL cell line SupB15-RT displays cross-resistance to all clinically available ABL-TKI and was established by us as a model to study mutation-independent TKI resistance in Ph+ALL. We have previously reported that the well-established mechanisms of TKI resistance are not involved in conferring the TKI resistant phenotype in SupB15-RT [12]. Our unique model is relevant because it emulates the mutation-independent TKI resistance, which is present at 20 % of the patients without clarified mechanisms. The second interesting characteristic is that this resistance is independent of BCR-ABL as evidenced by the TKI-induced dephosphorylation of BCR-ABL, these cells do not undergo apoptosis.

By comparative gene expression profiling, we identified 29 genes that were differentially expressed in the parental SupB15 cells (SupB15-WT) and the derivative resistant SupB15-RT line. Several of these genes have been implicated in transcriptional regulation in a variety of malignant cell types.

The ATX gene was of particular interest because it displayed the highest degree of upregulation in SupB15-RT cells and has been reported to be involved in oncogenesis and to influence migratory behavior, survival, growth and invasion in breast cancer and other solid tumors [17], [18], [29], [30], [34], [35]. ATX is overexpressed in various human cancers and plays a major role in downstream signaling pathways of several oncogenes [15], [19]. The oncogenic effects of ATX are mediated via LPA, which in turn activates diverse LPA receptor (LPAR) signaling pathways including PI3K, Ras, MAPK, Rho and NFκβ [16], [19], [36], [37], [38]. In hematological malignancies, data on the role of ATX-LPA signaling are very limited. Several studies have shown that LPA stimulates cell proliferation and protects leukemic cells, e.g. in CLL, from apoptosis by upregulating expression of LPA receptors [36], [39], [40], [41]. ATX is increased in Hodgkin's lymphoma cells [42], and has been suggested to be useful as a tumor marker in follicular lymphoma [43]. A study has just demonstrated that a deregulation of ATX contributes to the pathogenesis of FLT3-ITD acute myeloid leukemia (AML) [44]. Our study is the first study reporting a functional role of the ATX-LPA axis in Ph+ ALL and its involvement in resistance to TKI.

Both ATXα and ATXβ were transcriptional overexpressed in SupB15-RT vs. SupB15-WT with absence of the alpha isoform and only low level expression of ATXβ in the imatinib sensitive SupB15-WT. We showed that migration of SupB15-RT cells was 2-fold higher than migration of the SupB15-WT, raising the possibility that this difference in migratory behavior could be attributable to the difference in ATX expression. We compared these two isoforms in terms of there ability to modulate migration and proliferation of leukemic cells. This association suggested that ATXα may be responsible for the enhanced migration of SupB15-RT vs. SupB15-WT cells. The migration effect of ATXα was confirmed by BaF3 cells which were engineered to coexpress $p185^{BCR-ABL}$ and the alpha or beta isoform of ATX ($Ba/F3^{p185/ATX\alpha}$, $Ba/F3^{p185/ATX\beta}$). Our data suggest that ATXα but not ATXβ enhances migration of SupB15-RT cells.

To date the structure and the mode of action of ATX isoforms have not been conclusively established [45]. The differential localization of ATXα and ATXβ could be a result of differential splicing, as the precursor form of ATX is known to have two cleavage sites [14], [15], [46]. SupB15-WT cells do not express ATXα transcript, consistent with absence of ATXα in the conditioned medium by either WB analysis or in agreement with enzyme activity assays. Our observation that ATXβ is not secreted into the culture medium by $Ba/F3^{Mock/ATX\beta}$ or $Ba/F3^{p185/ATX\beta}$ cells is consistent with the hypothesis that ATXβ exists in these cells only in a non-secretable precursor form. Houben et al. also showed that ATX isoforms use distinct mechanisms to ensure spatially restricted LPA production and signaling [46]. The absence of ATX in the immediate extracellular environment limits enzymatic generation of the signaling molecule LPA, resulting in a lack of LPAR stimulation. This could explain why SupB15-WT and $Ba/F3^{p185/ATX\beta}$ cells show reduced migratory capacity compared with SupB15-RT and $Ba/F3^{p185/ATX\alpha}$. Taken together, our observations indicate that secretion of ATX could be a stimulus for migration of the leukemic cells.

Vidot et al. showed that LPA mediates resistance to cytotoxic chemotherapy [32]. We hypothesized that upregulation of ATX and the resulting enhanced production of LPA could be a general mechanism by which cells protect themselves against anti-proliferative and pro-apoptotic activity of anti-cancer agents. Our results confirm that resistance of SupB15-RT cells to TKI is mediated partially by ATX. This is also in agreement with the results of our experiments demonstrating that expression of both ATXα and ATXβ in $Ba/F3^{p185}$ cells mitigates resistance to the antiproliferative effects of imatinib.

Our comparison of SupB15-WT and SupB15-RT cells revealed that LPAR1 was expressed in SupB15-WT cells but not in SupB15-RT cells, whereas LPAR2 and LPAR3 were expressed in both SupB15-WT and SupB15-RT cells. These data suggest that the LPA induced signal transduction in SupB15-RT cells is not mediated by LPAR1 [42], but possibly involves one of the other LPAR, e.g. LPAR2 which is highly related to LPAR1 [16], [40]. The other possible explanation for the downregulation of LPAR1 is the existence of a feedback loop in which the production of LPA is enhanced by ATX, but the LPA effect is counteracted by LPA induced downregulation of LPAR, e.g. LPAR1.

Conclusions

In summary, our study is the first to report an upregulation of ATX and a role of ATX in mutation-independent TKI resistance in Ph+ ALL cells. This appears to involve LPA induced signaling through LPAR which also stimulate proliferation and migration of Ph+ ALL cells. ATX and LPA receptors are attractive therapeutic targets [16], [40], as their inhibition could counteract one of the mechanisms of resistance to ABL-directed TKI in Ph+ ALL.

Competing interests

The authors have declared that no competing interests exist.

Oliver Ottmann is an endowed Professor of the Deutsche José Carreras Foundation.

Funding: This work is supported by the deutsche José Carreras Leukämie-Stiftung (Germany), the A.L.A.R.M.E., the Fondation de France and the Société française d'hématologie (France).

Author's contributions

Conceived and designed the experiments: AS-T, AR, MR
Performed the experiments: AS-T, AR, PNGM, TR, SB, HP
Analyzed the data: AS-T, AR; HP, WKH, MR
Wrote the paper: AS-T, AR, OGO

Acknowledgements

We wish to thank Harald Rieder (Department of Genetics, University Marburg, Germany) for FISH analysis.

References

1. Chalandon Y, Thomas X, Hayette S, Cayuela JM, Abbal C, et al. Randomized study of reduced-intensity chemotherapy combined with imatinib in adults with Ph-positive acute lymphoblastic leukemia. Blood 2015; Apr 15: epub ahead of print
2. Faderl S, Jeha S, Kantarjian HM.The biology and therapy of adult acute lymphoblastic leukemia. Cancer 2003;98:1337-1354.
3. Ottmann OG, Druker BJ, Sawyers CL, Goldman JM, Reiffers J, et al. A phase 2 study of imatinib in patients with relapsed or refractory Philadelphia chromosome-positive acute lymphoid leukemias. Blood 2002;100:1965-1971.
4. Tanguy-Schmidt A, Rousselot P, Chalandon Y, Cayuela JM, Hayette S, et al. Long-term follow-up of the imatinib GRAAPH-2003 study in newly diagnosed patients with de novo Philadelphia chromosome-positive acute lymphoblastic leukemia: a GRAALL study. Biol Bone Marrow Transplant 2013;19:150-155.
5. Gruber F, Mustjoki S, Porkka K. Impact of tyrosine kinase inhibitors on patient outcomes in Philadelphia chromosome-positive acute lymphoblastic leukaemia. Br J Haematol 2009;145:581-597.
6. Gorre ME, Mohammed M, Ellwood K, Hsu N, Paquette R, et al. Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification. Science 2001;293:876-880.
7. Pfeifer H, Wassmann B, Pavlova A, Wunderle L, Oldenburg J, et al. Kinase domain mutations of BCR-ABL frequently precede imatinib-based therapy and give rise to relapse in patients with de novo Philadelphia-positive acute lymphoblastic leukemia (Ph+ ALL). Blood 2007;110:727-734.
8. Pfeifer H., Lange T, Wystub S, Wassmann B, Maier J, et al. Prevalence and dynamics of bcr-abl kinase domain mutations during imatinib treatment differ in patients with newly diagnosed and recurrent bcr-abl positive acute lymphoblastic leukemia. Leukemia 2013;27:1254-1262.
9. Goldman JM, Melo JV. Chronic myeloid leukemia-advances in biology and new approaches to treatment. N Engl J Med 2003;349:1451-1464.
10. O'Hare T, Eide CA, Deininger MW. Bcr-Abl kinase domain mutations, drug resistance, and the road to a cure for chronic myeloid leukemia. Blood 2007;110:2242-2249.

11. Hofmann WK, de Vos S, Elashoff D, Gschaidmeier H, Hoelzer D, et al. Relation between resistance of Philadelphia-chromosome-positive acute lymphoblastic leukaemia to the tyrosine kinase inhibitor STI571 and gene-expression profiles: a gene-expression study. Lancet 2002;359:481-486.

12. Koyama N, Koschmieder S, Tyagi S, Portero-Robles I, Chromic J, et al. Inhibition of phosphotyrosine phosphatase 1B causes resistance in BCR-ABL-positive leukemia cells to the ABL kinase inhibitor STI571. Clin Cancer Res 2006;12:2025-2031.

13. Stracke ML, Krutzsch HC, Unsworth EJ, Arestad A, Cioce V, et al. Identification, purification, and partial sequence analysis of autotaxin, a novel motility-stimulating protein. J Biol Chem 1992;267:2524-2529.

14. Giganti A, Rodriguez M, Fould B, Moulharat N, Coge F, et al. Murine and human autotaxin alpha, beta, and gamma isoforms: gene organization, tissue distribution, and biochemical characterization. J Biol Chem 2008;283:7776-7789.

15. Boutin JA, Ferry G. Autotaxin. Cell Mol Life Sci 2009;66:3009-3021.

16. Choi JW, Herr DR, Noguchi K, Yung YC, Lee CW, et al. LPA receptors: subtypes and biological actions. Annu Rev Pharmacol Toxicol 2010;50:157-186.

17. Umezu-Goto M, Kishi Y, Taira A, Hama K, Dohmae N, et al. Autotaxin has lysophospholipase D activity leading to tumor cell growth and motility by lysophosphatidic acid production. J Cell Biol 2002;158:227-233.

18. Nam SW, Clair T, Kim YS, McMarlin A, Schiffmann E, et al. Autotaxin (NPP-2), a metastasis-enhancing motogen, is an angiogenic factor. Cancer Res 2001;61:6938-6944.

19. vanMeeteren LA, Moolenaar WH. Regulation and biological activities of the autotaxin-LPA axis. Prog Lipid Res 2007;46:145-160.

20. Grignani F, Kinsella T, Mencarelli A, Valtieri M, Riganelli D, et al. High-efficiency gene transfer and selection of human hematopoietic progenitor cells with a hybrid EBV/retroviral vector expressing the green fluorescence protein. Cancer Res 1998;58:14-19.

21. Romanski A, Bacic B, Bug G, Pfeifer H, Gul H, et al. Use of a novel histone deacetylase inhibitor to induce apoptosis in cell lines of acute lymphoblastic leukemia. Haematologica 2004;89:419-426.

22. Komor M, Guller S, Baldus CD, de Vos S, Hoelzer D, et al. Transcriptional profiling of human hematopoiesis during in vitro lineage-specific differentiation. Stem Cells 2005; 23: 1154-1169.

23. Grez M, Becker S, Saulnier S, Knoss H, Ott MG, et al. Gene therapy of chronic granulomatous disease. Bone Marrow Transplant 2000;25:99-104.

24. Zheng X, Beissert T, Kukoc-Zivojnov N, Puccetti E, Altschmied J, et al. Gamma-catenin contributes to leukemogenesis induced by AML-associated translocation products by increasing the self-renewal of very primitive progenitor cells. Blood 2004;103:3535-3543.

25. Pane F, Intrieri M, Quintarelli C, Izzo B, Muccioli GC, et al. BCR/ABL genes and leukemic phenotype: from molecular mechanisms to clinical correlations. Oncogene 2002; 21: 8652-8667.

26. Steelman LS, Pohnert SC, Shelton JG, Franklin RA, Bertrand FE, et al. STAT, Raf/MEK/ERK, PI3K/Akt and BCR-ABL in cell cycle progression and leukemogenesis. Leukemia 2004;18:189-218.

27. Jilani I, Kantarjian H, Gorre M, Cortes J, Ottmann OG, et al. Phosphorylation levels of BCR-ABL, Crkl, AKT, STAT5 in imatinib-reistant chronic myeloid leukemia cells implicate alternative pathway usage as asurvival strategy. Leuk Res.2008;32:643-649.

28. Hashimoto T, Okudaira S, Igarashi K, Hama K, Yatomi Y, et al. Identification and biochemical characterization of a novel autotaxin isoform, ATXdelta, with a four-amino acid deletion.J Biol chem 2012;151:89-97.

29. Yang SY, Lee J, Park CG, Kim S, Hong S, et al. Expression of autotaxin (NPP-2) is closely linked to invasiveness of breast cancer cells. Clin Exp Metastasis 2002;19:603-608.

30. Liu S, Umezu-Goto M, Murph M, Lu Y, Liu W, et al. Expression of autotaxin and lysophosphatidic acid receptors increases mammary tumorigenesis, invasion, and metastases. Cancer Cell 2009;15:539-550.

31. Beissert T, Puccetti E, Bianchini A, Guller S, Boehrer S, et al. Targeting of the N-terminal coiled coil oligomerization interface of BCR interferes with the transformation potential of BCR-ABL and increases sensitivity to STI571. Blood 2003;102:2985-2993.

32. Vidot S, Witham J, Agarwal R, Greenhough S, Bamrah HS, et al. Autotaxin delays apoptosis induced by carboplatin in ovarian cancer cells. Cell Signal 2010;22:926-935.

33. Nimmanapalli R, Bhalla K. Mechanisms of resistance to imatinibmesylate in Bcr-Abl-positive leukemias. Curr Opin Oncol 2002;14:616-620.

34. Hama K, Aoki J, Fukaya M, Kishi Y, Sakai T, et al. Lysophosphatidic acid and autotaxin stimulate cell motility of neoplastic and non-neoplastic cells through LPA1. J Biol Chem 2004;279:17634-1739.

35. Song J, Clair T, Noh JH, Eun JW, Ryu SY, et al. Autotaxin (lysoPLD/NPP2) protects fibroblasts from apoptosis through its enzymatic product, lysophosphatidic acid, utilizing albumin-bound substrate. Biochem Biophys Res Commun 2005;337:967-975.

36. Hu X, Haney N, Kropp D, Kabore AF, Johnston JB, et al. Lysophosphatidic acid (LPA) protects primary chronic lymphocytic leukemia cells from apoptosis through LPA receptor activation of the anti-apoptotic protein AKT/PKB. J Biol Chem 2005;280:9498-9508.

37. Lee J, Duk Jung I, Gyo Park C, Han JW, Young Lee H. Autotaxin stimulates urokinase-type plasminogen activator expression through phosphoinositide 3-kinase-Akt-necrosis factor kappa B signaling cascade in human melanoma cells. Melanoma Res 2006;16:445-452.

38. Lin ME, Herr DR, Chun J. Lysophosphatidic acid (LPA) receptors: signaling properties and disease relevance. Prostaglandins Other Lipid Mediat 2010;91:130-138.

39. Rosskopf D, Daelman W, Busch S, Schurks M, Hartung K, et al. Growth factor-like action of lysophosphatidic acid on human B lymphoblasts. Am J Physiol 1998;274:C1573-C1582.

40. Kumar SA, Hu X, Brown M, Kuschak B, Hernandez TA, et al. (2009) Lysophosphatidic acid receptor expression in chronic lymphocytic leukemia leads to cell survival mediated though vascular endothelial growth factor expression. Leuk Lymphoma 2009;50:2038-2048.

41. Satoh Y, Ohkawa R, Nakamura K, Higashi K, Kaneko M, et al. Lysophosphatidic acid protection against apoptosis in the human pre-B-cell line Nalm-6. Eur J Haematol 2007;78: 510-517.

42. Baumforth KR, Flavell JR, Reynolds GM, Davies G, Pettit TR, et al. Induction of autotaxin by the Epstein-Barr virus promotes the growth and survival of Hodgkin lymphoma cells. Blood 2005;106:2138-2146.

43. Masuda A, Nakamura K, Izutsu K, Igarashi K, Ohkawa R, et al. Serum autotaxin measurement in haematological malignancies: a promising marker for follicular lymphoma. Br J Haematol 2008;143:60-70.

44. Ortlepp C, Steudel C, Heidreich C, Koch S, Jacobi A, et al. Autotaxin is expressed in FLT3-ITD positive acute myeloid leukemia and hematopoietic stem cells and promotes cell migration and proliferation. Exp Hematol 2013;41:444-461.

45. Hausmann J, Perrakis A, Moolenaar WH. (2013) Struture-function relationships of autotaxin, a secreted lysophospholipase D. Advances in Biological Regulation 2013;53:112-117.

46. Houben AJ, van Wijk XMR, van Meeteren LA, van Zeijl L, van de Westerlo EMA et al.The polybasic Insertion in Autotaxin αconfers Specific Binding to Heparin and Cell Surface Heparan Sulfate Proteoglycans. J Biol Chem 2013;288:510-519.

Figure legends

Figure 1: Characterization of SupB15-WT and SupB15-RT cells.

(A) Proliferation was determined by cell counting. Cells were counted 72 h after imatinib treatment (0 µM, 0.5µM, 2µM, 5µM). Data are shown as mean of fold-variation of proliferation +/- SD (n=3). **(B)** Apoptosis was assessed using Annexin V/PI staining and measured by FACS after 72 h of imatinib treatment with the indicated concentration (0 µM, 0.5µM, 2µM, 5µM). Data are shown as mean of fold variation of apoptosis +/- SD (n=3). **(C)** Apoptosis was assessed using Annexin V/PI staining and measured by FACS analysis after TKI treatment (72 h) with the indicated TKI concentrations (imatinib, nilotinib, dasatinib). Data are shown as mean +/- SD (n=3). **(D)** Signaling pathway of BCR-ABL in SupB15-WT and SupB15-RT cells. Whole cell lysates from imatinib treated SupB15-WT (left panel) and SupB15-RT cells (right panel) were probed with antibodies (phosphorylated and non-phosphorylated forms) against AKT, STAT5.

Figure 2: ATX expression in SupB15-WT and SupB15-RT cells.

(A) Comparison of the gene expression profiles of SupB15-WT and SupB15-RT was assessed by microarray technology. **(B)** Q-PCR analysis of ATX expression in SupB15-WT and SupB15-RT cells was compared to expression of the housekeeping gene GAPDH and fold change was determined by the ΔΔCt method. Results are represented as mean of $2^{-\Delta\Delta CT}$ values +/- SD (n=3). **(C)** RT-PCR analysis of ATXα and ATXβ, two ATX isoforms in SupB15-WT and SupB15-RT cells. Actin is given as internal control of PCR. **(D)** Western blot analysis of ATX protein in concentrated conditioned medium (supernatant, 50x) of 1 x 10^7 SupB15-WT and SupB15-RT cells under reducing and non-reducing conditions.

Figure 3: Impact of ATX expression on proliferation

(A) WB analysis of ATXα and ATXβ in cellular extracts and in concentrated conditioned medium (50x, supernatant) of infected Ba/F3 ($Ba/F3^{Mock/Mock}$, $Ba/F3^{Mock/ATX\alpha}$ and $Ba/F3^{Mock/ATX\beta}$,$Ba/F3^{p185/Mock}$, $Ba/F3^{p185/ATX\alpha}$ and $Ba/F3^{p185/ATX\beta}$) cells. Cells were treated with imatinib for 40 h before lysates and supernatants were prepared. Imatinib treatment of the cells was indicated by +/- label. **(B)** Proliferation of Ba/F3 cells co-infected with mock, ATXα or ATXβ and mock ($Ba/F3^{Mock/Mock}$, $Ba/F3^{Mock/ATX\alpha}$ and $Ba/F3^{Mock/ATX\beta}$) or p185 ($Ba/F3^{p185/Mock}$, $Ba/F3^{p185/ATX\alpha}$ and $Ba/F3^{p185/ATX\beta}$), with or without imatinib treatment. Proliferation was determined by cell counting using trypan blue dye exclusion assay. Results represent the mean proliferation index at day 3 +/- SD (n=3).

Figure 4: ATX activity in SupB15 and Ba/F3 cells.

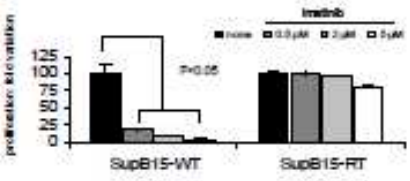
(A) Transmigration of leukemic cells: number of SupB15-WT and SupB15-RT cells migrating through a transwell in 24 h is given. The bar graphs represent the mean +/-SD (n=3). **(B)** Enzymatic activity of ATX in supernatant of SupB15-WT and SupB15-RT was measured using FS-3 substrate. Bar graphs show fluorescence change as mean of triplicates +/- SD (n=3). **(C)** Enzymatic activity of ATX in supernatant of $Ba/F3^{p185/Mock}$, $Ba/F3^{p185/ATX\alpha}$ and $Ba/F3^{p185/ATX\beta}$ was measured using FS-3 substrate. Bar graphs show fluorescence change as mean of triplicates +/- SD (n=3). **(D)** Enzymatic activity of ATX in cellular lysate of $Ba/F3^{p185/Mock}$, $Ba/F3^{p185/ATX\alpha}$ and $Ba/F3^{p185/ATX\beta}$ was measured using FS-3 substrate. Bar graphs show fluorescence change as mean of triplicates +/- SD (n=3).

Figure 5: LPA-ATX axis in SupB15-WT and SupB15-RT cells

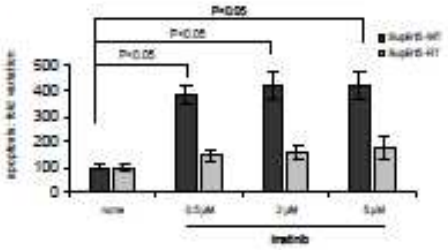
(A) Effect of LPA on proliferation of SupB15-WT. The SupB15-WT cells were treated with the indicated concentration of LPA for 72 h and proliferation was determined by trypan blue dye exclusion of viable cells. **(B)** Analysis of LPA receptors (LPAR1-3) in imatinib treated SupB15-WT and SupB15-RT cells. The expression of the LPAR was assessed by PCR following reverse transcription of RNA. The PCR analysis of β-Actin was used as a control. Data shown are representative of three independent experiments.

Figure 1

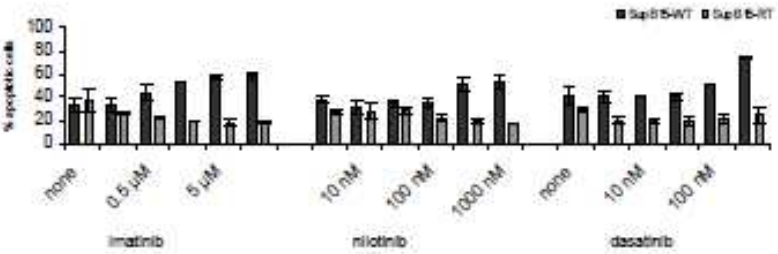
A



B



C



D

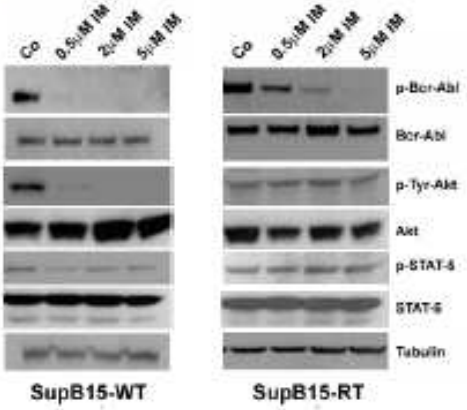
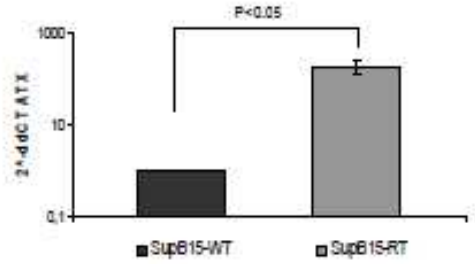


Figure 2

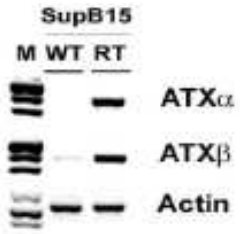
A

SupB15-WT Normalized	SupB15-RT Normalized	Common	Gustomark	Map
0.5	0.6	[illegible]	[illegible]	[illegible]
0.4	1.5	TX2	[illegible]	[illegible]
0.2	1.5	DSRRB	[illegible]	[illegible]
0.4	1.2	CX3_LEF_LEF_TC	[illegible]	[illegible]
1.9	16.2	ATX_PERIS_PD-ALPHA	[illegible]	[illegible]
0.4	1.2	MCEC	[illegible]	[illegible]
0.4	1.4	CEBPD	[illegible]	[illegible]
0.1	1.2	MEEA6	[illegible]	[illegible]
0.7	2.2	TRAM2	[illegible]	[illegible]
0.2	0.4	cDNA DKFZp686D153	[illegible]	[illegible]
0.3	1.9	NOX4	[illegible]	[illegible]
0.8	1.9	GABRB1	[illegible]	[illegible]
0.4	1.2	DNH3	[illegible]	[illegible]
1.1	0.1	HSUT5200	[illegible]	[illegible]
12.8	6.8	OAT1, TBAR1, TABR-1	[illegible]	[illegible]
1.8	0.7	BZF-C	[illegible]	[illegible]
0.9	1.9	DnZ	[illegible]	[illegible]
2.8	0.4	ITGB4	[illegible]	[illegible]
4.5	1.9	SCMB2	[illegible]	[illegible]
1.5	0.3	RTS, RDC/G6	[illegible]	[illegible]
4.3	1.0	DAZ3	[illegible]	[illegible]
3.9	0.5	AC133, CD133	[illegible]	[illegible]
1.9	0.5	KIAA0781	[illegible]	[illegible]
1.8	0.9	KLF5IR2	[illegible]	[illegible]
3.1	1.0	PTHR	[illegible]	[illegible]
1.8	0.9	TYDP1	[illegible]	[illegible]

B



C



D

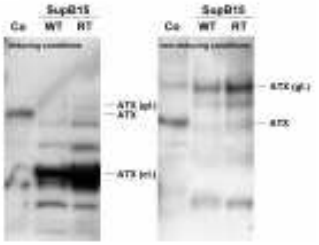
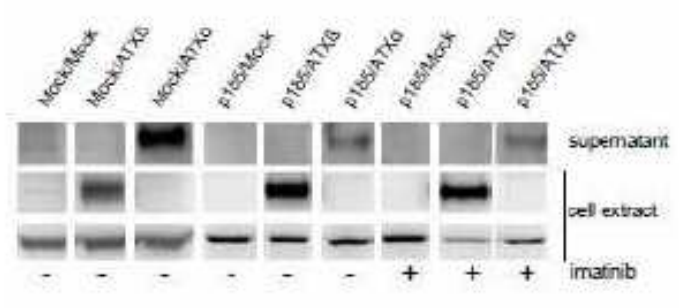


Figure 3

A


Mock/Mock
Mock/ATXδ
Mock/ATXα
p185/Mock
p185/ATXδ
p185/ATXα
p185/Mock
p185/ATXδ
p185/ATXα
supernatant
cell extract
imatinib
- - - - - - + + +

B

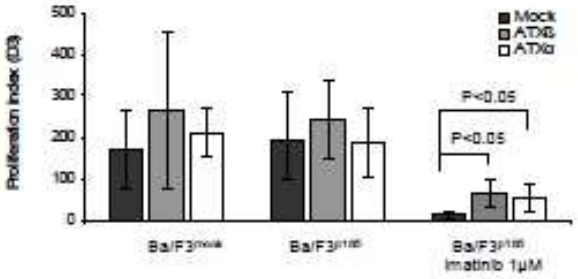
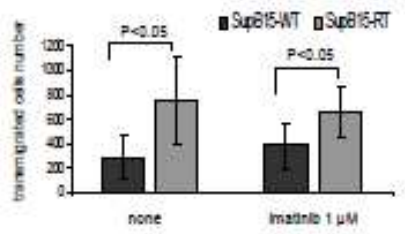
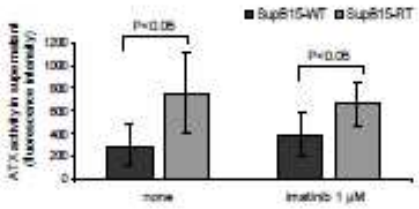

Proliferation index (OD)
500
400
300
200
100
0
Mock
ATXδ
ATXα
P<0.05
P<0.05
Ba/F3mock
Ba/F3p185
Ba/F3p185
imatinib 1µM

Figure 4

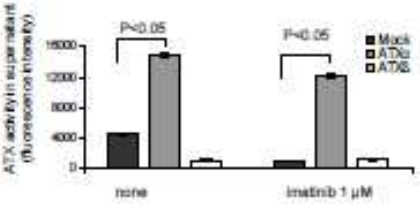
A



B



C



D

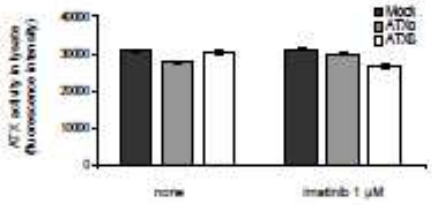
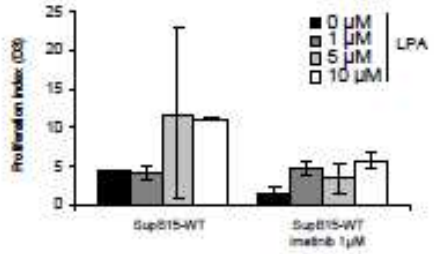
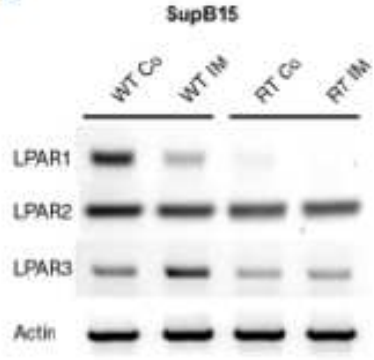


Figure 5

A


Proliferation Index (OD)
25
20
15
10
5
0
0 µM
1 µM
5 µM
10 µM
LPA
SupB15-WT
SupB15-WT
imatinib 1µM

B


SupB15
WT Co
WT IM
RT Co
RT IM
LPAR1
LPAR2
LPAR3
Actin

II – Les LAL à Ph1

3 - FTY 720: potentiel agent thérapeutique dans les LAL à Ph1

Article n° 4
Schmidt-Tanguy A et al. The immunomodulator FTY720 is a potential therapeutic agent in acute lymphoblastic leukemia.

Des progrès thérapeutiques certains ont lieu concernant le pronostic des LAL à Ph1. La stratégie associant ITK et allogreffe de CSP est actuellement le traitement de référence des LAL àPh1. Cependant, devant la persistance de rechutes, la mortalité liée à la allogreffe de CSH et l'existence des mécanismes de résistance, de nouvelles pistes thérapeutiques doivent être envisagées.
Le fingolimod est un agent immunomodulateur, composé synthétique issu de la modification d'un immunosuppresseur naturel. Interférant dans le trafic cellulaire des lymphocytes T, il est utilisé en transplantation d'organes. Par l'intermédiaire de l'activation de la phosphatase PP2A, le fingolimod induit une apoptose cellulaire dans des hémopathies malignes à cellules B, indépendamment de l'expression du Bcl2.

Nous avons étudié l'effet du fingolimod sur 5 lignées cellulaires humaines primaires de LAL BCR-ABL positif, sur 5 lignées cellulaires humaines primaires de LAL BCR-ABL négatif et sur une lignée de LAL BCR-ABL positif avec une résistance non mutationnelle à l'imatinib. Pour le modèle de résistance aux ITK, nous avons utilisé la lignée cellulaire SupB15RT obtenue par exposition croissante progressive à l'imatinib de la lignée SupB15W (LAL pro-B BCR-ABL positif sensible à l'imatinib). La lignée cellulaire SupB15RT présente une résistance croisée aux ITK de seconde génération sans implication de mécanismes de résistance connus (absence de mutations du domaine kinase, d'amplification du BCR-ABL, de surexpression protéique ou mécanisme d'efflux).

Les cellules sont traitées par le fingolimod avec une concentration de 1 à 10µM pendant 7 jours. La viabilité est déterminée par coloration de trypan, l'apoptose évaluée en cytométrie en flux. La phosphorylation de Abl est analysée par western blot.
Le fingolimod induit une apoptose cellulaire dose dépendante dans 4 des 6 LAL Bcr-Abl positif et dans 3 des 5 LAL BCR-ABL négatif. Dans SupB15W, le fingolimod induit une apoptose majeure (75%) avec une prédominance d'apoptose tardive (53%) et inhibe la croissance. Dans SupB15RT, l'apoptose est moindre (39%) et précoce (27%).

La phosphorylation d'Abl, substrat connu de la PP2A n'est pas modifiée par le fingolimod dans les lignées SupB15W et SupB15RT.
Nous avons ainsi démontré que le Fingolimod est un inducteur d'apoptose dans nos modèles de LAL à Ph1. Le fingolimod est un agent antiprolifératif dans les LAL BCR-ABL positif qui abroge partiellement la résistance à l'imatinib. Il pourrait être utilisé dans le traitement des LAL à Ph1. Le mécanisme de mort cellulaire semble différer en cas de sensibilité ou de résistance à l'imatinib et mériterait d'être étudié.

Le manuscrit est en préparation. Ce travail sera prochainement soumis dès que l'analyse statistique sera finalisée.

Title

The immunomodulator FTY720 is a potential therapeutic agent in acute lymphoblastic leukemia.

Authors

[1,3] Schmidt-Tanguy A., [2]Romanski A., [1]Orvain C., [2]Badura S., [2]Wystub S., [1]Hunault-Berger M., [2]Ottmann O.G.

[1]Department of Hematology, University of Angers, France
[2]Department of Hematology/Oncology, University of Frankfurt, Germany
[3]CRCNA, INSERM U892, Angers, France

Correspondence: O.G. Ottmann
Address : Institute of Cancer & Genetics
Cardiff University School of Medicine
Institute of Medical Genetics Building
Heath Park
Cardiff CF14 4XN
E-mail: ottmanno@cf.ac.uk

Funding

This work is supported by the deutsche José Carreras Leukämie-Stiftung (Germany), the A.L.A.R.M.E., the Fondation de France and the Société française d'hématologie (France).

Competing interests

The authors declare that no competing interests.

Abstract

Despite continuous progress in the therapy of adult acute lymphoblastic leukemia (ALL), most patients eventually relapse and survival rates after relapse remain poor. Among prognostic factors, the Bcr-Abl fusion transcript confers a poor outcome. Although treatment results have improved significantly following front-line therapy based on the ABL tyrosine kinase inhibitor (TKI), with complete remission rates of approximately 95%, the majority of patients who do not undergo allogeneic stem cell transplantation (SCT) relapses within the first two years of treatment.

FTY720 is a synthetic compound poduced by the modification of a natural immunosuppressor. This immunomodulator interferes with T-cell trafficking and is used in organ transplantation. Recent studies indicate that FTY720 induces apoptosis through activation of the serine/threonine phosphatase PP2A pathway in a variety of B-cell malignancies independently of Bcl-2 expression, but the precise mechanisms of action of this compound are incompletely understood.

We examined the potential effect of FTY720 in long-term cultured primary human Ph+ and Ph- ALL cells and in a Ph+ ALL cell line displaying non-mutational imatinib-resistance. We used 7 long-term cultured primary human ALL cells obtained from patients with Bcr-Abl positive (n=4) and Bcr-Abl negative cells (n=3), and 7 acute leukaemia cell lines (4 ALL with Bcr-Abl positive (n=2) and Bcr-abl negative (n=2), chronic myeloid leukaemia in blast crisis (n=1), acute myeloblastic leukemia (n=2)). In addition, as a model of imatinib resistance we used the $SupB15_R$ cell line which was derived from the previously well characterized Bcr-Abl positive B-precursor $SupB15_W$ cell line by gradually increasing the exposure to imatinib. SupB15RT cells are cross-resistant to the second generation TKI with no evidence of commonly implicated mechanisms of imatinib resistance, e.g. Bcr-Abl gene amplification, point mutations in the TKD mutations, or Bcr-Abl overexpression. Cells were treated with 1 to 10 µM FTY720 for a period of 7 days. Cell viability was determined by trypan cell counting and apoptosis was measured using the Annexin V-FITC apoptosis kit. Abl and PP2A C phosphorylation was analyzed by western blot.

FTY720 induced apoptosis in a dose-dependant manner in 4 cases of Ph+ ALL cell lines (n=4/6) and in 3 Ph- ALL cell lines (n=3/5). The maximum apoptotic effect was observed after 48 hours. In SUP-B15W, FTY720 had a dramatic effect with 75% of apoptosis, principally late apoptosis (53 %) after 48 hours, and suppressed growth with low concentration (2.5 µM). In imatinib-resistant SUP-B15R, we showed apoptotic and antiproliferative effects after 48 hours with less apoptosis (39%) and mostly early apoptosis (27%).The potential effect of FTY720 on phosphorylation of Bcr-Abl, a known substrate of PP2A, was examined by Western blotting, demonstrating that in SUP-B15W and in SUP-B15R cells, the level of Abl and PP2A phosphorylation is not modified by FTY720.

In conclusion, the immunomodulator FTY720 is a potent inducer of apoptosis and an antiproliferative agent in ALL. It partially abrogates mutational-independent imatinib resistance and may be of potential use as a therapeutic agent in Ph+ ALL. The mechanism by which FTY720 induces cell death appears to differ between imatinib-sensitive and imatinib-resistant Ph+ ALL cells, and is currently being investigated.

Introduction

Despite the continuous progress in the treatment of adult acute lymphoblastic leukemia (ALL), the cure rate of adult ALL remains unsatisfactory as approximately 40% of adults diagnosed with ALL relapse following treatment.[1,2] Indeed, even if prognostic factors have been clarified, and treatment is now stratified according to these prognostic factors, intensive therapy is associated with serious acute and late complications and many patients still relapse. Survival rates after relapse have been consistently low.[3-4] Resistance in ALL have shown to involve an altered expression of the Bcl-2 family member that prevents apoptosis and aberrant signalling through the PI3K and Ras pathways.[5]

Among poor prognostic factors, the Bcr-Abl fusion transcript confers poor outcome.[6,7] Although treatment results have improved significantly following front-line therapy based on the ABL tyrosine kinase inhibitor (TKI), with complete remission rates of approximately 95%, the majority of patients who do not undergo allogeneic stem cell transplantation (SCT) relapses within the first two years of treatment.[2,8] More potent second generation tyrosine kinase inhibitors are also highly effective as initial therapy but do not appear to abrogate the development of resistance.[9]

New therapies including agents with alternative mechanisms of action are needed in frontline therapy for very-high-risk patients and in salvage regimens in the treatment of adult ALL.

The FTY720 is a synthetic structural analog of sphingosine related to the naturally-ocuring drug Myriocin (ISP-1), which was isolated from cultured filtrates of the ascomycete *Isaria sinclai*. FTY720 is phosphorylated by sphingosine kinase 2, The phosphorylated compound is a potent agonist of 4 shingosine-1-phosphate (S1P) receptors which disrupts the sphingolipid pathway. FTY720 is soluble in water or in ethanol, it has a high oral bioavailability.[10,11] FTY720 is a novel immunomodulator with a unique mode of action by modulating chemotactic responses and lymphocyte trafficking and redistributing lymphocytes from blood to the thymus and secondary lymphoid organs, although there is still some debate as to whether FTY720 induces immunosuppressive effect through its antagonist or agonistic effect on the S1P pathway.[11,12] FTY720 blocks T-cell proliferation and has been shown to prolong the survival of skin, heart, or liver allograft in animal models of transplantation. Thus, FTY720 may be a useful tool for the prevention of transplant rejection and a new therapeutic approach for immune diseases.[12] Recent studies indicate an additional role for FTY720 by inducing cell apoptosis in various types of cancer cell lines.[13] Several other mechanisms of action are reported but are incompletely understood. FTY720 induces apoptosis in T blood lymphocytes by a Bcl2-dependent mechanism. It appears that it also induces caspase-dependent apoptosis in other various cancers.[13-16] On the other hand, in a variety of B-cell malignancies, it induces apoptosis through activation of the PP2A pathway, independent of FAS, caspase, or Bcl-2 expression possibly through increasing intracellular ceramide concentration. Loss or reduced expression of PP2A is associated with various malignancies as it is considered to be a tumor suppressor. Its loss of function is essential for chronic myeloid leukemia (CML) blastic transformation.[14,17,18]

Based upon the apoptotic potential of FTY720, we report for the first time the effects of FTY720 in a large panel of ALL primary cells and cell lines with the aim of determining the potential antileukemic effect of FTY720 in Ph- and Ph+ ALL cells, examining the ability of FTY720 to overcome non-mutational TKI-resistance, and determining whether the activity is indeed mediated by activation of PP2A or by other mechanisms. For that, we worked on 14 models of acute leukemia primary cells and cell lines including long-term cultured primary human ALL cells, unique *in vitro* models of primary AL, and a Ph+ ALL cell line displaying non-mutational imatinib-resistance.

Thus, we report that the immunomodulator FTY720 has a potent *in vitro* activity in a variety of Ph- and Ph+ ALL. It partially abrogates mutation-independent TKI-resistance.

Methods

Cells lines and chemical reagents
Cells were cultured at 37°C in 5% CO_2 in humidified atmosphere.
Long-term cultured human ALL cells, BV, CM, KW, PH, HP, KR, and RL, were obtained from Bart A. Nijmeijer at the department of Hematology of Leiden University Medical Center. Cells were cultured in serum-free medium consisted of IMDM (*Lonza, Verviers, Belgium*) supplemented with 1 µg/ml bovine insulin, 5×10^{-5} M ß-mercaptoethanol (both *Sigma, St Louis, MO, USA*), 200 µg/ml Fe^{3+}-saturated human apo-transferrin (*Invitrogen, Karlsruhe, Germany)*, 0.6% human serum albumin (*Sanquin, Amsterdam, The Netherlands*), 2 mM L-glutamine (*Lonza*), and 20 µg/ml cholesterol (*Sigma*; first dissolved in ethanol at a concentration of 20 mg/ml). K562, MV4-11, KG-1, Nalm 6, SEM, and SUP-B15W were provided by the German Collection of Microorganisms and Cell Cultures (*DSMZ, Braunschweig, Germany*). K562, MV4-11, KG-1, Nalm 6, SEM were maintained in RPMI 1640 supplemented with 10% fetal calf serum (FCS), 1% Glutamine and 1% Penicillin/Streptomycin (40 U/ml Penicillin, 40 µg/ml Streptomycin) (all from *Invitrogen*). SUP-B15W were maintained in same medium but supplemented with 15% FCS. To select the tyrosine kinase inhibitor-resistant phenotype, SupB15W were cultured with gradually increasing concentrations of imatinib, dissolved in 0.1 N HCl and stocked at 10 µM solution at 4°C) for 6 months. Starting concentration was 0.05 µM imatinib increased every 10 to 14 days by 0.05 µM. The treated cells, designated SUP-B15RT, were able to grow in up to 5µM imatinib and were then maintained in the presence of 1µM imatinib. Parental SupB15W cells were maintained without imatinib.
The characteristics of the cell lines are recapitulated in table 1 and in figure 1.
Cells were treated with FTY720 (1 to 10 µM) for 7 days.
FTY720 (fingolimod) and Imatinib were provided by Novartis (*Basel, Switzerland)*.

Western blotting
Western blotting was done according to widely used protocols using the following antibodies: monoclonal rabbit anti-PP2A A antibody, anti-PP2A B antibody, anti-PP2A C antibody, anti-phospho-c-Abl (Tyr245)-antibody (*Cell Signaling Technologies, Frankfurt, Germany*), polyclonal rabbit anti-phospho-PP2A C (Y307) antibody (*RnDsystems*), monoclonal mouse anti-c-Abl (clone 24-11)-antibody (α-Abl), polyclonal rabbit anti-c-Abl -antibody (*Santa Cruz, Heidelberg, Germany)*. Secondary polyclonal goat-anti-rabbit-antibody and polyclonal goat-anti-mouse-antibody were purchased from *Dianova GmbH, (Hamburg, Germany)*. Blocking was done in TBS containing 0.1% Tween 20 (TBS-T) with 5% low-fat dry milk; washing was carried out in TBS-T. Antibody incubations were done in either 0.5% low-fat dry milk or TBS-T.

Proliferation assay
Viability of cells was determined by the trypan blue dye exclusion. Cells were counted by using a Neubauer chamber.

Analysis of apoptosis

For the analysis of apoptosis, cells were stained with annexin V conjugated to flourescein isothiocyanate (FITC) and propidium iodide (PI) using the Annexin-V-FLUOS Staining kit according to the manufacturer's instructions (*Roche, Mannheim, Germany*). Briefly, 1.10^6 cells were analyzed by flow cytometry using a FACScanII Becton Dickinson *(Becton Dickinson, Heidelberg, Germany)*. Apoptotic cells were identified as annexinV+ and/or PI- cells. Early apoptotic cells were identified as annexinV+ and PI- cells and late apoptotic cells as annexinV+ and PI+ cells. Cells excluding both FITC and PI were considered viable.

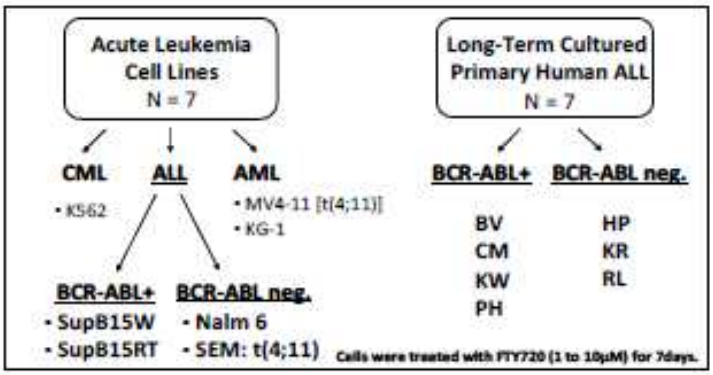
Statistical analysis of data

Data are expressed as mean ± SD and were compared using a paired Student's *t* test. Differences were considered significant when $P < 0.05$. All experiments were done at least twice.

Table 1: Characteristics of primary cells and cell lines

		Malignancies	Cytogenetics	Ploidy	Imatinib-sensitivity
Long-Term Cultured Primary human ALL	BV	Ph+ ALL	t(9;22)	46	+++
	CM	Ph+ ALL	t(9;22)	46	+++
	HP	Ph- ALL	t(13;16)	47	no
	KW	Ph+ ALL	t(9;22)	48	+++
	KR	Ph- ALL	t(1;19)	46	no
	PH	Ph+ ALL	t(9;22)	46	+++
	RL	Ph-ALL	t(1;19)	46	no
Acute Leukemia Cell Lines	K562	CML BC	t(9;22)	65	+++
	KG-1	AML1	complex karyotype	45	no
	MV4-11	AML5	t(4;11)	48	no
	Nalm 6	Ph- ALL	t(5;12)	46	no
	SEM	Ph- ALL	t(4;11)	45	no
	SUP-B15W	Ph+ ALL	t(9;22)	46	+++
	SUP-B15R	Ph+ ALL	t(9;22)	46	no

Figure 1: Diagram of the 14 cell models

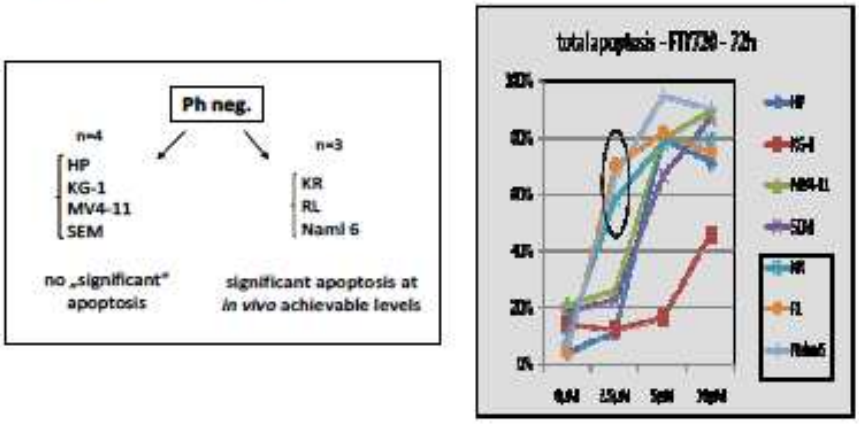


Results

FTY720 is a potent inducer of apoptosis in Ph- ALL.

FTY720 induces apoptosis in 3 out of 5 models of Ph- ALL cells in a dose-dependent manner (figure 2). Treatment with increasing concentrations of FTY720 (0.- 10 µM) for 0 to 420 h or 7 days led to a dose-dependent growth inhibition followed by cell death. The apoptotic effect was observed after 72 hours for concentrations as low as 2.5 µM.

Figure 2: Apoptosis in Ph- ALL treated with FTY720

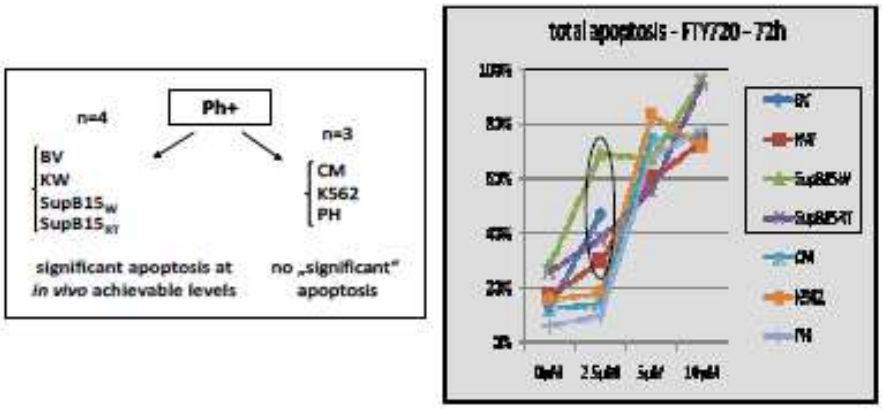


Apoptosis was assessed using Annexin V/PI staining and measured by FACS after 72 h of FTY 720 treatment with the indicated concentration (0 µM, 2.5 µM, 5 µM, 10 µM).Data are shown as mean of fold variation of apoptosis +/- SD (n=2).

FTY720 is a potent inducer of apoptosis in Ph+ ALL.

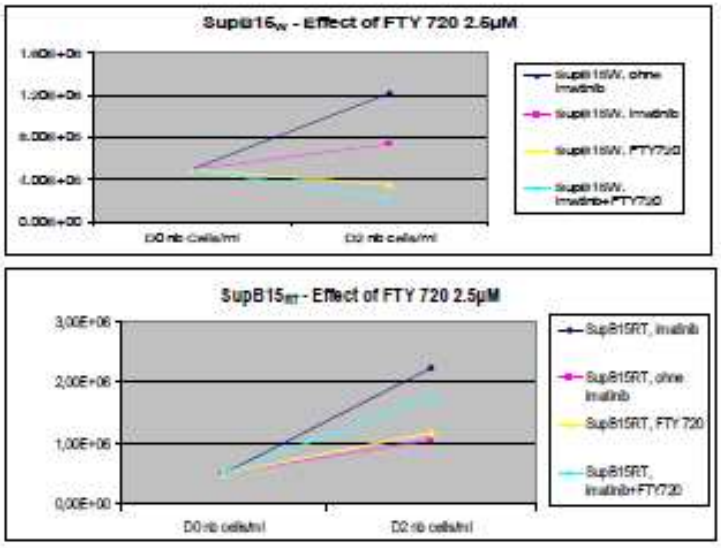
FTY720 induces apoptosis in 4 out of 6 models Ph+ ALL cells in a dose-dependent manner (figure 3). Treatment with increasing concentrations of FTY720 (0.- 10 µM) for 0 to 420 h or 7 days led to a dose-dependent growth inhibition followed by cell death. The apoptotic effect was observed after 72 hours for concentrations as low as 2.5 µM.
In SupB15W, FTY720 has a dramatic effect with 75% of apoptosis, principally late apoptosis (53 %) after 48 hours, and suppresses growth with concentrations as low as 2.5 µM (figures 3 and 4). In imatinib-resistant SupB15RT, we show apoptotic and antiproliferative effects after 48 hours with the different experimental conditions (figures 3 and 4).

Figure 3: Effect of FTY720 on apoptosis in Ph+ ALL



Apoptosis was assessed using Annexin V/PI staining and measured by FACS after 72 h oh FTY 720 treatment with the indicated concentration (0 µM, 2.5 µM, 5 µM, 10 µM). Data are shown as mean of fold variation of apoptosis +/- SD (n=2).

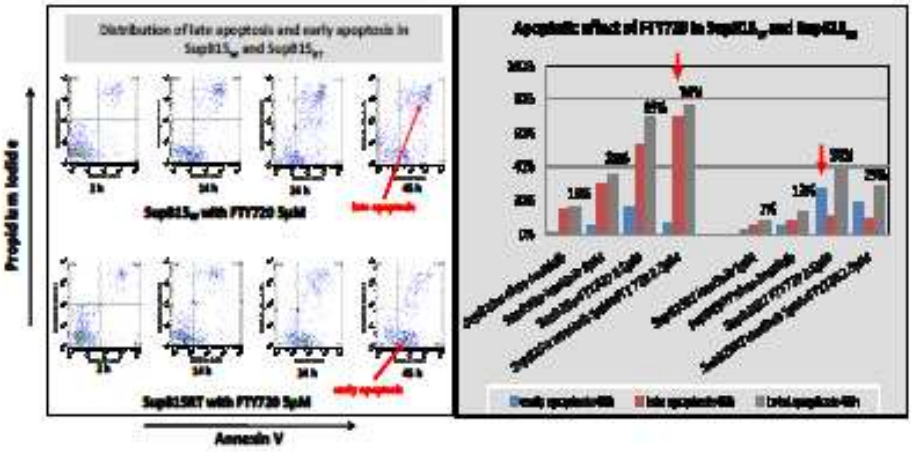
Figure 4: Effect of FTY720 on proliferation in Ph+ ALL



Proliferation was determined by cell counting. Cells were counted 48 h after imatinib treatment (1 µM) and/or FTY 720 treatment (2.5 µM). Data are shown as mean of fold variation of apoptosis +/- SD (n=2

However the apoptotic effect is different with less apoptosis (39%), with mostly early apoptosis (27%), in contrast to SupB15W cells which present 53 % of late apoptosis (figure 5).

Figure 5: Distribution of late and early apoptosis in SupB15W and SupB15RT cells

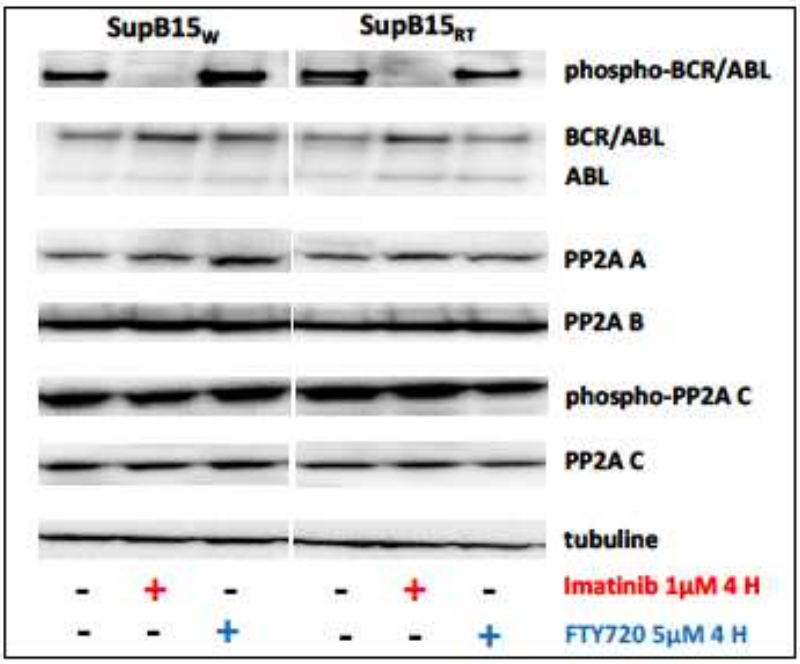


Apoptosis was assessed using Annexin V/PI staining and measured by FACS 48 h after imatinib treatment (1 µM) and/or FTY 720 treatment (2.5 µM). Data are shown as mean of fold variation of apoptosis +/- SD (n=2).

Activation of PP2A is not required for FTY720-induced cell death

The potential effect of FTY720 on phosphorylation of Bcr-Abl, a known substrate of PP2A, was examined by Western blotting, demonstrating that in SupB15W and in SupB15RT cells, the level of Abl phosphorylation is not modified by FTY720 (figure 6). Neither was the phosphorylation of PP2A.

Figure 6 : Levels of phosphorylated Bcr-Abl and PP2A in SupB15W and in SupB15RT cells



Cells were treated with imatinib (1 µM) and / or FTY 720 (5 µM) for 4 h before lysates were prepared. Whole cell lysates were probed with antibodies (phosphorylated and non-phosphorylated forms) against BCR/ABL, ABL, PP2A A, PP2A B, PP2A C and tubuline.

Discussion

FTY720 is a modified natural immuno-suppressive agent which is a synthetic structural analog of sphingosine. This agent disrupts the sphingolipid pathway, thereby interfering with T-cell activation, trafficking, and survival. Its use has been mostly investigated in organ transplantation and immunotherapy.[11,12] Several studies have reported the role of FTY720 in T-cell trafficking and in cancer. [19,20] When administrated at doses at least 10-fold higher than required for immunomodulation, FTY720 inhibits tumor growth, angiogenesis, and metastasis in various carcinoma models.[19,20] In particular, a few studies showed FTY720 to have activity against a number of haematological malignancies including multiple myeloma, chronic lymphocytic leukaemia, mantle cell lymphoma, acute myeloblastic leukemia, CML and ALL cells models.[15,16,21-27] However, systematic analysis of the effect of FTY720 in acute lymphoblastic leukaemia has not been performed.

We investigate for the first time the effect of FTY720 in vitro in a large panel of ALL cell lines. In most of the in vitro experiments we used 2.5 µM because this concentration is within normal range in primary hematopoietic progenitors, whereas it activates PP2A, induces apoptosis, and inhibits cell growth in Bcr-Abl primary cells and cells lines within 6 hours of treatment. Its inhibitory effects on Ph+ cell growth occurs at nanomolecular and low molecular concentrations that reportedly do not impair the viability of normal human myeloid and lymphoid cells.[10-12,16,20,28,29] Thus, we confirmed for the first time in a large panel of ALL primary cells and cell lines, that FTY720 is a potent inducer of apoptosis in ALL.

The anti-leukemic activity of FTY720 has only been described in a few studies in chronic myeloid leukamia and only one Ph+ALL cell model.[15,22,26,27,30] In our study, we show that FTY720 is a potent inducer of apoptosis and antiproliferative agent in Ph+ ALL. Moreover, we report that it partially abrogates mutation-independent imatinib-resistance.

In a variety of B-cell malignancies, FTY720 induces apoptosis through activation of the PP2A, independent of FAS or Bcl-2 expression.[13,17,18,26,31] However, precise mechanisms of action are incompletely understood.[14,20] We demonstrate that growth inhibition and induction of apoptosis by FTY720 do not depend on the interference with bcr-abl oncogenic activity which contrasts with the results of the study of P.Neviani.[18] The FTY720 antiproliferative and proapoptotic effects are not preceded by a decrease of bcr-abl and PP2A activity expression. Moreover, FTY720 induces cell death by different and unclear mechanism between imatinib-sensitive and -resistant Ph+ ALL cells. The FTY720 mechanism of action remains to be elucidated, perhaps by induced Akt dephosphorylation.

In conclusion, ours results show that FTY720 may be of potential use as a therapeutic agent in Ph+ and Ph- ALL, in particular in mutation-independent ITK-resistant Ph+ ALL. FTY720 could be part of new therapies for very-high-risk patients and in salvage regimens in the treatment of adult ALL.

Acknowledgements

We wish to thank Bart A. Nijmeijer at the department of Hematology of Leiden University Medical Center for the long-term cultured human ALL cells, BV, CM, KW, PH, HP, KR, and RL.

Author's contributions

Conceived and designed the experiments: AST, AR, OOG

Performed the experiments: AST, AR, SB, SW

Analyzed the data: AST, AR

Wrote the paper: AST, AR, MH,OC ,OG

References

1. Inaba H, Greaves M, Mullighan CG. Acute lymphoblastic leukaemia. Lancet. 2013;381:1943-1955

2. Jabbour E, O'Brien S, Konopleva M, Kantarjian H. New insights into the pathophysiology and therapy of adult acute lymphoblastic leukemia. Cancer. 2015;121:2517-2528

3. Rowe JM. Optimal management of adults with ALL. Br J Haematol. 2009;144:468-483

4. Jeha S. New therapeutic strategies in acute lymphoblastic leukemia. Semin Hematol. 2009;46:76-88

5. Faderl S, Jeha S, Kantarjian HM. The biology and therapy of adult acute lymphoblastic leukemia. Cancer. 2003;98:1337-1354

6. Hunger SP, Mullighan CG. Redefining ALL classification: toward detecting high-risk ALL and implementing precision medicine. Blood. 2015;125:3977-3987

7. Pullarkat V, Slovak ML, Kopecky KJ, Forman SJ, Appelbaum FR. Impact of cytogenetics on the outcome of adult acute lymphoblastic leukemia: results of Southwest Oncology Group 9400 study. Blood. 2008;111:2563-2572

8. Ohanian M, Cortes J, Kantarjian H, Jabbour E. Tyrosine kinase inhibitors in acute and chronic leukemias. Expert Opin Pharmacother. 2012;13:927-938

9. O'Hare T, Deininger MW, Eide CA, Clackson T, Druker BJ. Targeting the BCR-ABL signaling pathway in therapy-resistant Philadelphia chromosome-positive leukemia. Clin Cancer Res. 2011;17:212-221

10. Chiba K, Yanagawa Y, Kataoka H, Kawaguchi T, Ohtsuki M, Hoshino Y. FTY720, a novel immunosuppressant, induces sequestration of circulating lymphocytes by acceleration of lymphocyte homing. Transplant Proc. 1999;31:1230-1233

11. Adachi K, Chiba K. FTY720 story. Its discovery and the following accelerated development of sphingosine 1-phosphate receptor agonists as immunomodulators based on reverse pharmacology. Perspect Medicin Chem. 2008;1:11-23

12. Chiba K. FTY720, a new class of immunomodulator, inhibits lymphocyte egress from secondary lymphoid tissues and thymus by agonistic activity at sphingosine 1-phosphate receptors. Pharmacol Ther. 2005;108:308-319

13. Chen L, Luo LF, Lu J, Li L, Liu YF, Wang J, Liu H, Song H, Jiang H, Chen SJ, Luo C, Li KK. FTY720 induces apoptosis of M2 subtype acute myeloid leukemia cells by targeting sphingolipid metabolism and increasing endogenous ceramide levels. PLoS One. 2014;9:e103033

14. Pitman MR, Woodcock JM, Lopez AF, Pitson SM. Molecular targets of FTY720 (fingolimod). Curr Mol Med. 2012;12:1207-1219

15. Wallington-Beddoe CT, Hewson J, Bradstock KF, Bendall LJ. FTY720 produces caspase-independent cell death of acute lymphoblastic leukemia cells. Autophagy. 2011;7:707-715

16. Li D, Zhang Y, Hu X, Cao W, Huang W. Role of extracelluar regulated protein kinases in FTY720-induced apoptosis of leukemia cell lines HL-60 and U937. J Huazhong Univ Sci Technolog Med Sci. 2004;24:45-47

17. Pippa R, Dominguez A, Christensen DJ, Moreno-Miralles I, Blanco-Prieto MJ, Vitek MP, Odero MD. Effect of FTY720 on the SET-PP2A complex in acute myeloid leukemia; SET binding drugs have antagonistic activity. Leukemia. 2014;28:1915-1918

18. Neviani P, Harb JG, Oaks JJ, Santhanam R, Walker CJ, Ellis JJ, Ferenchak G, Dorrance AM, Paisie CA, Eiring AM, Ma Y, Mao HC, Zhang B, Wunderlich M, May PC, Sun C, Saddoughi SA, Bielawski J, Blum W, Klisovic RB, Solt JA, Byrd JC, Volinia S, Cortes J, Huettner CS, Koschmieder S, Holyoake TL, Devine S, Caligiuri MA, Croce CM, Garzon R, Ogretmen B, Arlinghaus RB, Chen CS, Bittman R, Hokland P, Roy DC, Milojkovic D, Apperley J, Goldman JM, Reid A, Mulloy JC, Bhatia R, Marcucci G, Perrotti D. PP2A-activating drugs selectively eradicate TKI-resistant chronic myeloid leukemic stem cells. J Clin Invest. 2013;123:4144-4157

19. Zhang L, Wang H, Zhu J, Ding K, Xu J. FTY720 reduces migration and invasion of human glioblastoma cell lines via inhibiting the PI3K/AKT/mTOR/p70S6K signaling pathway. Tumour Biol. 2014;35:10707-10714

20. Zhang L, Wang HD, Ji XJ, Cong ZX, Zhu JH, Zhou Y. FTY720 for cancer therapy (Review). Oncol Rep. 2013;30:2571-2578

21. Alinari L, Mahoney E, Patton J, Zhang X, Huynh L, Earl CT, Mani R, Mao Y, Yu B, Quinion C, Towns WH, Chen CS, Goldenberg DM, Blum KA, Byrd JC, Muthusamy N, Praetorius-Ibba M, Baiocchi RA. FTY720 increases CD74 expression and sensitizes mantle cell lymphoma cells to milatuzumab-mediated cell death. Blood. 2011;118:6893-6903

22. Kiyota M, Kuroda J, Yamamoto-Sugitani M, Shimura Y, Nakayama R, Nagoshi H, Mizutani S, Chinen Y, Sasaki N, Sakamoto N, Kobayashi T, Matsumoto Y, Horiike S, Taniwaki M. FTY720 induces apoptosis of chronic myelogenous leukemia cells via dual activation of BIM and BID and overcomes various types of resistance to tyrosine kinase inhibitors. Apoptosis. 2013;18:1437-1446

23. Liu Q, Alinari L, Chen CS, Yan F, Dalton JT, Lapalombella R, Zhang X, Mani R, Lin T, Byrd JC, Baiocchi RA, Muthusamy N. FTY720 shows promising in vitro and in vivo preclinical activity by downmodulating Cyclin D1 and phospho-Akt in mantle cell lymphoma. Clin Cancer Res. 2010;16:3182-3192

24. Liu Q, Zhao X, Frissora F, Ma Y, Santhanam R, Jarjoura D, Lehman A, Perrotti D, Chen CS, Dalton JT, Muthusamy N, Byrd JC. FTY720 demonstrates promising preclinical activity for chronic lymphocytic leukemia and lymphoblastic leukemia/lymphoma. Blood. 2008;111:275-284

25. Mani R, Chiang CL, Frissora FW, Yan R, Mo X, Baskar S, Rader C, Klisovic R, Phelps MA, Chen CS, Lee RJ, Byrd JC, Baiocchi R, Lee LJ, Muthusamy N. ROR1-targeted delivery

of OSU-2S, a nonimmunosuppressive FTY720 derivative, exerts potent cytotoxicity in mantle-cell lymphoma in vitro and in vivo. Exp Hematol. 2015;43:770-774 e772

26. Neviani P, Santhanam R, Oaks JJ, Eiring AM, Notari M, Blaser BW, Liu S, Trotta R, Muthusamy N, Gambacorti-Passerini C, Druker BJ, Cortes J, Marcucci G, Chen CS, Verrills NM, Roy DC, Caligiuri MA, Bloomfield CD, Byrd JC, Perrotti D. FTY720, a new alternative for treating blast crisis chronic myelogenous leukemia and Philadelphia chromosome-positive acute lymphocytic leukemia. J Clin Invest. 2007;117:2408-2421

27. Wallington-Beddoe CT, Don AS, Hewson J, Qiao Q, Papa RA, Lock RB, Bradstock KF, Bendall LJ. Disparate in vivo efficacy of FTY720 in xenograft models of Philadelphia positive and negative B-lineage acute lymphoblastic leukemia. PLoS One. 2012;7:e36429

28. Kataoka H, Ohtsuki M, Shimano K, Mochizuki S, Oshita K, Murata M, Sugahara K, Sato N, Hoshino Y, Chiba K. Immunosuppressive activity of FTY720, sphingosine 1-phosphate receptor agonist: II. Effect of FTY720 and FTY720-phosphate on host-versus-graft and graft-versus-host reaction in mice. Transplant Proc. 2005;37:107-109

29. Kiuchi M, Adachi K, Tomatsu A, Chino M, Takeda S, Tanaka Y, Maeda Y, Sato N, Mitsutomi N, Sugahara K, Chiba K. Asymmetric synthesis and biological evaluation of the enantiomeric isomers of the immunosuppressive FTY720-phosphate. Bioorg Med Chem. 2005;13:425-432

30. Zonta F, Pagano MA, Trentin L, Tibaldi E, Frezzato F, Trimarco V, Facco M, Zagotto G, Pavan V, Ribaudo G, Bordin L, Semenzato G, Brunati AM. Lyn sustains oncogenic signaling in chronic lymphocytic leukemia by strengthening SET-mediated inhibition of PP2A. Blood. 2015;125:3747-3755

31. Ruvolo PP. The Interplay between PP2A and microRNAs in Leukemia. Front Oncol. 2015;5:43

D - Discussion

Les LAL de l'adulte sont dues à une série d'altérations successives combinatoires et représentent de ce fait des situations très hétérogènes avec une survie à long terme différente selon l'âge et les groupes pronostiques désormais focalisés sur les facteurs de risque de rechute: l'obtention de la RC est en effet la règle. Des progrès significatifs sont venus de l'utilisation des protocoles de chimiothérapie d'inspiration pédiatrique. Cependant, la survie à long terme n'est que de 40 à 50 % et plus de 50 % de patients rechutent. Affiner les groupes pronostiques et adapter le traitement en conséquence reste d'actualité.[5,18,71,74,75,79,85,113]

Dans les LAL T, la classification pronostique s'appuie sur les avancées récentes dans l'identification des anomalies moléculaires, anomalies qui dérégulent la différenciation, les étapes de contrôle au niveau du cycle cellulaire, la prolifération ou la survie. Deux grands types sont distingués, les anomalies de type 1 et les anomalies de type 2. Les premières, mutuellement exclusives et probablement fondatrices, sont corrélées au stade du blocage de maturation des lymphocytes T. Les secondes ne sont pas corrélées à un stade précis de maturation. Pour elles, plus d'une trentaine sont décrites, redondantes entre elles et avec les anomalies de type 1.[5,18,45,53,54,74,75,79,113] Les anomalies de NOTCH1/ FBXW7 font partie des lésions prédominantes : leur présence est associée à un bon pronostic.[67,114,115]
Les mutations de RAS appartiennent aux anomalies de type 2 et ont été mis en évidence dans seulement 2 % des LAL de l'enfant, sans impact pronostique.[116] Dans notre étude, nous montrons que les mutations de RAS sont fréquentes chez l'adulte (10%) et associées à un mauvais pronostic. L'expression de PTEN peut être régulée par de nombreux mécanismes. Chez l'enfant, les délétions de PTEN étaient connues comme associées à un mauvais pronostic même si l'impact pronostique est débattu. En revanche les mutations de PTEN ne sont pas décrites comme ayant un impact pronostic chez l'enfant.[117-119] Peu d'études se sont intéressées à PTEN chez l'adulte et son impact pronostique n'a pas été rapporté. Nous montrons premièrement dans notre cohorte que les anomalies de PTEN chez l'adulte sont de 12% et qu'elles soient constituées de délétions ou de mutations. Chez l'adulte, la fréquence des anomalies de PTEN, impliqué dans la régulation du cycle cellulaire, est peut-être liée au fait que, les LAL T des adultes présentent souvent un blocage de maturation plus immature par rapport à celles de l'enfant. Deuxièmement nous rapportons qu'elles sont associées à un mauvais pronostic.
Le taux de leucocytes élevés au diagnostic, la persistance d'une maladie résiduelle après l'induction et l'absence de mutation de la voie NOTCH 1 sont des facteurs de mauvais pronostic reconnus : en leur présence, une

intensification thérapeutique par allogreffe de CSH est classiquement envisagée.[67,70,87,120]
La combinaison des anomalies des voies NOTCH1/FBXW7/RAS/PTEN nous a permis d'identifier, dans le groupe des LAL T Notch1 mutées jusqu'ici considérées comme de bon pronostic, un sous-groupe de mauvais pronostic qui en fait est péjoratif et sans doute insuffisamment traité.
Ces résultats sont pris en compte dans le design du prochain protocole du GRAALL 2015. La stratification thérapeutique est basée sur les sous-groupes, les LAL T de bon pronostic (NOTCH muté et absence d'anomalies de RAS et PTEN) les LAL T de mauvais pronostic (absence de mutation de NOTCH ou mutations de RAS ou anomalies de PTEN) associés aux facteurs pronostiques connus, le taux de leucocytes au diagnostic et la MRD.

L'implication de ces voies permet d'envisager de nouvelles thérapeutiques ciblées avec l'utilisation d'inhibiteurs, dont la place reste à définir face à l'intensification thérapeutique par allogreffe, traitement efficace mais grevé d'une toxicité importante. Le démembrement de sous-groupes de pronostics différents doit se poursuivre et affiner les repérages actuels, «à charge comme à décharge».
En effet, même dans la nouvelle classification NOTCH/FBWX7/RAS/PTEN, 15% des patients considérés comme de bon pronostic rechutent et sont probablement insuffisamment traités. Une nouvelle entité récemment décrite, les ETP-ALL correspond peut-être à ce sous-groupe de patients.[50,51]
A contrario, 40 % des patients LAL T de mauvais pronostic ne rechutent pas. Ce qui incite à repérer parmi ces patients ceux qui pourraient bénéficier d'une thérapeutique ciblée à la place de l'allogreffe.

La question de la place de l'allogreffe est également observée avec attention dans les LAL à Ph1 depuis l'utilisation des ITK, laquelle a permis d'atteindre des taux de RC proche de 95% et une survie à 12 mois de 75%.[96,121] Cependant le suivi médian, dans la plupart des études rapportées, n'excédait pas 2 ans.
Dans le protocole GRAAPH 2003, nous avons pu confirmer que ce bénéfice, des ITK associés à la chimiothérapie en première ligne de traitement, se maintenait à long terme, avec un suivi médian de 46 mois, ce que d'autres équipes ont également montré.[70,76,122]
Ces résultats combinées conduisent à s'interroger sur la dose de chimiothérapie réellement indispensable lorsqu'il y a une association aux ITK, ce afin de limiter la toxicité lié au traitement.
La faisabilité de la diminution d'intensité de dose de chimiothérapie vient d'être confirmée avec les résultats du protocole GRAAPH 2005 du groupe du GRAALL : la survie à long terme des patients qui reçoivent une chimiothérapie minime est proche de 40% sans différence significative avec la chimiothérapie standard.[123]

Les résultats du GRAAPH 2005 ont aussi répondu à la question soulevée par les résultats du GRAAPH 2003 en confirmant que la survie des patients autogreffés atteint 80%, survie non différente de celle des patients allogreffés avec des effectifs toutefois restreints. Dans le GRAAPPH 2003, l'allogreffe permettait une survie prolongée même en cas de MRD positive avant greffe. Cependant la faible incidence des rechutes dans ce groupe de patients était contrebalancée par une mortalité liée au traitement majeur. Dans le groupe des patients autogreffés, la faible mortalité liée au traitement contrebalançait un risque de rechute, certes non négligeable mais très diminué en cas de MRD négative.
Ces résultats sont confirmés dans le protocole GRAAPH 2005, avec une survie identique entre les patients allogreffés ou autogreffés dès lors que la MRD est négative et le taux de leucocytes bas au diagnostic, deux facteurs pronostiques reconnus.[94,124,125] L'allogreffe conserve toute sa place si un seul de ces critères n'est pas obtenu. Ces résultats sont pris en compte dans le design du prochain protocole du GRAAPH 2015, qui laisse le choix aux centres investigateurs entre la réalisation d'une allogreffe ou d'une autogreffe avec maintenance par ITK dans ce sous-groupe de patients.

Cependant, avec une survie à long terme d'environ 50%, le devenir des patients n'est pas satisfaisant, du fait de la toxicité du traitement, notamment de l'allogreffe et des rechutes, et ce malgré l'utilisation d'une maintenance par ITK. En effet, malgré le développement d'ITK de seconde, troisième et quatrième génération, la réponse n'est pas durable : des résistances aux ITK préexistent ou se développent précocément.[76,77,91,123,126-128] Plusieurs mécanismes existent, qui peuvent s'additionner : mutations ponctuelles de BCR-ABL, amplification du gène BCR-ABL, surexpression protéique de BCR-ABL, perte d'activité de BCR-ABL ou résistance multidrogue par surexpression de glycoprotéine P. A la rechute environ 80% des patients développent une résistance secondaire aux ITK, principalement par mutations du domaine kinase. De plus, les résistances primaires sont multifactorielles et non totalement élucidées.[20,91-94]
Tout comme les LAL T, les LAL à Ph1 représentent une pathologie hétérogène. Le transcrit BCR-ABL est souvent associé à de nombreuses anomalies cytogénétiques, ce qui explique pourquoi l'inhibition de la voie du BCR-ABL n'est pas suffisante pour inhiber la prolifération cellulaire contrairement à la LMC. [27,34,35,39,129]

En étudiant un modèle cellulaire de LAL à Ph1 résistant aux ITK sans mécanisme connu, nous avons démontré l'implication d'ATX dans la résistance aux ITK. ATX, protéine extracellulaire à activité enzymatique, synthétise deux lipides biologiques, le LPA et la S1P, grâce à son activité lysoPLD.[100-102] La sécrétion d´ATX est augmentée dans différentes pathologies tumorales avec une implication dans la régulation du système immunitaire, la croissance tumorale et la dissémination métastatique par action sur la mobilité tissulaire, la

prolifération tissulaire et l´apoptose.[106,130] Dans les hémopathies malignes, seules six études rapportent son implication. ATX augmenterait la croissance et la viabilité des cellules de lymphome de Hodgkin lié au virus EBV, sa sécrétion est augmentée dans les lymphomes anaplasiques, ATX serait un marqueur tumoral dans le lymphome folliculaire. Il est décrit une surexpression génique d'ATX chez des patients présentant une LMC résistante à l'imatinib. Son rôle est également évoqué dans la migration de cellules de LAM présentant une mutation de FLT-3 (Fms-like tyrosine kinase 3). Enfin, ATX protège les cellules de la lignée cellulaire Nalm-6 (lignée cellulaire de LAL B) de l'apoptose.[105,106,109,131-134]

Ainsi, notre travail est le premier à démontrer qu' ATX augmente la prolifération et la migration des blastes dans les LAL à Ph1 et diminue la réponse à l'imatinib, ouvrant ainsi la possibilité à l'utilisation d'inhibiteurs de l'axe ATX/LPA/LPAR.[108,112,135-137] Ce mécanisme est indépendant du BCR-ABL et il sera nécessaire de le comprendre. ATX augmente probablement la synthèse de LPA protégeant ainsi les cellules de la cytotoxicité de la chimiothérapie ou des thérapeutiques ciblées comme cela a été montré par l'équipe de Vidot.[104]

Notre hypothèse est que la surexpression d'ATX entraîne une dérégulation de l'équilibre de l'activation des 6 récepteurs du LPA, récepteurs et des 6 récepteurs de S1P (deuxième lipide synthétisé par ATX), lesquels sont impliqués dans les voies de signalisation PI3K, RAS, MAPK, Rho et NFkB.[138] Dans notre modèle de résistance, le récepteur au LPA, LPAR1 n'est plus exprimé. Cette hypothèse reste à démontrer et il sera nécessaire de tester les inhibiteurs sur des modèles murins de LAL à Ph1 afin de valider cette nouvelle voie thérapeutique.

Devant l'implication de l'ATX/LPA/LPAR dans notre modèle de LAL à Ph1 résistante aux ITK, nous nous sommes intéressés à un agent thérapeutique, le fingolimod. Le fingolimod est un immunomodulateur, utilisé en transplantation d'organes et en neurologie, impliquant l'activation des récepteurs à la S1P, S1PR.[51,139-142] Normalement, par l'intermédiaire de l'activation de la phosphatase PP2A, le fingolimod induit une apoptose cellulaire dans des hémopathies malignes à cellules B, indépendamment de l'expression du Bcl2.[140,143-153]

Dans notre travail, nous avons démontré que dans les LAL à Ph1, le fingolimod est un inducteur d'apoptose, un agent antiprolifératif et qu'il abroge partiellement la résistance à l'imatinib, ce qui laisse espérer son utilité dans le traitement des LAL à Ph1. Compte tenu de l'implication de voies de signalisation indépendantes de BCR-ABL dans la leucémogénèse des LAL à Ph1 ainsi que dans les mécanismes de résistance aux ITK, avoir accès à des thérapeutiques ne ciblant pas le BCR-ABL est intéressant. Nous n'avons pas identifié son mécanisme d'action et seulement observé qu'il n'impliquait pas la phosphorylation d'ABL, substrat connu de la PP2A. Son potentiel d'action en

hématologie a été montré dans quelques études avec des effets différents, tant sur l'activation de PP2A que sur le mécanisme d'apoptose. [140,143-153]
D'autres études récentes montrent actuellement l'implication de nouveaux mécanismes dans l'oncogénèse des LAL à Ph1 - par exemple celle de l'implication du facteur d'initiation 4E ou de la voie STAT /JAK - et décrivent le rôle de nouvelles thérapeutiques telles que la ribavirine dans l'inhibition de la voie liée au facteur 4E ou les inhibiteurs de Jak.[138,154,155]
Etablir une cartographie des anomalies géniques comparables à celle des LAL T permettrait d'identifier les sous-groupes de patients qui pourraient tirer bénéfice des alternatives thérapeutiques ou combinaison thérapeutiques (ITK et autres cibles).

E- Conclusion

La meilleure compréhension de la leucémogénèse, l'identification de groupes pronostiques et le développement de nouvelles thérapeutiques notamment ciblées a permis de progresser dans le diagnostic et les soins des LAL de l'adulte, tant en terme de survie que de qualité de vie.
Il demeure le défi de leur utilisation dans des modèles pragmatiques dans le respect des grands équilibres socioéconomiques.

Bibliographie

1. Swerldow W et al. WHO Classification of tumours of Haematopoietic and Lymphoid Tissues. IARC; 2008

2. Vardiman JW et al. The World Health Organization (WHO) classification of tumors of the hematopoietic and lymphoid tissues: an overview with emphasis on the myeloid neoplasms. Chem Biol Interact 2010;184:16-20

3. Belot A et al. Cancer incidence and mortality in France over the period 1980-2005. Rev Epidemiol Sante Publique 2008;56:159-175

4. Siegel R et al. Cancer statistics, 2013. CA Cancer J Clin 2013;63:11-30

5. Inaba H et al. Acute lymphoblastic leukaemia. Lancet 2013;381:1943-1955

6. Cornell RF et al. Adult acute leukemia. Dis Mon 2012;58:219-238

7. Kim AS et al. Childhood acute lymphocytic leukemia and perspectives on risk assessment of early-life stage exposures. Mutat Res 2006;613:138-160

8. Buffler PA et al. Environmental and genetic risk factors for childhood leukemia: appraising the evidence. Cancer Invest 2005;23:60-75

9. Preston DL et al. Leukemia, lymphoma and multiple myeloma, 1950-1987. Radiat Res 1994;137:S68-97

10. Sawyers CLet al. Leukemia and the disruption of normal hematopoiesis. Cell 1991;64:337-350

11. Hanahan D et al. Hallmarks of cancer: the next generation. Cell 2011;144:646-674

12. Sonnenschein C et al. The aging of the 2000 and 2011 Hallmarks of Cancer reviews: a critique. J Biosci 2011;38:651-663

13. Mullighan CG. The molecular genetic makeup of acute lymphoblastic leukemia. Hematology 2012; 2012:389-396

14. Hunger SP et al. Redefining ALL classification: toward detecting high-risk ALL and implementing precision medicine. Blood 2015;125:3977-3987

15. Moorman AV. The clinical relevance of chromosomal and genomic abnormalities in B-cell precursor acute lymphoblastic leukaemia. Blood Rev 2012;26:123-135

16. Mullighan CG et al. Genome-wide analysis of genetic alterations in acute lymphoblastic leukaemia. Nature 2007;446:758-764

17. http://atlasgeneticsoncology.org//index.html:Atlas of Genetics and Cytogenetics in Oncology and Haematology

18. Jabbour E et al. New insights into the pathophysiology and therapy of adult acute lymphoblastic leukemia. Cancer 2015;121:2517-2528

19. Faderl S et al. The biology and therapy of adult acute lymphoblastic leukemia. Cancer 2003;98:1337-1354

20. Kurzrock R et al. Philadelphia chromosome-positive leukemias: from basic mechanisms to molecular therapeutics. Ann Intern Med 2003;138:819-830

21. Advani AS et al. Bcr-Abl variants: biological and clinical aspects. Leuk Res 2002;26:713-720

22. Score J et al. Analysis of genomic breakpoints in p190 and p210 BCR-ABL indicate distinct mechanisms of formation. Leukemia 2010;24:1742-1750

23. Pane F et al. BCR/ABL genes and leukemic phenotype: from molecular mechanisms to clinical correlations. Oncogene 2002;21:8652-8667

24. Verstovsek S et al. Neutrophilic-chronic myeloid leukemia: low levels of p230 BCR/ABL mRNA and undetectable BCR/ABL protein may predict an indolent course. Cancer 2002;94:2416-2425

25. van Rhee F et al. p190 BCR-ABL mRNA is expressed at low levels in p210-positive chronic myeloid and acute lymphoblastic leukemias. Blood 1996;87:5213-5217

26. Chiarella P et al. BCR/ABL1 fusion transcripts generated from alternative splicing: implications for future targeted therapies in Ph+ leukaemias. Curr Mol Med 2012;12:547-565

27. Cilloni D et al. Molecular pathways: BCR-ABL. Clin Cancer Res 2012;18:930-937

28. Hassan AQ et al. Allosteric inhibition of BCR-ABL. Cell Cycle 2010;9:3710-3714

29. Melo JV. The molecular biology of chronic myeloid leukaemia. Leukemia 1996;10:751-756

30. Hazlehurst LA et al. Signaling networks associated with BCR-ABL-dependent transformation. Cancer Control 2009;16:100-107

31. O'Hare T et al. The BCR-ABL35INS insertion/truncation mutant is kinase-inactive and does not contribute to tyrosine kinase inhibitor resistance in chronic myeloid leukemia. Blood 2011;118:5250-5254

32. Zheng X et al. Reciprocal t(9;22) ABL/BCR fusion proteins: leukemogenic potential and effects on B cell commitment. PLoS One 2009;4:e7661

33. Chang KH et al. Vav3 collaborates with p190-BCR-ABL in lymphoid progenitor leukemogenesis, proliferation, and survival. Blood 2012;120:800-811

34. Gillis LC et al. Gads (Grb2-related adaptor downstream of Shc) is required for BCR-ABL-mediated lymphoid leukemia. Leukemia 2013;27:1666-1676

35. Wu N et al. PECAM-1 is involved in BCR/ABL signaling and may downregulate imatinib-induced apoptosis of Philadelphia chromosome-positive leukemia cells. Int J Oncol 2013;42:419-428

36. Steelman LS et al. Contributions of the Raf/MEK/ERK, PI3K/PTEN/Akt/mTOR and Jak/STAT pathways to leukemia. Leukemia 2008;22:686-707

37. Hu Y et al. Requirement of Src kinases Lyn, Hck and Fgr for BCR-ABL1-induced B-lymphoblastic leukemia but not chronic myeloid leukemia. Nat Genet 2004;36:453-461

38. Tavernier E et al. HSP90 inhibition results in apoptosis of Philadelphia acute lymphoblastic leukaemia cells: an attractive prospect of new targeted agents. J Cancer Res Clin Oncol 2012;138:1753-1758

39. Jaso J et al. Prognostic significance of immunophenotypic and karyotypic features of Philadelphia positive B-lymphoblastic leukemia in the era of tyrosine kinase inhibitors. Cancer 2011;117:4009-4017

40. Mullighan CG. The genomic landscape of acute lymphoblastic leukemia in children and young adults. Hematology 2014;2014:174-180

41. Mullighan CG. Molecular genetics of B-precursor acute lymphoblastic leukemia. J Clin Invest 2012;122:3407-3415

42. Katz AJ et al. Acute lymphoblastic leukemia: an assessment of international incidence, survival, and disease burden. Cancer Causes Control 2015;26:1627-1642

43. Aifantis I et al. Molecular pathogenesis of T-cell leukaemia and lymphoma. Nat Rev Immunol 2008;8:380-390

44. De Keersmaecker K et al. Genetic insights in the pathogenesis of T-cell acute lymphoblastic leukemia. Haematologica 2005;90:1116-1127

45. Durinck K et al. Novel biological insights in T-cell acute lymphoblastic leukemia. Exp Hematol 2015;43:625-639

46. Graux C et al. Cytogenetics and molecular genetics of T-cell acute lymphoblastic leukemia: from thymocyte to lymphoblast. Leukemia 2006;20:1496-1510

47. Bene MC et al. Proposals for the immunological classification of acute leukemias. European Group for the Immunological Characterization of Leukemias (EGIL). Leukemia 1995;9:1783-1786

48. Asnafi V et al. Age-related phenotypic and oncogenic differences in T-cell acute lymphoblastic leukemias may reflect thymic atrophy. Blood 2004;104:4173-4180

49. Asnafi V et al. Analysis of TCR, pT alpha, and RAG-1 in T-acute lymphoblastic leukemias improves understanding of early human T-lymphoid lineage commitment. Blood 2003;101:2693-2703

50. Coustan-Smith E et al. Early T-cell precursor leukaemia: a subtype of very high-risk acute lymphoblastic leukaemia. Lancet Oncol 2009;10:147-156

51. Zhang J et al. The genetic basis of early T-cell precursor acute lymphoblastic leukaemia. Nature 2012;481:157-163

52. Van Vlierberghe P et al. Molecular-genetic insights in paediatric T-cell acute lymphoblastic leukaemia. Br J Haematol 2008;143:153-168

53. Van Vlierberghe P et al. The molecular basis of T cell acute lymphoblastic leukemia. J Clin Invest 2012;122:3398-3406

54. Van Vlierberghe P et al. Prognostic relevance of integrated genetic profiling in adult T-cell acute lymphoblastic leukemia. Blood 2013;122:74-82

55. Van der Meulen J et al. The epigenetic landscape of T-cell acute lymphoblastic leukemia. Int J Biochem Cell Biol 2014;53:547-557

56. Clappier E et al. The C-MYB locus is involved in chromosomal translocation and genomic duplications in human T-cell acute leukemia (T-ALL), the translocation defining a new T-ALL subtype in very young children. Blood 2007;110:1251-1261

57. Soulier J et al. HOXA genes are included in genetic and biologic networks defining human acute T-cell leukemia (T-ALL). Blood 2005;106:274-286

58. Ben Abdelali R et al. The prognosis of CALM-AF10-positive adult T-cell acute lymphoblastic leukemias depends on the stage of maturation arrest. Haematologica 2013;98:1711-1717

59. Zhou MH et al. NUP214 fusion genes in acute leukemia (Review). Oncol Lett 2014;8:959-962

60. Cayuela JM et al. Disruption of the multiple tumor suppressor gene MTS1/p16(INK4a)/CDKN2 by illegitimate V(D)J recombinase activity in T-cell acute lymphoblastic leukemias. Blood 1997;90:3720-3726

61. Ellisen LW et al. TAN-1, the human homolog of the Drosophila notch gene, is broken by chromosomal translocations in T lymphoblastic neoplasms. Cell 1991;66:649-661

62. Mullighan CG. Mutations of NOTCH1, FBXW7, and prognosis in T-lineage acute lymphoblastic leukemia. Haematologica 2009;94:1338-1340

63. Paganin M et al. Molecular pathogenesis and targeted therapies for NOTCH1-induced T-cell acute lymphoblastic leukemia. Blood Rev 2011;25:83-90

64. Tzoneva G et al. Recent advances on NOTCH signaling in T-ALL. Curr Top Microbiol Immunol 2012;360:163-182

65. Thompson BJ et al. The SCFFBW7 ubiquitin ligase complex as a tumor suppressor in T cell leukemia. J Exp Med 2007;204:1825-1835

66. O'Neil J et al. FBW7 mutations in leukemic cells mediate NOTCH pathway activation and resistance to gamma-secretase inhibitors. J Exp Med 2007;204:1813-1824

67. Asnafi V et al. NOTCH1/FBXW7 mutation identifies a large subgroup with favorable outcome in adult T-cell acute lymphoblastic leukemia (T-ALL): a Group for Research on Adult Acute Lymphoblastic Leukemia (GRAALL) study. Blood 2009;113:3918-3924

68. Kumar V et al. Notch and NF-kB signaling pathways regulate miR-223/FBXW7 axis in T-cell acute lymphoblastic leukemia. Leukemia 2014;28:2324-2335

69. Sanghvi VR et al. Characterization of a set of tumor suppressor microRNAs in T cell acute lymphoblastic leukemia. Sci Signal 2014;7:ra111

70. Bassan R et al. Modern therapy of acute lymphoblastic leukemia. J Clin Oncol 2011;29:532-543

71. Medd PG et al. Evidence for a GVL effect following reduced-intensity allo-SCT in ALL: a British Society of Blood and Marrow Transplantation study. Bone Marrow Transplant 2013;48:982-987

72. Sive JI et al. Outcomes in older adults with acute lymphoblastic leukaemia (ALL): results from the international MRC UKALL XII/ECOG2993 trial. Br J Haematol 2012;157:463-471

73. Hunger SP et al. Acute Lymphoblastic Leukemia in Children. N Engl J Med 2015;373:1541-1552

74. Huguet F et al. Pediatric-inspired therapy in adults with Philadelphia chromosome-negative acute lymphoblastic leukemia: the GRAALL-2003 study. J Clin Oncol 2009;27:911-918

75. Litzow MR. Finally moving forward in adult ALL. Blood 2015;125:2453-2454

76. Fielding AK et al. UKALLXII/ECOG2993: addition of imatinib to a standard treatment regimen enhances long-term outcomes in Philadelphia positive acute lymphoblastic leukemia. Blood 2014;123:843-850

77. Fielding AK et al. Allogeneic haematopoietic stem cell transplant in Philadelphia-positive acute lymphoblastic leukaemia. Bone Marrow Transplant 2008;41:447-453

78. Curran E et al. How I treat acute lymphoblastic leukemia in older adolescents and young adults. Blood 2015;125:3702-3710

79. Litzow MR et al. How I treat T-cell acute lymphoblastic leukemia in adults. Blood 2015;126:833-841

80. Mathisen MS et al. Emerging drugs for acute lymphocytic leukemia. Expert Opin Emerg Drugs 2014;19:37-50

81. Mathisen MS et al. Treatment of adult acute lymphoblastic leukemia (ALL) with a focus on emerging investigational and targeted therapies. Oncology (Williston Park) 2012;26:851-859

82. Rowe JM. Prognostic factors in adult acute lymphoblastic leukaemia. Br J Haematol 2010;150:389-405

83. Beldjord K et al. Oncogenetics and minimal residual disease are independent outcome predictors in adult patients with acute lymphoblastic leukemia. Blood 2014;123:3739-3749

84. Dhedin N et al. Role of allogeneic stem cell transplantation in adult patients with Ph-negative acute lymphoblastic leukemia. Blood 2015;125:2486-2496; quiz 2586

85. Garand R et al. Flow cytometry and IG/TCR quantitative PCR for minimal residual disease quantitation in acute lymphoblastic leukemia: a French multicenter prospective study on behalf of the FRALLE, EORTC and GRAALL. Leukemia 2013;27:370-376

86. Ghazavi F et al. Molecular basis and clinical significance of genetic aberrations in B-cell precursor acute lymphoblastic leukemia. Exp Hematol 2015;43:640-653

87. Gokbuget N et al. Adult patients with acute lymphoblastic leukemia and molecular failure display a poor prognosis and are candidates for stem cell transplantation and targeted therapies. Blood 2012;120:1868-1876

88. Larson S et al. Progress in the treatment of adults with acute lymphoblastic leukemia. Curr Opin Hematol 2008;15:400-407

89. Fava C et al. The choice of first-line chronic myelogenous leukemia treatment. Ann Hematol 2015;94 Suppl 2:S123-131

90. Fava C et al. Update on emerging treatments for chronic myeloid leukemia. Expert Opin Emerg Drugs 2015;20:183-196

91. Leoni V et al. Tyrosine kinase inhibitors in BCR-ABL positive acute lymphoblastic leukemia. Haematologica 2015;100:295-299

92. Ohanian M et al. Tyrosine kinase inhibitors in acute and chronic leukemias. Expert Opin Pharmacother 2012;13:927-938

93. O'Hare T et al. Targeting the BCR-ABL signaling pathway in therapy-resistant Philadelphia chromosome-positive leukemia. Clin Cancer Res 2011;17:212-221

94. Pfeifer H et al. Kinase domain mutations of BCR-ABL frequently precede imatinib-based therapy and give rise to relapse in patients with de novo Philadelphia-positive acute lymphoblastic leukemia (Ph+ ALL). Blood 2007;110:727-734

95. Yang K et al. Mechanisms of resistance to BCR-ABL TKIs and the therapeutic strategies: A review. Crit Rev Oncol Hematol 2015;93:277-292

96. de Labarthe A et al. Imatinib combined with induction or consolidation chemotherapy in patients with de novo Philadelphia chromosome-positive acute lymphoblastic leukemia: results of the GRAAPH-2003 study. Blood 2007;109:1408-1413

97. Ravandi F et al. First report of phase 2 study of dasatinib with hyper-CVAD for the frontline treatment of patients with Philadelphia chromosome-positive (Ph+) acute lymphoblastic leukemia. Blood 2010;116:2070-2077

98. Clappier E et al. NOTCH1 and FBXW7 mutations have a favorable impact on early response to treatment, but not on outcome, in children with T-cell acute lymphoblastic leukemia (T-ALL) treated on EORTC trials 58881 and 58951. Leukemia 2010;24:2023-2031

99. Mansour MR et al. Prognostic implications of NOTCH1 and FBXW7 mutations in adults with T-cell acute lymphoblastic leukemia treated on the MRC UKALLXII/ECOG E2993 protocol. J Clin Oncol 2009;27:4352-4356

100. Stracke ML et al. Identification, purification, and partial sequence analysis of autotaxin, a novel motility-stimulating protein. J Biol Chem 1992;267:2524-2529

101. Stracke ML et al. Autotaxin, tumor motility-stimulating exophosphodiesterase. Adv Enzyme Regul 1997;37:135-144

102. Umezu-Goto M et al. Autotaxin has lysophospholipase D activity leading to tumor cell growth and motility by lysophosphatidic acid production. J Cell Biol 2002;158:227-233

103. Tania M et al. Autotaxin: a protein with two faces. Biochem Biophys Res Commun 2010;401:493-497

104. Vidot S et al. Autotaxin delays apoptosis induced by carboplatin in ovarian cancer cells. Cell Signal 2010;22:926-935

105. Federico L et al. Autotaxin, a lysophospholipase D with pleomorphic effects in oncogenesis and cancer progression. J Lipid Res 2015; Epub ahead of print

106. Gotoh M et al. Controlling cancer through the autotaxin-lysophosphatidic acid receptor axis. Biochem Soc Trans 2012;40:31-36

107. Lee HY et al. Autotaxin promotes motility via G protein-coupled phosphoinositide 3-kinase gamma in human melanoma cells. FEBS Lett 2002;515:137-140

108. Liu S et al. ATX-LPA receptor axis in inflammation and cancer. Cell Cycle 2009;8:3695-3701

109. Masuda A et al. Serum autotaxin measurement in haematological malignancies: a promising marker for follicular lymphoma. Br J Haematol 2008;143:60-70

110. Jonkers J et al. Mammary tumorigenesis through LPA receptor signaling. Cancer Cell 2009;15:457-459

111. Nam SW et al. Autotaxin (ATX), a potent tumor motogen, augments invasive and metastatic potential of ras-transformed cells. Oncogene 2000;19:241-247

112. Teo K et al. The role and therapeutic potential of the autotaxin-lysophosphatidate signalling axis in breast cancer. Biochem J 2014;463:157-165

113. Hoelzer D et al. Chemoimmunotherapy in acute lymphoblastic leukemia. Blood Rev 2012;26:25-32

114. Ben Abdelali R et al. Pediatric-inspired intensified therapy of adult T-ALL reveals the favorable outcome of NOTCH1/FBXW7 mutations, but not of low ERG/BAALC expression: a GRAALL study. Blood 2011;118:5099-5107

115. Weng AP et al. Activating mutations of NOTCH1 in human T cell acute lymphoblastic leukemia. Science 2004;306:269-271

116. Perentesis JP et al. RAS oncogene mutations and outcome of therapy for childhood acute lymphoblastic leukemia. Leukemia 2004;18:685-692

117. Gutierrez A et al. High frequency of PTEN, PI3K, and AKT abnormalities in T-cell acute lymphoblastic leukemia. Blood 2009;114:647-650

118. Zuurbier L et al. The significance of PTEN and AKT aberrations in pediatric T-cell acute lymphoblastic leukemia. Haematologica 2012;97:1405-1413

119. Bandapalli OR et al. NOTCH1 activation clinically antagonizes the unfavorable effect of PTEN inactivation in BFM-treated children with precursor T-cell acute lymphoblastic leukemia. Haematologica 2013;98:928-936

120. Goldstone AH et al. In adults with standard-risk acute lymphoblastic leukemia, the greatest benefit is achieved from a matched sibling allogeneic transplantation in first complete remission, and an autologous transplantation is less effective than conventional consolidation/maintenance chemotherapy in all patients: final results of the International ALL Trial (MRC UKALL XII/ECOG E2993). Blood 2008;111:1827-1833

121. Lee S et al. The effect of first-line imatinib interim therapy on the outcome of allogeneic stem cell transplantation in adults with newly diagnosed Philadelphia chromosome-positive acute lymphoblastic leukemia. Blood 2005;105:3449-3457

122. Ribera JM et al. Concurrent intensive chemotherapy and imatinib before and after stem cell transplantation in newly diagnosed Philadelphia chromosome-positive acute lymphoblastic leukemia. Final results of the CSTIBES02 trial. Haematologica 2010;95:87-95

123. Chalandon Y et al. Randomized study of reduced-intensity chemotherapy combined with imatinib in adults with Ph-positive acute lymphoblastic leukemia. Blood 2015;125:3711-3719

124. Ravandi F et al. Minimal residual disease assessed by multi-parameter flow cytometry is highly prognostic in adult patients with acute lymphoblastic leukaemia. Br J Haematol 2015; Epub ahead of print

125. Stirewalt DL et al. Predictors of relapse and overall survival in Philadelphia chromosome-positive acute lymphoblastic leukemia after transplantation. Biol Blood Marrow Transplant 2003;9:206-212

126. Carpenter PA et al. Prophylactic administration of imatinib after hematopoietic cell transplantation for high-risk Philadelphia chromosome-positive leukemia. Blood 2007;109:2791-2793

127. Radich J et al. Detection of bcr-abl transcripts in Philadelphia chromosome-positive acute lymphoblastic leukemia after marrow transplantation. Blood 1997;89:2602-2609

128. Sanford DS et al. The role of ponatinib in Philadelphia chromosome-positive acute lymphoblastic leukemia. Expert Rev Anticancer Ther 2015;15:365-373

129. Bixby D et al. Mechanisms of resistance to tyrosine kinase inhibitors in chronic myeloid leukemia and recent therapeutic strategies to overcome resistance. Hematology 2009; 2009:461-476

130. Tokuhara Y et al. A New Enzyme Immunoassay for the Quantitative Determination of Classical Autotaxins (ATXalpha, ATXbeta, and ATXgamma) and Novel Autotaxins (ATXdelta and ATXepsilon). PLoS One 2015;10:e0130074

131. Baumforth KR et al. Induction of autotaxin by the Epstein-Barr virus promotes the growth and survival of Hodgkin lymphoma cells. Blood 2005;106:2138-2146

132. Ortlepp C et al. Autotaxin is expressed in FLT3-ITD positive acute myeloid leukemia and hematopoietic stem cells and promotes cell migration and proliferation. Exp Hematol 2013;41:444-461 e444

133. Satoh Y et al. Lysophosphatidic acid protection against apoptosis in the human pre-B-cell line Nalm-6. Eur J Haematol 2007;78:510-517

134. Yuelling LM et al. Autotaxin (ATX): a multi-functional and multi-modular protein possessing enzymatic lysoPLD activity and matricellular properties. Biochim Biophys Acta 2008;1781:525-530

135. Peyruchaud O. Novel implications for lysophospholipids, lysophosphatidic acid and sphingosine 1-phosphate, as drug targets in cancer. Anticancer Agents Med Chem 2009;9:381-391

136. Valentine WJ et al. (S)-FTY720-vinylphosphonate, an analogue of the immunosuppressive agent FTY720, is a pan-antagonist of sphingosine 1-phosphate GPCR signaling and inhibits autotaxin activity. Cell Signal 2010;22:1543-1553

137. Choi JW et al. Biological roles of lysophospholipid receptors revealed by genetic null mice: an update. Biochim Biophys Acta 2008;1781:531-539

138. Kalender Atak Z et al. High accuracy mutation detection in leukemia on a selected panel of cancer genes. PLoS One 2012;7:e38463

139. Adachi K et al. FTY720 story. Its discovery and the following accelerated development of sphingosine 1-phosphate receptor agonists as immunomodulators based on reverse pharmacology. Perspect Medicin Chem 2008;1:11-23

140. Chiba K et al. FTY720, a novel immunosuppressant, induces sequestration of circulating lymphocytes by acceleration of lymphocyte homing. Transplant Proc 1999;31:1230-1233

141. Chiba K. FTY720, a new class of immunomodulator, inhibits lymphocyte egress from secondary lymphoid tissues and thymus by agonistic activity at sphingosine 1-phosphate receptors. Pharmacol Ther 2005;108:308-319

142. Kiuchi M et al. Asymmetric synthesis and biological evaluation of the enantiomeric isomers of the immunosuppressive FTY720-phosphate. Bioorg Med Chem 2005;13:425-432

143. Kiyota M et al. FTY720 induces apoptosis of chronic myelogenous leukemia cells via dual activation of BIM and BID and overcomes various types of resistance to tyrosine kinase inhibitors. Apoptosis 2013;18:1437-1446

144. Alinari L et al. FTY720 increases CD74 expression and sensitizes mantle cell lymphoma cells to milatuzumab-mediated cell death. Blood 2011;118:6893-6903

145. Chen L et al. FTY720 induces apoptosis of M2 subtype acute myeloid leukemia cells by targeting sphingolipid metabolism and increasing endogenous ceramide levels. PLoS One 2014;9:e103033

146. Liu Q et al. FTY720 shows promising in vitro and in vivo preclinical activity by downmodulating Cyclin D1 and phospho-Akt in mantle cell lymphoma. Clin Cancer Res 2010;16:3182-3192

147. Liu Q et al. FTY720 demonstrates promising preclinical activity for chronic lymphocytic leukemia and lymphoblastic leukemia/lymphoma. Blood. 2008;111:275-284

148. Wallington-Beddoe CT et al. Disparate in vivo efficacy of FTY720 in xenograft models of Philadelphia positive and negative B-lineage acute lymphoblastic leukemia. PLoS One 2012;7:e36429

149. Wallington-Beddoe CT et al. FTY720 produces caspase-independent cell death of acute lymphoblastic leukemia cells. Autophagy 2011;7:707-715

150. Pippa R et al. Effect of FTY720 on the SET-PP2A complex in acute myeloid leukemia; SET binding drugs have antagonistic activity. Leukemia 2014;28:1915-1918

151. Neviani P et al. PP2A-activating drugs selectively eradicate TKI-resistant chronic myeloid leukemic stem cells. J Clin Invest 2013;123:4144-4157

152. Neviani P et al. FTY720, a new alternative for treating blast crisis chronic myelogenous leukemia and Philadelphia chromosome-positive acute lymphocytic leukemia. J Clin Invest 2007;117:2408-2421

153. Pitman MR et al. Molecular targets of FTY720 (fingolimod). Curr Mol Med 2012;12:1207-1219

154. Shi F et al. Ribavirin Inhibits the Activity of mTOR/eIF4E, ERK/Mnk1/eIF4E Signaling Pathway and Synergizes with Tyrosine Kinase Inhibitor Imatinib to Impair Bcr-Abl Mediated Proliferation and Apoptosis in Ph+ Leukemia. PLoS One 2015;10:e0136746

155. Appelmann I et al. Janus kinase inhibition by ruxolitinib extends dasatinib- and dexamethasone-induced remissions in a mouse model of Ph+ ALL. Blood 2015;125:1444-1451

Résumé

Les leucémies aiguës (LA) sont un groupe hétérogène d'hémopathies malignes dues à la transformation oncogénique clonale des cellules souches hématopoïétiques (CSH).
On distingue les LA myéloblastiques et lymphoblastiques (LAL). Les LAL sont classées selon le type de précurseur lymphoïde atteint, leur degré de maturité et leurs anomalies cytogénétiques.
Le traitement permettant d'obtenir 80 à 90 % de rémission complète (RC) comporte une chimiothérapie d'induction, une consolidation et une intensification (intensification retardée ou allogreffe de CSH selon la situation pronostique). Néanmoins la survie globale à long terme n'est que de 40 à 50 %, du fait de la survenue de rechutes et de la toxicité des traitements.
Différents groupes pronostiques basés sur la cytogénétique et la biologie moléculaire se dégagent, pouvant bénéficier de thérapeutiques adaptées.
Dans les LAL à chromosome philadelphie (LAL à Ph), antérieurement de mauvais pronostic, les inhibiteurs de tyrosine kinase (ITK) permettent d'obtenir 80% de RC avec cependant un taux de rechute non négligeable.
Nous avons démontré qu'une intensification thérapeutique par autogreffe chez des patients avec une maladie résiduelle indétectable permettait une survie à long terme prolongée avec une toxicité moindre que celle de l'allogreffe. En montrant l'implication de l'autotaxine dans les mécanismes de résistance aux ITK dans les LAL à Ph, nous ouvrons la voie à l'utilisation potentielle de nouvelles thérapeutiques.
Dans les LAL T, groupe considéré de bon pronostic, un tiers des patients rechute. Nous avons démontré que l'absence de mutation de Notch et/ou FBXW7 ou la présence de mutations de RAS ou PTEN était de mauvais pronostic identifiant un sous-groupe de LAL T dont le traitement devait être renforcé. Nos travaux ont ainsi contribué à l'identification des groupes pronostiques dans les LAL et à l'adaptation des traitements afin d'améliorer les chances de survie.

Mots clefs

Leucémie aiguë lymphoblastique
LAL à Ph1
LAL T
résistance aux inhibiteurs de tyrosine kinase
mutations Notch, FBXW7, RAS et PTEN
groupe pronostique

Abstract

Acute leukemias are a heterogeneous groups of malignant hematological diseases due to the clonal oncogenic transformation of hematopoietic stem cells (HSTs). We distinguish acute myeloblastic leukaemia from acute lymphoblastic leukemia (ALL). ALLs are classified according to the type of lymphoid precursor affected, its degree of maturity, and with associated cytogenetic abnormalities.
Treatment incorporating induction therapy, consolidation, and intensification – delayed intensification or allogeneic stem cell transplantation (SCT) according to prognostic factors – enable 80 to 90 % of complete remission (CR). Nevertheless, long-term overall survival is only 40 to 50% because of relapse and treatment-related toxicity. Different prognostic groups based on cytogenetic abnormalities and molecular biology are emerging and patients from each prognostic group can benefit from adapted therapies.
In chromosome Philadelphia-positive ALL (Ph+ ALL) which used to be of particular bad prognosis, tyrosine kinase inhibitors (TKIs) enables 80% of CR but with a high-relapse risk. We demonstrated that high-dose therapy followed by autologous SCT enables prolonged long-term survival with less drug-related toxicity as compared to allogeneic SCT in patients with undetectable minimal residual disease. By showing the implication of autotaxine in the resistance to TKIs in Ph+ LAL, we enable the use of novel therapeutics in clinical practice.
T-cell ALL is considered of poor prognosis as one third of patients relapse. In this group of patients we showed that the absence of a Notch and/or a FBXW7 mutation or the presence of mutations in RAS or PTEN identified a subgroup of patients in whom the treatment must be intensified. Our research has contributed to the identification of prognostic groups in ALL and to the adjustment of treatment according to potential survival.

Keywords:

Acute lymphoblastic leukaemia
Ph + ALL
T-cell ALL
Resistance to tyrosine kinase inhibitors
Notch, FBXW7, RAS, and PTEN mutations
Prognostic groups