RIIP

RENCONTRES EN IMMUNOLOGIE & IMMUNOTHERAPIE PRATIQUES

29 et 30 SEPTEMBRE 2022

UIC-P - Espaces Congrès
16, rue Jean Rey - 75015 Paris

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Sous l'égide de :

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RENCONTRES EN IMMUNOLOGIE & IMMUNOTHERAPIE PRATIQUES

Hémopathies Lymphoïdes et autoimmunité quels liens ?

Thierry MARTIN

Immunologie Clinique Médecine interne

CRMR maladies auto-immunes systémiques rares RESO

Hôpitaux Universitaires de Strasbourg

29 et 30 SEPTEMBRE 2022

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Sous l'égide de :

- 7-10% des LLC s'accompagnent à un moment de leur évolution d'une maladie autoimmune

- Les plus fréquentes: anémies hémolytiques et thrombopénies (AHAI/PTI)

- 30-40% avant ou au moment du diagnostic

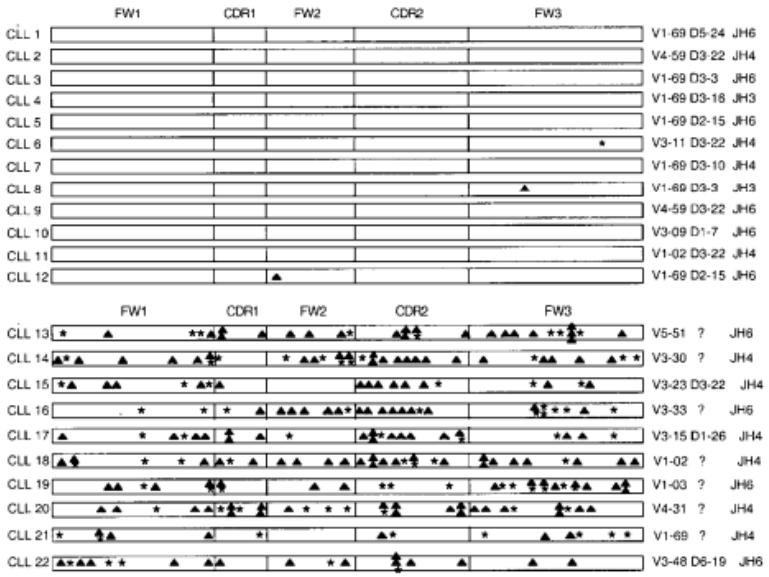
- Évolution ne suit pas forcément celle de la lymphoprolifération

Moreno et al, Blood 2010
Shanafelt et al J Clin Oncol 2011

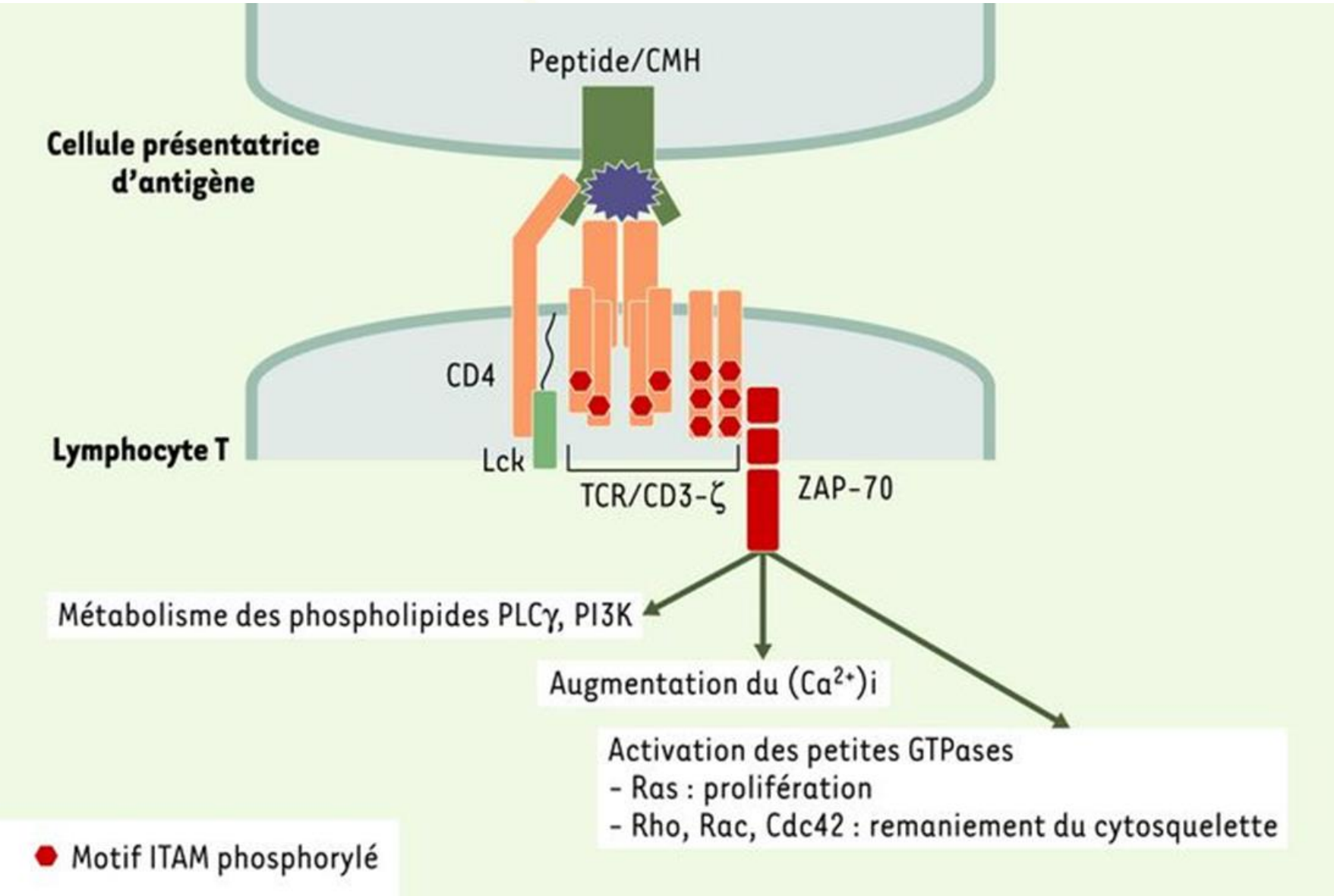
Mécanismes ?

Dans la majorité des cas les autoAc sont des IgG polyclonales
TJ Kipps, Blood 1993

- **Facteurs pronostiques LLC:**
  - Régions VH non mutées
  - Expression anormale de la kinase ZAP70



- **ZAP70:**
  - Zeta-chain-associated protein of 70 kDa
  - Tyrosine kinase impliquée dans le signaling du TCR
  - Équivalent de SYK dans les LyB


Peptide/CMH
Cellule présentatrice d'antigène
CD4
Lymphocyte T
Lck
TCR/CD3-ζ
ZAP-70
Métabolisme des phospholipides PLCγ, PI3K
Augmentation du (Ca2+)i
Activation des petites GTPases
- Ras : prolifération
- Rho, Rac, Cdc42 : remaniement du cytosquelette
Motif ITAM phosphorylé

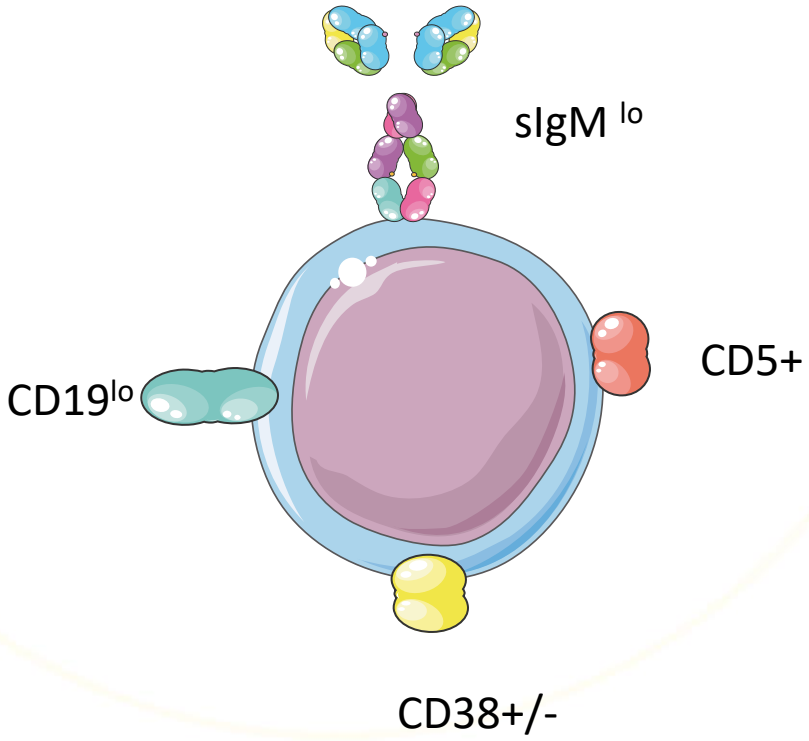
Au cours de la LLC, l'expression de ZA70 est fortement associée au développement de l'autoimmunité (p<0,0001)

- **290 patients**
- **46 (15%) cytopénies AI (CAI)**
- **37 (80%) ZAP70+**
  - *Zanotti et al Am J Hematol 2010*

63 patients : 19 CAI toutes ZAP70+

- Ghergus et al Blood revision

- La cellule de LLC



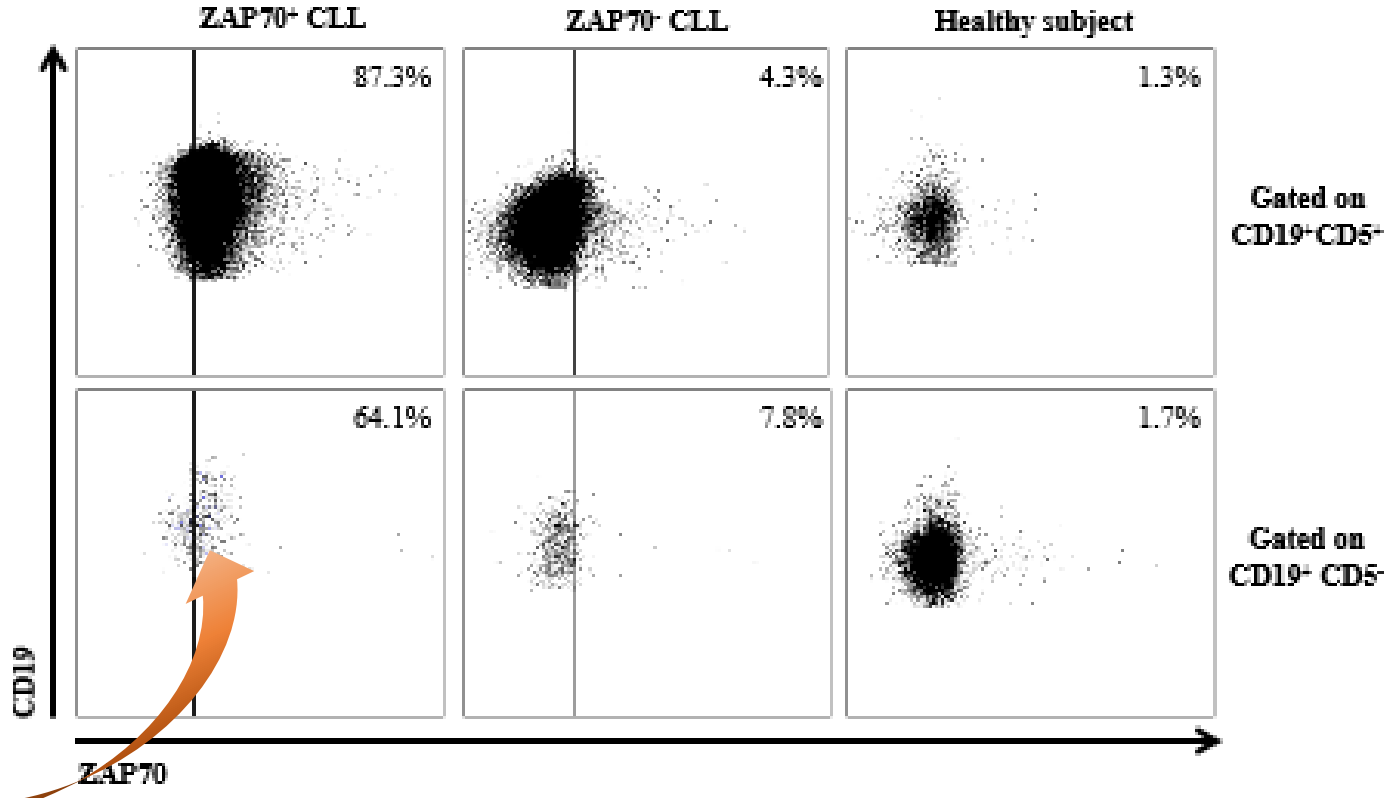
Immunophénotypage permet de déterminer le score de Matutes

Un point est accordé à chaque item

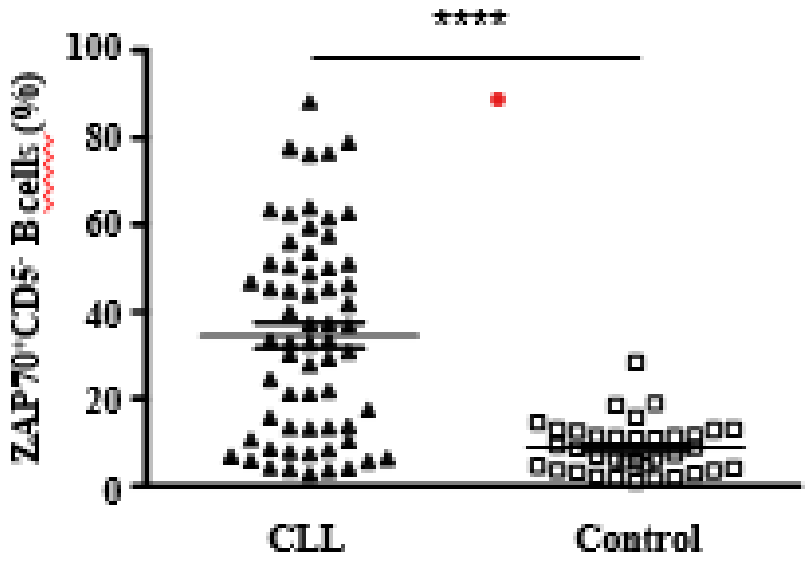
	1 point	0 point
Ig de surface	Expression faible	Expression forte
Expression du CD5	+	-
Expression du CD23	+	-
Expression du CD79b	Faible ou nulle	Forte
Expression de FMC7	-	+

Score de Matutes = 4 ou 5 =>> LLC

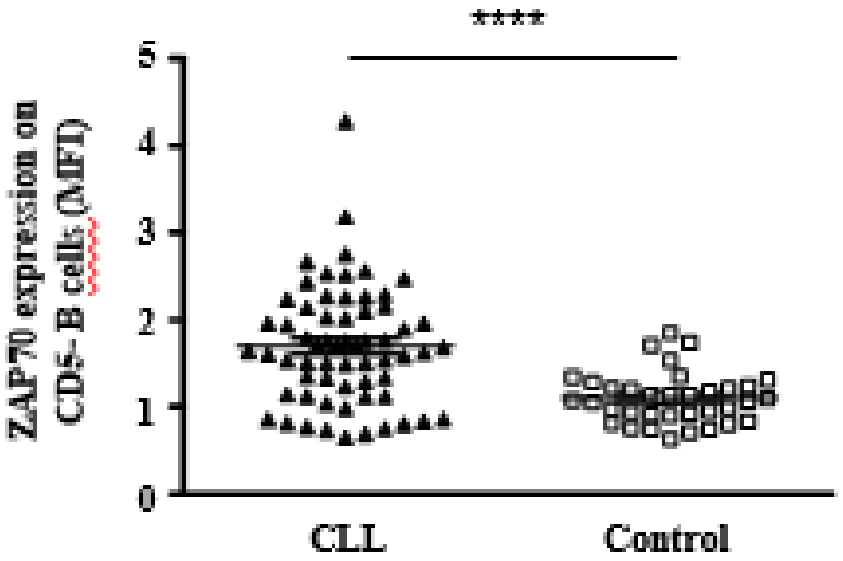
- LLC ZAP70+ vs ZAP70-



Ly B CD5- mais ZAP70+


****
100
80
60
40
20
0
ZAP70+CD5- B cells (%)
CLL
Control

CD5+

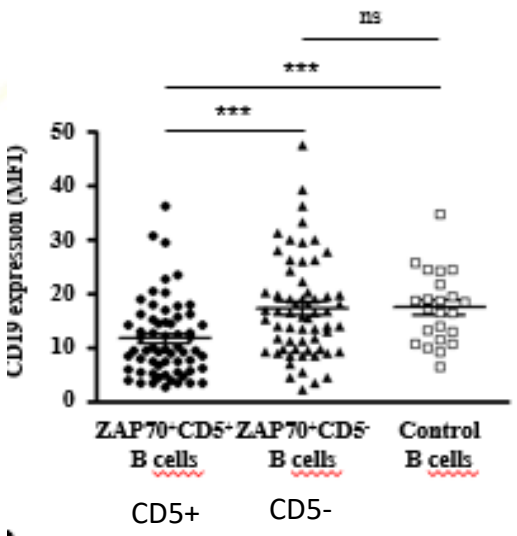

C
****
5
4
3
2
1
0
ZAP70 expression on CD5- B cells (MFI)
CLL
Control

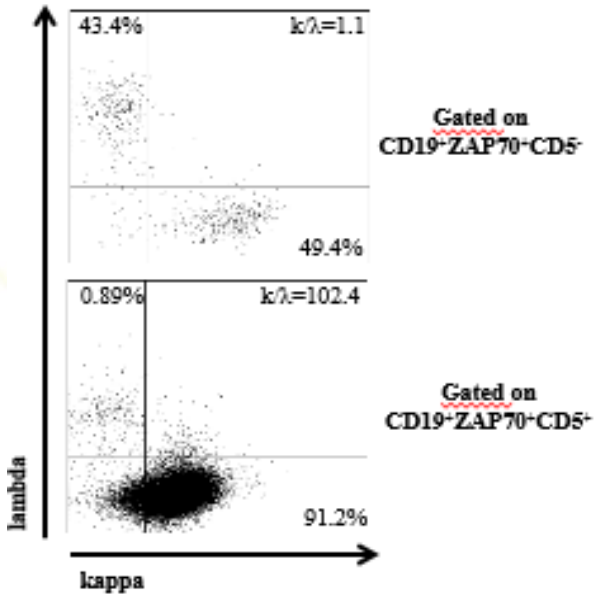
E

CD5-

- LyB tumoral qui a perdu CD5

- LyB non tumoral mais qui exprime ZAP70





Les LyB CD5- mais ZAP70+ ont des niveaux normaux de CD19

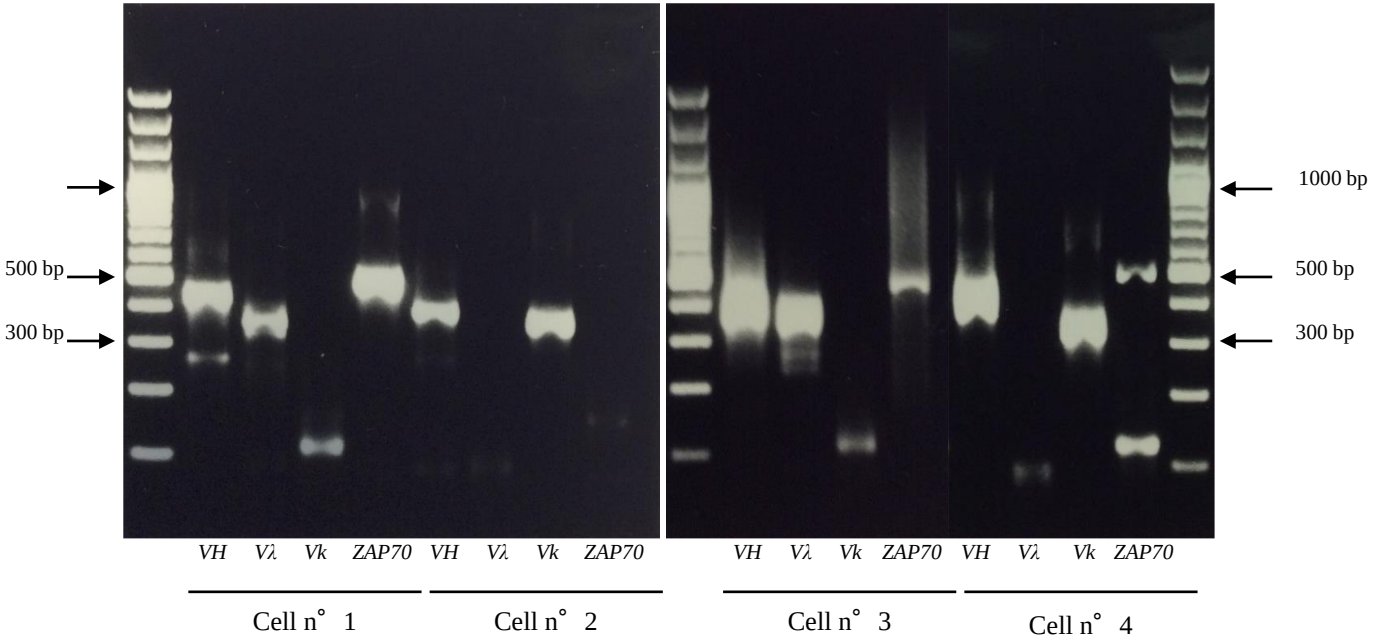
Les LYB CD5-ZAP70+ sont pour l'essentiel des cellules non tumorales

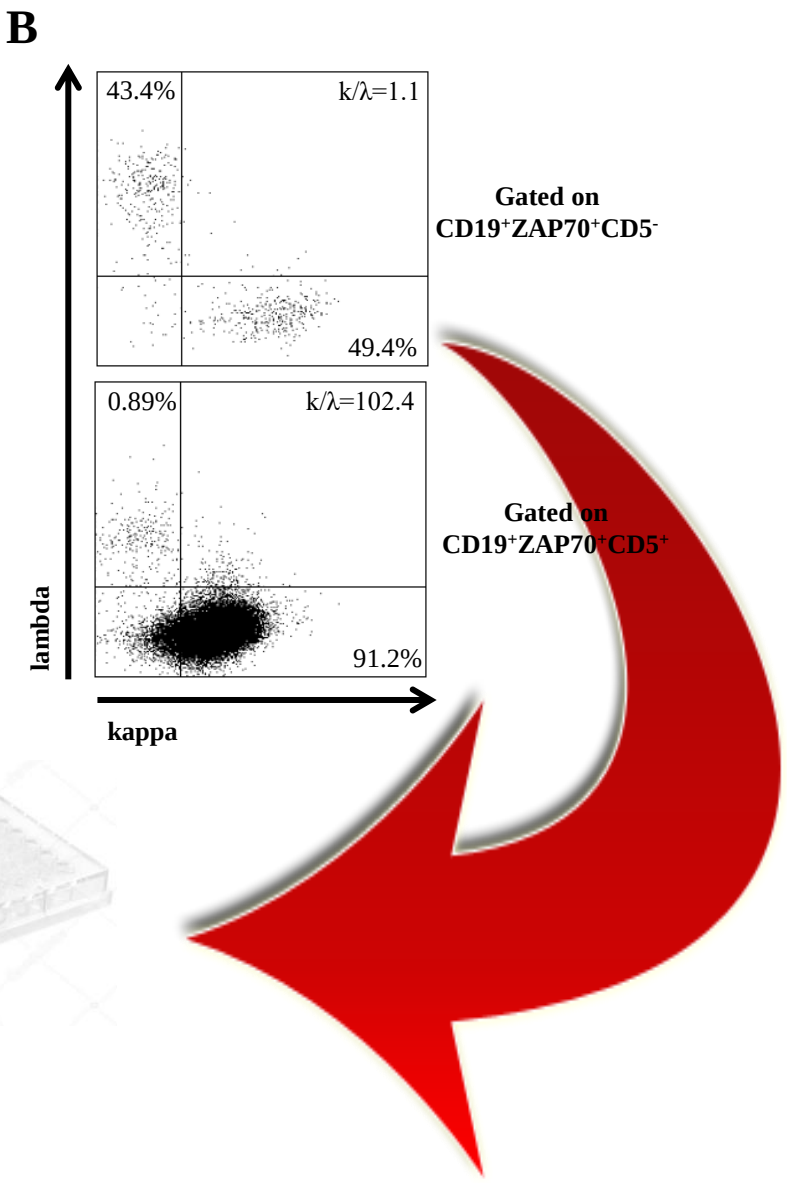
Ghergus D… Martin T, Blood revision

En résumé:

- Certaines LLC B expriment la kinase ZAP70
- Il existe une corrélation entre cette expression et la survenue de manifestations auto-immunes
- Les patients atteints de LLC ZAP70+ ont des LYB CD5- apparemment normaux, polyclonaux, qui expriment aussi ZAP70
- **L'expression de ZAP70 dans les LyB serait un phénomène antérieur à la transformation néoplasique**

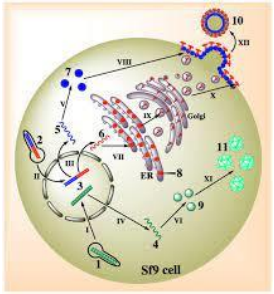
Séquençage des régions V confirme la polyclonalité





Single cell culture

RT-PCR
VH-VL cloning



mAb

Les LyB CD5- sont souvent autoréactifs

Patient	rAb	MAIPA	IAT	ANA pattern (IFA)	Hep-2 (ELISA)	Polyreactivity (ELISA)
1	1-1	-	-	-	-	-
	1-2	-	-	-	-	-
	1-3	-	-	-	-	-
	1-4	-	-	-	**Positive**	-
	1-5	-	-	**Cytoplasmic**	**Positive**	-
	1-6	-	-	-	**Positive**	-
2	2-1	-	-	-	-	-
	2-2	-	-	**Nuclear**	**Positive**	-
	2-3	-	-	**Nuclear + cytoplasmic**	-	**Positive**
	2-4	-	-	-	**Positive**	**Positive**
	2-5	-	-	-	-	-
3	3-1	-	-	**Endoplasmic reticulum**	-	-
	3-2	-	-	-	-	-
4	4-1	**Positive**	-	**Cytoplasmic**	**Positive**	**Positive**
	4-2	-	-	-	-	**Positive**
5	5-1	-	-	**Cytoplasmic**	**Positive**	**Positive**
6	6-1	-	-	-	-	-
7	7-1	-	-	-	-	-

En résumé:

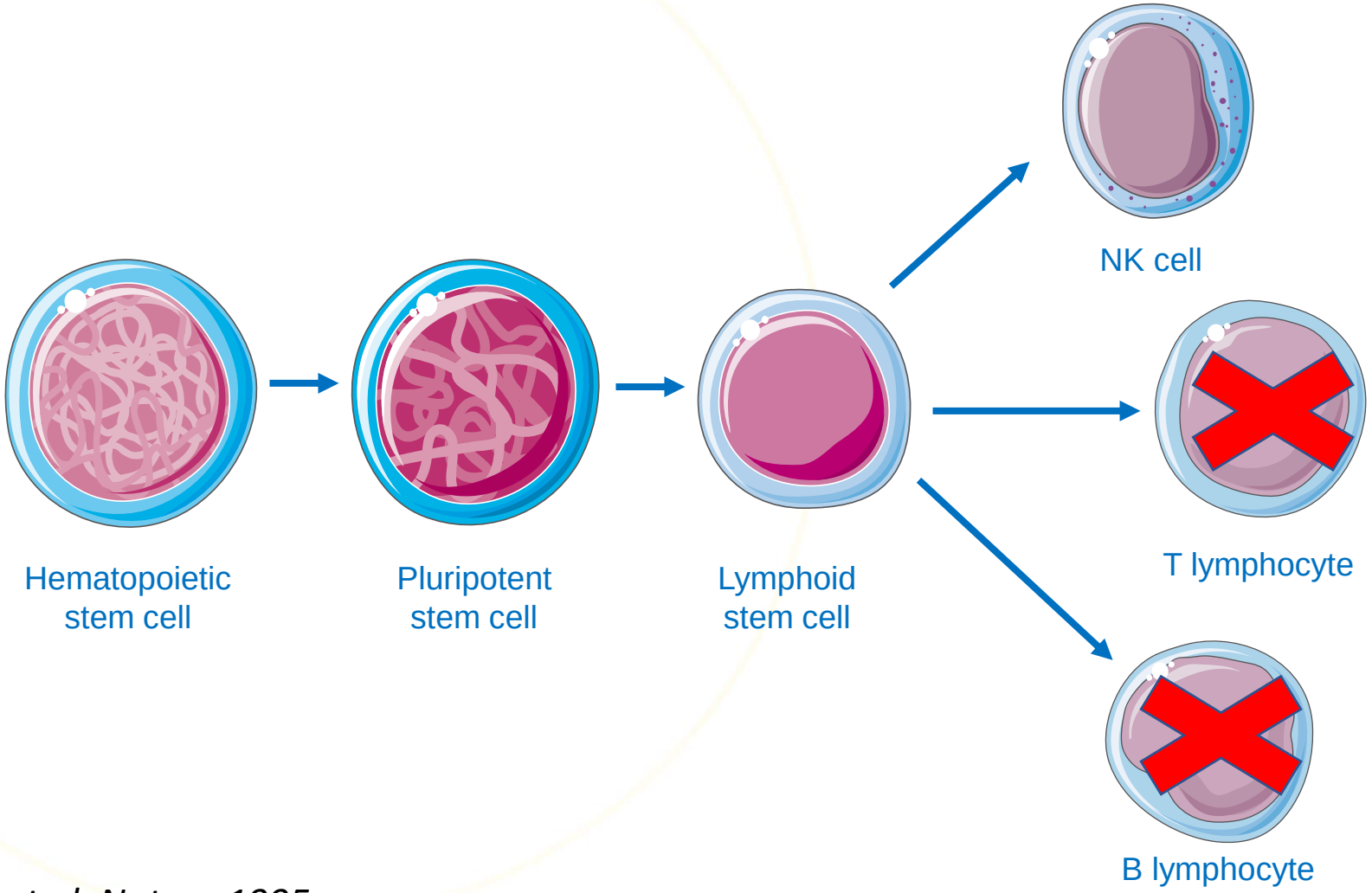
- Certaines LLC B expriment la kinase ZAP70
- Il existe une corrélation entre cette expression et la survenue de manifestations auto-immunes
- Les patients atteints de LLC ZAP70+ ont des LYB CD5- apparemment normaux, polyclonaux, qui expriment aussi ZAP70
- L'expression de ZAP70 dans les LyB serait un phénomène antérieur à la transformation néoplasique
- **Les LyB ZAP70+ CD5- sont polyclonaux, différents du clone tumoral et fréquemment autoréactifs**

Questions en suspens

- l'expression de ZAP70 dans les LyB est elle impliquée:
  - Dans la rupture de tolérance ?
  - Dans la transformation néoplasique ?

Les LyB de LLC ZAP70 sont souvent autoréactifs

Dühren Von Minden M et al, Nature 2012
Hervé M et al, JCI 2005



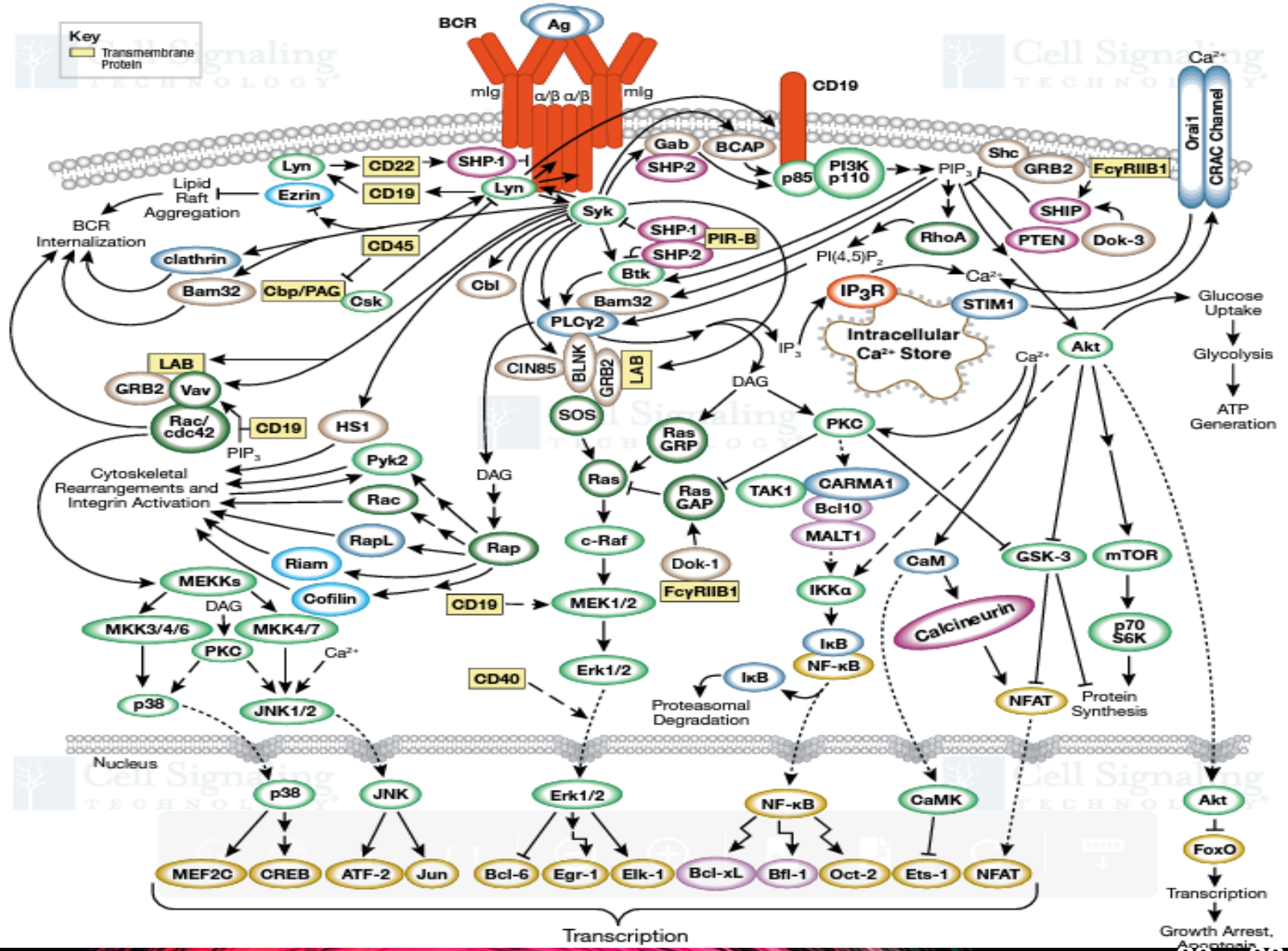
ZAP70-KO

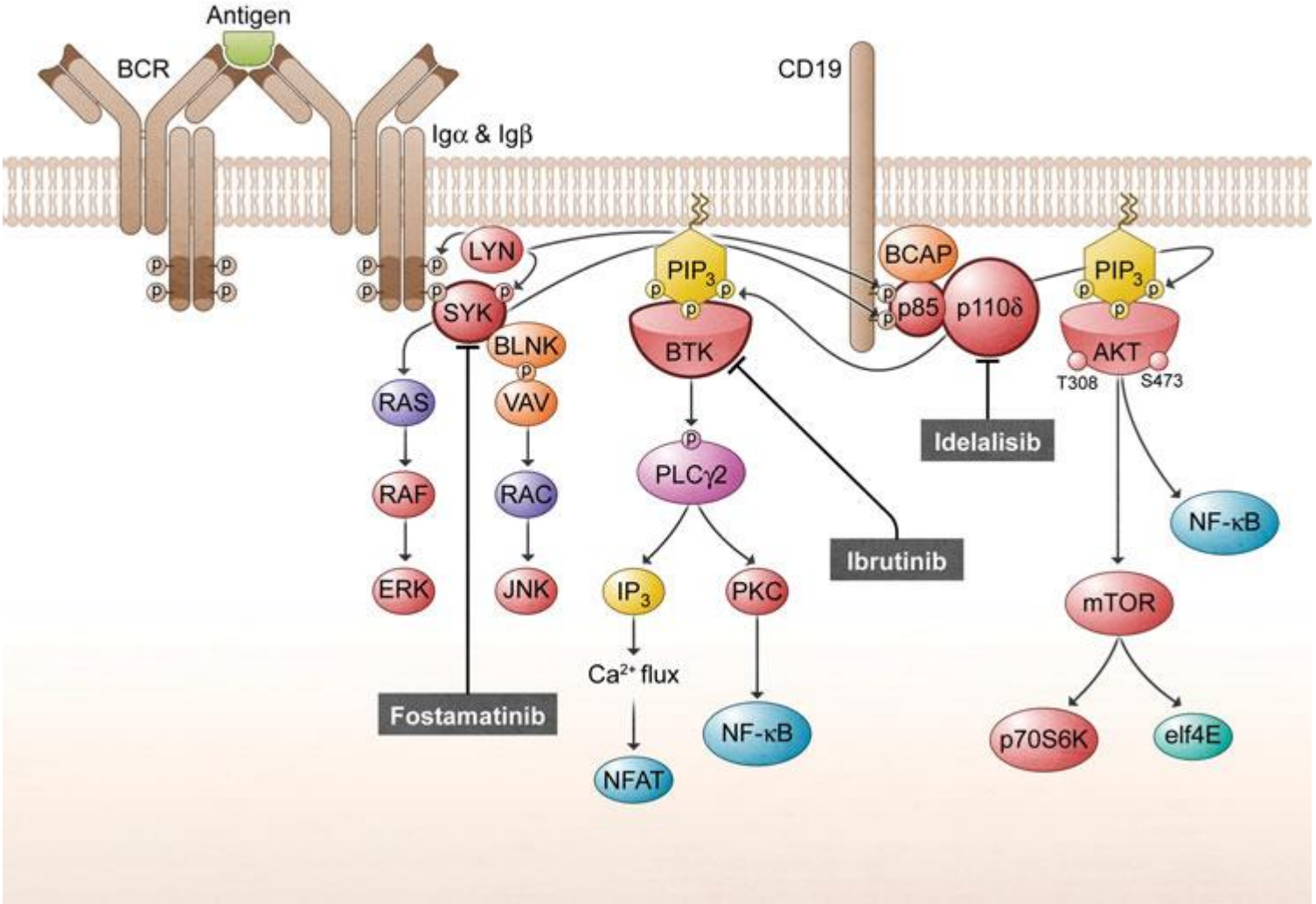
SYK-KO

Negishi et al, Nature 1995
Cheng et al, Nature 1995

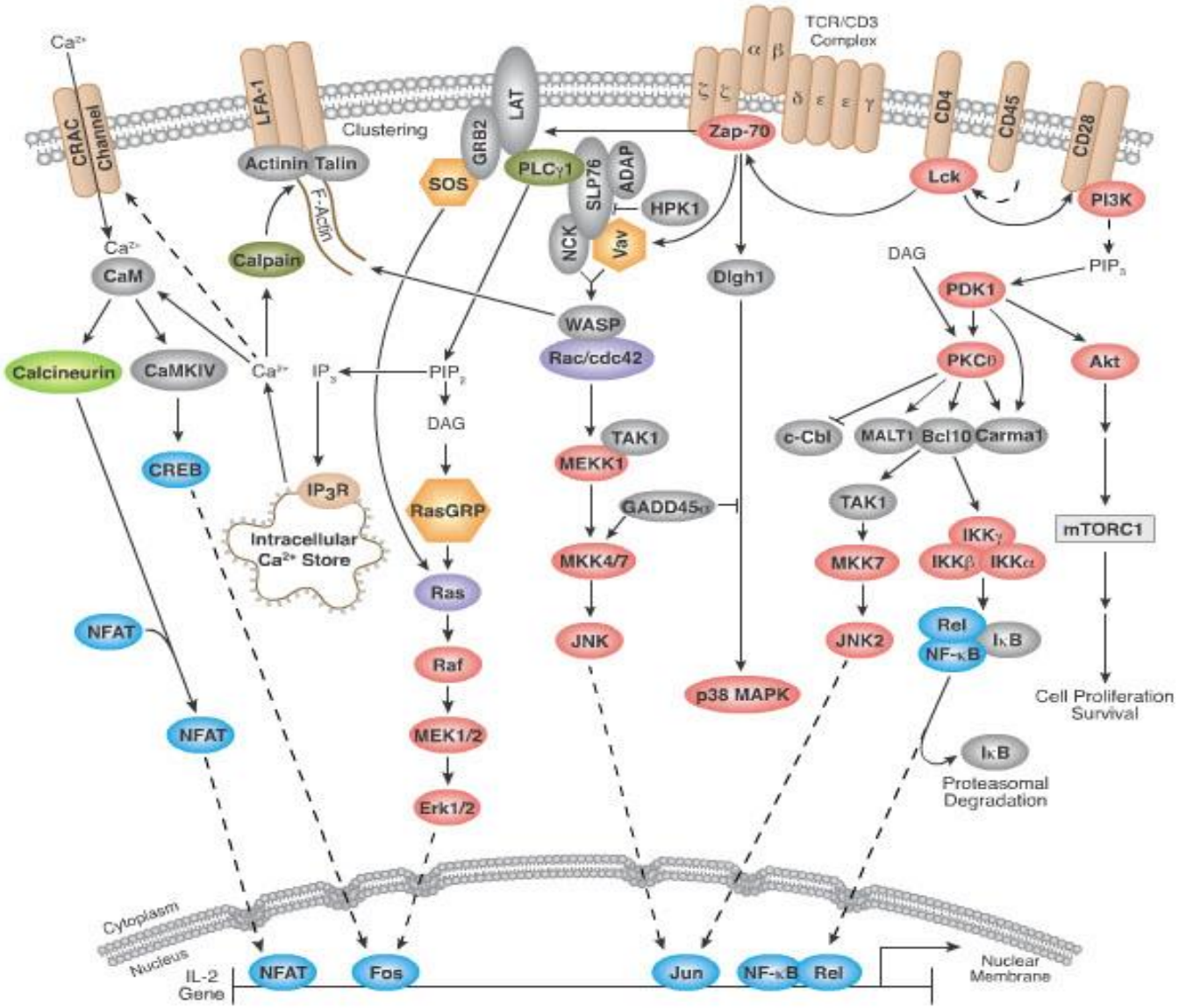
SYK

B Cell Receptor Signaling




Antigen
BCR
Igα & Igβ
CD19
LYN
SYK
BLNK
VAV
RAS
RAF
ERK
RAC
JNK
PIP3
BTK
PLCγ2
IP3
PKC
Ca2+ flux
NFAT
NF-κB
BCAP
p85
p110δ
AKT
T308
S473
mTOR
p70S6K
elf4E
Fostamatinib
Ibrutinib
Idelalisib

T Cell Receptor Signaling



Expression of ZAP-70 is associated with increased B-cell receptor signaling in chronic lymphocytic leukemia

Liguang Chen, George Widhopf, Lang Huynh, Laura Rassenti, Kanti R. Rai, Arthur Weiss, and Thomas J. Kipps

We examined isolated leukemia B cells of patients with chronic lymphocytic leukemia (CLL) for expression of zeta-associated protein 70 (ZAP-70). CLL B cells that have nonmutated immunoglobulin variable region genes (V genes) expressed levels of ZAP-70 protein that were comparable to those expressed by normal blood T cells. In contrast, CLL B cells that had mutated immunoglobulin variable V genes, or that had low-level expression of CD38, generally did not express detectable amounts of ZAP-70 protein. Leukemia cells from identical twins with CLL were found discordant for expression of ZAP-70, suggesting that B-cell expression of ZAP-70 is not genetically predetermined. Ligation of the B-cell receptor (BCR) complex on CLL cells that expressed ZAP-70 induced significantly greater tyrosine phosphorylation of cytosolic proteins, including p72^{Syk}, than did similar stimulation of CLL cells that did not express ZAP-70. Also, exceptional cases of CLL cells that expressed mutated immunoglobulin V genes and ZAP-70 also experienced higher levels tyrosine phosphorylation of such cytosolic proteins following BCR ligation. Following BCR ligation, ZAP-70 underwent tyrosine phosphorylation and became associated with surface immunoglobulin and CD79b, arguing for the involvement of ZAP-70 in BCR signaling. These data indicate that expression of ZAP-70 is associated with enhanced signal transduction via the BCR complex, which may contribute to the more aggressive clinical course associated with CLL cells that express nonmutated immunoglobulin receptors. (Blood. 2002;100:4609-4614)

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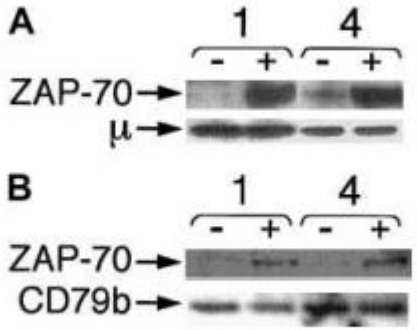
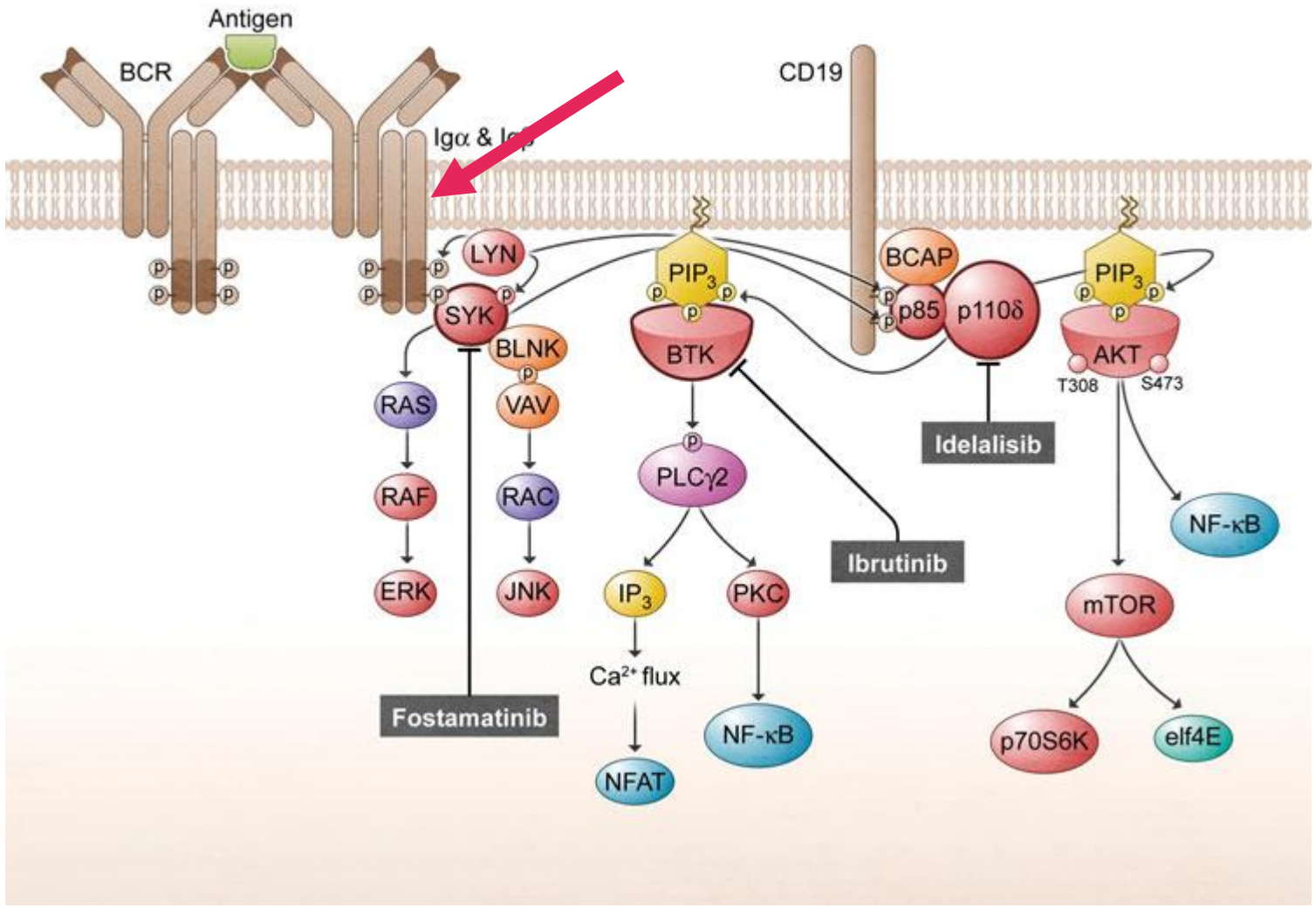


Figure 6. Association of ZAP-70 with IgM and CD79b in CLL B cells after BCR ligation. Lysates were made before and 10 minutes after BCR ligation with anti-μ


Antigen
BCR
Igα & Igβ
CD19
LYN
SYK
BLNK
VAV
RAS
RAF
ERK
RAC
JNK
PIP3
BTK
PLCγ2
IP3
PKC
Ca2+ flux
NFAT
NF-κB
BCAP
p85
p110δ
AKT
T308
S473
mTOR
p70S6K
elf4E
Fostamatinib
Ibrutinib
Idelalisib

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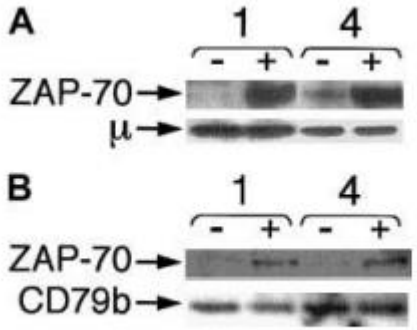


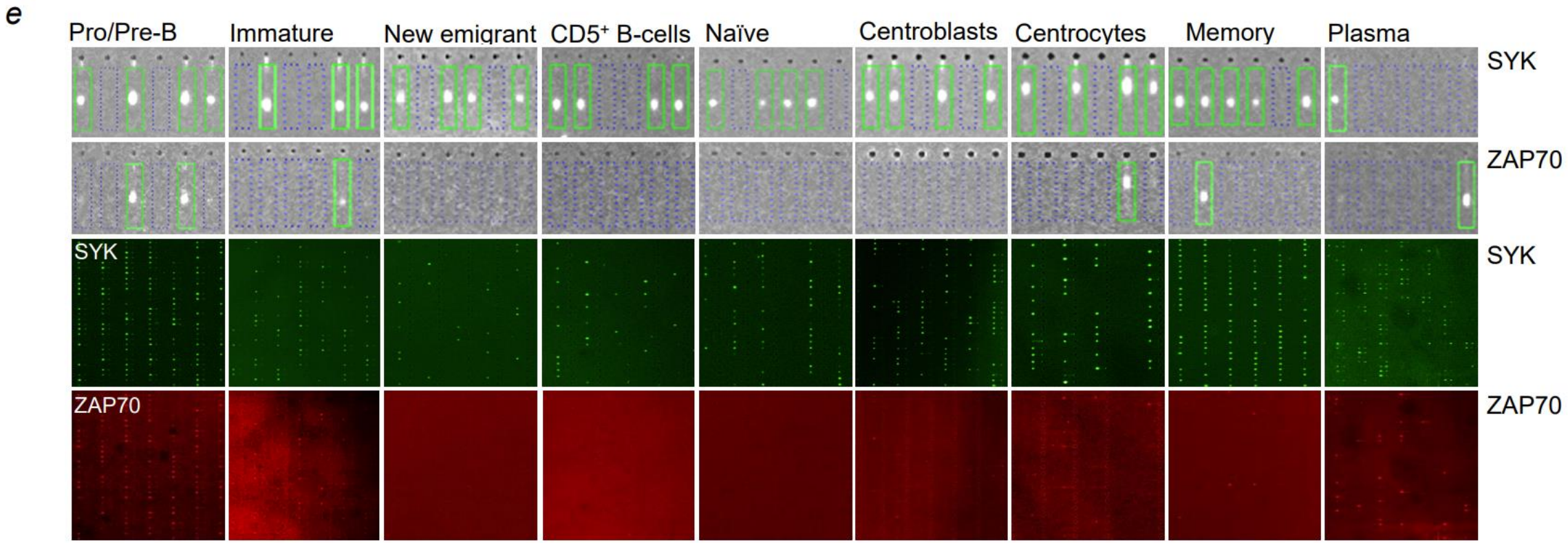
Figure 6. Association of ZAP-70 with IgM and CD79b in CLL B cells after BCR ligation. Lysates were made before and 10 minutes after BCR ligation with anti-μ

- ZAP70 et SYK ont des fonctions similaires mais sont normalement lignées B/T spécifiques

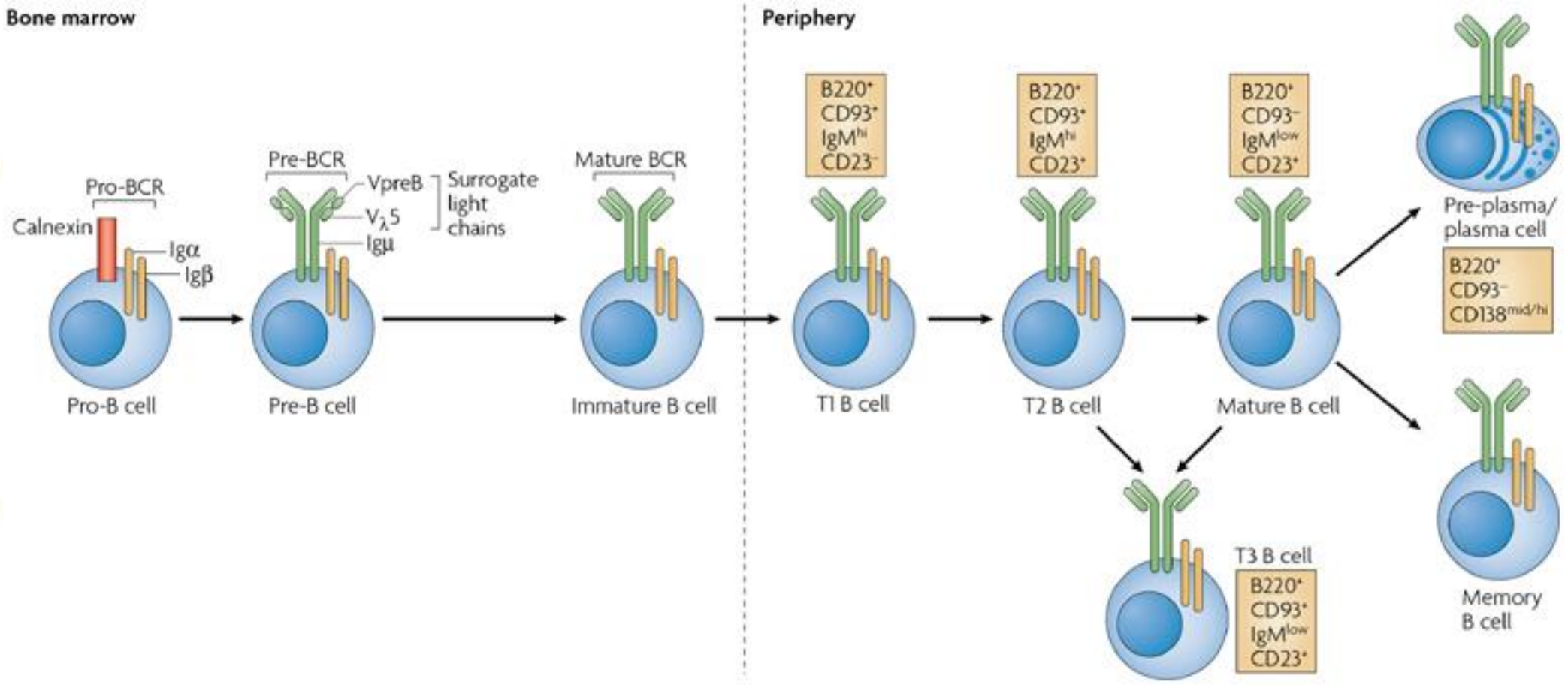
- 40-50% des LLC B expriment ZAP70, facteur de mauvais pronostic

- L’engagement du BCR des LyB LLC ZAP70+ active ZAP70 et certaines voies du BCR

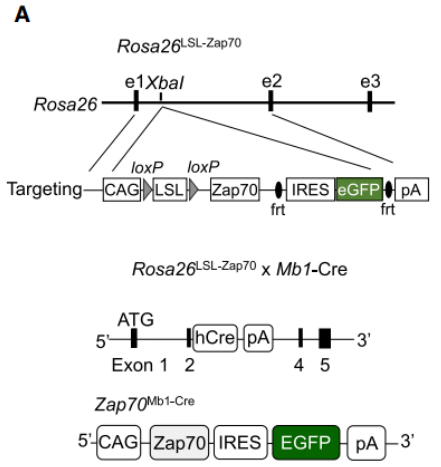
Expression de ZAP70 dans LyB normaux ?



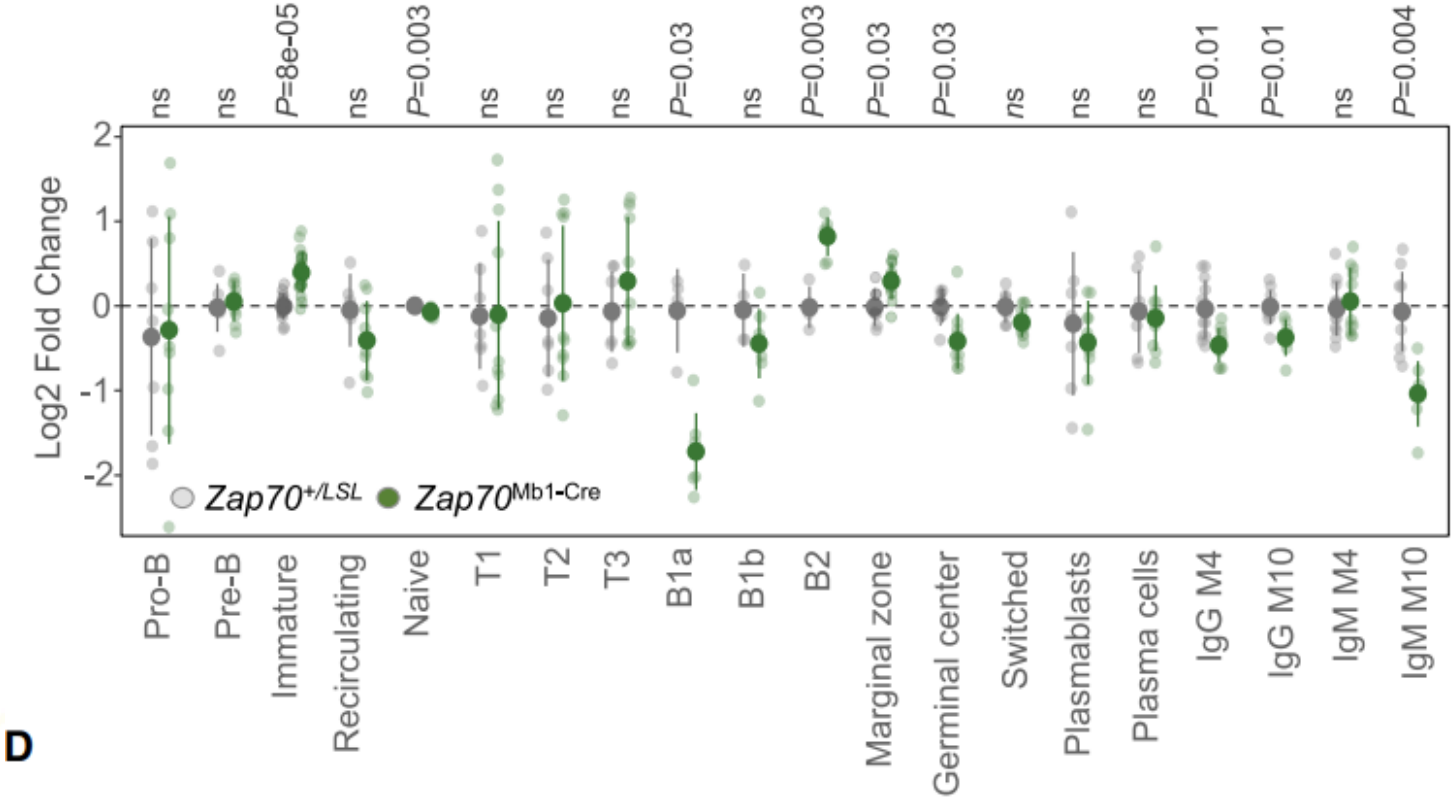
Sadras T… MartinT, Molecular Cell 2021


Bone marrow
Periphery
Pro-BCR
Calnexin
Igα
Igβ
Pre-BCR
VpreB
Vλ5
Igμ
Surrogate light chains
Mature BCR
Pro-B cell
Pre-B cell
Immature B cell
B220+ CD93+ IgMhi CD23−
T1 B cell
B220+ CD93+ IgMhi CD23+
T2 B cell
B220+ CD93− IgMlow CD23+
Mature B cell
Pre-plasma/ plasma cell
B220+ CD93− CD138mid/hi
Memory B cell
T3 B cell
B220+ CD93+ IgMlow CD23+
Nature Reviews | Immunology

Un modèle de souris exprimant ZAP70 dans les LyB repoduit des anomalies observées dans la LLC

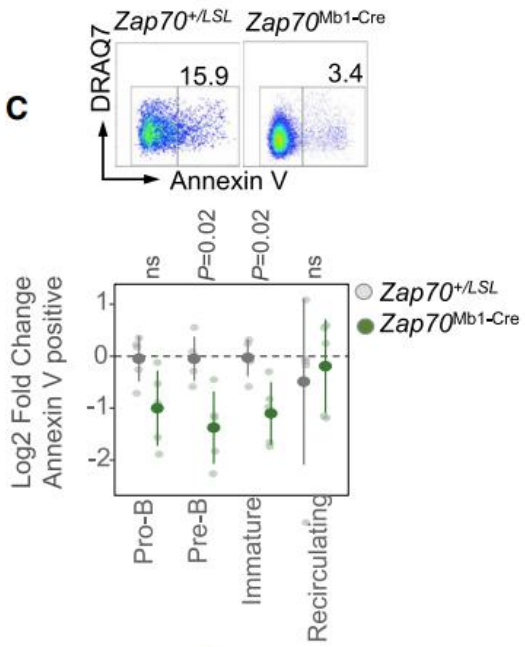


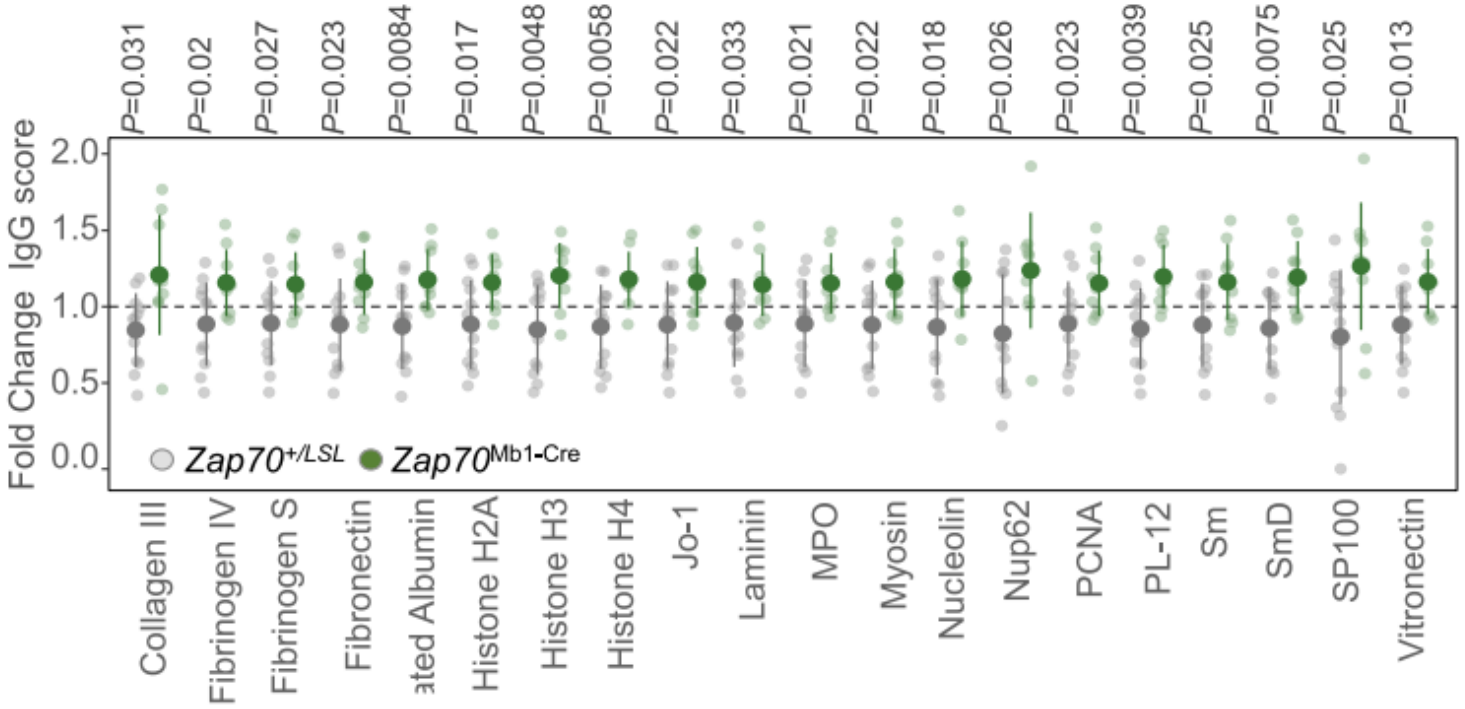
- Ne développent pas de lymphoprolifération maligne : Vie trop courte ?



- ↑Immatures
- ↘ B1 et ↑ MZ : signaling BCR
- Hypogammaglobulinémie

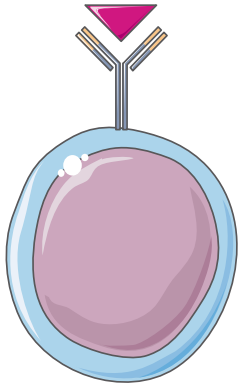
L'expression de ZAP70 dans les LyB entrave la tolérance centrale et la sélection négative des B

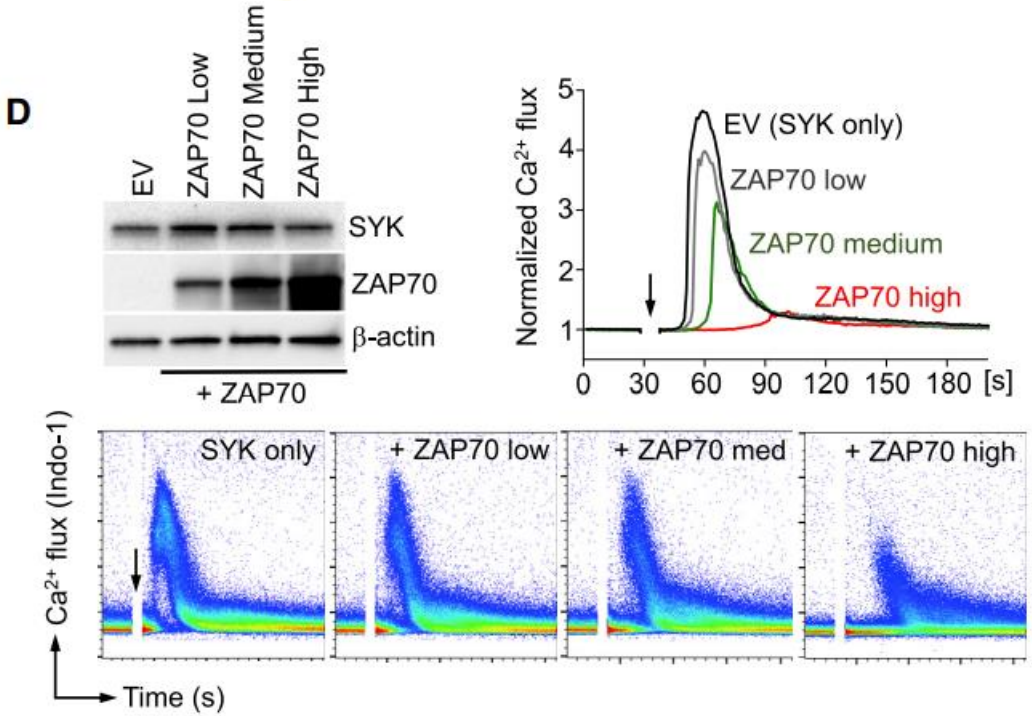




↘Apoptose spontanée des préB/immatures

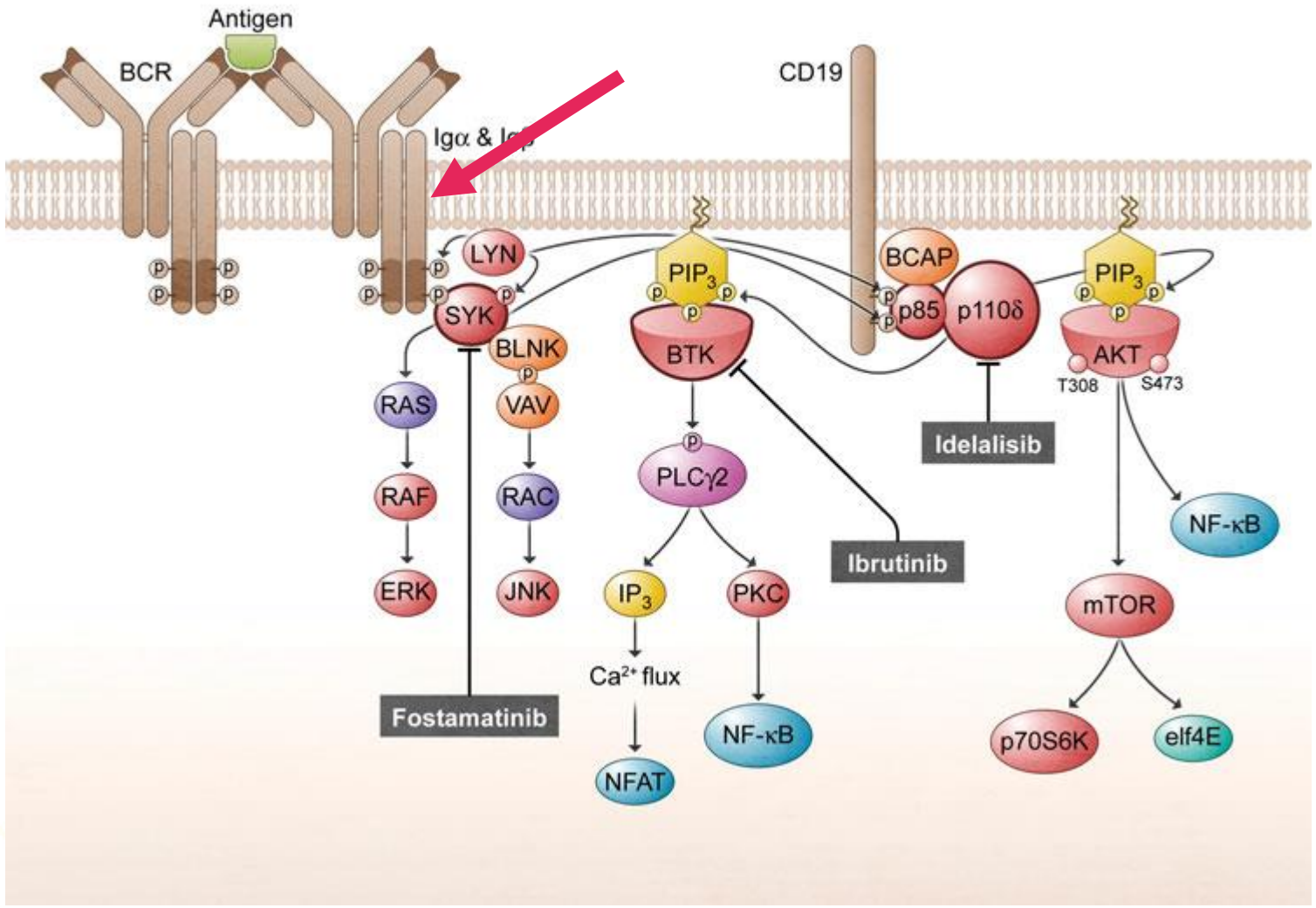
L'expression de ZAP70 dans le LyB inhibe le signaling du BCR



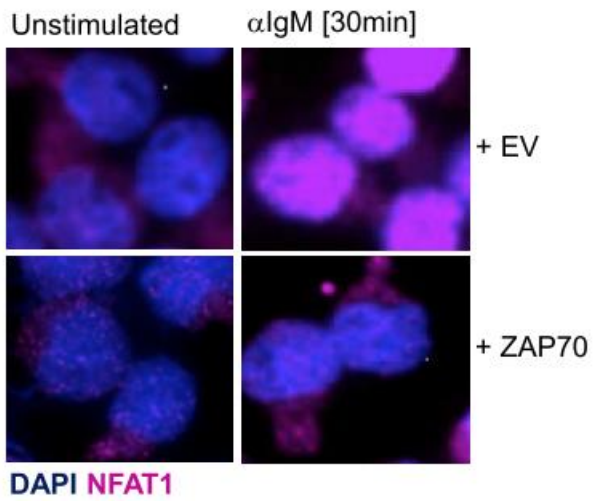


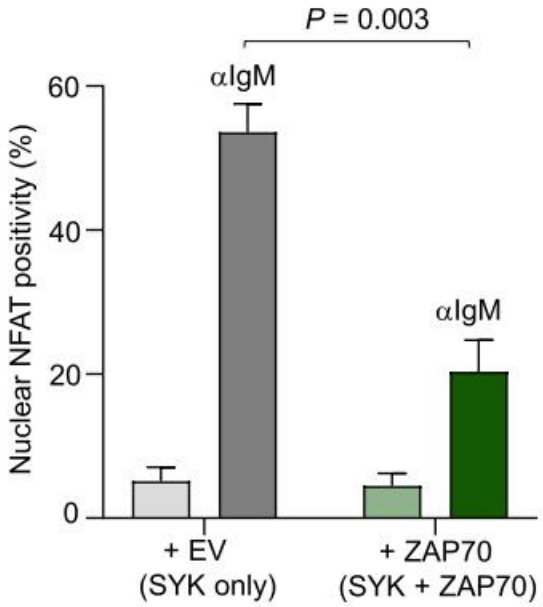
L'expression de ZAP70 dans le LyB in vivo

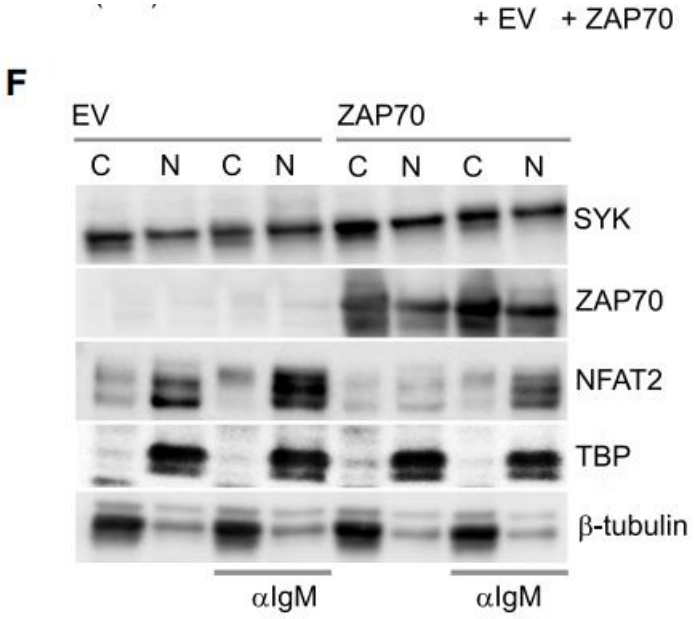
- Entraine une hypogammaglobulinémie
- Entraine des anomalies de tolérance centrale B
- Favorise un répertoire autoréactif
- Entrave le signal du BCR


Antigen
BCR
Igα & Igβ
CD19
LYN
SYK
BLNK
VAV
RAS
RAF
ERK
RAC
JNK
PIP3
BTK
PLCγ2
IP3
PKC
Ca2+ flux
NFAT
NF-κB
BCAP
p85
p110δ
AKT
T308
S473
mTOR
p70S6K
elf4E
Fostamatinib
Ibrutinib
Idelalisib

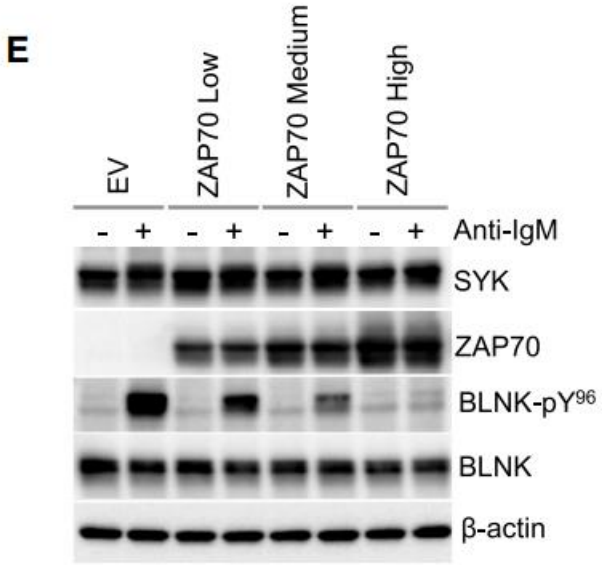
L'expression de ZAP70 entrave la translocation nucléaire de NFAT

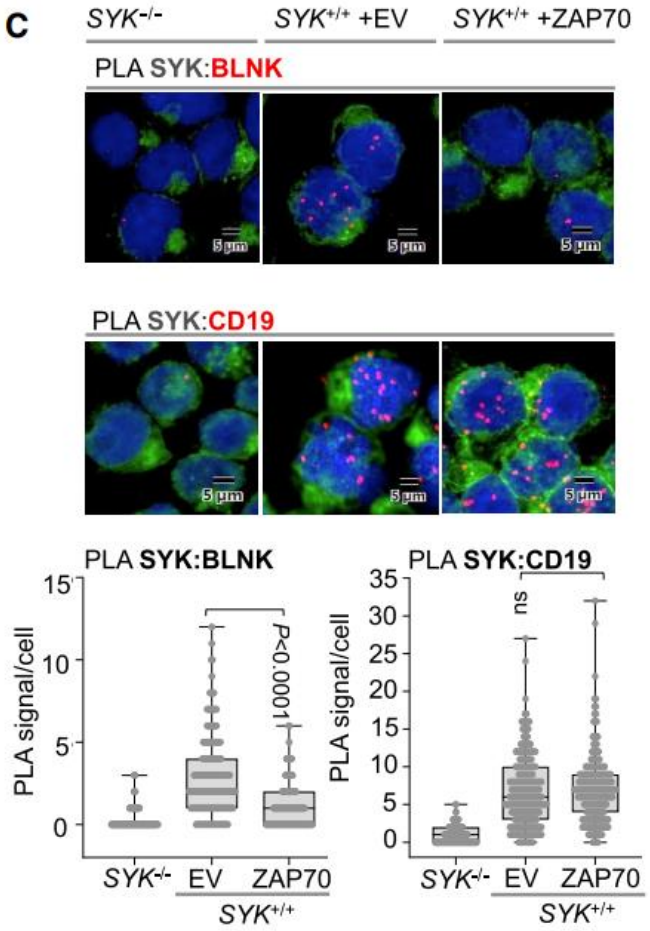




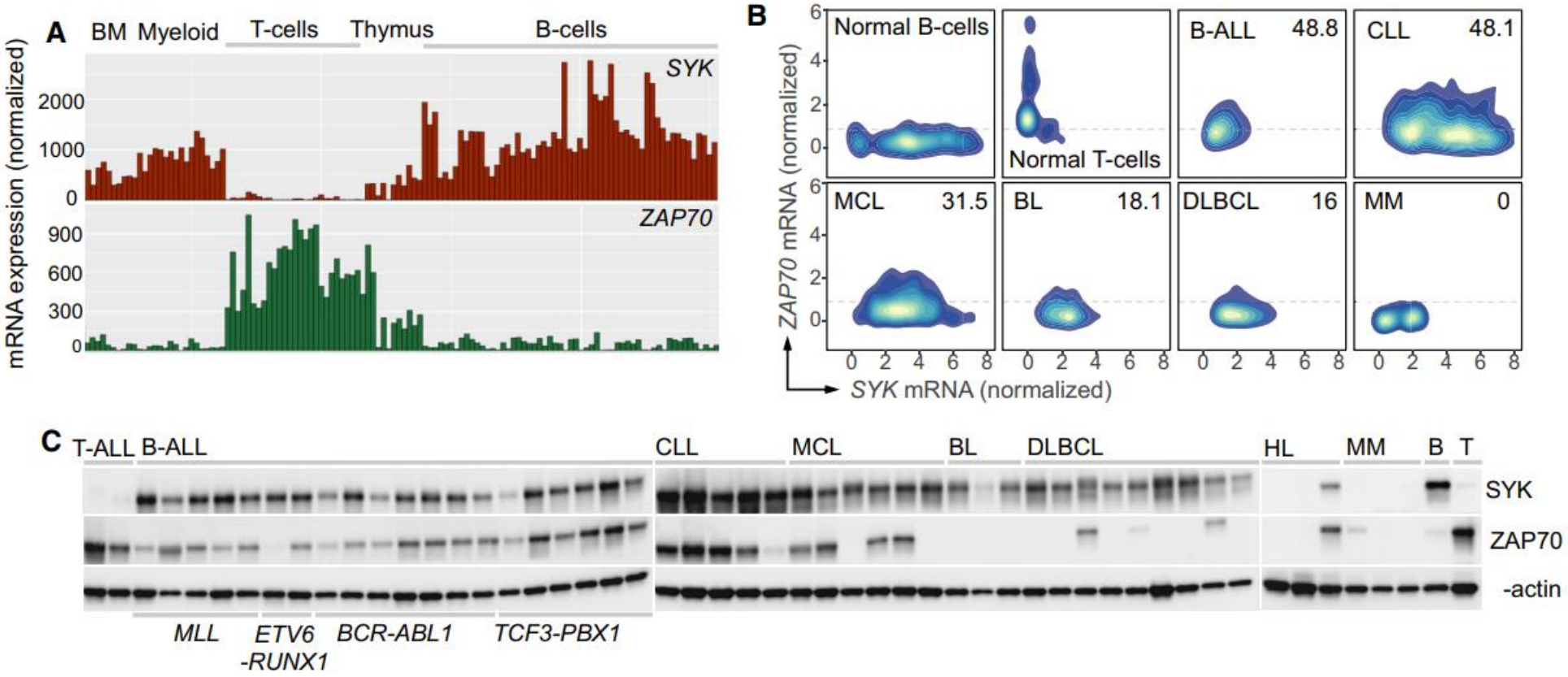


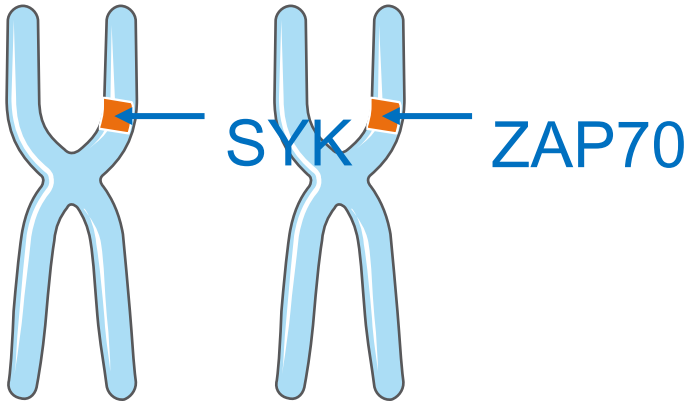
ZAP70 dévie l'activation de SYK de la voie BLNK-BTK vers PI3K-CD19





D'autres lymphoprolifération B malignes expriment ZAP70

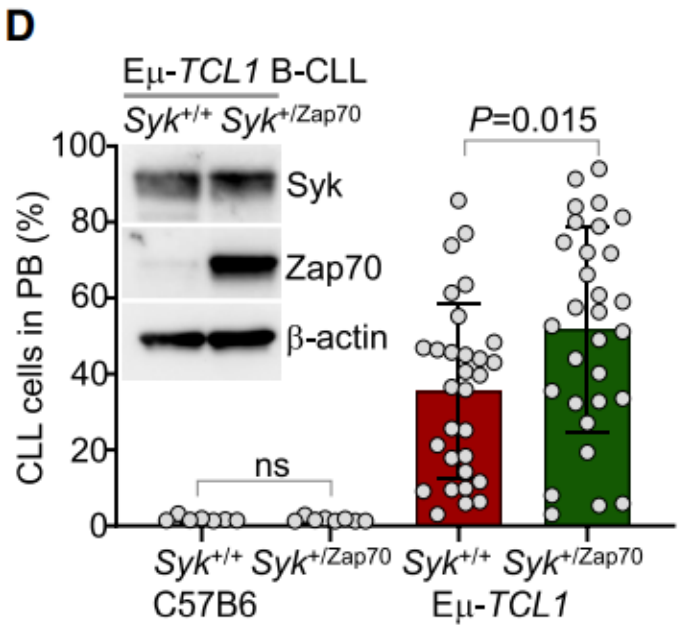


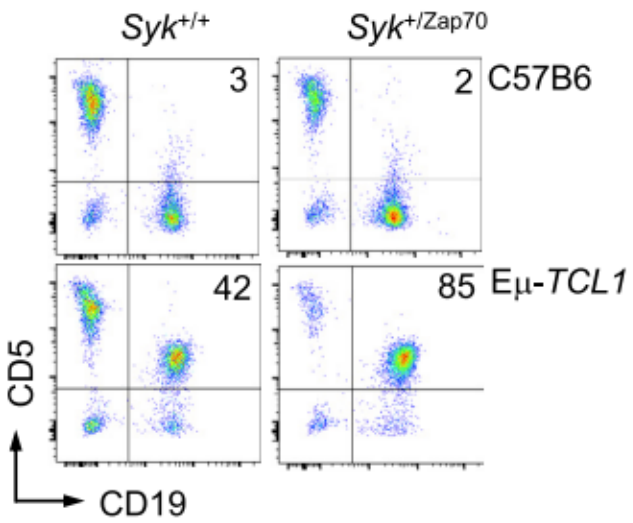


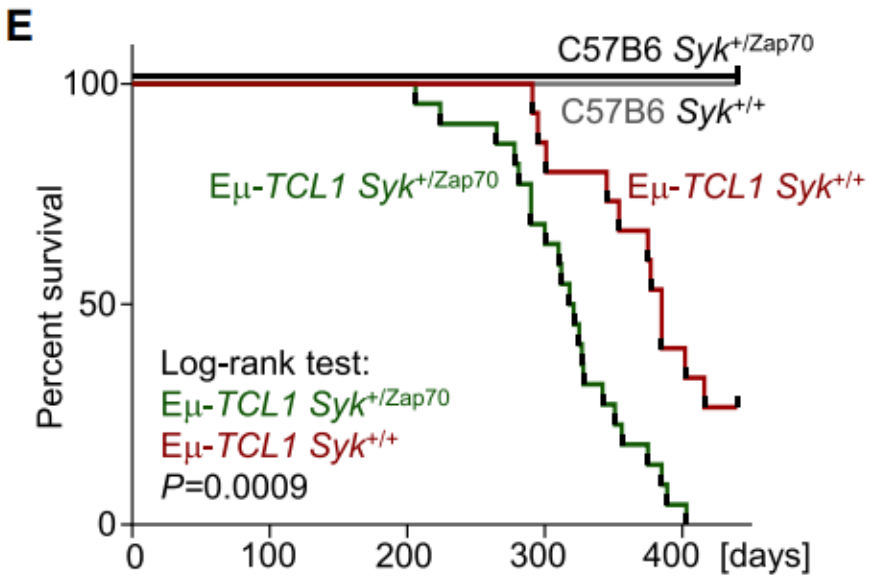
Eμ-TCL1 B-CLL

Bichi et al PNAS 2002

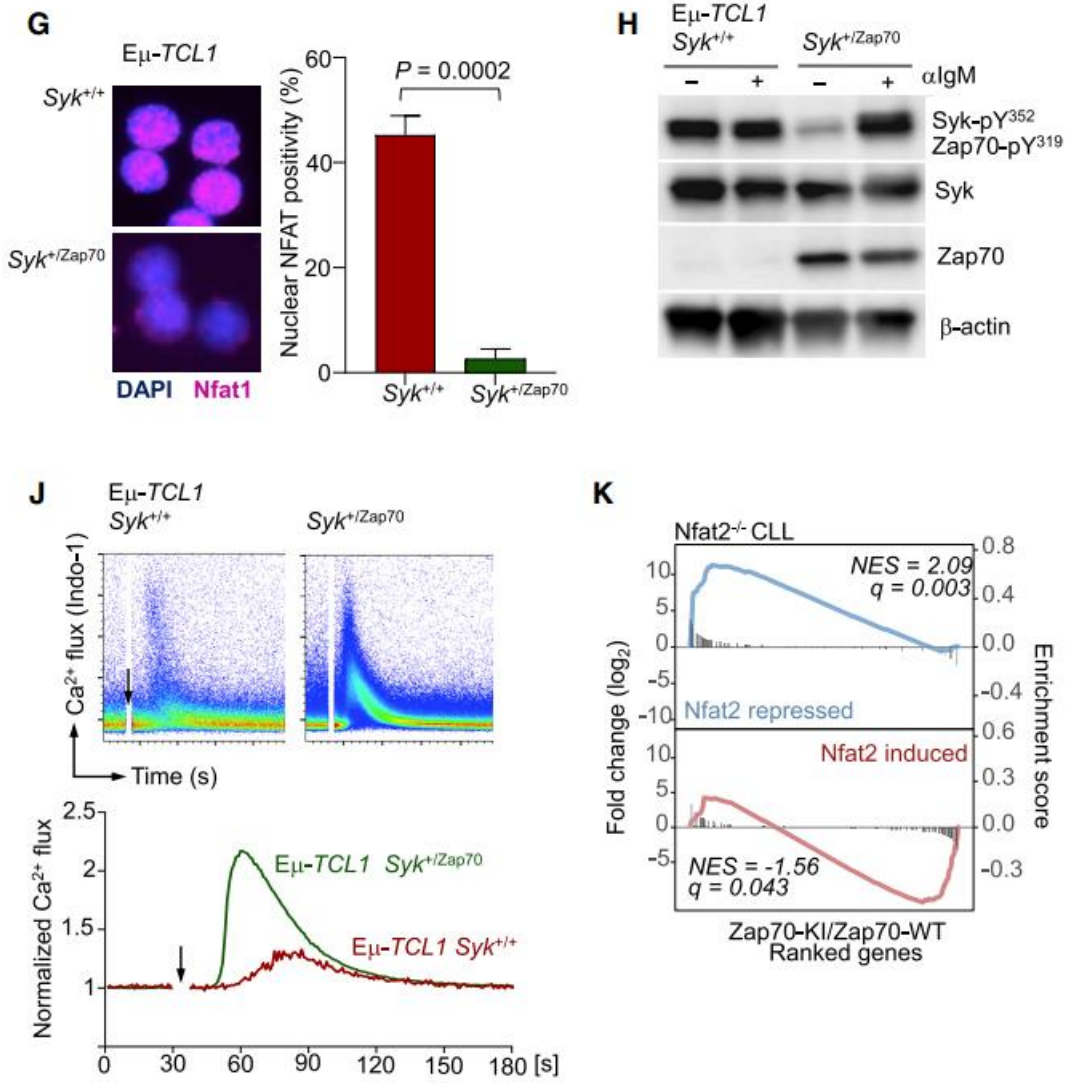
L'expression aberrante de ZAP70 contribue à la transformation néoplasique

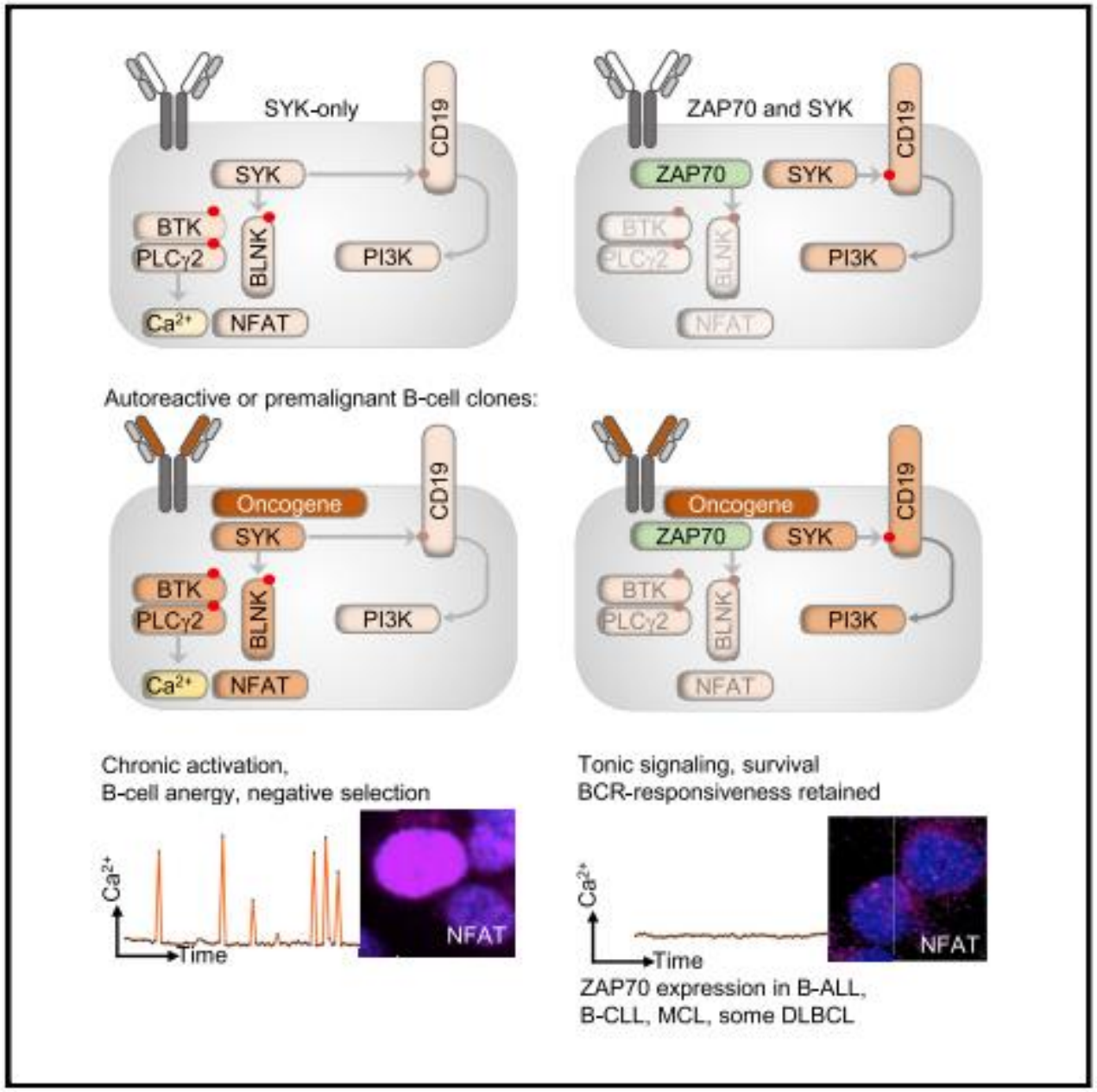






- Celulles de LLC ont phénotype anergique
- Activation NFAT rôle +++ dans induction anergie
- ZAP70 bloque voie NFAT et bloque l'anergie




SYK-only
CD19
SYK
BTK
PLCγ2
BLNK
PI3K
Ca2+
NFAT
ZAP70 and SYK
CD19
ZAP70
SYK
BTK
PLCγ2
BLNK
PI3K
NFAT
Autoreactive or premalignant B-cell clones:
Oncogene
CD19
SYK
BTK
PLCγ2
BLNK
PI3K
Ca2+
NFAT
Oncogene
CD19
ZAP70
SYK
BTK
PLCγ2
BLNK
PI3K
NFAT
Chronic activation,
B-cell anergy, negative selection
Ca2+
Time
NFAT
Tonic signaling, survival
BCR-responsiveness retained
Ca2+
Time
NFAT
ZAP70 expression in B-ALL,
B-CLL, MCL, some DLBCL

conclusions

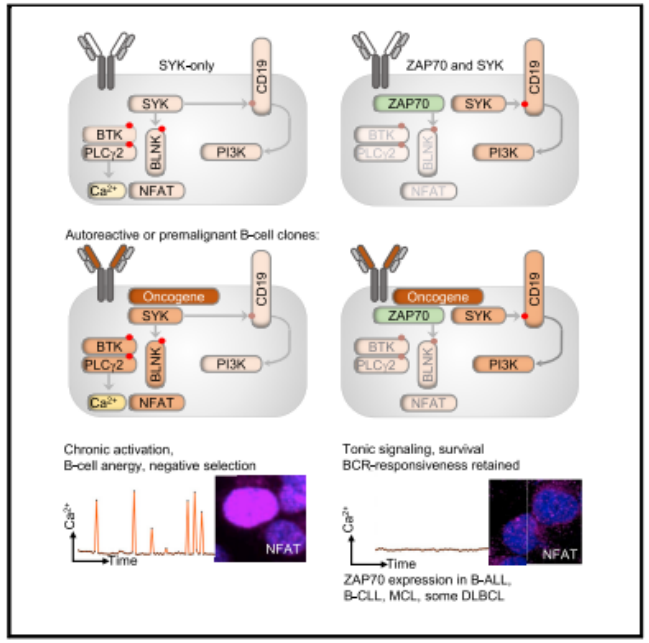
- ZAP70 est exprimée de façon aberrante dans une série de lymphoproliférations B malignes
- L'expression de ZAP70 interfère la sélection négative des B autoréactifs
- ZAP70 détourne SYK de l'anergie dépendante de NFAT vers des signaux de survie dépendants du signaling de PI3K
- ZAP70 prévient l'anergie dépendant de NFAT et accélère la transformation B
- ZAP70 peut être exprimée dans des B non tumoraux: rôle dans l'autoimmunité ?

Molecular Cell

Article

Developmental partitioning of SYK and ZAP70 prevents autoimmunity and cancer

Graphical abstract



Authors

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eric.meffre@yale.edu (E.M.), thierry.martin@chru-strasbourg.fr (T.M.), markus.muschen@yale.edu (M.M.)

In brief

Although SYK and ZAP70 are segregated in normal B and T lymphocytes, Sadras et al. show that aberrant ZAP70 coexpression is common across multiple B cell malignancies. By competing with SYK, ZAP70 diverts BCR signaling from NFAT to PI3K activation, which disables mechanisms of negative selection of autoreactive or premalignant B cell clones.

Highlights

- ZAP70 is aberrantly coexpressed with SYK in a broad range of B cell malignancies
- Forced Zap70 expression *in vivo* disrupts negative selection of autoreactive B cells
- ZAP70 diverts SYK from NFAT-dependent anergy to PI3K-dependent survival signaling
- Zap70 prevents NFAT-dependent anergy and accelerates B cell transformation