Supplementary Appendix

Adult T-ALL treatment protocols

Of the 431 adult T-ALLs included in our study, 225 were treated within the prospective multicenter LALA-94 or GRAALL-2003/2005 protocols. [1-3] The remaining adult T-ALL patients were treated according to LALA/GRAALL but off-protocol, on GRAALL pilot protocols or within the latest GRAALL05 protocol, for which clinical details have not yet been released, or on pediatric FRALLE or EORTC protocols [4, 5] and a few cases were treated on other regimens, including 4 TCR- *CALM-AF10*+ T-ALLs treated as AML and one as lymphoma (with CHOP).

ETP-ALL diagnosis

ETP-ALL diagnosis was based on its distinctive immunophenotype characterized by an absence (<5% positive lymphoblasts) of CD1a and CD8 expression, absence or weak CD5 expression with less than 75% positive lymphoblasts, and expression of one or more of the following myeloid or stem-cell markers on at least 25% of lymphoblasts: CD117, CD34, human leucocyte antigen (HLA)-DR, CD13 and CD33, as defined in pediatric ETP-ALL. [6] Immunophenotype analyses were performed centrally or reviewed and confirmed at Necker Hospital.

TCR-HOXA fluorescence in situ hybridization

We previously reported a *CALM-AF10* positive T-ALL (Table S2, patient UPN3749) with co-existing t(7;14)(p15;q11) leading to a *TCRδ-HOXA* fusion. [7] In this study we screened *CALM-AF10* positive cases for *TCRβ-HOXA* and *TCRδ-HOXA* rearrangements. FISH screening for *TCR-HOXA* rearrangements was performed using two different tetramethyl rhodamine-labeled BAC clones: RP11-114L10 and RP11-1084E14 for *TCRβ*, CTD-2552B11 and RP11-

1083M21 for *TCRδ* followed by hybridization with fluorescein isothiocyanate-labeled BAC clones RP11-1136C8 and 1132K14 covering the *HOXA* locus. *TCRδ-HOXA* or *TCRβ–HOXA* rearrangements generated fusion signals.

Statistical analyses

Binary variables were compared with the Fisher's exact test. The Mann-Whitney test was used for median comparisons. Estimation of event-free survival (EFS) and overall survival (OS) curves used the Kaplan Meier method [8] then compared by the log-rank test [9] . *P* values lower than 0.05 was considered to indicate statistical significance. All calculations were performed using the STATA/SE software, version 10.0 (Stata Corporation, College Station, TX, USA) and the R software, version 1.5.1 (The R Development Core Team, A Language and Environment Copyright, 2002).

Supplementary Tables

Table S1. ***CALM-AF10*** **real-time quantitative RT-PCR**

Primer/Probe	Locus	Primers and Probes sequence (5'-3')
CALM (Forward)	exon 16	GATGACTTGGATTCATCTTTAGCCA
CALM (Probe)	exon 16	TTGTGGGCAATCTTGGCATCGGA
CALM (Forward)	exon 19	TCCTCCACAAATGGGAAGTGTT
CALM (Probe)	exon 19	TGGAGGTCTCATGACAGGCTGGCTGT
AF10 (Reverse)	exon 4	GCCCAACCCCCATTATCTGT
AF10 (Reverse)	exon 7	CACAGTATTGGACATTATCGGCA
AF10 (Reverse)	exon 10	TGCAGTAGTATCTTCCAAGCGCT

We used the Abelson gene (*ABL*) as a housekeeping gene. cDNA was considered to be of acceptable quality for molecular analysis if the *ABL* Cycle threshold was <30. Two CALM-AF10 multiplex RTQ-PCR were performed. One using the forward primer and probe located in *CALM* exon 16 and the second using the forward primer and probe located in *CALM* exon 19. For both multiplex RTQ-PCR, 3 reverse primers were designed to anneal to *AF10* exons 4, 7 and 10 respectively. Reaction mixtures of 25 µl contained 12.5 µl of Mastermix Universal (Applied Biosystems), 0.3µM of each primer and 0.2µM of probe. The amplification protocol consisted of 10 min at 95°C, followed by 45 cycles of 15 s at 95°C and 1 min at 65°C. Real-time analysis was performed on the ABI PRISM 7900HT and all samples were tested in duplicate.

Table S2. Karyotypes of *CALM-AF10* positive T-ALLs

UPN	Karyotype
Adult cases	
4336	46,XX,t(10;11)(p12-13;q13-14)[18]
10740	48,XX,add(1)(p36.3),t(10;11)(p13;q21),+marx2[18]/46,XX[3]
4621	46,XY[16]
1334	46,XY,del (5)(q14q35),+9,t(11;17)(p12-13;q11),-15[20]
7962	46,XY,t(10;11)(p13;q14)[20]
9829	Failed
1488	45,XY,-7[19]
10533	46,XX,del(5)(q23q34),t(9;12)(p24;p13),t(10;11)(p12;q23)[11]/46,XX[9]
10562	n/a
9803	46,XX,t(1;13)(p33;q3?4),del(5)(q14q21),-8,del(9)(p12q2?), t(10;11)(p13;q21),del(15)(q2?3),+?20[22]/46,XX(3]
3471	46,XY[3]/46,XY,t(10;11)(p13;q14)[17]
7754	47,XX,-5,-7,?t(10;11)(p13;q21),-13,-14,-15,+6mars[18]
634	46,XY[20]
1439	46,XY,ins(10;11)(p14;q14q25)[15]
1666	46,XY,-6,t(10;11)(p14;q14-21),-20,+2mar[38]
4396	46,XX,t(10;11)(p12-13;q13-14)[10]
10354	n/a
2577	Failed
3188	46,XY,del(8)(q?22),t(10;11)(p13;q14)[4]/46,XY[2]
3472	46,XX[18]/46,XX,add(9)(p13),t(10;11)(p14;q14)[5]
3489	46,XY[4]/46,XY,t(10;11)(p13;q14)[11]
3749	46,XY[9]/46,XY,t(10;11)(p14;q21),t(7;14)(p15;q11),add(18)(q23)[11]
4158	45,X,-Y,del(1)(q41),del(11)(q21),add(14)(q32),i(17)(q?10)[12] /46,XY[14]
5105*	46,XX,add (1p?), add(5)(q?),del(7)(p?)[10]
5848*	Failed

7771	87-92,XXXX,der(1)dup(1)(q12qter)inv(1p1q)x2,inc[19]/46,XX[1]
7783	46,XY,t(10;11)(q13;q14)[2]/47,XY,idem,+8[13]/48,XY,idem,+8,+9[18]
8389	46,XY[25]
9266	46,XX[14]
9966	46,Y,der(?X)add(X)(p?)add(X)(q?),der(1)t(1;?5)(p10;?),der(5)t(5;5)[20]
Pediatric cases	
738	47,XX,+8,t(10;11)(p12-p14;q14-q21)[8]
10356	Failed
4516	46,XX,del(1)(p34),t(3;10;11)(q25;p12;q21),del(7)(p15p13),?del(22)(q13)[15]
1978	92,XXYY,t(10;11)(p14;q14),i(?17q)[13]
94	Failed
269	46,XX[20]
2726	Failed
4092	46,XX,t(10;11)(p13-14;q14-21)[13]
4105	46,XY,del(3)(q12q26),add(4)(q28),add(5)(q23),del(6)(q15),der(9)t(9;?)(p21;?),t(10;11)(p13;q14),-13,+mar[7]
4614	46,XY[20]
5259	Failed
8291	46,XY[20]
10255	n/a
10355	n/a
4562	46,XY[20]

Twenty T-ALLs were previously published under the same UPN (underlined)[10]. We previously reported the patient UPN3749 that had a t(10;11)(p14;q21)/*CALM-AF10* and a t(7;14)(p15;q11) leading to a *TCRδ-HOXA* fusion [7]. Here, FISH analyses identified the coexistence of *TCRβ-HOXA* and *CALM-AF10* in 2 cases UPN5105 and UPN5848 (indicated by asterisks).

Table S3. Characteristics and clinical course of *CALM-AF10* positive T-ALLs

UPN	Sex/age(y)	CA transcript	TCR subset	WBC	Clinical course	NOTCH1	FBXW7	ETP	cCD3	sCD3	CD1a	CD8	CD5	CD34	CD117	CD13	CD33	HLA-DR
Adult cases																		
4396£	F/20	3'	IMγ	49	CR-BMT, Rel, D (18 mo)	n/a	n/a	yes	81	0	0	0	3	95	67	81	70	41
1488£	M/23	5'	IMγ	9,4	NR, D (3 mo)	n/a	n/a	yes	97	4	1	2	44	92	0	3	60	9
9966*	M/23	3'	IMγ	94,4	CR, Rel, D (9 mo)	GL	GL	yes	98	0	0	0	51	84	70	0	22	0
7783*	M/23	n/a	IMγ	8,9	CR-BMT, Rel, D (24 mo)	GL	GL	yes	90	13	0	10	34	77	0	80	95	88
7962**	M/24	5'	IMδ	1,6	CR-BMT, D (6mo)	n/a	n/a	n/a	81	1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
1666$	M/24	3'	IMγ	10	CR, Rel, BMT, Rel, D (18 mo)	n/a	n/a	yes	97	0	4	3	32	0	3	58	89	n/a
4336£	F/25	3'	IMδ	220	CR-BMT, Rel, D (11 mo)	n/a	n/a	no	78	0	71	0	91	82	0	0	0	0
1439£	M/25	3'	IMγ	22	CR, Rel, D (18 mo)	GL	GL	yes	75	4	3	2	5	80	0	8	62	n/a
634*	M/26	5'	IMγ	11	CR, Rel, D (31 mo)	Mut	GL	yes	96	5	9	6	53	1	0	1	3	n/a
10562**	F/28	5'	IMγ	2,2	CR,Rel , D (11 mo)	GL	GL	yes	99	0	1	5	12	51	4	1	85	92
3188*	M/28	3'	IMγ	33	CR-BMT, Alive 50 mo	Mut	Mut	yes	98	1	0	1	48	11	41	1	0	1
3471*	M/29	n/a	IMγ	2	CR, Rel, D (22 mo)	GL	GL	yes	85	1	1	2	38	79	n/a	2	2	n/a
7754*	F/33	n/a	IMγ	1.2	Early died at in/auction	n/a	n/a	yes	77	1	9	1	41	77	94	93	0	1
3472*	M/37	3'	IMγ	3,3	CR, Rel, D (36 mo)	Mut	Mut	no	86	5	56	3	82	0	n/a	0	0	n/a
7771*	F/37	5'	IMγ	5,8	CR-BMT, Rel, D (23 mo)	GL	GL	yes	99	0	0	0	0	70	0	93	93	n/a
10533**	F/40	3'	IMγ	4,6	CR-BMT, Alive 24 mo	GL	GL	n/a	99	1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
1334**	M/43	5'	IMδ	280	CR, Rel, Lost to follow up (28 mo)	Mut	GL	yes	99	1	0	1	19	57	n/a	38	0	n/a
9803*	F/43	5'	IMγ	2,1	CR-BMT, Rel, D (13 mo)	n/a	n/a	yes	75	0	0	0	43	3	0	0	75	1
4158*	M/43	5'	IMγ	4,5	CR,Rel, D (48 mo)	GL	GL	yes	73	0	9	0	8	5	0	1	6	97
9829*	M/46	5'	IMδ	3,2	CR, Alive 29 mo	GL	GL	no	99	2	4	44	96	1	4	1	2	2
10740#	F/17	3'	IM TCR neg	181	CR, Alive 36 mo	GL	Mut	no	91	1	1	1	98	63	1	1	1	46
10354&	M/17	5'	TCRγδ+	n/a	CR-BMT, Alive 131 mo	Mut	GL	no	99	76	97	97	98	3	1	0	0	1
2577**	M/20	3'	TCRγδ+	30	CR, Alive 64 mo	n/a	n/a	no	87	85	90	0	98	0	0	0	0	0
3749*	M/29	5'	TCRγδ+	42	CR, Alive 93 mo	GL	Mut	no	94	75	0	87	8	14	0	0	0	4
9266*	F/30	5'	TCRαβ+	6,6	CR-BMT, Alive 26 mo	GL	GL	no	99	91	16	4	96	72	0	76	44	n/a
8389*	M/32	3'	TCRαβ+	40,1	CR-BMT, Alive 43 mo	GL	GL	no	88	90	51	1	84	8	47	4	31	n/a

3489*	M/33	5'	TCRγδ+	105	CR-BMT, Alive 54 mo	GL	Mut	no	99	99	17	51	99	0	0	0	0	0
5105**	F/38	3'	TCRγδ+	20,9	CR, Alive 84 mo	Mut	GL	no	96	81	77	5	99	0	0	0	0	1
5848*	M/45	3'	TCRγδ+	56,6	CR, Alive 79 mo	Mut	GL	no	98	76	5	0	92	1	3	0	0	0
4621**	M/29	n/a	n/a	80,6	CR1, Rel, CR2-BMT, Rel, D (35 mo)	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Pediatric cases																		
738	F/12	3'	IMδ	124	CR1, Rel , CR2, Rel, D (105 mo)	n/a	n/a	yes	85	0	0	2	4	32	4	1	0	6
269	F/13	3'	IMγ	556	CR1, Rel, CR2, Rel, D (30 mo)	Mut	GL	n/a	96	5	n/a	2	98	0	0	0	0	n/a
4516	F/14	n/a	IMγ	7,9	CR, Lost to follow up (60 mo)	Mut	Mut	yes	99	0	0	0	45	47	0	57	34	20
1978	M/14	3'	IMδ	20	CR-BMT, Alive 146 mo	n/a	n/a	yes	75	5	4	5	61	81	0	89	92	95
4614	M/15	3'	IMγ	4,45	CR1, Rel, CR2-BMT, Alive 96 mo	n/a	n/a	n/a	91	2	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
94	M/3	5'	TCRγδ+	350	CR, Alive 115 mo	Mut	GL	no	98	99	1	20	99	0	0	0	0	0
10355	M/4	n/a	TCRγδ+	n/a	CR, Alive 47 mo	Mut	GL	no	96	94	67	56	96	0	0	4	82	2
2726	M/6	5'	TCRγδ+	60	CR-BMT, Alive 109 mo	GL	Mut	no	94	75	80	11	90	1	0	0	0	0
4562	M/7	n/a	TCRγδ+	77	CR, Alive 42 mo	n/a	n/a	no	98	91	93	86	97	0	0	0	0	n/a
4092	F/11	5'	TCRγδ+	39	CR, Alive 110mo	n/a	n/a	no	98	98	3	97	96	n/a	1	1	n/a	2
10356	M/14	3'	TCRγδ+	325	CR-BMT, GVHD ,D (38 mo)	GL	GL	no	96	98	18	n/a	99	67	0	0	23	n/a
8291	M/15	5'	TCRγδ+	131	CR, Alive 36 mo	n/a	n/a	no	87	75	86	55	95	n/a	0	5	0	n/a
4105	M/15	5'	TCRγδ+	46	CR-BMT, Alive 91 mo	GL	GL	no	99	64	0	0	99	90	85	96	50	n/a
10255	M/15	5'	TCRγδ+	n/a	CR, Alive 6 mo	n/a	n/a	no	98	64	1	7	94	0	0	1	1	1
5259	M/15	3'	TCRγδ+	n/a	Early Died,	Mut	GL	no	96	96	5	2	96	3	0	1	52	2

Twenty T-ALLs were previously published under the same UPN (underlined)[10]. Treated on * or according to ** LALA or GRAALL trials, as AML £, as non-Hodgkin's lymphoma, with CHOP $, on the pediatric FRALLE 2000 ALL protocol & or on the Children's Cancer Group ALL protocol #. Abbreviations= M: male; F: female; CA: *CALM-AF10;* n/a: not available; TCR: T-cell Receptor; IM: immature; WBC: white blood cell; CR: complete remission; Alive: in complete remission; Rel: relapse; BMT: bone marrow transplantation; D: died; GVHD: graft-versus-host disease; Mut: mutated; GL: germline; ETP: Early T-cell Precursor.

Supplementary Figures

Figure S1. Flow diagram showing molecular and immunophenotypic analyses performed in our cohort

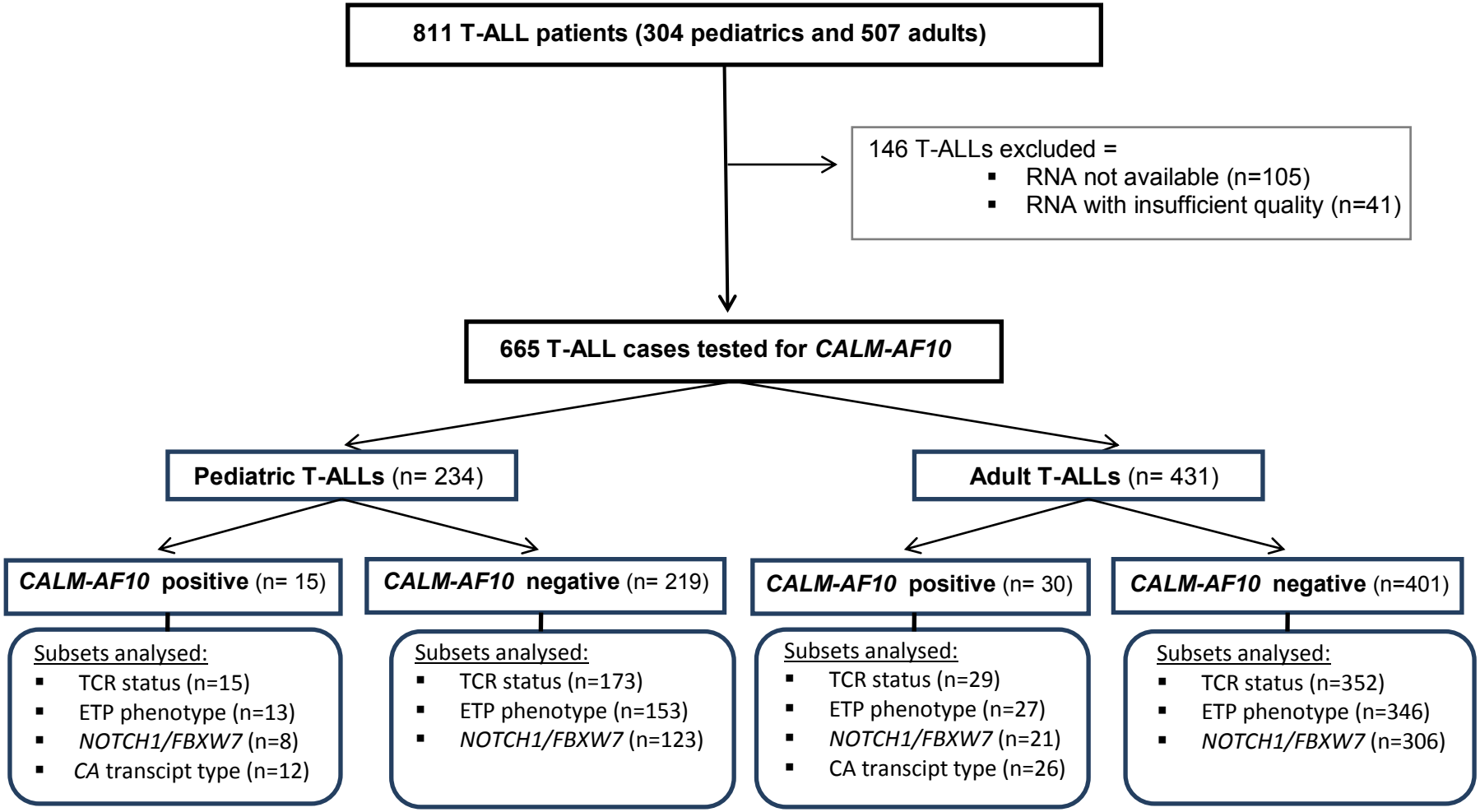
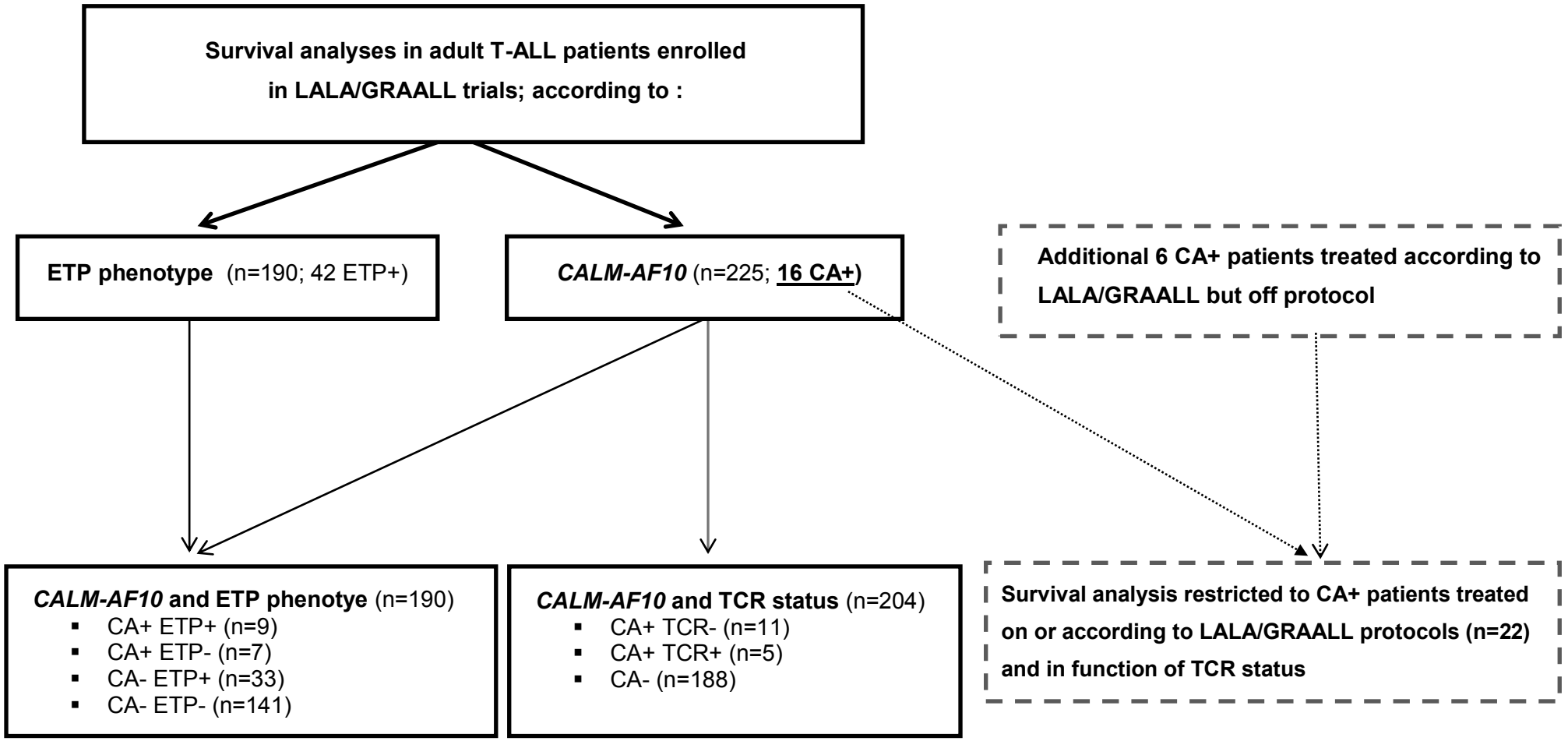
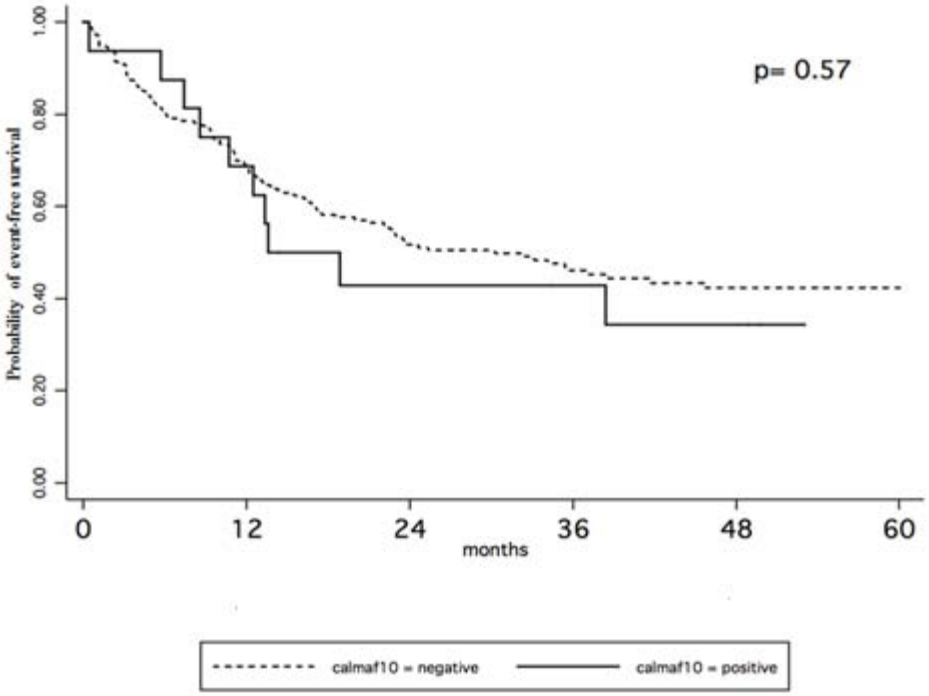


Figure S2. **Schematic representation of survival analyses performed in adult T-ALL patients treated on or according to LALA/GRAALL protocols**

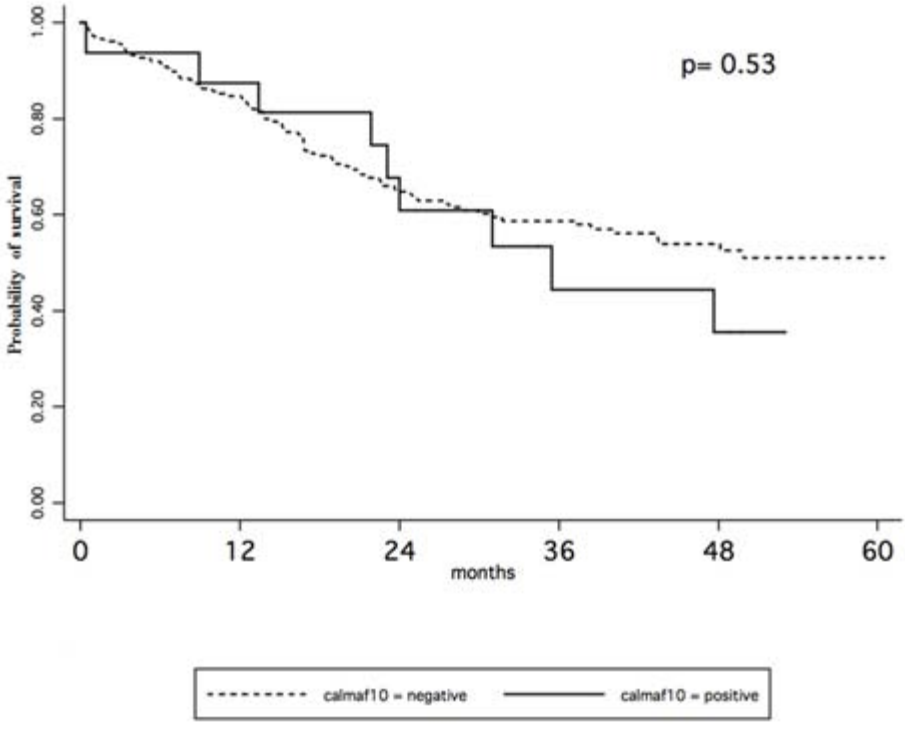


<u>Figure S3.</u> Prognostic impact of *CALM-AF10* in adult LALA/GRAALL T-ALLs. (A) event-free survival and (B) overall survival. No differences were found between the overall *CALM-AF10+* (full line) and *CALM-AF10-* (dotted line) group regarding 3-year event-free survival (43% [95%CI, 19-65] versus 46% [95%CI, 38-53]; p=0.57) and overall survival (44% [95%CI, 18-68] versus 59% [95%CI, 51-66]; p=0.53)

(A)

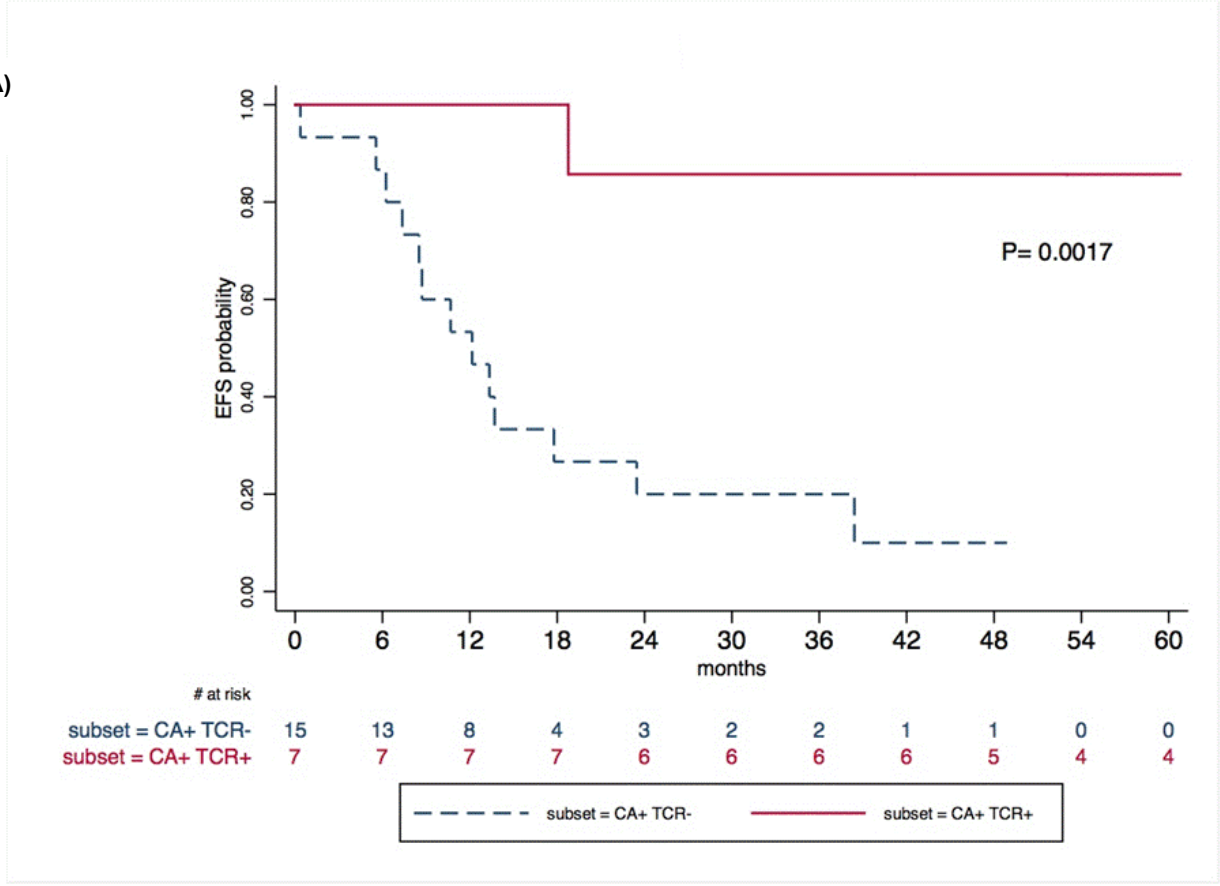


(B)

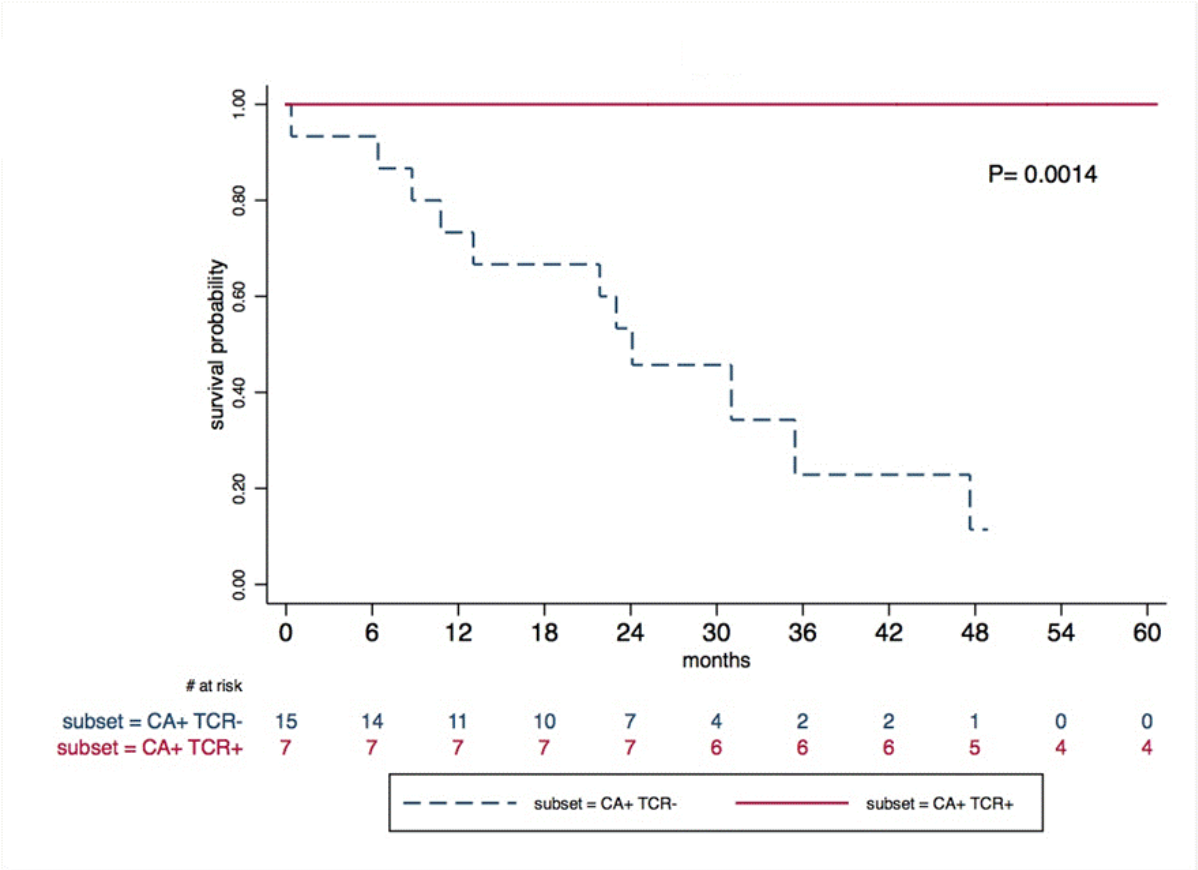


<u>Figure S4.</u> Impact of TCR status on event-free survival (A) and overall survival (B) of 22 *CALM-AF10*+ adult T-ALLs treated on or according to LALA/GRAALL protocols

(A)

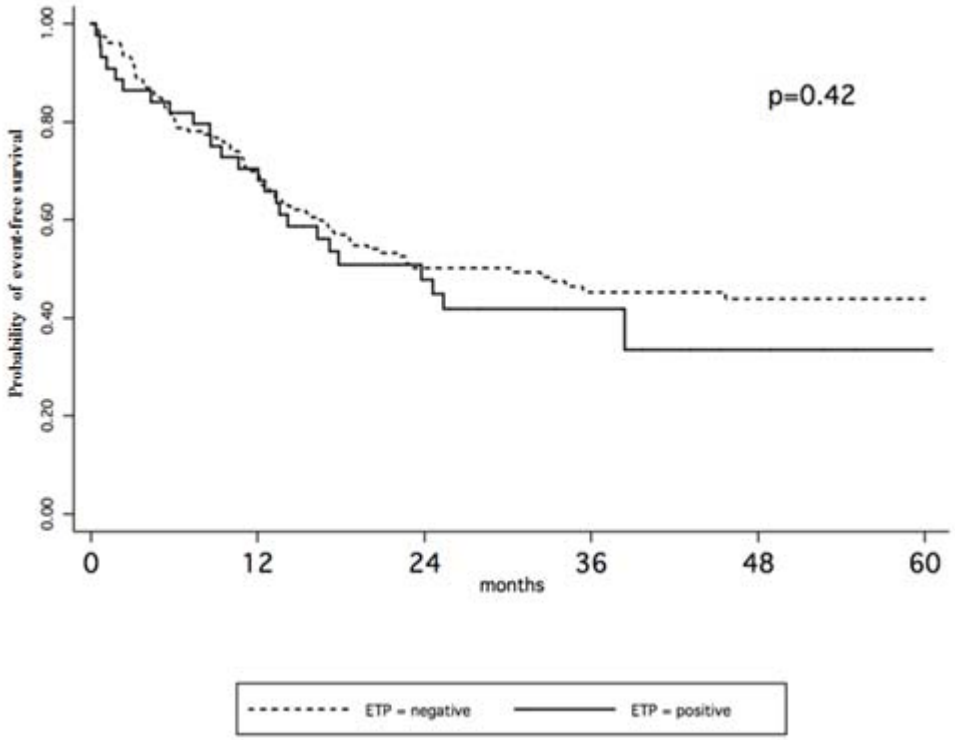


(B)

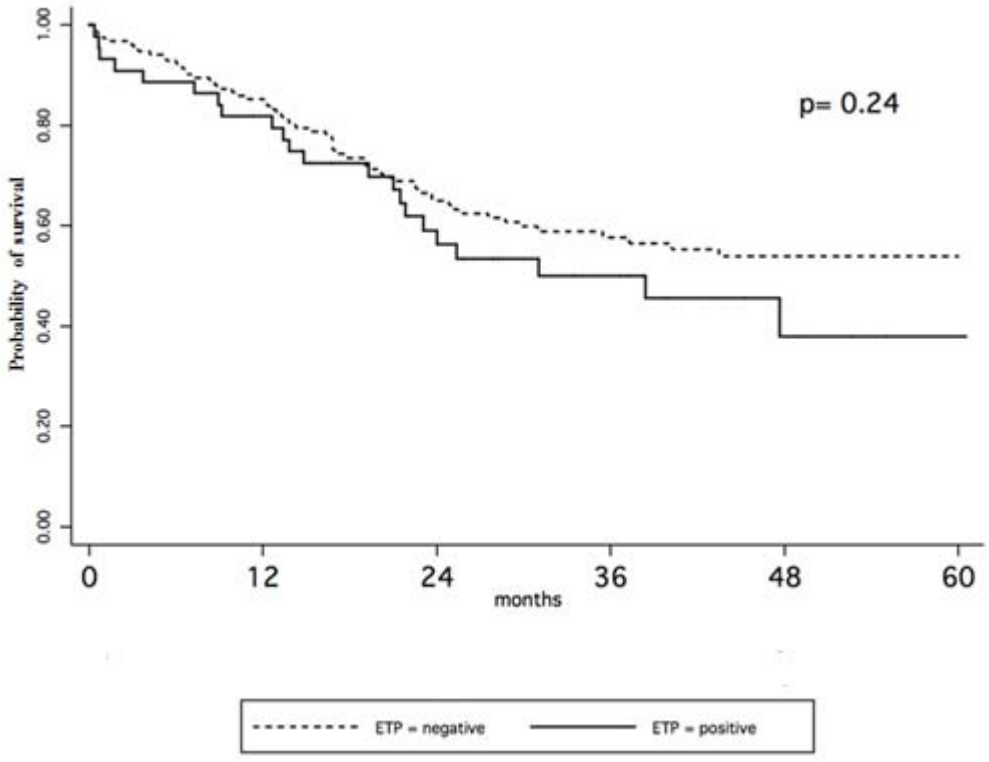


<u>Figure S5</u>. Prognostic impact of ETP phenotype in adult LALA/GRAALL T-ALLs. (A) event-free survival and (B) overall survival. No significant differences were found between ETP-ALL (full line) and non-ETP-ALL patients (dotted line) regarding 3-year EFS (42% [95%CI, 26-57] *versus* 45% [95%CI, 37-54]; p=0.42) and OS (50% [95%CI, 33-65] *versus* 58% [95%CI, 49-66]; p=0.24).

(A)



(B)



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