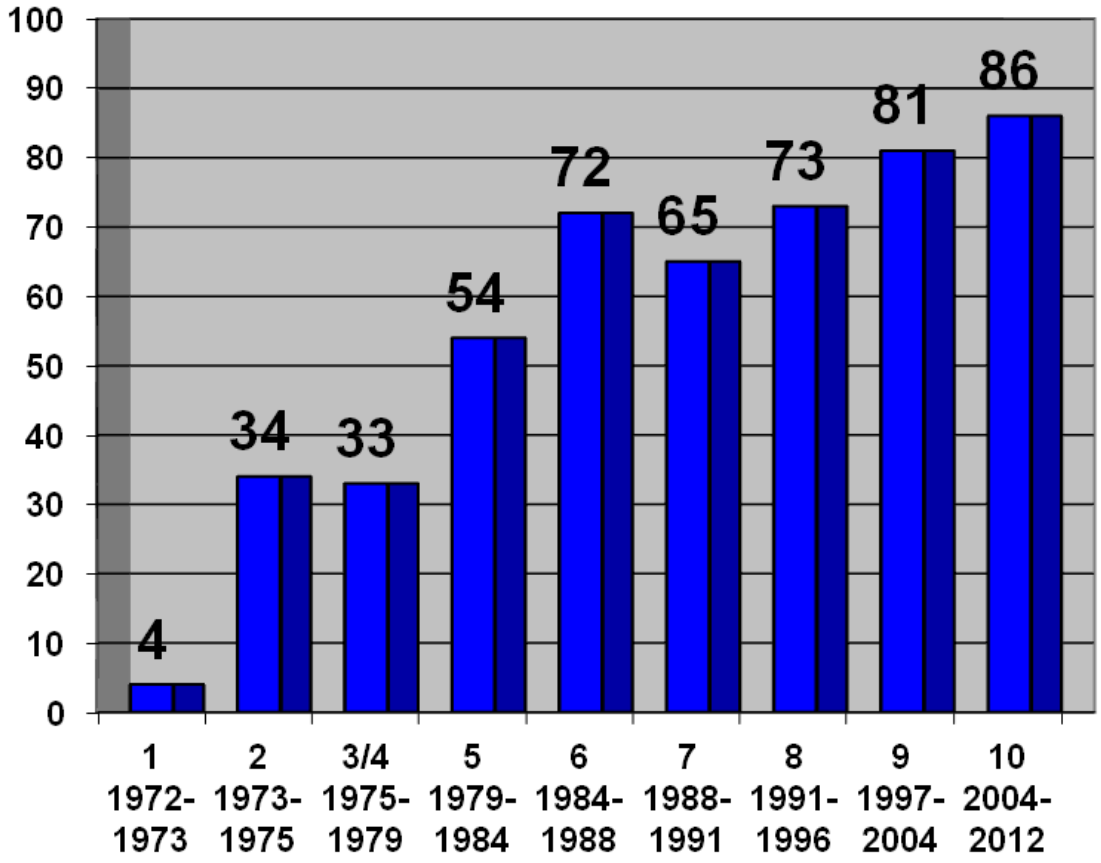
Improving treatment results in childhood acute lymphoblastic leukemia:

Approaches in lab & clinical research

Rob Pieters

5-year EFS of childhood ALL in the Netherlands



Chemotherapy elements

- Remission induction
- Consolidation/CNS treatment
- Reinduction/intensification
- Continuation/maintenance

Minimal residual disease (MRD)

- Rearrangements of Ig and TCT genes in early T-cell and B-cell differentiation leading to “junctional regions” unique for each lymphocyte
- ALL clone originates from one single lymphocyte
- Detection of clonal Ig/TCR rearrangements by PCR in >95% of childhood ALL
- MRD detection during treatment, sensitivity $<10^{e}$-4
- MRD end of induction and end of consolidation has strong prognostic value

Minimal Residual Disease and Outcome in ALL

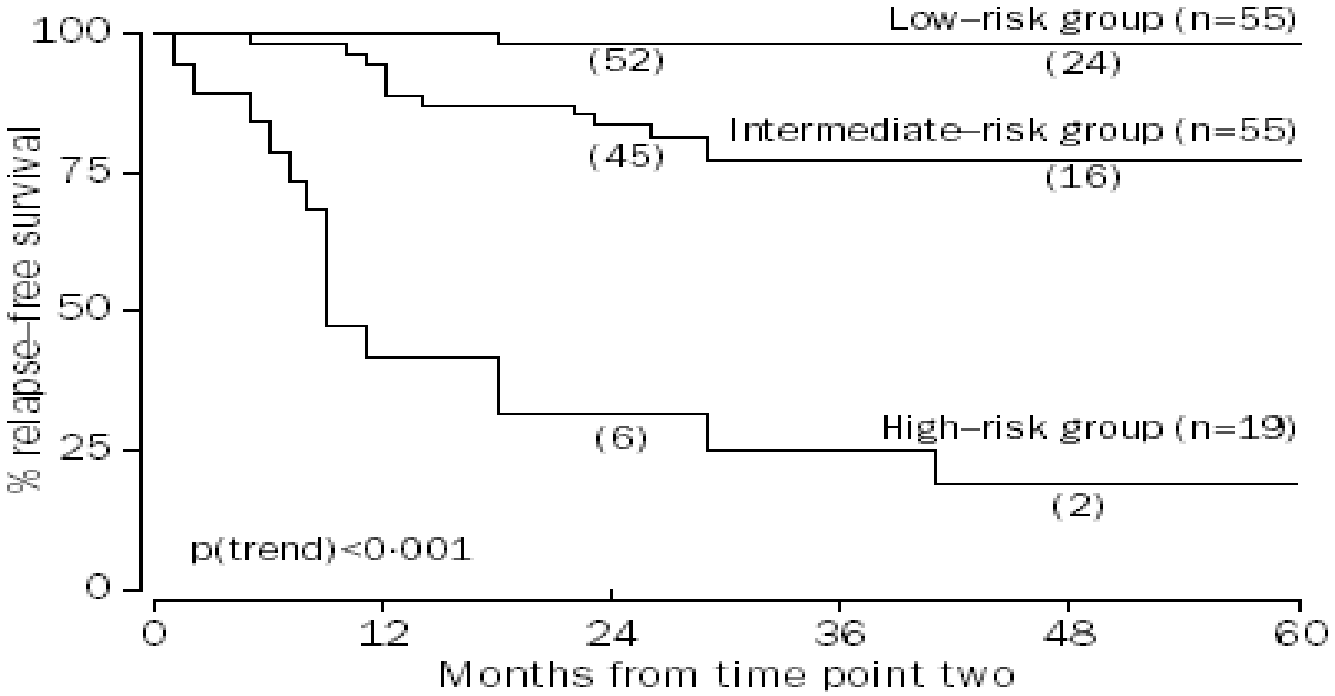
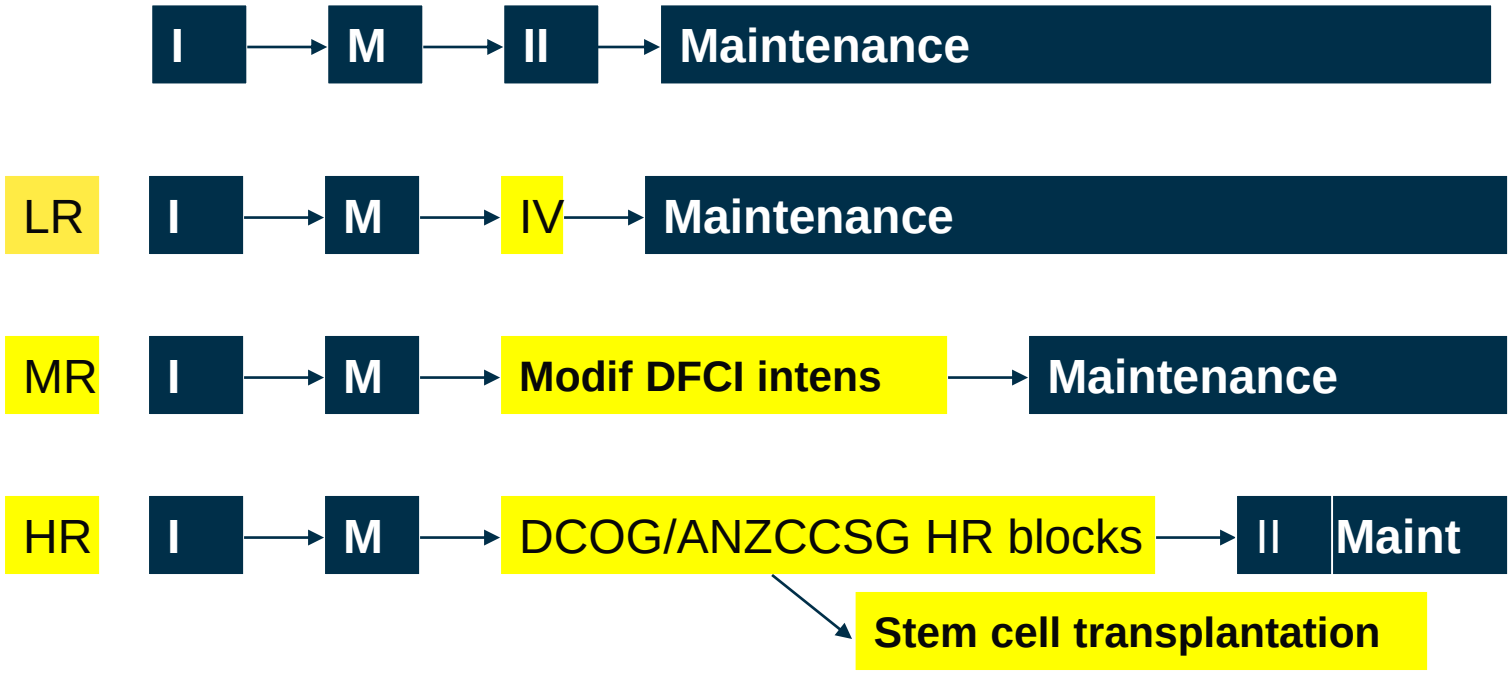


Figure 4: **Relapse-free survival of the three MRD-based risk groups, as defined by MRD information at time points one and two**

Van Dongen, Lancet 1998

ALL-10 protocol outlines



Pieters, J Clin Oncol 2016

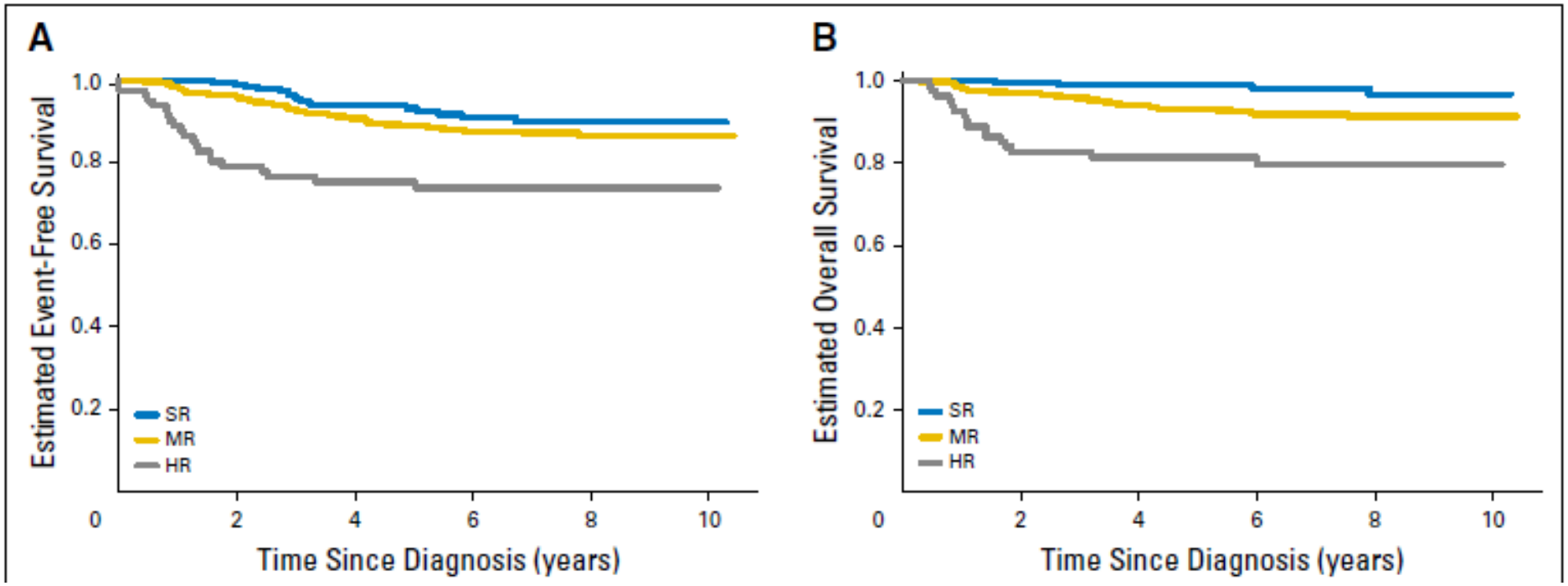
ALL-10 protocol outcome:

1. Therapy reduction in SR is safe; 5-yr survival 99%
2. Intensification in MR: 5yr EFS from 76% to 88%
3. Intensification in HR: 5yr EFS from 16% to 78%

Princess MÁXIMA center for pediatric oncology

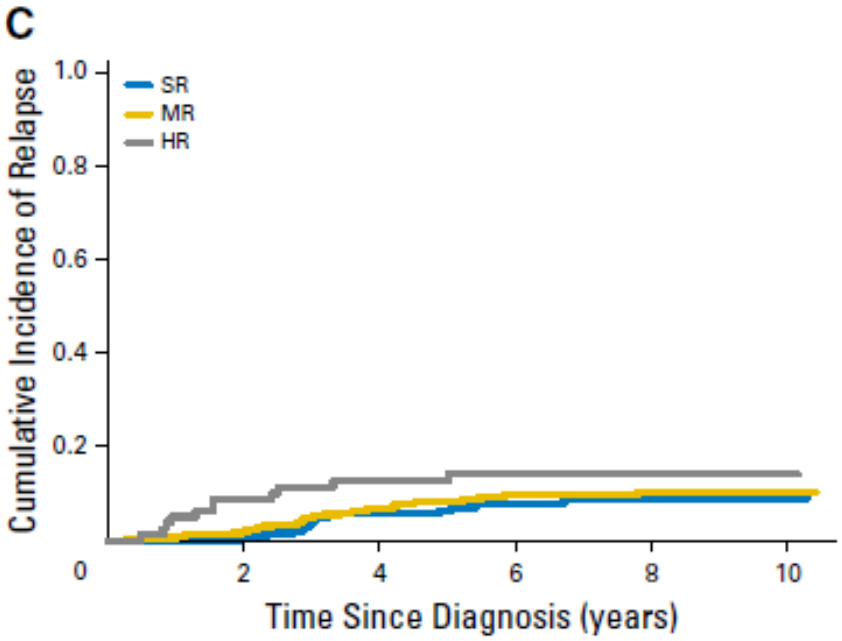
Event-free survival

Survival



Pieters, J Clin Oncol 2016

Relapse

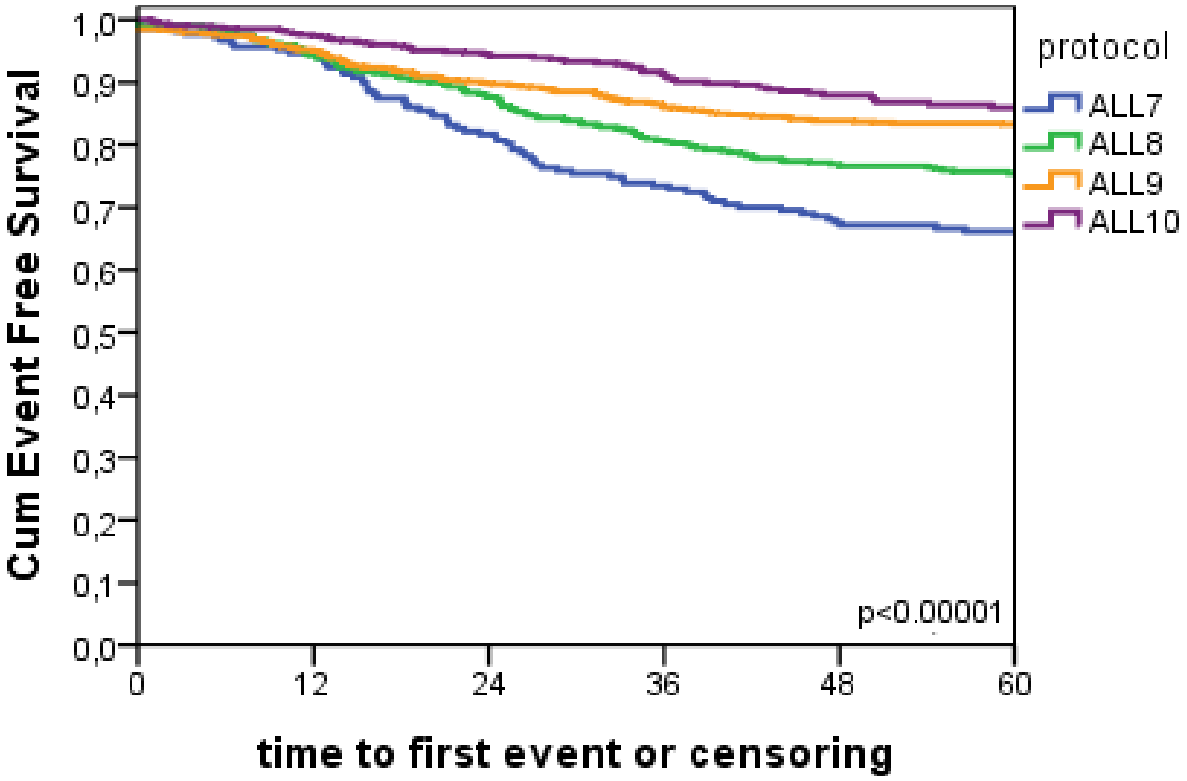


No. at risk

Time	0	2	4	6	8	10
SR	194	192	157	108	66	13
MR	490	470	377	250	132	21
HR	81	66	57	43	21	3

Pieters, J Clin Oncol 2016

EFS for ALL7, ALL8, ALL9, ALL10
1-15 yrs excl Ph+, excl Down syndrome



Pieters, J Clin Oncol 2016

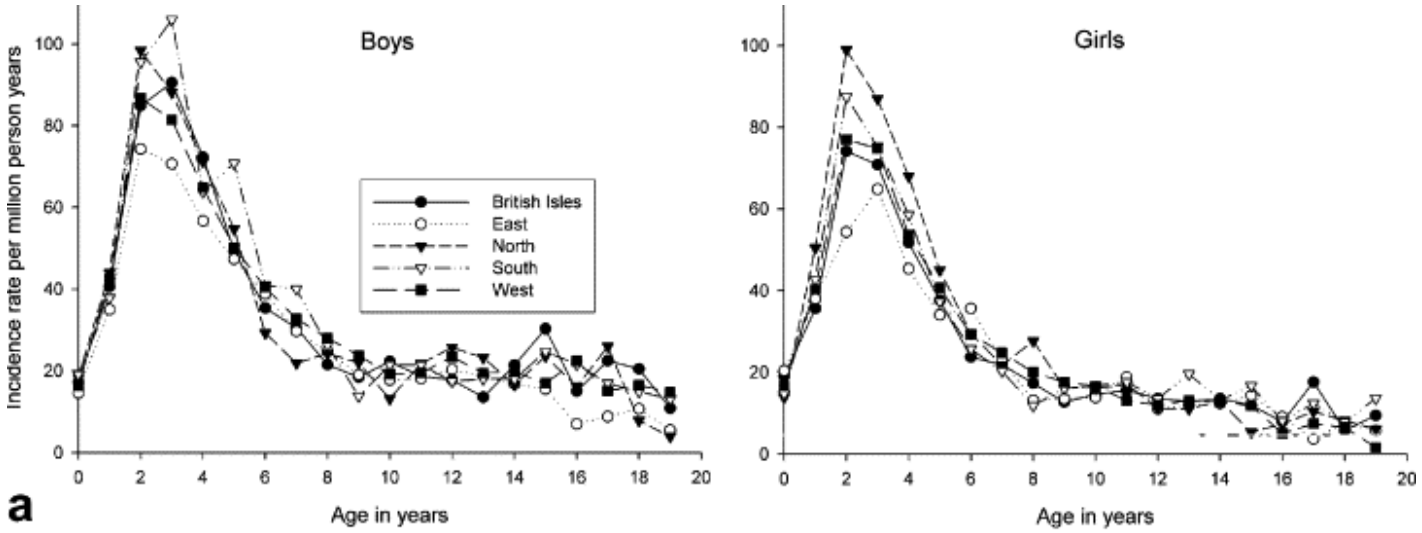
Osteonecrosis in ALL-10

- 1-4 yr 1.6%
- 5-9 yr 0.7%
- 10-14 yr 8.0%
- 15-18 yr 27.0%

Pieters, J Clin Oncol 2016

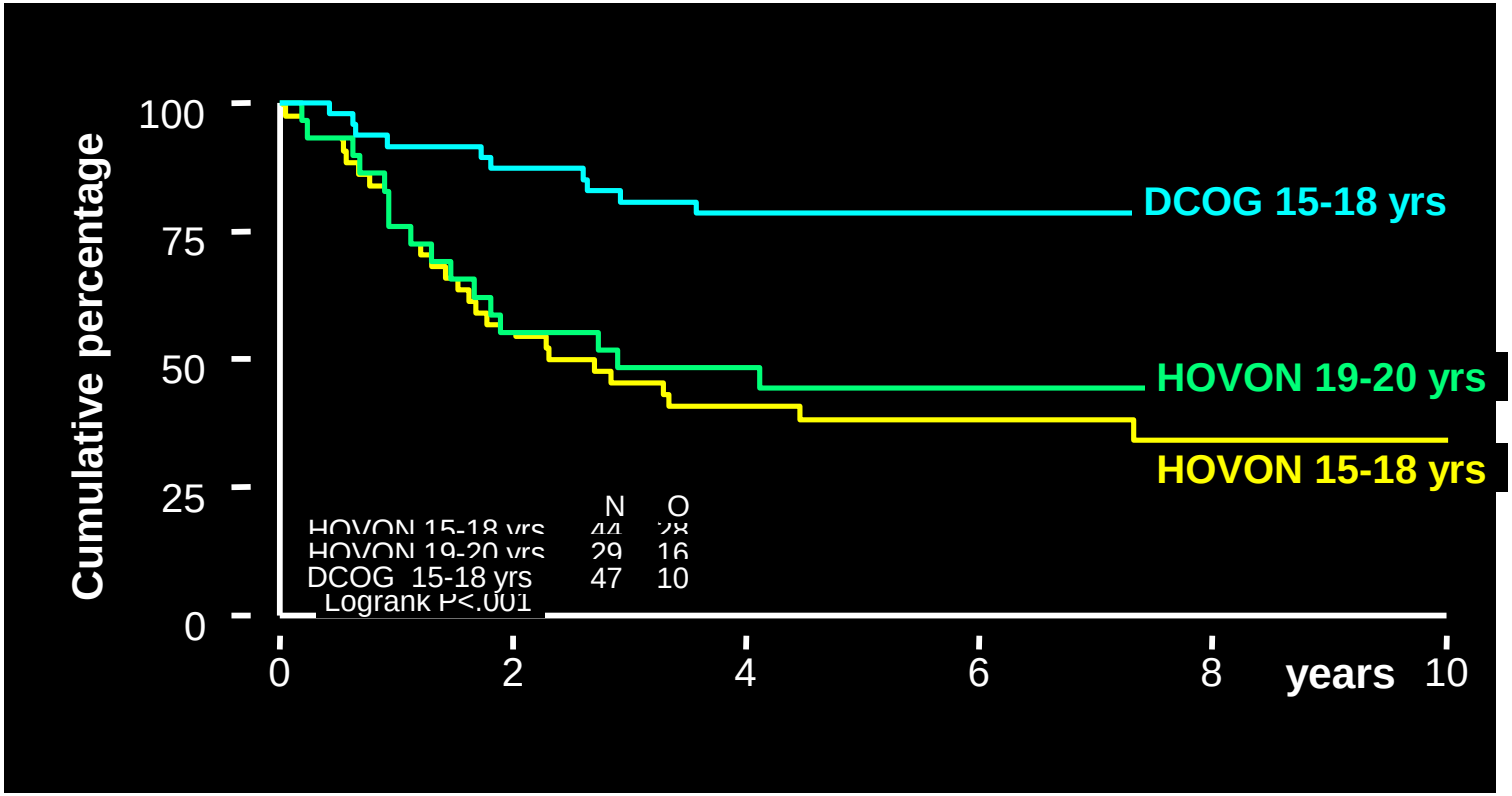
Pieters, Cancer 1987

Age-specific incidence rates of ALL among children 0-19 years



Coebergh, Eur J Cancer 2006

Outcome of adolescent ALL on pediatric DCOG vs adult HOVON protocol in the Netherlands



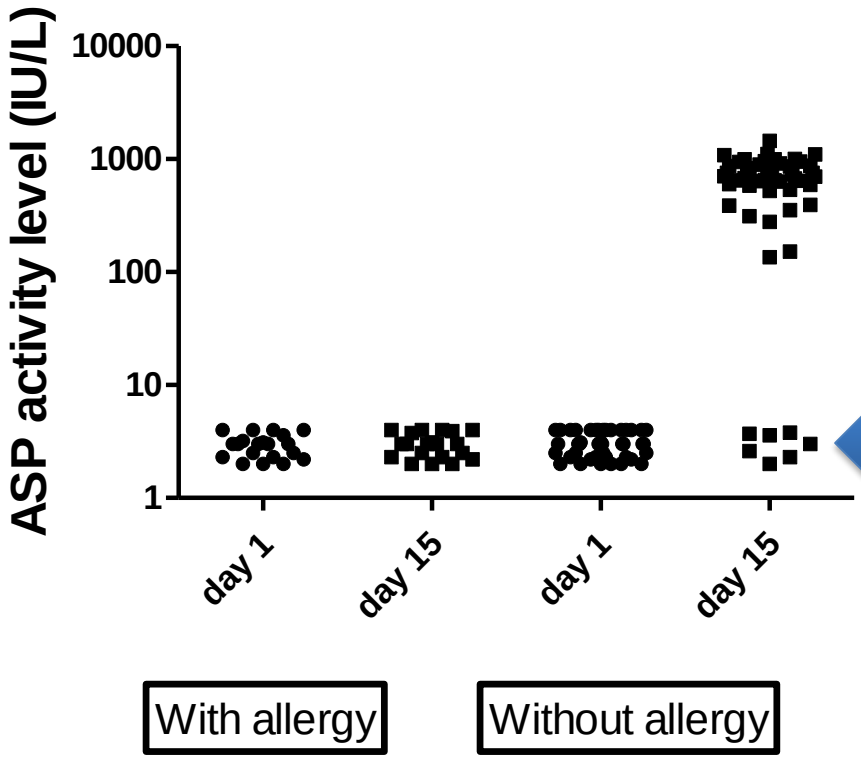
De Bont, Leukemia 2004

Intensification of asparaginase

	EFS (less intensive Asp)	EFS (more intensive Asp	difference	reference
Erwinase vs Coli Asp	60%	73%	significant	Duval 2002
Erwinase vs Coli Asp	78%	89%	significant	Moghrabi 2007
20 extra wks of Asp	79%	88%	Significant	Pession 2005
20 extra wks of Asp in IRG	72%	76%	not sign	Rizzari 2001
20 wks of Asp in T-ALL	55%	68%	Significant	Amylon 1999
20 wks of Asp in T-NHL	64%	78%	significant	Amylon 1999
> vs < 25 wks of Asp	73%	90%	significant	Silverman 2001

Pieters et al. **Cancer** 2011.

Asparaginase activity in patients with/without allergic reactions

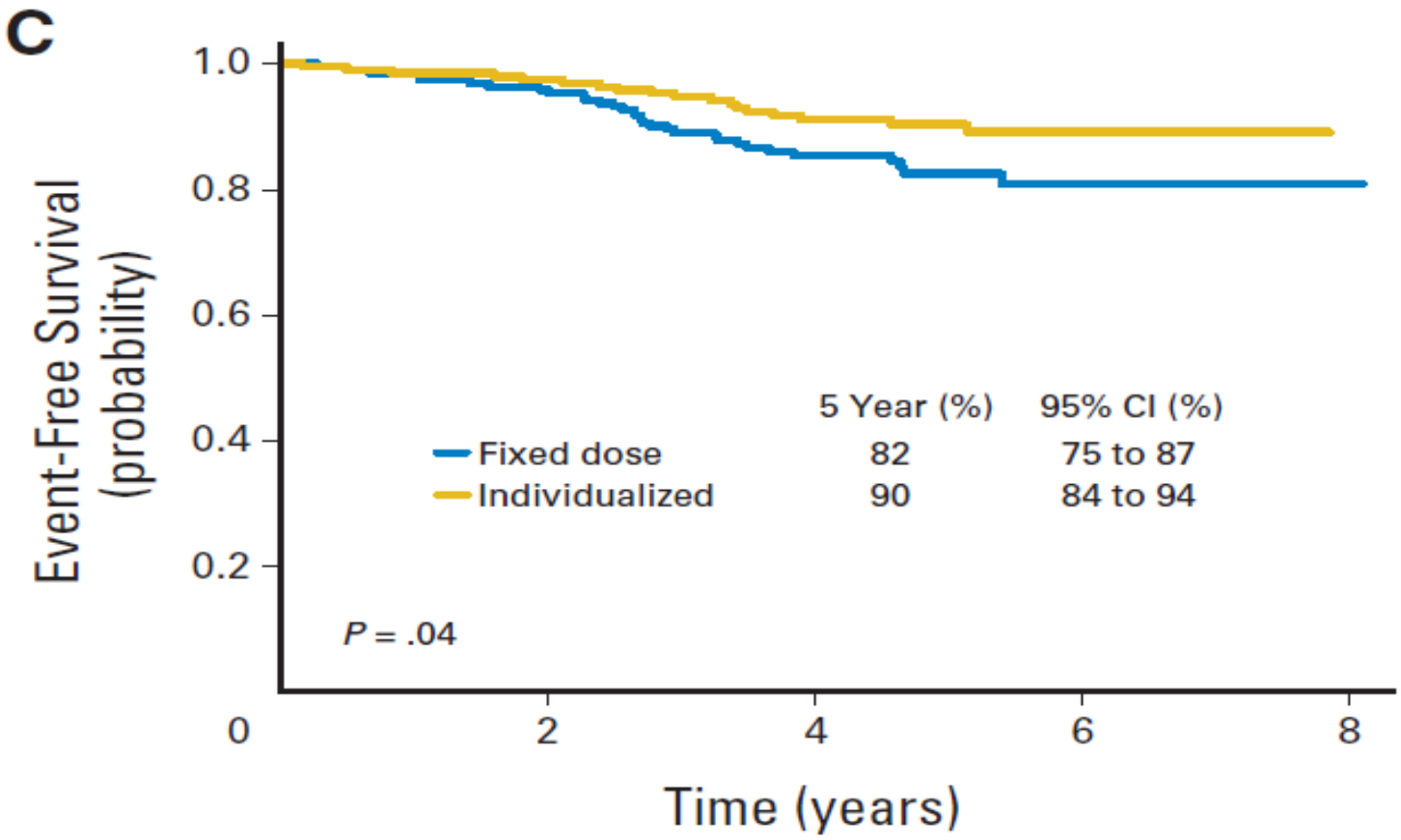


Too high levels

silent inactivation

Tong, Blood 2014

EFS for ALL patients randomized to fixed dosing of asparaginase *versus* individualized dosing (n=384)

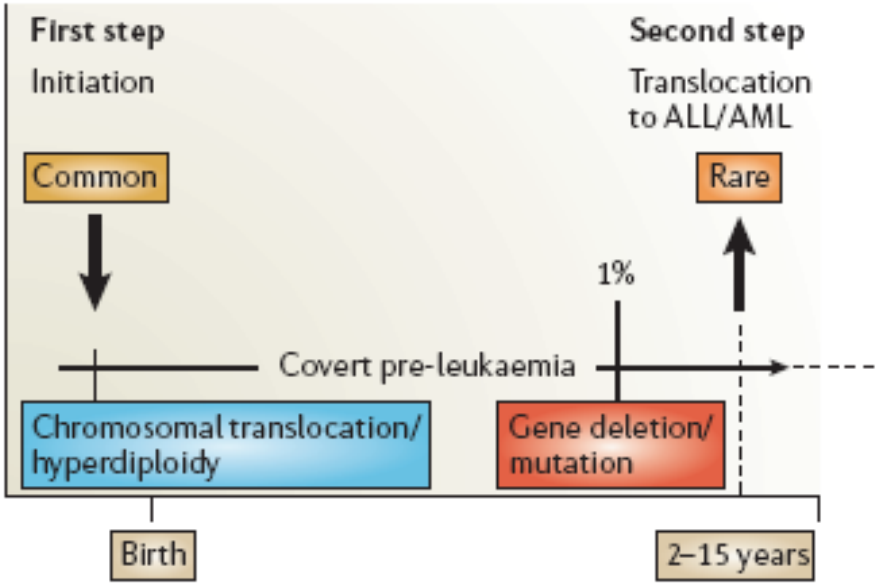


Vrooman, J Clin Oncol 2013

Genetic abnormalities in childhood leukemia

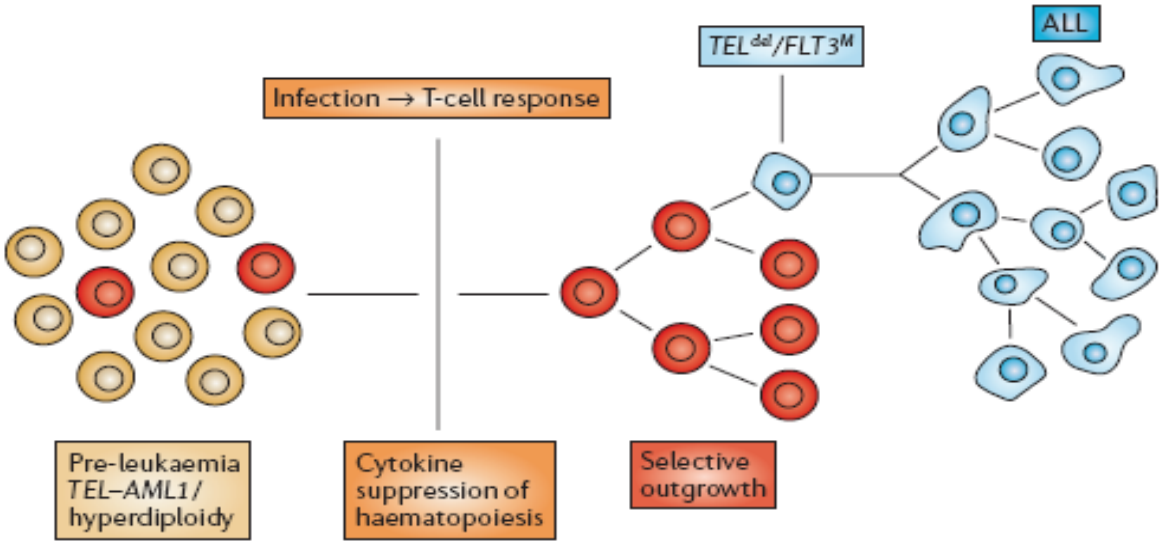
- Acquired
- Not hereditary
- Involved in leukemogenesis
- Related to outcome (poor and good)

Sequential events in the development of childhood leukemia



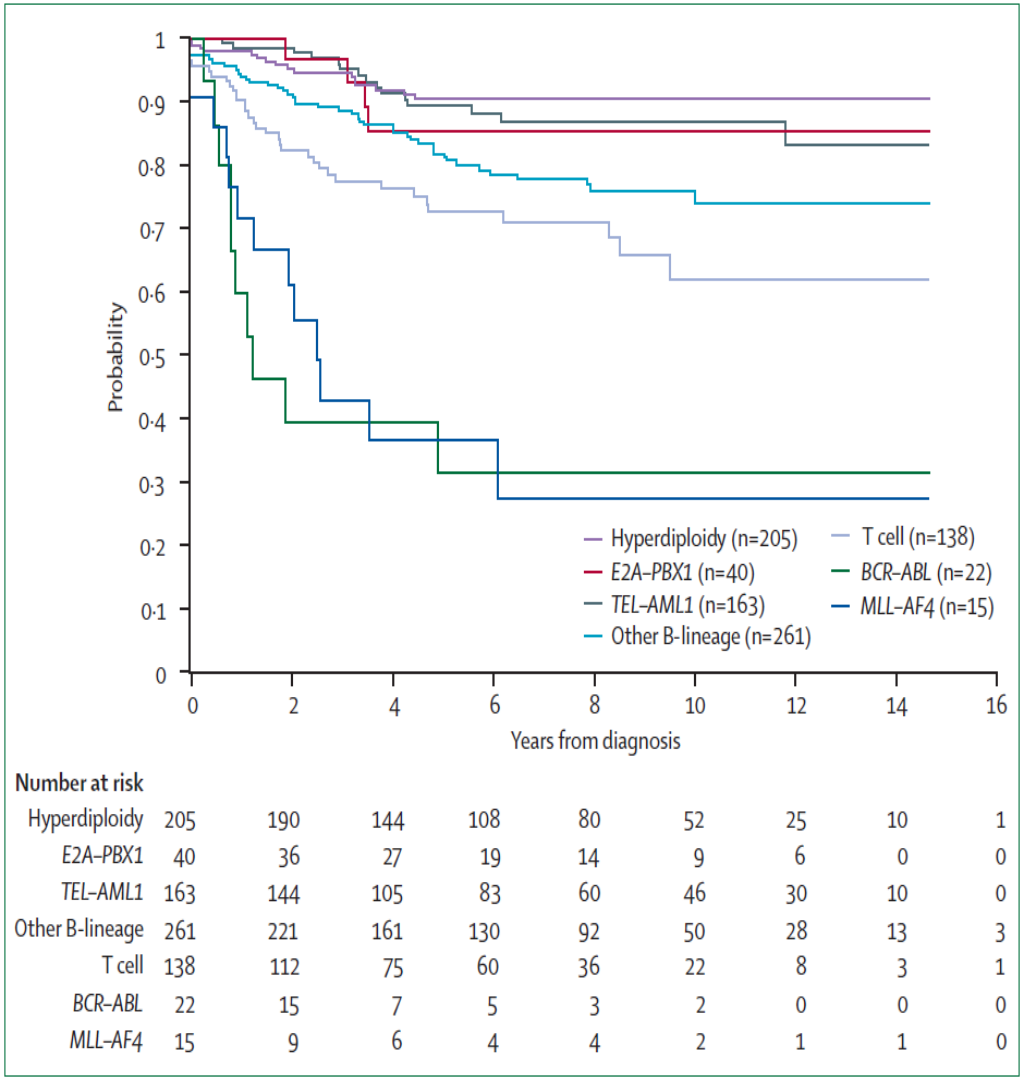
Greaves, Nat Rev Cancer 2006

Model for infection-derived proliferative stress in the selection of pre-leukemic cells



Greaves, Nat Rev Cancer 2006

Genetics and outcome in childhood ALL



Number at risk

Hyperdiploidy	205	190	144	108	80	52	25	10	1
E2A–PBX1	40	36	27	19	14	9	6	0	0
TEL–AML1	163	144	105	83	60	46	30	10	0
Other B-lineage	261	221	161	130	92	50	28	13	3
T cell	138	112	75	60	36	22	8	3	1
BCR–ABL	22	15	7	5	3	2	0	0	0
MLL–AF4	15	9	6	4	4	2	1	1	0

Figure 4: Kaplan-Meier analysis of event-free survival according to biological subtype of leukaemia

Pui, Lancet 2008

Genetic subtypes of childhood ALL

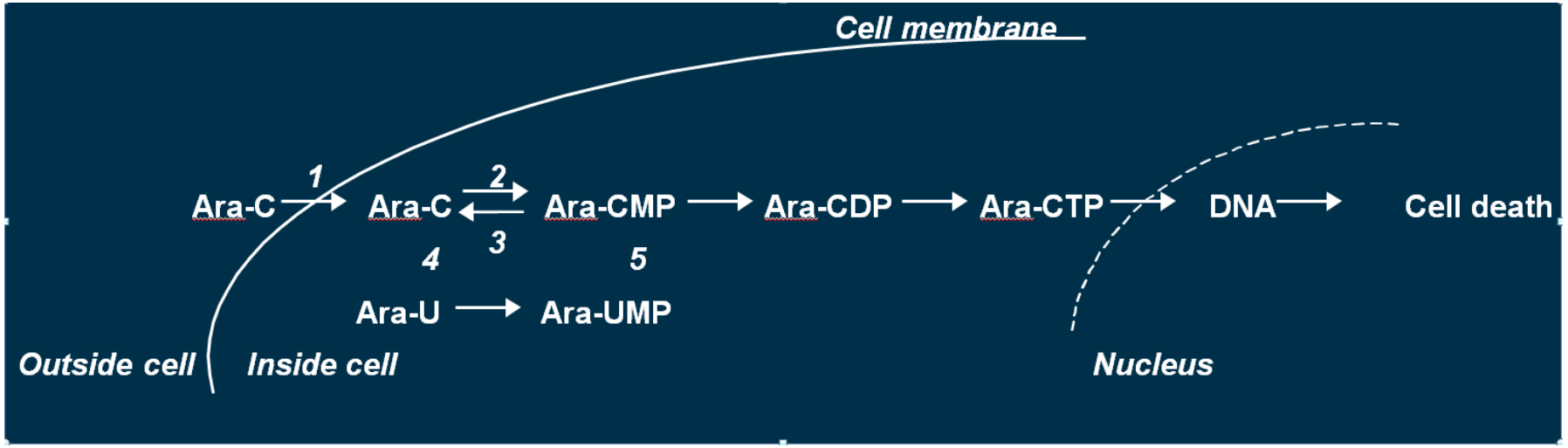
Subtype		Outcome
15%:	T-lineage ALL	intermediate
85%	B-Lineage ALL:	
~25%	*Hyperdiploidy (>50 chromosomes)*	*good*
~25%	*t(12;21) TEL/AML1 or ETV6-CBFA2 fusion*	*good*
~5%	*t(1;19) E2A-PBX1 fusion*	*good*
~3%	*t(9;22) BCR-ABL fusion (Philadelphia)*	*poor*
~5%	*MLL rearrangements*	*poor*
Others		*intermediate*

In vitro resistance/sensitivity of Infant ALL

Drug	Infants <1 year median (25th–75th)	n=	c/preB ⩾1 year median (25th–75th)	n=	Resistance ratio[a]	P-value
Prednisolone	>250 (0.30–>250)	41	0.43 (0.12–12.5)	373	>581	0.001
Dexamethasone	3.61 (0.05–>6.0)	11	0.07 (0.01–0.55)	241	54.8	0.040
Vincristine	0.55 (0.10–2.54)	37	0.69 (0.24–2.52)	369	0.80	0.088
L-Asparaginase	0.96 (0.35–1.43)	29	0.08 (0.01–1.04)	361	12.0	0.001
Daunorubicin	0.07 (0.03–0.12)	33	0.09 (0.06–0.17)	386	0.83	0.090
6-Mercaptopurine	201 (95.2–321)	12	97.9 (50.4–248)	280	2.05	0.110
6-Thioguanine	6.04 (5.23–10.1)	27	5.92 (3.80–9.10)	299	1.02	0.256
Cytarabine	0.27 (0.13–0.51)	35	0.49 (0.27–1.31)	291	0.54	0.001
2-CdA[b]	0.02 (0.01–0.03)	29	0.030 (0.02–0.14)	79	0.59	<0.001
Etoposide	1.04 (0.48–2.56)	17	1.50 (0.64–2.77)	162	0.70	0.305
Teniposide	0.28 (0.16–0.75)	11	0.25 (0.18–0.58)	227	1.12	0.786
4-HOO-ifosfamide	4.08 (1.93–5.66)	20	3.07 (1.24–5.23)	221	1.33	0.185

Pieters, Leukemia 1998; Ramakers-van Woerden, Leukemia 2004

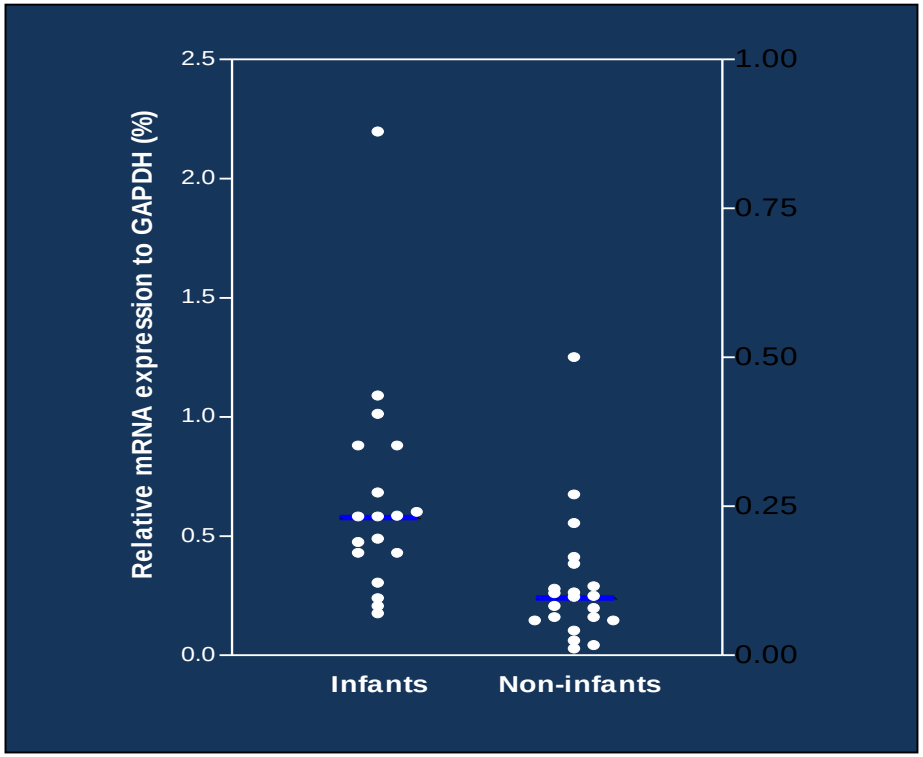
Ara-C Metabolism

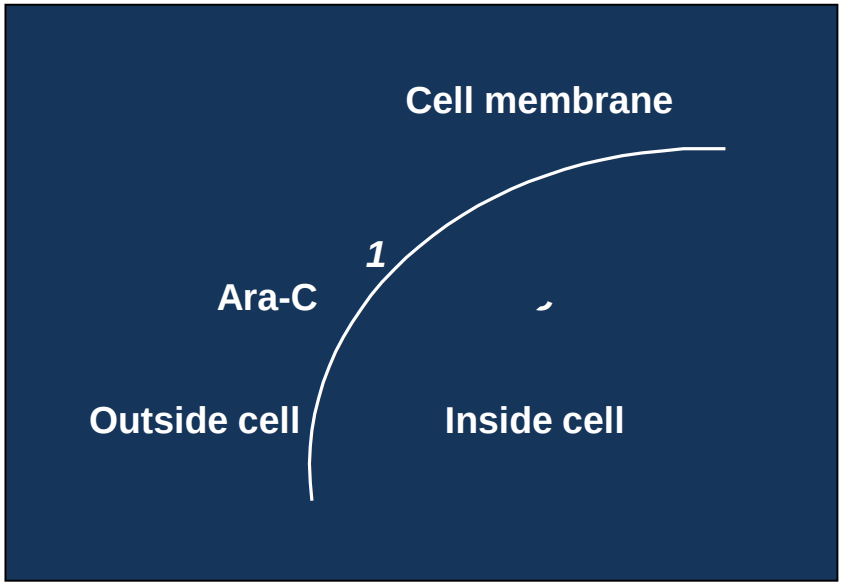


1. Equilibrative nucleoside transporter 1 (hENT1)
2. Deoxycytidine Kinase (dCK)
3. Pyrimidine Nucleotidase I (PN-I)
4. Cytidine Deaminase (CDA)
5. Deoxycytidylate Deaminase (dCMPD)

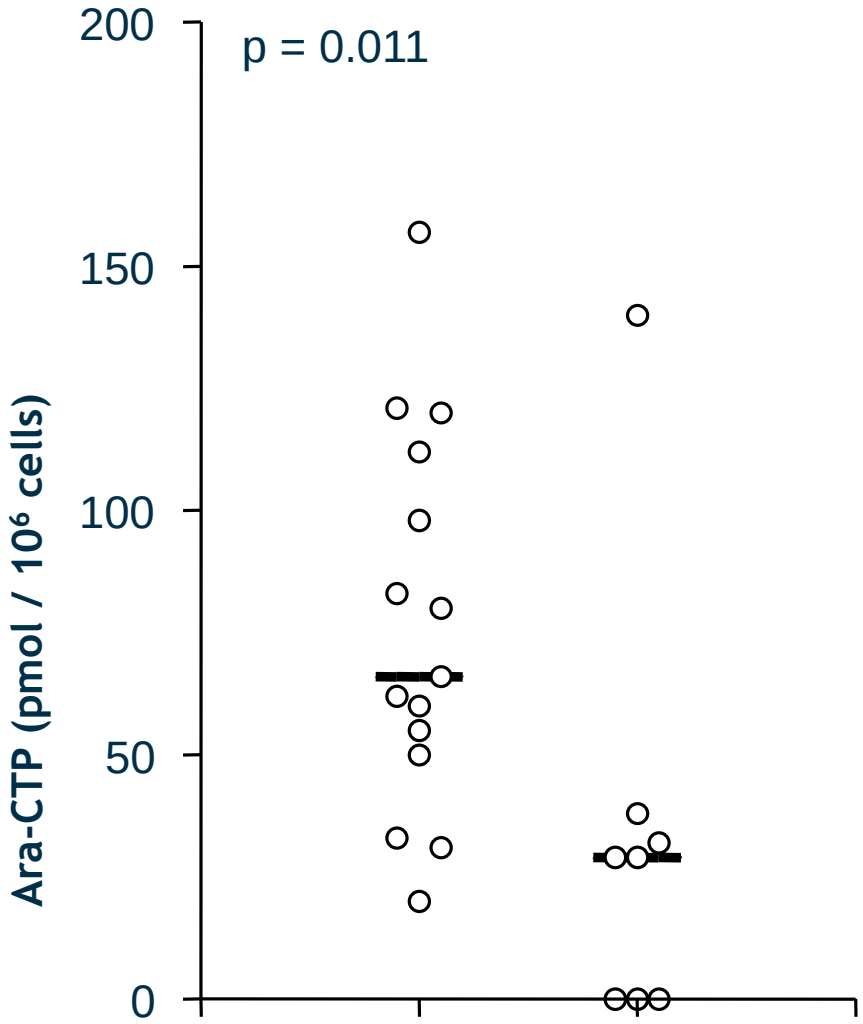
Stam, Blood 2002

AraC membrane transport system
Equilibrative nucleoside transporter 1 (hENT1)





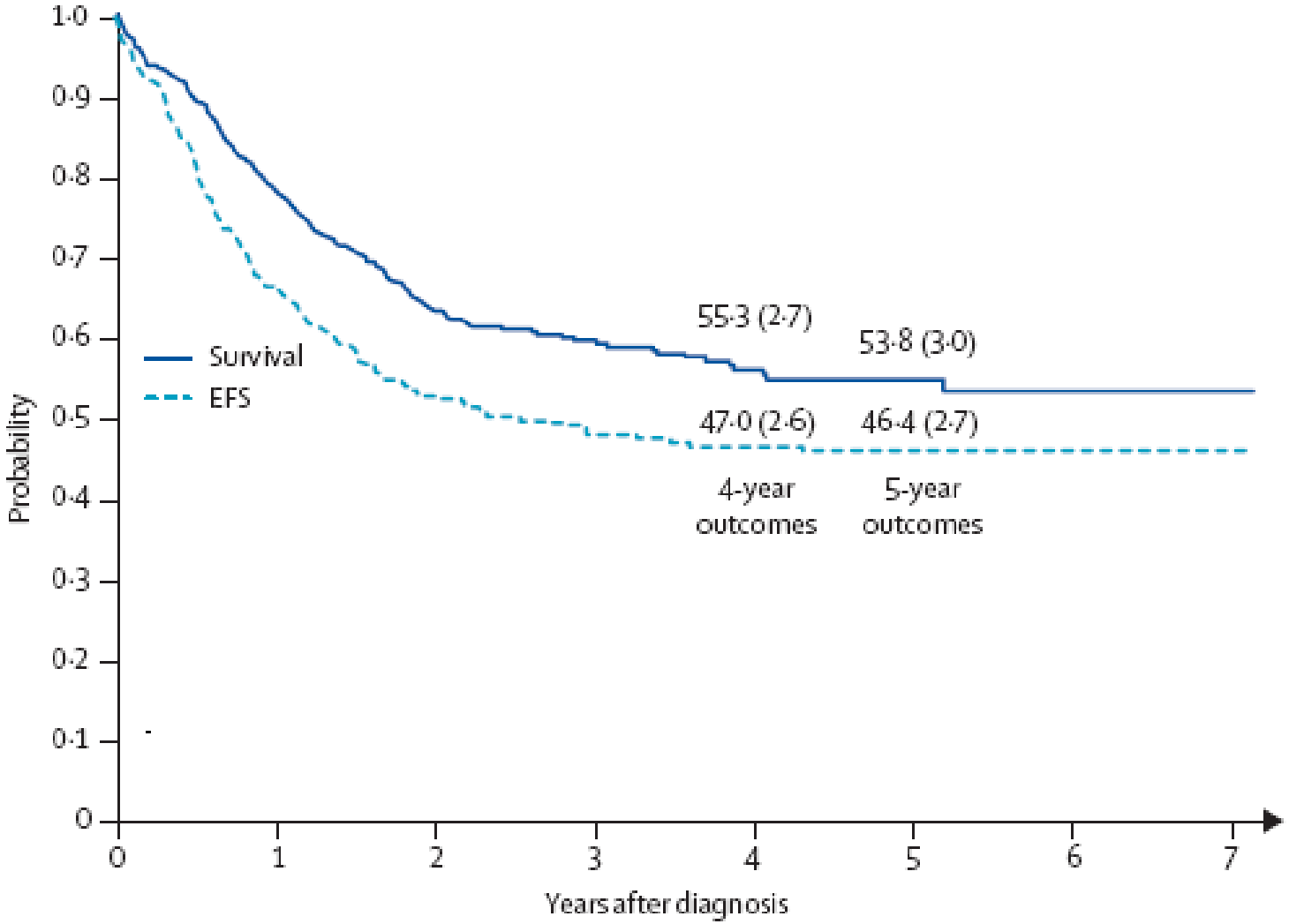
Stam, Blood 2002



Ara-CTP accumulation in infant and non-infant ALL cells

Stam, Blood 2002

Outcome of the Interfant-99 protocol



Pieters, Lancet 2007

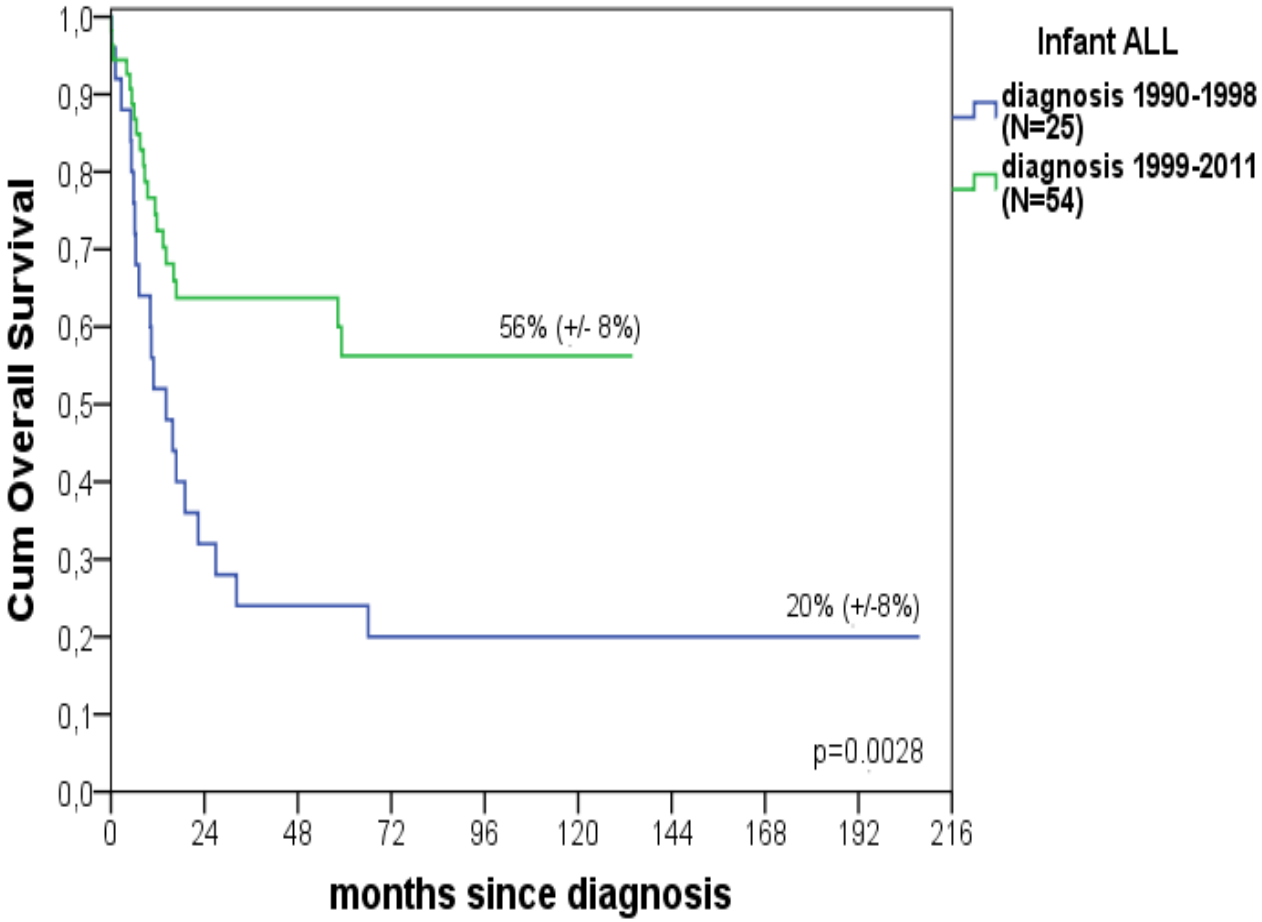
Outcome of treatment protocols for infant ALL

princessMÁXIMA center for pediatric oncology

	Date (year)	CR rate	EFS or survival timepoint	EFS rate (SE)	Survival rate (SE)	Patients enrolled
DFCI (1985–95)[3]	1997	96%	4 year	54% (11)	–	23
Interfant-99	2007	94%	4 year	47% (2·6)	55% (2·7)	482
AIEOP-91/95[4]	2006	96%	5 year	45% (95% CI 31–58)	–	52
BFM[5]	1999	95%	6 year	43% (5)	48% (6)	105
EORTC-CLCG[6]	1994	86%	4 year	43% (95% CI 24–62)	-	25
CCG-1953[7]	2006	97%	5 year	42% (9)	45% (6)	115
CCG-1883[8]	1999	97%	4 year	39% (4)	51% (4)	135
CCG-107[8]	1999	94%	4 year	33% (5)	45% (5)	99
UKALL-92[9]	2002	94%	5 year	33% (95% CI 23–44)	46% (95% CI 35–57)	86
POG 8493[10]	1997	93%	4 year	28% (5)	–	82
POG alternating drugs[11]	1998	94%	4 year	17% (8)	–	33

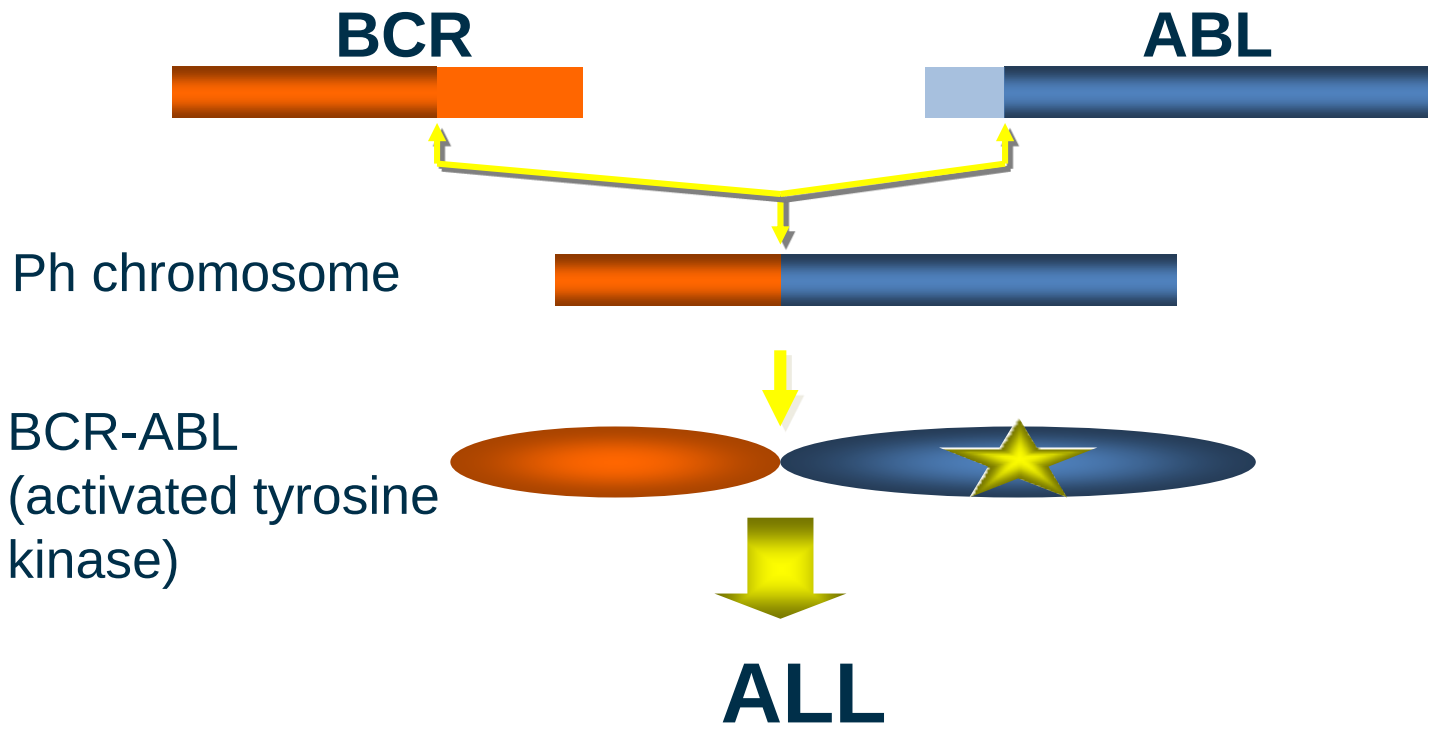
Pieters, Lancet 2007

Survival infant ALL before and after introduction of Interfant protocol



Pieters, Lancet 2007

The Philadelphia Chromosome t(9;22) or bcr-abl translocation



Addition of imatinib (abl tyrosine kinase inhibitor) in bcr-abl positive ALL

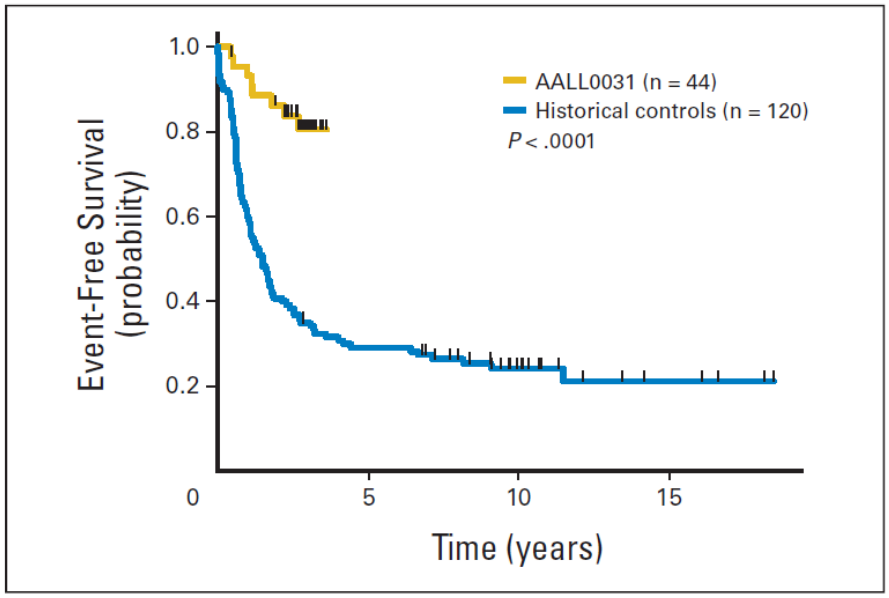
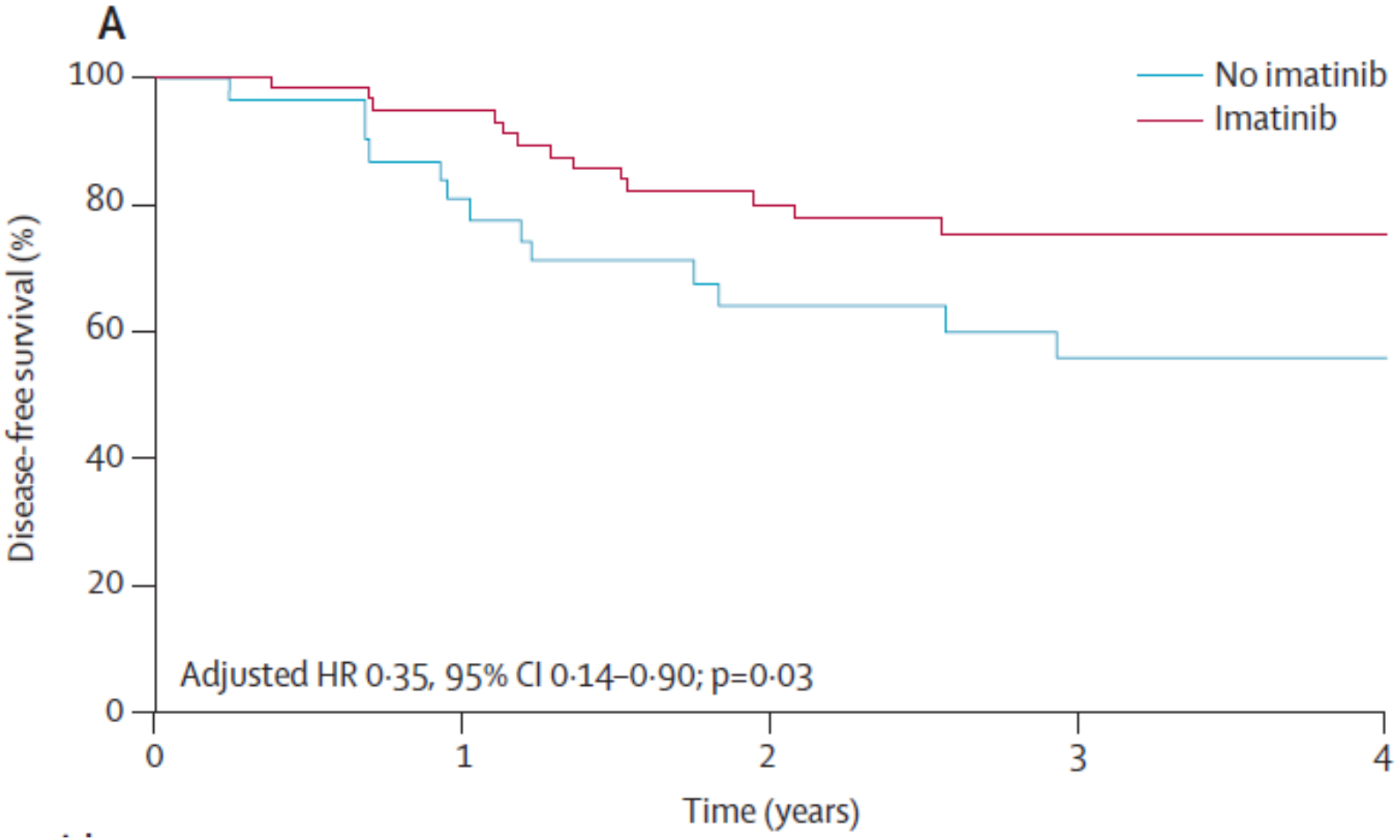


Fig 3. Early event-free survival in Philadelphia chromosome–positive acute lymphoblastic leukemia patients treated with imatinib. Treated patients in cohort 5 (n = 44) were compared with patients previously treated on Pediatric Oncology Group (POG) protocols ALinC 14, 15, and 16 from January 1986 through November 1999 (N = 120).

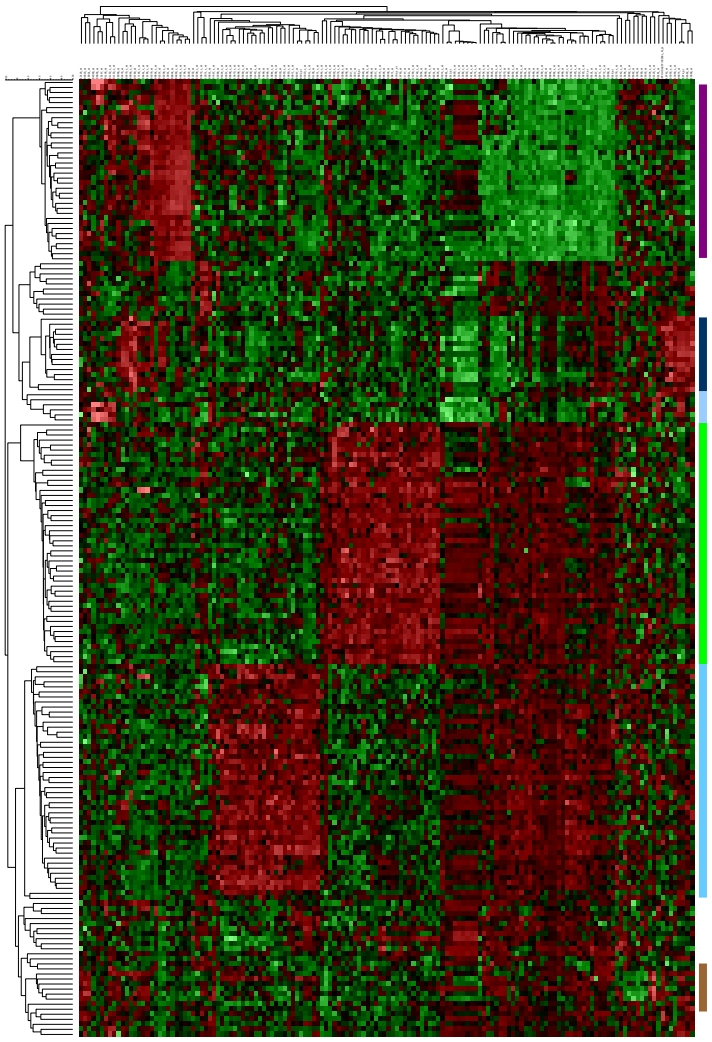
Schultz, J Clin Oncol 2009

DFS for good-risk Ph+ ALL patients as treated with imatinib (EsPhALL)



Biondi A, Lancet 2012

Discovery of BCR-ABL-like ALL



T-ALL

E2A-rearranged

MLL-rearranged

TEL-AML1

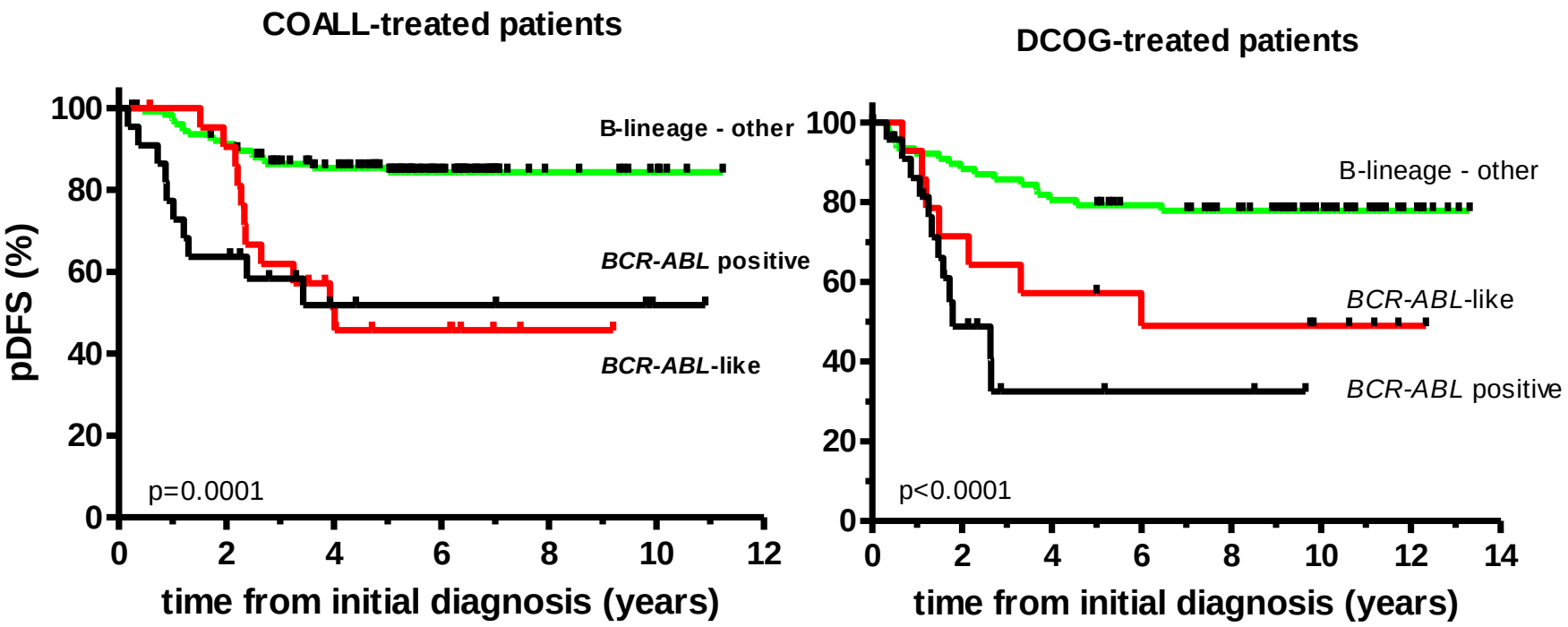
Hyperdiploid

BCR-ABL

5 real BCR-ABL
30 BCR-ABL like

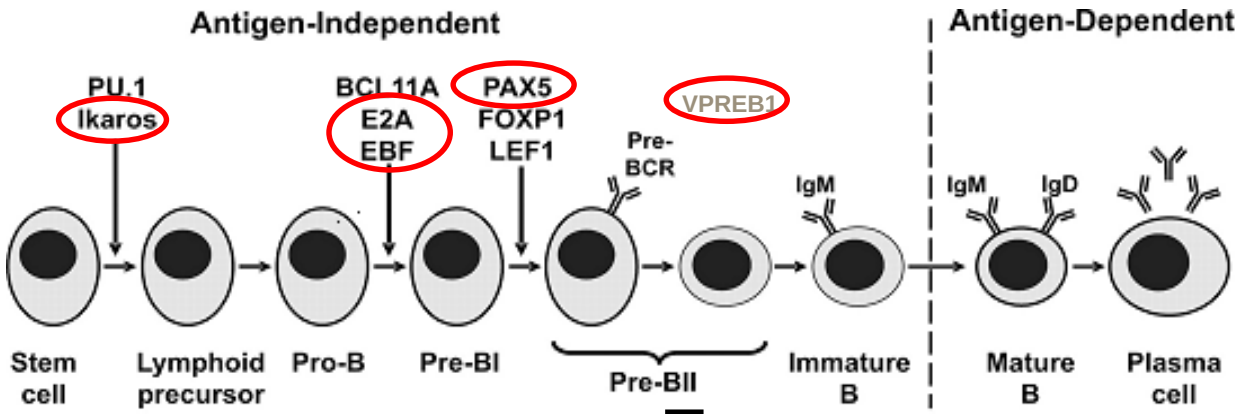
Den Boer et al. Lancet Oncology 2009

Unfavorable prognosis of BCR-ABL-like ALL



Den Boer et al. Lancet Oncology 2009

B-cell development



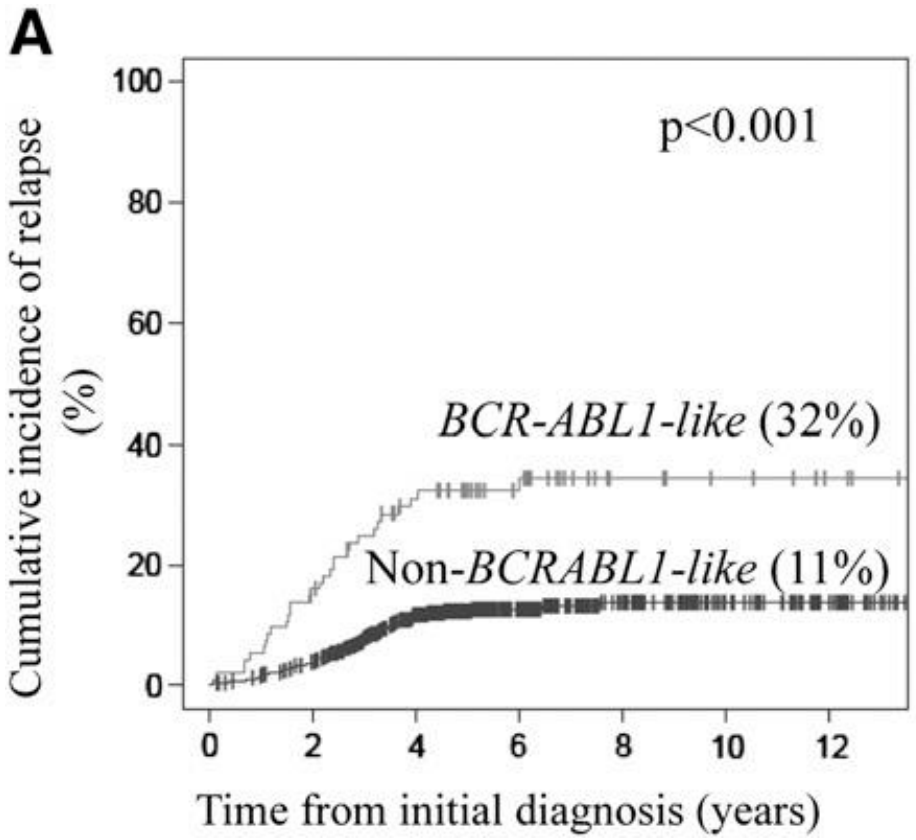
Adapted from Downing and Mullighan, 2006

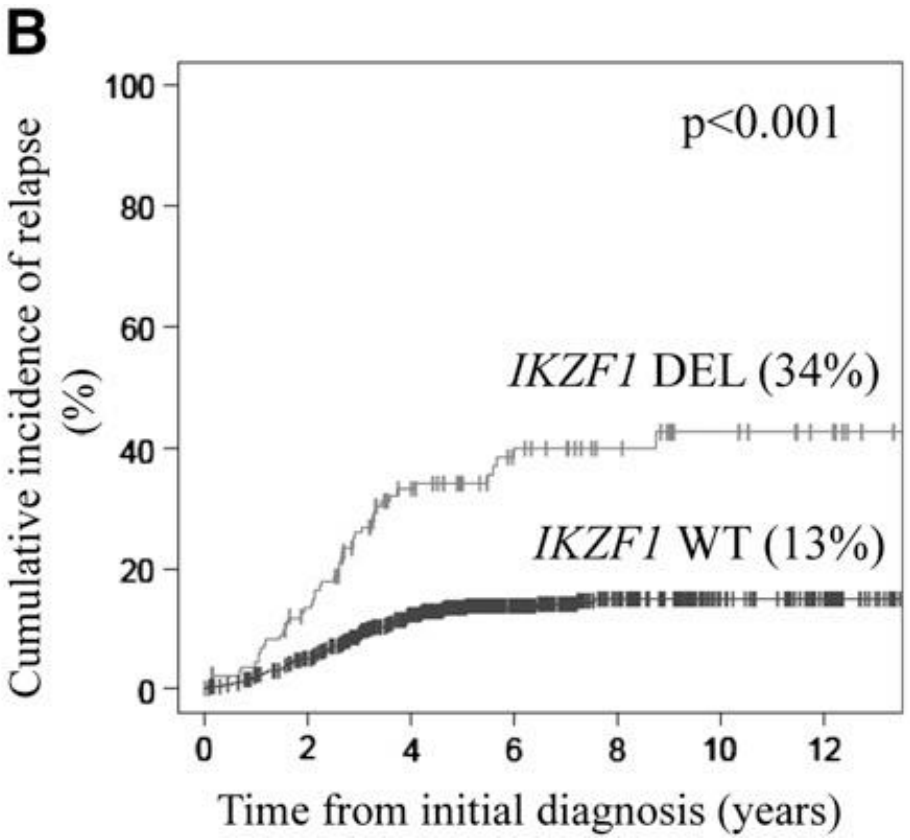
Abnormalities in B-cell development genes:

	BCR-ABL-like	*BCR-ABL*+	B-other
Total	36/44 82% P<0.001	12/15 80% P<0.01	9/25 36%

Den Boer et al. Lancet Oncology 2009

Prognostic value of BCR-ABL like ALL and IKZF1 deletions





Van der Veer, Blood 2013

Frequency of identified tyrosine kinase fusion genes

Marker	*BCR-ABL1*-like (n=77)	Remaining B-other (n=76)
***ABL1/ABL2* fusion**	**3.9%**	0%
ZMIZ1-ABL1	1	
FOXP1-ABL1	1	
RCSD1-ABL2	1	
***PDGFRB* fusion**	**5.2%**	0%
EBF1-PDGFRB	4	
***CSF1R* fusion**	**2.6%**	0%
SSBP2-CSF1R	2	
***JAK2* fusion**	**6.5%**	0%
PAX5-JAK2	3	
BCR-JAK2	1	
TERF2-JAK2	1	
CRLF2* high expression	15.6%	15.8%
PAR1 deletion**	10.5%	10.7%

12% with ABL-1 class fusions

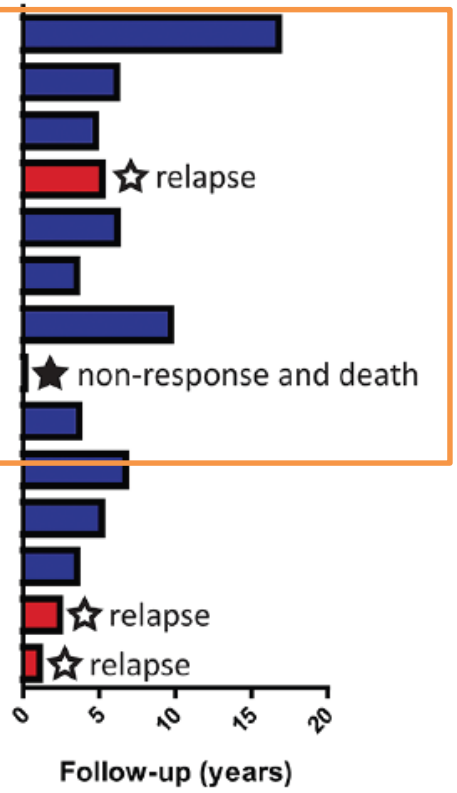
6% with JAK2 fusions

Boer JM, Oncotarget 2016

BCR-ABL1-like tyrosine kinase fusion cases

A

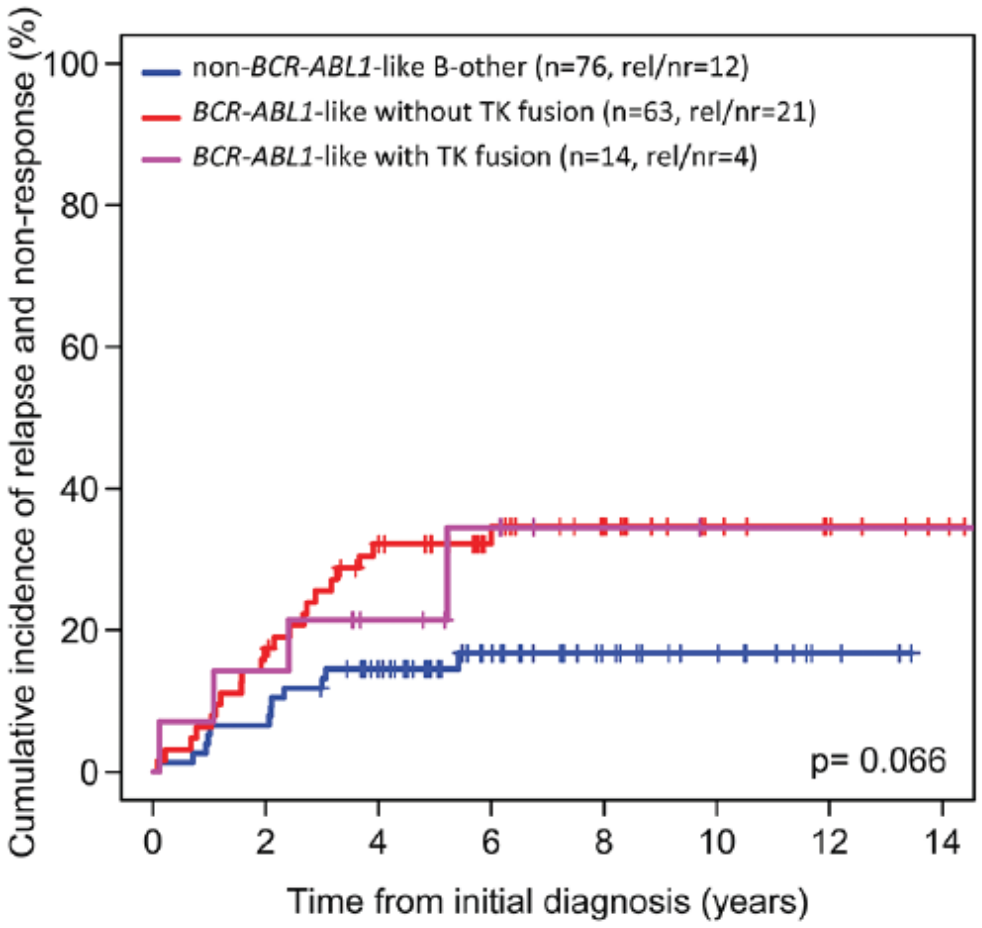
Case	Sex	Protocol	Pred	Resp	MRD TP1	MRD TP2	IKZF1 del	TK fusion
R32	M	ALL8-HR*	poor	LR	nd	nd	WT	*EBF1-PDGFRB*
A288	M	ALL10-HR*	poor	LR	high	high	DEL4-7	*EBF1-PDGFRB*
A472	M	ALL10-HR*	good	CR	high	high	WT	*EBF1-PDGFRB*
A428	M	ALL10-MR	good	unkn	high	low	DEL1-8	*EBF1-PDGFRB*
A123	F	COALL03-HR	nd	CR	int	nd	DEL1-8	*SSPB2-CSF1R*
A526	M	ALL10-HR*	good	CR	high	high	DEL2-7	*SSBP2-CSF1R*
A91	M	ALL9-SR	nd	LR	nd	nd	WT	*ZM1Z1-ABL1*
A26	M	ALL9-HR	nd	NR	nd	nd	DEL2-8	*FOXP1-ABL1*
A530	M	ALL10-HR*	good	unkn	high	high	DEL2-3	*RCSD1-ABL2*
A31	F	ALL9-HR	nd	CR	int	low	DEL1	*PAX5-JAK2*
A286	F	ALL10-SR	good	CR	low	low	WT	*PAX5-JAK2*
A204	M	COALL03-HR	nd	CR	high	nd	DEL2-7	*PAX5-JAK2*
A214	M	COALL03-HR*	nd	LR	high	nd	WT	*TERF2-JAK2*
A216	M	COALL03-HR*	nd	CR	int	nd	DEL2-3	*BCR-JAK2*



Boer JM, Oncotarget 2016

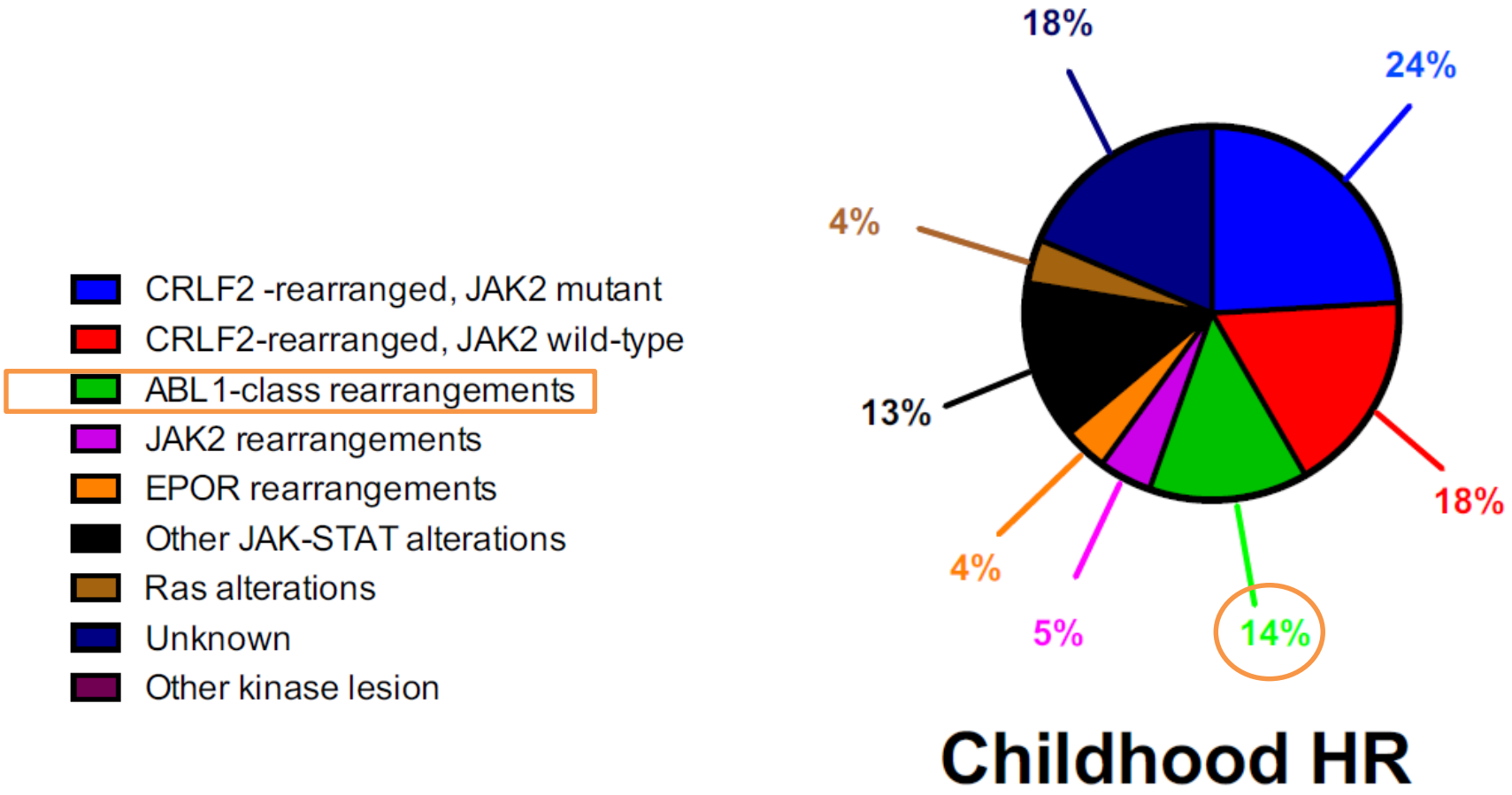
BCR-ABL1-like tyrosine kinase fusion cases

B



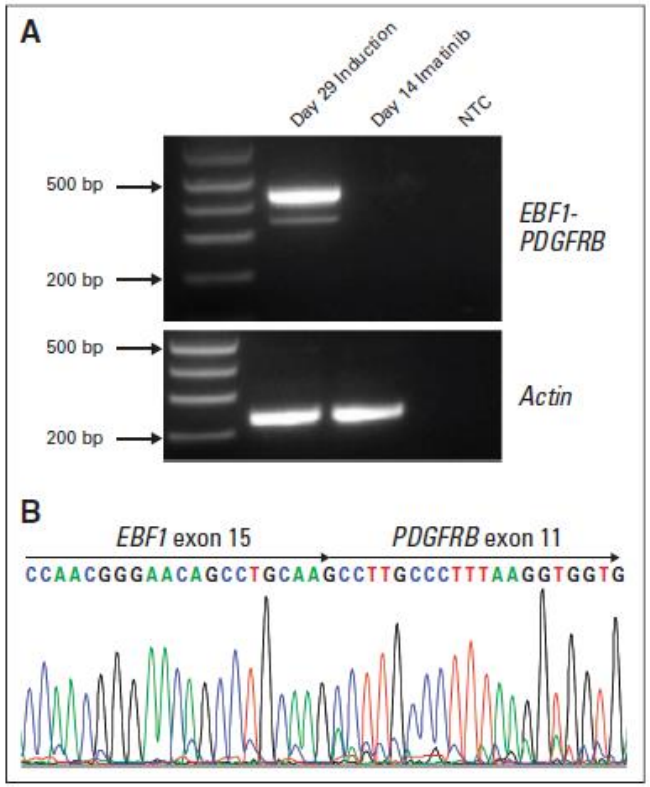
Boer JM, Oncotarget 2016

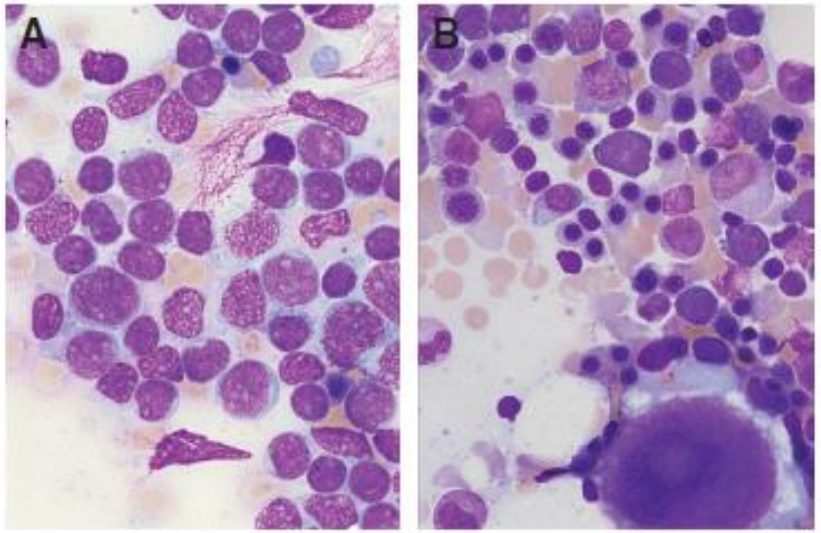
Distribution of Ph-like ALL subgroups among with children with HR ALL



Tran TH, Hematology 2016

Imatinib induces CR in refractory ALL case with EBF1-PDGFRB





Weston, J Clin Oncol 2013

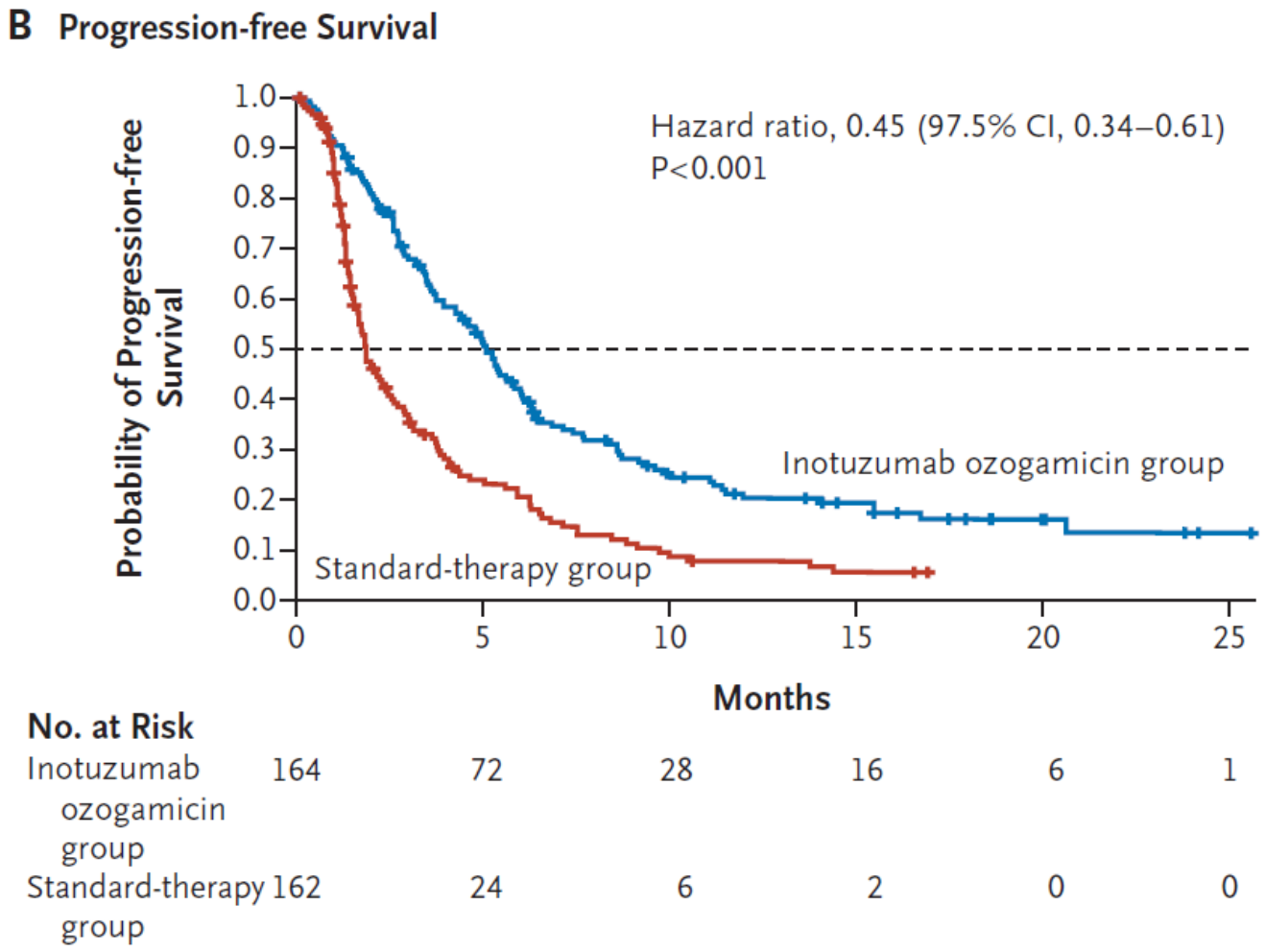
Cell surface antigen targets in pediatric ALL

princessMÁXIMA center for pediatric oncology

		Therapeutic monoclonal antibodies		
Surface antigen	ALL subtype	Unconjugated	Conjugated	Bispecific
CD19	B-lineage		SAR3419	Blinatumomab
CD20	B-lineage	Rituximab, Ofatumumab, Obinutuzumab, Veltuzumab, AME 133	^{90}Y-ibritumomab tiuxetan, ^{131}I-tositumomab	
CD22	B-lineage	Epratuzumab	CAT-3888, CAT-8015, Inotuzumab ozogamicin, ^{90}Y-epratuzumab	
CD33	B-lineage		Gemtuzumab ozogamicin	
CD40	B-lineage	Dacetuzumab, Lucatumumab		
HLA-DR	B-lineage	Apolizumab, Milatuzumab		
CD52	B-lineage T-lineage	Alemtuzumab		

Barth M, Br J Hem 2012

Inotuzumab in adult ALL: Duration of progression-free survival



Kantarjian HM, NEJM 2016

Blinatumomab in children with relapsed ALL:
39% CR
20% MRD negative

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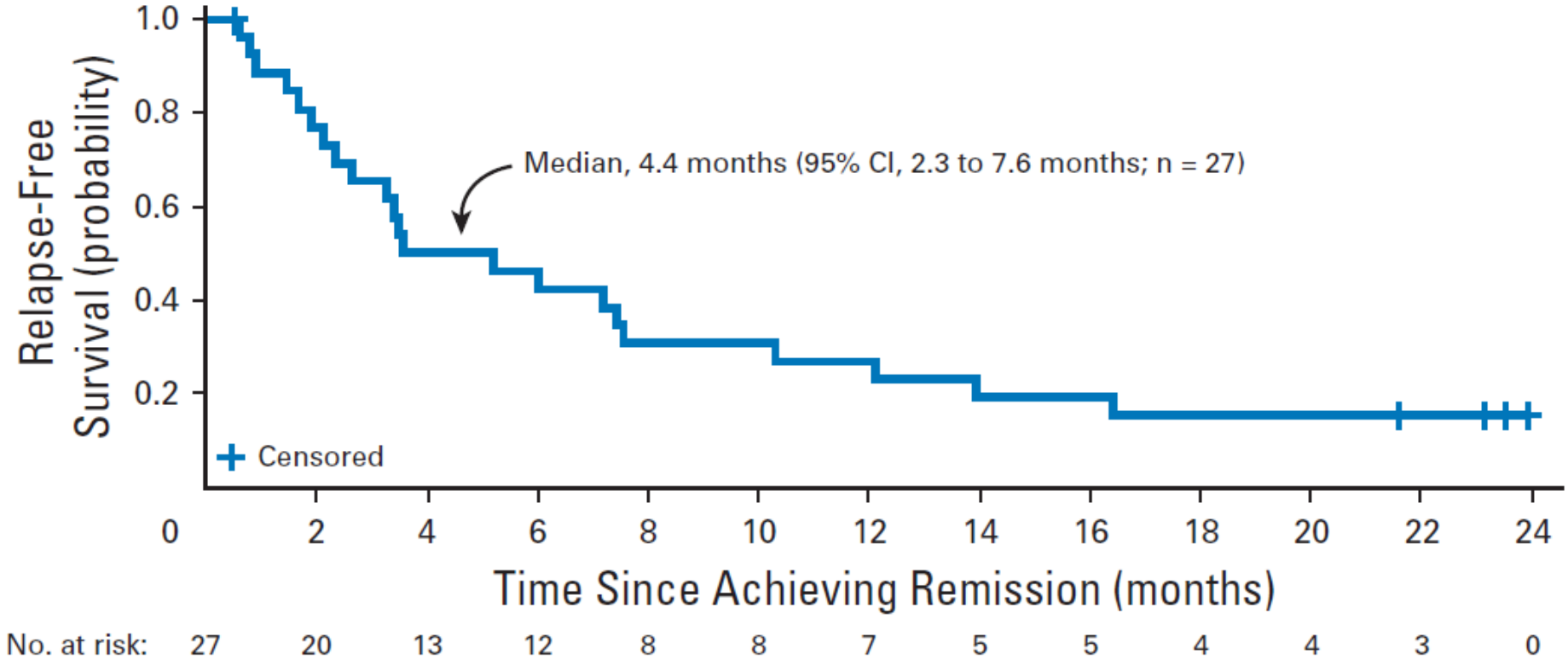
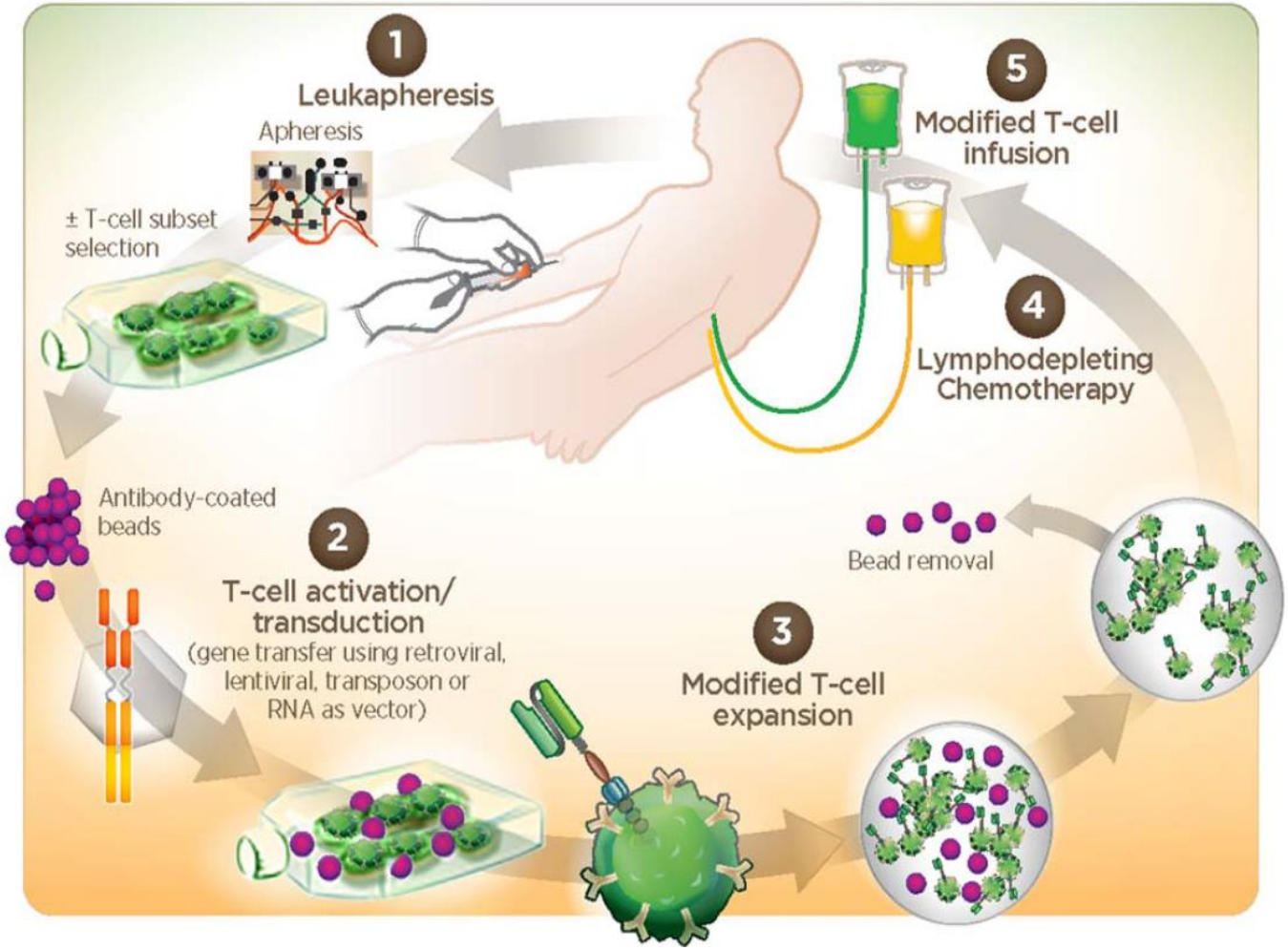
disease-free survival for cases with CR



Von Stackelberg A, JCO 2016

Manufacturing overview of CTL019



Frey NV, Am J Hemat 2016

CD19 CAR clinical trials in ALL

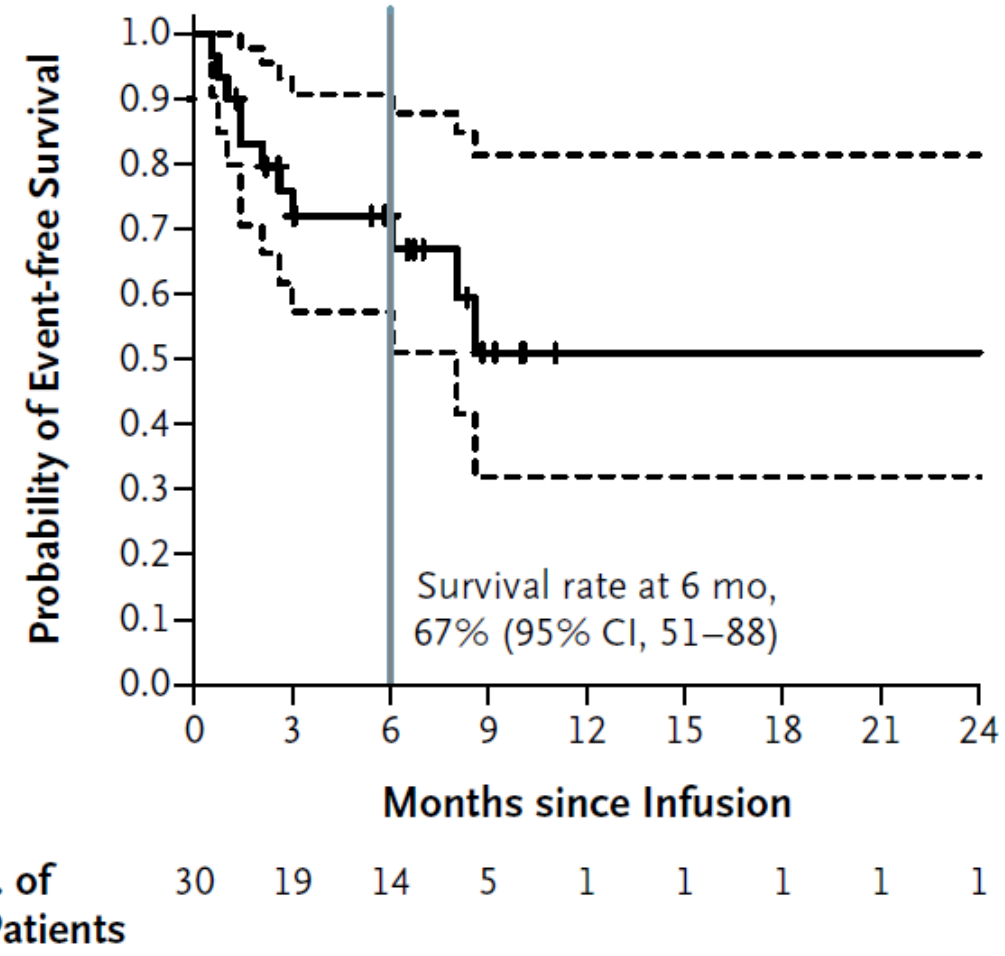
princessMÁXIMA
center for pediatric oncology

Center	CAR design	Manufacture method	Vector	No. of pts	Dose (cells/kg)	CR rate	Persistence
MSKCC	19/28/z	CD3/CD28 beads	Retrovirus	5	$1.5–3 \times 10^6$	100 %	1–2 m
MSKCC	19/28/z	CD3/CD28 beads	Retrovirus	16	3×10^{6a}	88 %	2–3 m
NCI	19/28/z	CD3/CD28 beads + IL-2	Retrovirus	20	$1–3 \times 10^{6b}$	70 %	≤68 days
CHOP/Penn	19/BB/z	CD3/CD28 beads	Lentivirus	2	$1.2–14 \times 10^6$	100 %	NR
CHOP/Penn	19/BB/z	CD3/CD28 beads	Lentivirus	30	$0.76–20.6 \times 10^6$	90 %	24 m

Singh N, Curr Treat Opinions Oncol 2016

CTL019: Probability of EFS at 6 months

A



Maude SL, NEJM 2014

ALL: improving cure rate by research

- Minimal residual disease (MRD) monitoring
- Therapeutic drug monitoring
- Specific protocols for genetic subtypes
- Targetable genetic lesions
- Immunotherapies