EARLY-AGE LEUKEMIA WITH *KMT2A-r* AND ITS ASSOCIATION WITH MATERNAL EXPOSURES AND GENE VARIANTS

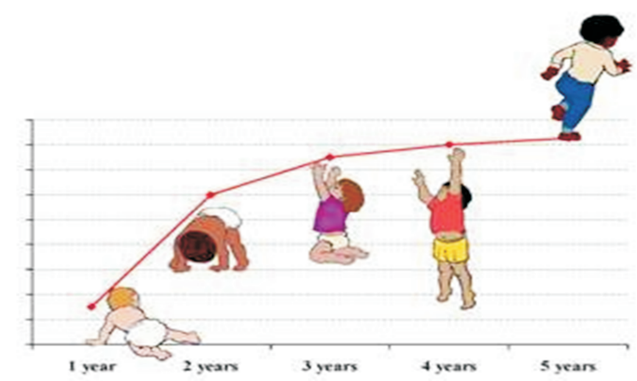
PIs) Maria S.Pombo-de-Oliveira, MD,Ph.D, Ana Rossini,PhD

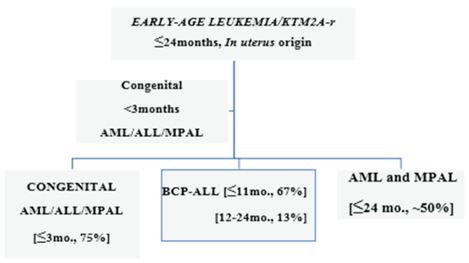
Co-participants, Orlando S.Louzada Neto, Ph.D student, Gisele Delapicola Brisson, Ph.D student; Francianne G Andrade, Ph.D; Ingrid Sardou-Cezar; Bruno Lopes,Ph.D; Pedro H. Cardoso, Mcs student, Paulo Chagas Neto, and the

Brazilian Collaborative Study Group of Infant Acute Leukemia*

Background

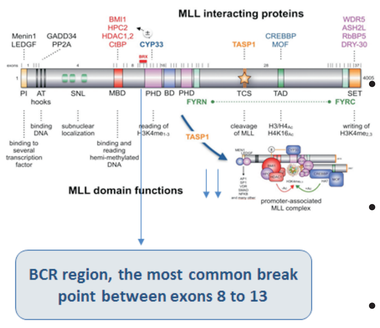
ACUTE LEUKEMIA - AGE - SOMATIC ABNORMALITIES





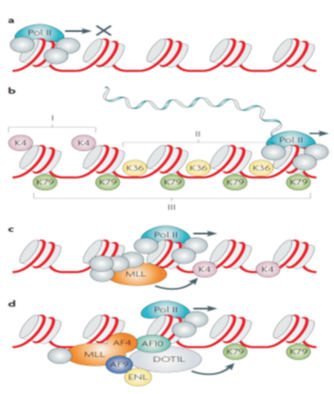
Epidemiological studies reveal that infant BCP-ALL has a peak incidence of 6-months of age, whereas, i-AML has a peak at 12 months of age at leukemia diagnosis. This time-frame supports the premise that leukemia initiated and accumulated additional genomic lesions during fetal life. Previous case-control study showed that maternal exposure previous and/or during pregnancy to hormones was associated with EAL risk in offspring

KMT2A OR *MLL* GENE (HISTONE-LYSINE N-METHYLTRANSFERASE 2A) ASSOCIATED WITH LEUKEMOGENESIS AND THE BIOLOGICAL PLAUSABILITY

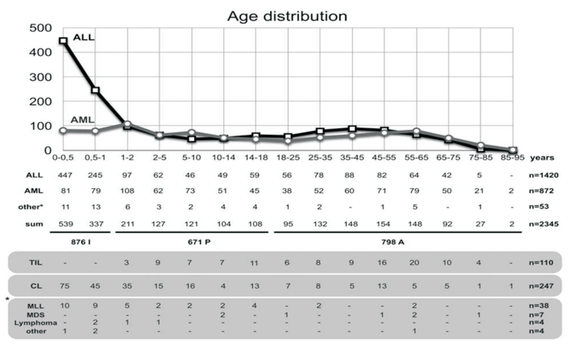


Rolf Marschalek, Ph.D.
http://dx.doi.org/10.3343/alm.2016.36.2.85

- *KMT2A* is highly expressed in embryonal tissue. Hypothesis derived from the fact that Topo-II inhibitor chemotherapy is associated with t-AML and the *KMT2A-MLL* cleavage mechanism (Potter *et al*, 1984);
- H3K4me3 is a histone mark associated with promoters and early transcribed regions of active genes. Topo-II cleaves DNA to generate a double-strand break (DSB), and reseals it so relaxing DNA strand in transcriptional events;
- The H3K4 demetilation in the SET domain is critical to initiation and early elongation to relaxing chromatin and give access to transcriptional driver process;
- *KMT2A-MLL* is frequently transcribed in close proximity to the main partner genes (*AFF1* or *MLLT3*), thus increasing the probability of rearrangements since a Topo-IIβ poison inhibits the DNA relegation step (Sondka et al. 2012)



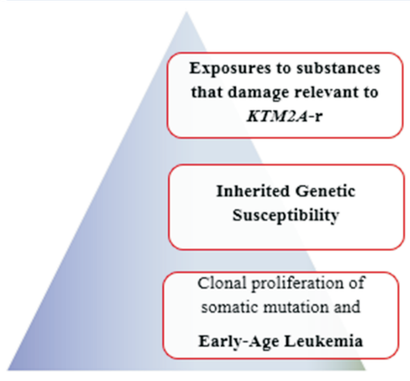
FREQUENCY DISTRIBUTION OF *KMT2A-R* AND PARTNER GENES IN LEUKEMIA ACCORDING TO AGES



- The precise localization of genomic breakpoints within the *KMT2A or MLL gene* and the involved translocation partner genes (TPGs) have been determined overtime;
- NINE rearrangements account for more than 90% of all llegitimate recombinations of the *MLL* : *AFF1/AF4, MLLT3/AF9, MLLT1/ENL, MLLT10/AF10, partial tandem duplications (MLL PTDs), ELL, MLLT4/AF6, EPS15 and MLLT11/AF1Q.*
- There was an age-dependent breakpoint shift with breakpoints in intron 11, which is associated with poorer outcome, being more common in younger patients while breakpoints in intron 9 predominate in older patients.
- The molecular characterization of MLL breakpoints suggests different leukemias aetiologies in different age-strata

Meyer et al. Leukemia (2018) 32, 273–284; doi:10.1038/leu.2017.213

Rational



Topo-II inhibitor chemotherapy is associated with t-AML with aberrant *KMT2A-MLL* cleavage;

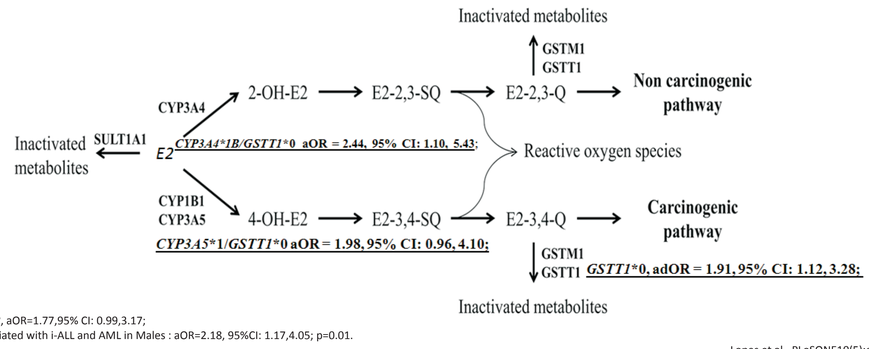
Foetus is vulnerable to toxic effects of maternal intakes in a closed environment (uterus);

Animal model´ studies have demonstrated substancies transplacental perfusion during pregnancy exposure;

Previous case-control study showed that maternal exposure to hormones was associated with EAL risk in offspring;

The gene variants in the estrogen metabolism (phase I and II enzymes) alter the production of quinones and reactive oxygen species, which damage DNA modulating the risk estrogen carcinogenic pathaway .

The DNA repair genomic system efficiency ensures the genomic stability if single strand breaks (BER,NER) and/or double strand breaks (NHEJ) would occur after xenobiotics exposures.



*CYP1B1*3/SULT1rs9282861 *2*, aOR=1.77,95% CI: 0.99,3.17;
SULT1A1(c.638G>A) was associated with i-ALL and AML in Males : aOR=2.18, 95%CI: 1.17,4.05; p=0.01.

Lopes et al., PLoSONE10(5):e0127308.doi:10.1371/2015

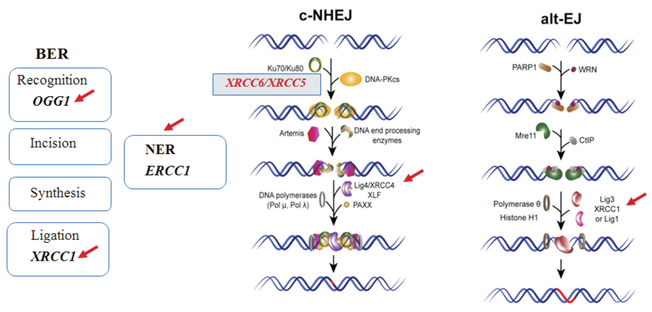
Acknowledgments

Brazilian Collaborative Study Group of Infant Acute Leukemia*

(*) The pediatritians, Renato Guedes, Sidnei Epelman (Hospital Santa Marcelina, São Paulo); Gustavo Ribeiro Neves (Hospital Sarina Rolin, Sorocaba, São Paulo), Teresa Cristina Oliveira (Hospital Amaral Carvalho, Jahu,São Paulo), Isis Maria Quezado Magalhães (Hospital da Criança de Brasília José Alencar, Brasília), Eloisa Cartaxo Fialho (Hospital Napoleão Laureano, João Pessoa,Paraíba), Patricia C. de Brito, Anna Carolina S. Dias, (Hospital Araújo Jorge, Goiana,Goiás), Eni de Carvalho, Juliana T. Costa, Luciana N. S. Souza (Hospital Martagão Gesteira, Salvador, Bahia), Marcelo S. Santos (Centro de Tratamento Onco-Hematológico Infantil de Mato Grosso do Sul, Campo Grande, Mato Grosso do Sul) and Teresa Cristina Cardoso-Fonseca (Santa Casa de Misericórdia de Itabuna, Bahia) that send samples and clinical data.

We would like to thank Bruno A.A. Gonçalves, Gisele M. Vasconcelos, and Eugenia T. Granado, Filipe V. S. Bueno, Elda P. Noronha (Programa de Hematologica Oncologia Pediátrico, Instituto Nacional de Câncer, Rio de Janeiro), for technical support. To Prof. Cintia B. Santos Rebouças for advices *in silico* analysis.

KEY-POINTS



Global genomic GG-BER is initiated by the recognition of damage-induced DNA helix distortions, and TC-BER is initiated by stalling of RNA polymerase II at a lesion. Following lesion recognition, the presence of DNA damage is verified (*OGG1*), structure-specific endonucleases are recruited to incise the damaged strand on both sides of the lesion and thereby excise the damage along with short flanking sequences. The excised strand is repaired by gap-filling DNA synthesis using the intact complementary strand as a template, in the ligation process (*XRCC1*).

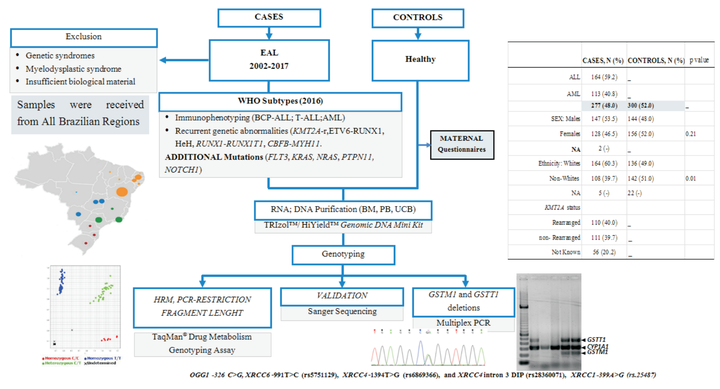
Extensive chromatin remodelling facilitates the DNA-damage detection steps of GG-BER and TC-BER, which results in restarting of transcription after repair and restoration of the original chromatin configuration.

BER deficiency is exemplary of the severe consequences of DNA damage. Defects in BER genes cause cancer predisposition.

Aim

To test if gene variants on the base excision repair (BER), NER and non-homolog end-joing (NHEJ) repair pathways were associated with EAL with *KMT2A-r* .

STUDY DESIGN



	CASES, N (%)	CONTROLS, N (%)	p value
ALL	164 (59.2)	_	
AML	113 (40.8)	_	
	277 (48.0)	300 (52.0)	_
SEX: Male	147 (53.5)	144 (48.0)	
Female	128 (46.5)	156 (52.0)	0.21
NA	2 (-)	_	
Ethnicity: White	164 (60.3)	136 (49.0)	
Non-Whites	108 (39.7)	142 (51.0)	0.01
NA	5 (-)	22 (-)	
KMT2A status			
Rearranged	110 (40.0)	_	
non- Rearranged	111 (39.7)	_	
Not Known	56 (20.2)	_	

The gene variants of BER,NER system and risk association with early-age leukemia with *KMT2A*-r according to cell subtypes

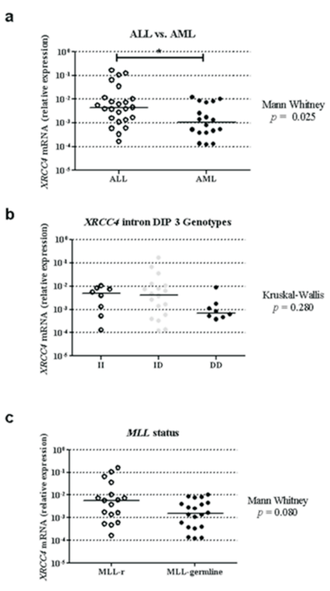
	GENE VARIANTS	EAL aOR (95% CI), *p*	i-ALL aOR (95% CI), *p*	AML aOR (95% CI), *p*
BER	***OGG1- 326C>G***			
	Dominat (CCxCG=GG)	0.92 (0.47-1.83), 0.83	1.03 (0.57-1.86), 0.91	1.40 (0.52-3.79). 0.50
	Recessive (CC=CGxGG)	2.58 (0.76-8.71), 0.12	3.16 (0.84-11.9), 0.08	1.80 (0.20-15.8), 0.59
	Aditive (CC=GGxCG)	1.16 (0.66-2.02), 0.59	1.08 (0.57-2.05), 0.80	1.45 (0.56-3.77), 0.44
NER	***ERCC1- 354 T>C***			
	Dominat (CCxCT+TT)	1.24 (0.82-1.81), 0.29	0.93 (0.45-1.88), 0.85	1.88 (0.64-5.48), 0.25
	Recessive (CC=CTxTT)	1.31 (0.71-2.41),0.37	1.26 (0.47-3.34), 0.64	2.46 (0.76-7.97), 0.13
	Aditive (CC=TTxCT)	1.50 0.79-2.83), 0.25	1.14 (0.39-3.35), 0.80	3.17 (0.80-12.5), 0.11
	ERCC1- 1516 C>A			
	Dominat (CCxCA+AA)	**0.54 (0.31-0.96), 0.03**	**0.46 (0.22-0.96), 0.03**	0.54 (0.23-1.28), 0.16
	Recessive (CC=CAxAA)	**0.79 (0.75-0.83), 0.01**	_	_
	Aditive (CC=AAxCA)	**0.76 (0.71-0.83), 0.01**	**2.80 (1.04-7.53), 0.04**	0.46 (0.09-2.36), 0.35

EAL, early-age leukemia; i-ALL, infant acute lymphoblastic leukemia; AML, acute myeloid leukemia; aOR, adjusted odd ratio for ethnicity; BER, base-excision repair; NER , nucleotide excision repair

The gene variants of at-EJ,NHEJ system and risk association with early-age leukemia with *KMT2A-r* according to cell subtypes

	GENE VARIANTS	EAL aOR (95% CI), *p*	i-ALL aOR (95% CI), *p*	AML aOR (95% CI), *p*
alt-EJ	***XRCC1-399 A>G***			
	Dominat (AAxAG=GG)	1.30 (0.90-1.92), 0.20	1.01 (0.62-1.66),0.94	**1.72 (1.03-2.87), 0.04**
	Recessive (AA=AGxGG)	**3.02 (1.64-5.56), 0.01**	0.79 (0.28-2.23), 0.66	**6.30 (3.25-12.2), <0.01**
	Aditive (AA=GGxAG)	**3.02 (1.59-5.73), 0.01**	0.83 (0.28-2.40), 0.70	**5.78 (2.86-11.6), 0.01**
NHEJ	***XRCC4- 1394 T>G***			
	Dominat (TTxTG=GG)	0.55 (0.24-1.30), 0.20	**0.30 (0.09-1.00), 0.04**	1.50 (0.50-4.65),0.51
	Recessive (TT=TGxGG)	1.90 (0.20-21.2), 0.51	_	8.30 (0.70-93.7), 0.17
	Aditive (TT=GGxTG)	0.60 (0.30-1.32), 0.28	0.31 (0.09-1.08), 0.05	1.80 (0.61-5.10), 0.34
	***XRCC4* Intron 3DIP**	aOR (95% CI), *p*	aOR (95% CI)	aOR (95% CI)
	Dominat	1.38 (0.81-2.34),0.23	1.70 (0.91-3.04),0.12	0.64 (0.26-1.57), 0.33
	Recessive	1.64 (0.95-2.86),0.10	**2.23 (1.17-4.25), 0.01**	0.74 (0.24-2.28), 0.60
	Aditive	1.45 (0.85-2.50), 0.20	1.50 (0.82-2.70),0.21	1.60 (0.68-3.76), 0.27

EAL, early-age leukemia; i-ALL, infant acute lymphoblastic leukemia; AML, acute myeloid leukemia; aOR, adjusted odd ratio for ethnicity; al-EJ, alternative non-homologous end-joining; NHEJ, canonical non-homologous end-joining.



Due to the observed association of *XRCC4* and i-ALL, we evaluated the bone marrow *XRCC4* mRNA expression levels by qPCR in a case-case approach: a) a higher expression of *XRCC4* in ALL than in AML (p = 0.025) was found; b) differential expression was observed according to the three different genotypes of the *XRCC4* intron 3 DIP; c) *KMT2A*-r showed higher *XRCC4* expression than *KMT2A* germline samples (p = 0.080).

In silico analysis was performed by using the bioinformatics tool Human Splicing Finder version 3.1 software (http://www.umd.be/HSF3/index) to test the potential functional impact of the indel polymorphism at *XRCC4* splicing sites. The model show that three changes on intron 3, caused by the deletion of 30 pb: (i) activation of an intronic cryptic donor site, (ii) alteration of a splicing silencer site and (iii) the creation of a splicing enhancer site. From those changes, we have speculated that *XRCC4* intron 3 DIP have a potential impact on splicing and function of the protein (data not shown).

Conclusion

The preliminary results suggest that *XRCC1* variant is strongly associated with i-AML, whereas *OGG1* variant is associated with i-ALL in a recessive model. Although we still need to test more samples to confirm these preliminary results;

Null association between *XRCC6* -991 T>C variant and EAL risk was found in any analyzed parameter; The *XRCC4* -1394 T>G variant demonstrated a protective effect on *KMT2A*-r only among i-ALL cases under the dominant model; i-ALL with *KMT2A*-r was associated with *XRCC4* intron 3 DIP (domint model IIxID genotype). The functional impact of the *XRCC4* intron 3 tested *in silico* predicted that the deletion allele is potentially associated with the activation of a 5-cryptic splice site in intron 3, which could retain part of this intron on the mRNA and modifying the protein structure; These data suggest the influence of the 30-bpdeletion allele over the XRCC4 protein or its repair function.

To the best of our knowledge, this study is the first to report gene variants in the BER,NER and NHEJ repair system associated with childhood leukemia with *KMT2A*-r and additional studies are necessary to evaluate the functional influence of these variants in the pathogeneses of EAL with *KMT2A*-r.

Projeto Gráfico: Setor de Edição e Informação Técnico-Científica / INCA