ALL-ICP, a simple and comprehensive method to detect chromosome abnormalities in Acute Lymphocytic Leukemia

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Interphase Chromosome Profiling

INTRODUCTION

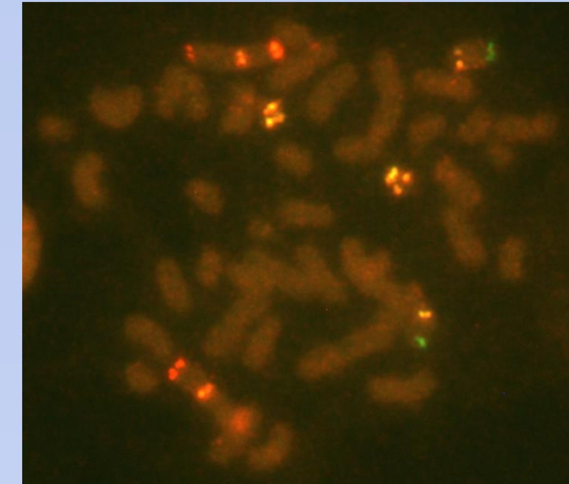
B- and T-cell acute lymphocytic leukemia (ALL) affects both children and adults. Cytogenetic and recent molecular genetic findings at diagnosis constitute important, independent prognostic factors in all age groups. Due to the typically poor morphology of the chromosome preparations, conventional cytogenetic analysis can miss one or more recurrent abnormalities and most ALL FISH panels only target select abnormalities. Therefore, a simple, fast, and comprehensive improvement to karyotype analysis would greatly enhance the diagnostic capabilities of clinical service as well as research laboratories for ALL.

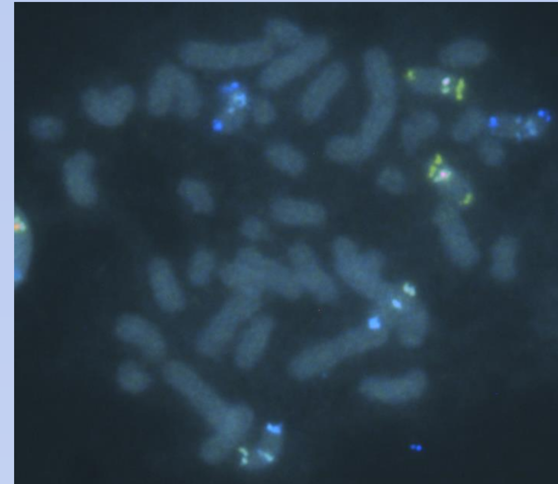
MATERIALS & METHODS

We recently developed and validated a novel molecular technology, Interphase Chromosome Profiling (ICP) (Babu et al., 2017). We tested a variation (ALL-ICP) of our original method for interrogating interphase cells and metaphase chromosomes in ALL samples. The design consisted of a multiplex approach with analysis of six chromosomes per hybridization site. Telomeres and pericentromeric regions on each chromosome are targeted and the resulting fluorescent signals are spectrally distinct and easily recognizable from each other using two dual filter sets from Chroma. Overnight hybridization is done on one slide in four areas and whenever possible, 20 metaphase spreads are analyzed for each chromosome. When 20 metaphases are unavailable, interphase nuclei were used to complete the 20-cell analysis. Thirty-two ALL samples were studied blindly by the laboratory that developed the technology. Two institutions provided cell pellets with known cytogenetic and FISH results.

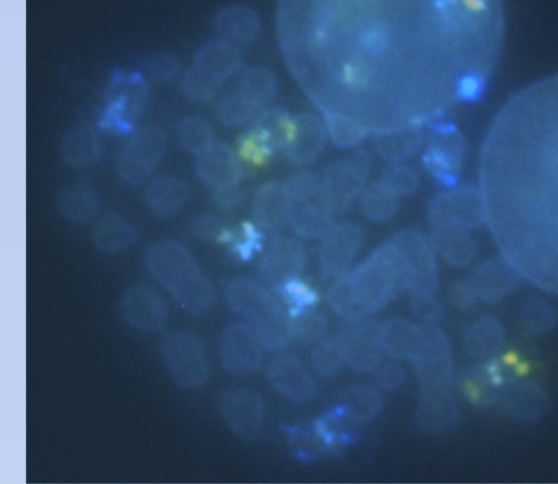
RESULTS

Sample Number	Cytogenetic Findings	ICP Results	Additional changes found and/or characterized by ALL-ICP
1	Hyperdiploid, +der	Concordant +	Characterized the derivative
2	Add(5q), inv(7)	Inv missed	Add origin 15q
3	Tetraploid, add(3p), 6(p) 1-3 mar	Concordant +	Deletion 14q, markers origin 13q and 10q
4	Complex abnormalities	Concordant +	14q deletion and dim 14q
5	Complex, add and interstitial Deletions	Missed interstitial deletions	Add origin 19q
6	Complex, add(5q), add(17q), interstitial deletions	Concordant + Missed inst. Deletions	Add origin 5q and 8q resulting in duplications
7	Complex with markers	Concordant +	One mar is der(11)
8	-X and 6q deletion – 1 cell	Missed deletion	t(12;21) and 14q deletion
9	Hyperdiploid, i(7q)	Concordant	None
10	Hyperdiploid, add(19p)	Concordant +	Add origin 12q
11	Hyperdiploid	Concordant +	+9 is der(9)t(8;9); +15 is +18
12	dic(9;20),+21	Concordant	None
13	t(1;19)(q23;p13.3)	Concordant +	14q deletion
14	t(1;19)(q23;p13.3)	Concordant	None
15	t(9;22)(q34;q11.2)	Concordant +	Homozygous 14q deletion
16	t(9;22)(q34;q11.2)	Concordant +	14q deletion
17	+i(21)(q10)x2	Concordant +	t(5;10), 16q and 22q deletions
18	Hyperdiploid	Concordant +	+14 is der(14)t(14;21), deletions (subtelomere) 18p and 15q
19	del(13)(q14q34)	Missed deletion	t(12;21), 14q deletion
20	Hypodiploid, t(9;22), dup(3q)	Missed duplication	None
21	Hyperdiploid with dup(1q)	Missed duplication	del(3p) and 13 identified as der(3); deletion 16p and -20
22	t(6;22)(q23;q12)	Concordant +	t(12;21) and multiple rearrangements involving chromosomes 6, 9, 8, 10
23	Complex with variant t(9;22)	Concordant +	12 p deletion
24	add(21)(q22)	Missed RUNX1 amplification	21q deletion
25	+X,+21c	Concordant +	21q deletion, 14q deletion, der(15)
26	del (12)(p11.2p13)	Concordant	None
27	Variant t(9;22)	Concordant +	Iso dic(17q)
28	del(6)(q13q27)	Missed deletion	None
29	Complex with add(10q)	Concordant +	Clarified add(10q) as iso dic(10q)
30	Deletions 9p and 6q	Missed deletions	-19
31	add (6q)	Concordant +	Add origin 5q; der(19)t(1;19), 14q deletion
32	Hyperdiploid	Concordant +	10p and 21q deletions

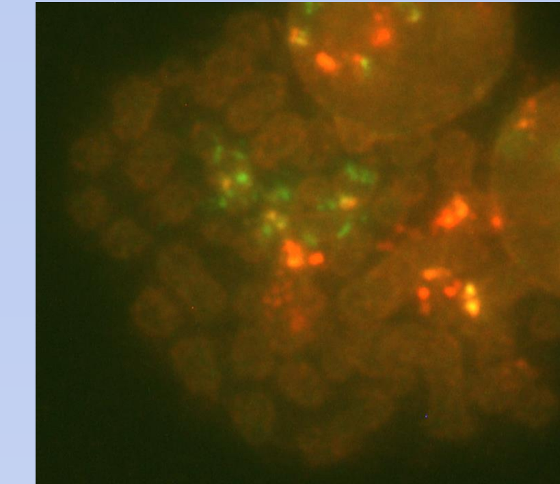




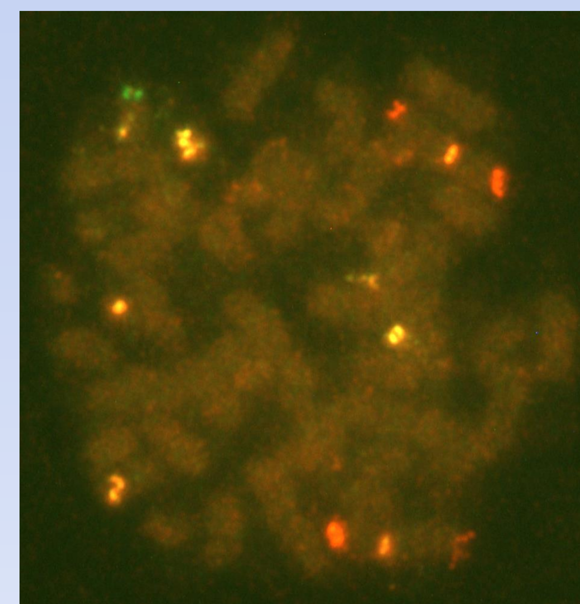
Normal Chromosomes – Mix 1



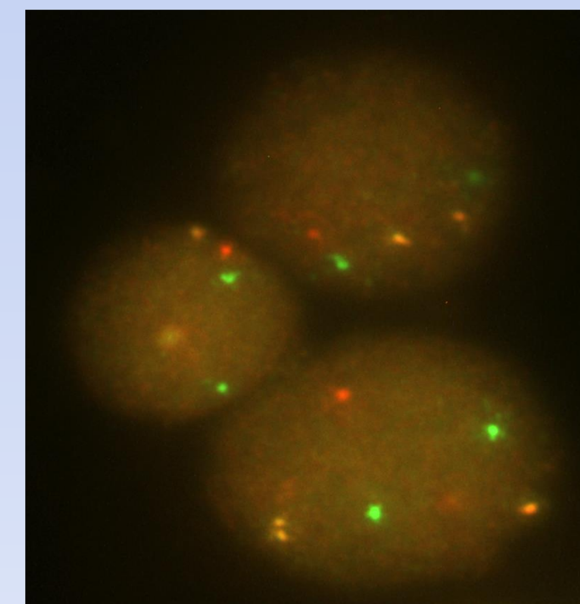
Trisomy 6



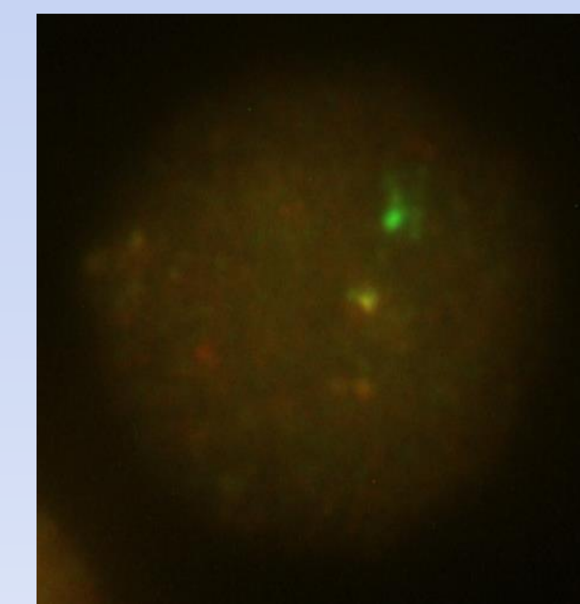
Trisomy 4, 10



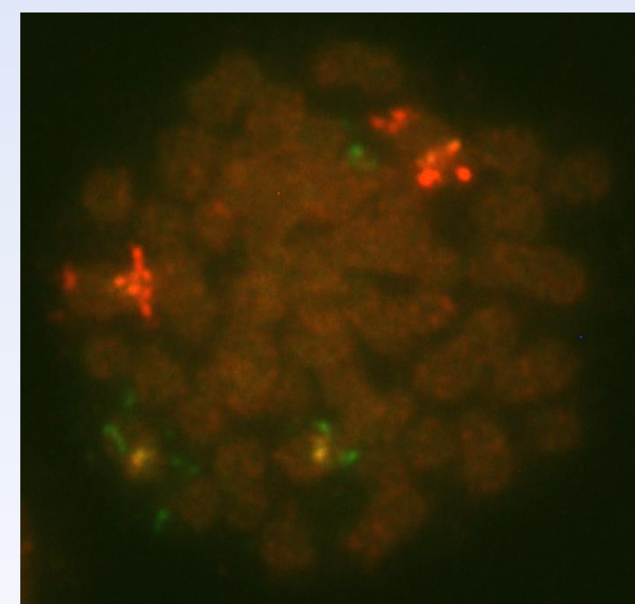
14q32 (IgH) deletion

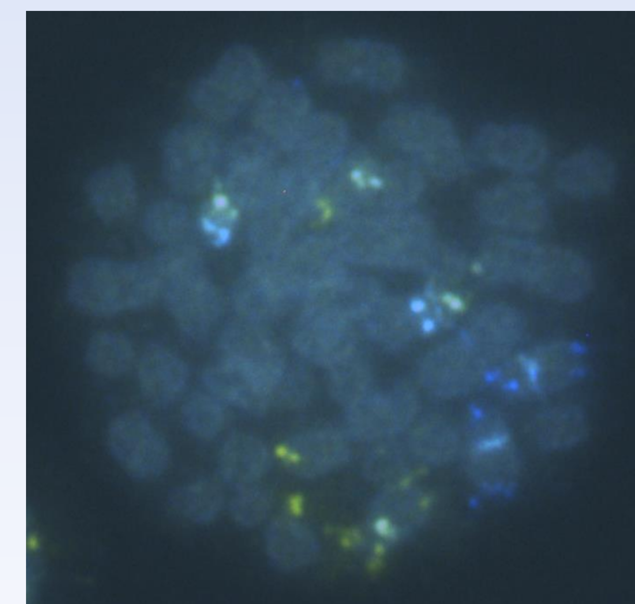


9p21.3(p16) deletion

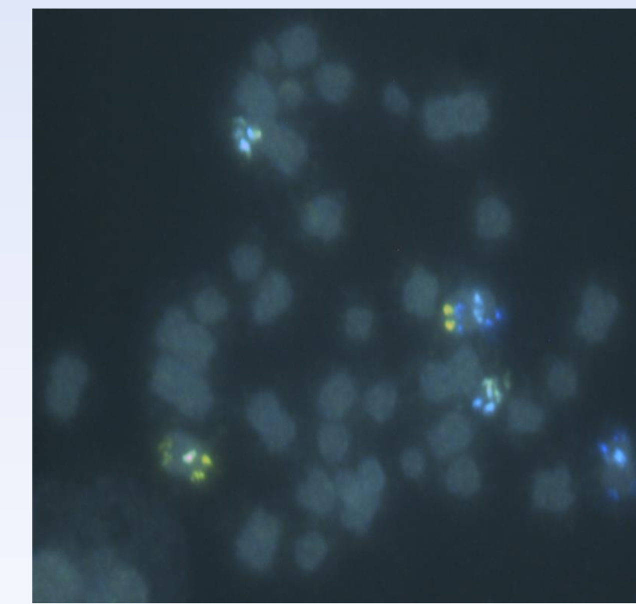


6q21 deletion, homozygous p16 deletion

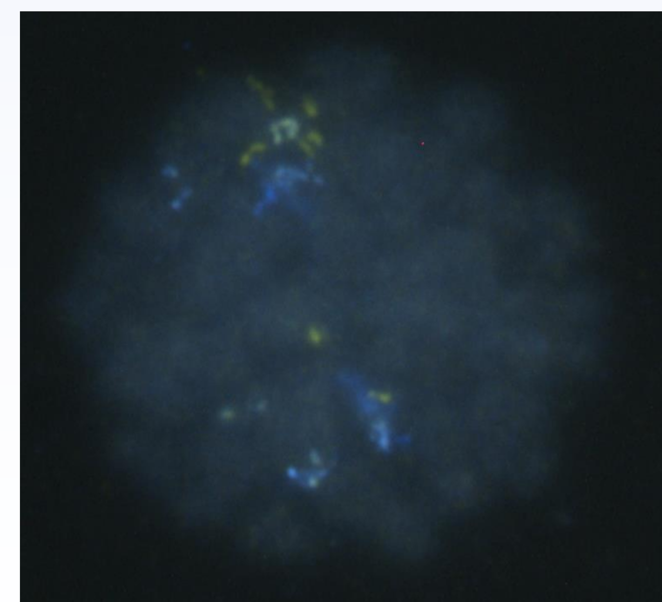


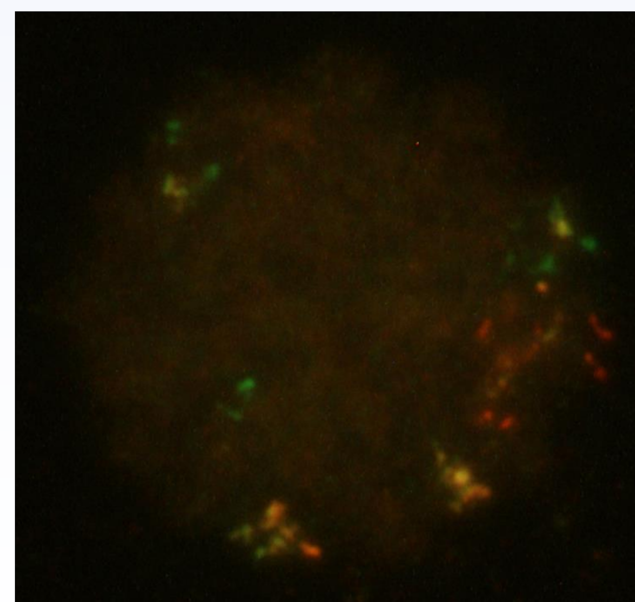


t(5;10)(p;q)

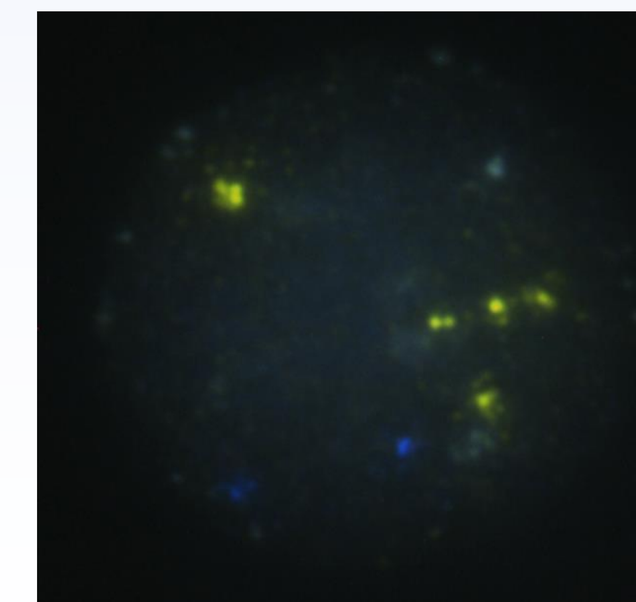


der(6)t(5;6)(q;q)





t(12;21)(p13;q22)



RUNX1 amplification

CHROMOSOME COLOR SCHEME

Color Pattern	Mix 1	Mix 2	Mix 3	Mix 4	Mix 5 *
Red	2	1	4	7	9
Green	14	12	10	16	6
R/G Hybrid	22	17	Y	13	13
Gold	8	X	5	3	21
Aqua	9	19	6	18	X
Y/A Hybrid	11	21	20	15	

*This probe set was developed and tested *after abstract submission*, for deletions and duplications

CONCLUSION

- All numerical abnormalities identified by karyotype or FISH are detected by ALL-ICP.
- ALL-ICP refined the breakpoints and clarified marker and derivative chromosomes.
- Novel 14q32 (IgH) deletions and several previously unknown sub-telomere deletions were identified.
- There appears to be an association between t(9;22) and 14q deletions – nearly 50% with this deletion had the translocation.
- An additional probe set identified all common deletions and amplification of RUNX1.
- All known and new balanced translocations were detected.
- ALL-ICP detected additional abnormalities in 87% of the cases.

ALL-ICP is a technically simple method that saves valuable time, and with the added probes will detect all clinically relevant recurrent B- and T-cell chromosome abnormalities. These attributes, supported by the study results, make ALL-ICP ideal for the genetic diagnostic workup of Acute Lymphocytic Leukemia. The novel telomeric deletions identified in this study, especially IGH deletions may have clinical significance and warrants further studies.

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