# Abstract

Bone marrow-level oxygen tension enables enhanced and sustained growth of 3 new pediatric acute lymphoblastic leukemia cell lines

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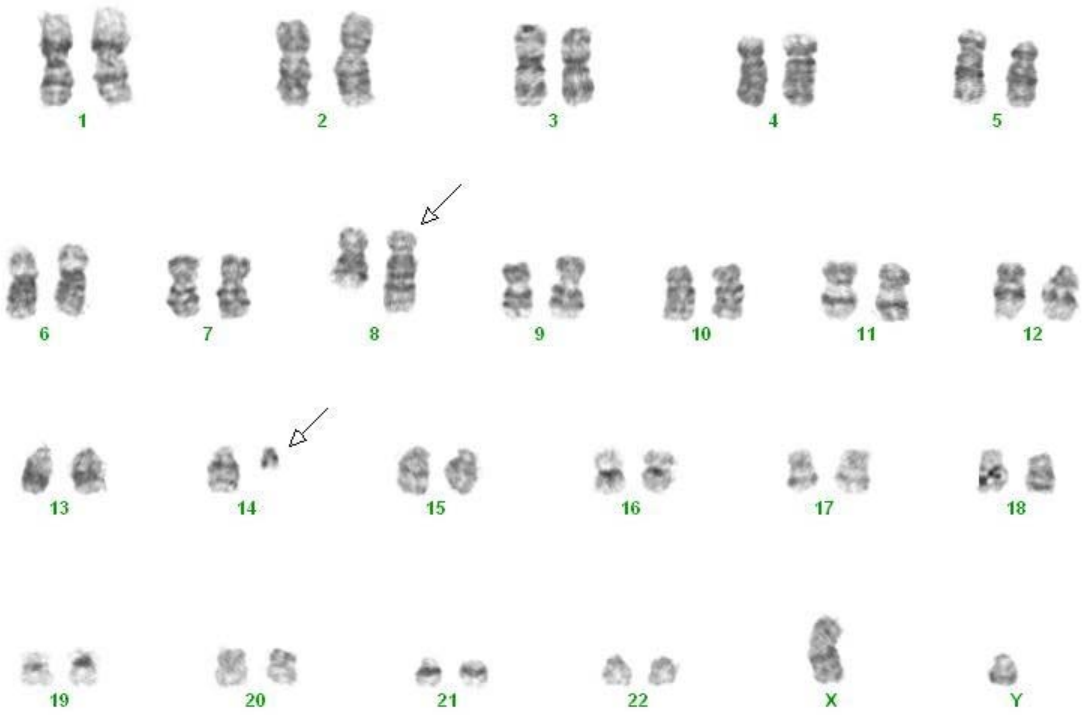
Many pediatric acute lymphoblastic leukemia (ALL) cell lines demonstrate genetic instability and are cultured under non-physiological selection pressures. We have established 3 new ALL cell lines in Iscove's MDM, 20% fetal bovine serum, insulin, transferrin, and selenous acid, and maintained them in both 20% $O_2$ (typical cell culture conditions, *i.e*., room air) and 5% $O_2$ (bone marrow-level oxygen tension) from the time of their initial isolation. Karyotypes, immunophenotypes by flow cytometry, and drug sensitivities (effective concentrations giving 90% cell kill ($EC_{90}$) by the DIMSCAN fluorescence imaging cytotoxicity assay) are shown in the table. Karyotypes and immunophenotypes of cell lines closely matched those of patient samples, and genotyping by examination of DNA short tandem repeats (STR) confirmed that cell lines matched the patient sample or were unique from all other cell lines in the lab. Sufficient expansion to enable wide distribution was possible. Higher growth rates were observed in 2 of the cell lines grown in 5% $O_2$ as demonstrated by decreased doubling times (29 h *vs*. 19 h for COG-LL-317; 62 h *vs.* 48 h for COG-LL-319) and by the increased clonogenic ability of 317 and 319 cells plated in 5% $O_2$ in limiting dilution assays ($p<0.001$). Higher levels of cell growth were due, at least in part, to increased rates of division, since faster growing cells exhibited both increased BrdU incorporation and a higher number of cell divisions as shown by loss of the dye CFSE after each mitotic cycle. Unlike many commonly available ALL cell lines that have a high level of DNA microsatellite alterations (MSA) (eg., NALM-6, MOLT-4, Jurkat), little or no MSA was observed in our lines. NOD/SCID mice xenografted with 317, 319, or 332 cells survived for 6.5, 9, and 4.5 weeks, respectively. These new pediatric ALL cell lines will provide novel models for biological and preclinical therapeutic studies, and will enable exploration of the molecular and biological differences in ALL cell lines cultured in "standard" culture conditions *versus* bone marrow-level physiological oxygen tension.

**Cell Line Characteristics**

| | **COG-LL-317** | **COG-LL-319** | **COG-LL-332** |
|---|---|---|---|
| **Pt / status / site / survival of xenografted NOD/SCID mice** | 2 y.o. male / 2nd relapse / BM / 4.5 weeks | 13 y.o. female / diagnosis / blood / 9 weeks | 10 y.o. male / 1st relapse / BM / 4.5 weeks |
| **Immunophenotype** | $sCD3^+CD7^+CD8^+$ (T cell ALL) | $CD10^+CD19^+CD22^+$ (pre-B cell ALL) | $sCD3^-CD7^+CD8^+$ (T cell ALL) |
| **Karyotype** | 46, XY, t(8;14)(q24.1;q11.2) | 46, XX, der(7)t(1;7)(q32;q36), del(9)(p11.2), der(19)t(1;19)(q23;p13.3) | 46, XY, t(1;9)(p34;q34) |
| **4-HC ( g/ml) ($EC_{90}$)** | 0.88 | 1.4 | 2.7 |
| **L-asparaginase (IU/ml)** | 0.23 | 2.2 | 11.7 |
| **Dexamethasone ( M)** | 3.3 | 24.5 | >400 |
| **Doxorubicin (nM)** | 5.7 | 34.5 | >400 |
| **Etoposide (nM)** | 49.1 | 202.5 | >400 |
| **Vincristine (ng/ml)** | 2.2 | 22.8 | 19.9 |

# Karyotypes of new COG leukemia cell lines

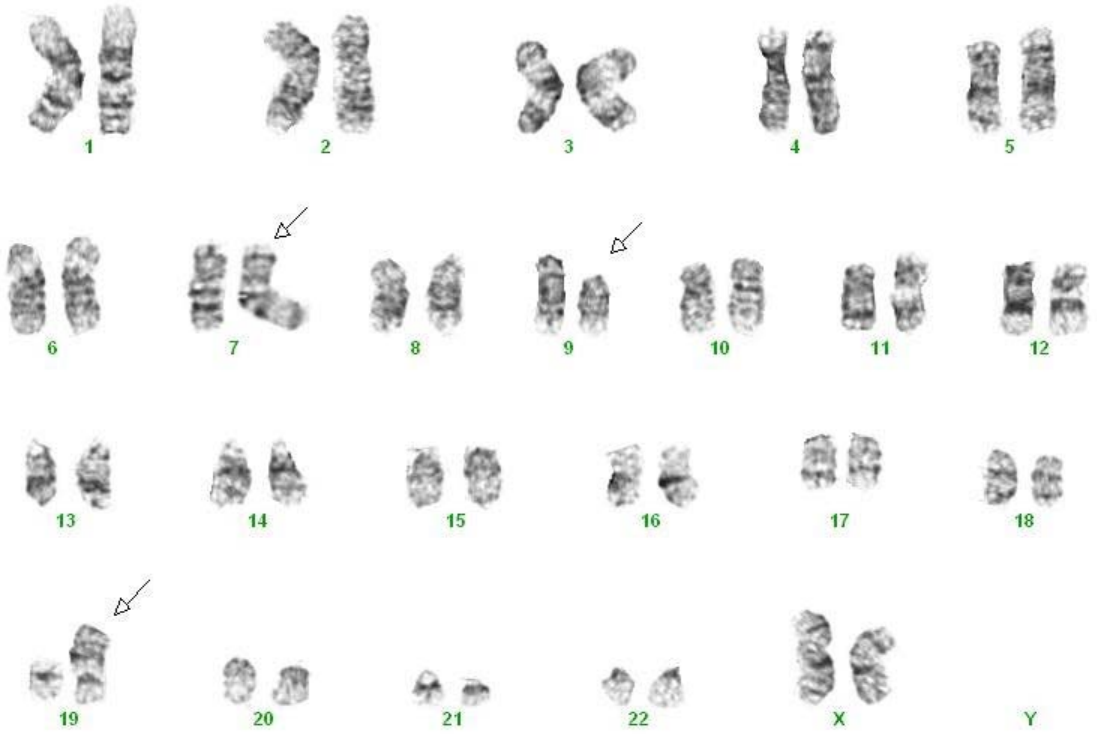
## COG-LL-317h




**46,XY,t(8;14)(q24.1;q11.2)**

The karyotype of COG-LL-317h T cell acute lymphoblastic leukemia cells, as identified by GTG banding techniques (Wu_SQ *et al*., 2003, *J Pediatr Hematol Oncol,* 25: 520-525) and described according to the International System for Human Cytogenetic Nomenclature (Mitelman F, ed. *An International System for Human Cytogenetic Nomenclature.* Basel, Switzerland: S. Karger, 1995).

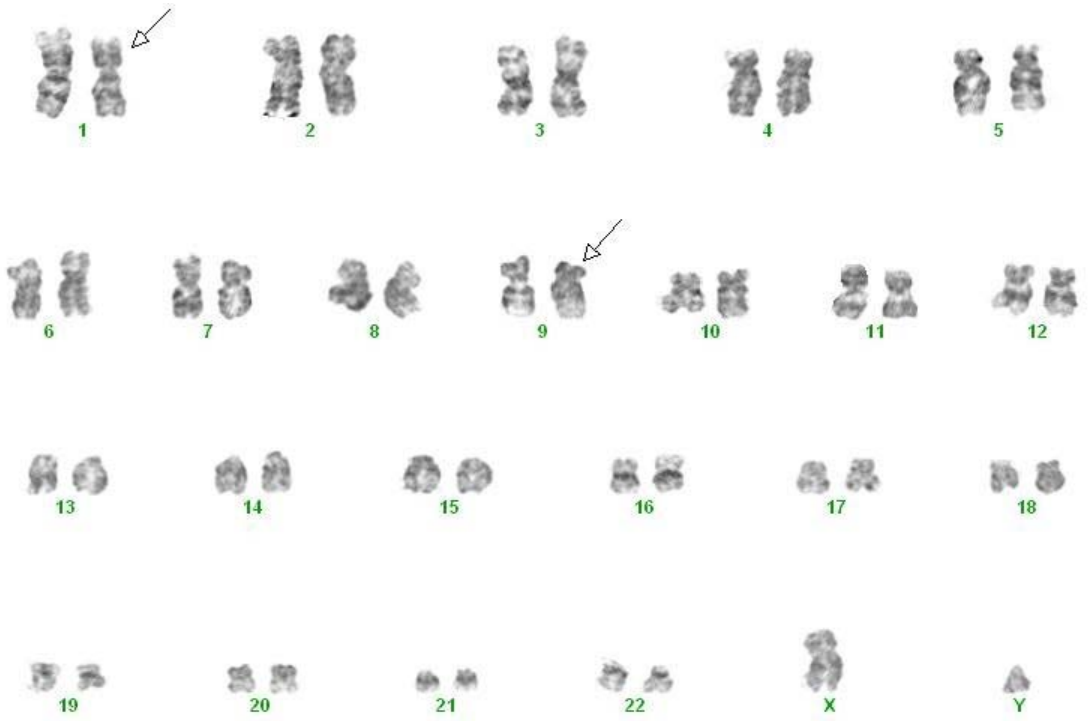
**COG-LL-319h**




**46,XX,der(7)t(1;7)(q32;q36),del(9)(p11.2),der(19)t(1;19)(q23;p13.3)**

The karyotype of COG-LL-319h pre-B cell acute lymphoblastic leukemia cells, as identified by GTG banding techniques (Wu_SQ *et al*., 2003, *J Pediatr Hematol Oncol,* 25: 520-525) and described according to the International System for Human Cytogenetic Nomenclature (Mitelman F, ed. *An International System for Human Cytogenetic Nomenclature.* Basel, Switzerland: S. Karger, 1995).

**COG-LL-332h**




**46,XY,t(1;9)(p34;q34)**

The karyotype of COG-LL-332h T cell acute lymphoblastic leukemia cells, as identified by GTG banding techniques (Wu_SQ *et al*., 2003, *J Pediatr Hematol Oncol,* 25: 520-525) and described according to the International System for Human Cytogenetic Nomenclature (Mitelman F, ed. *An International System for Human Cytogenetic Nomenclature.* Basel, Switzerland: S. Karger, 1995).