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RAS oncogene mutations and outcome of therapy for childhood acute lymphoblastic leukemia

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Activating mutations in the *RAS* oncogenes are among the most common genetic alterations in human cancers, including patients with acute lymphoblastic leukemia (ALL). We sought to define the frequency and spectrum, and possible prognostic importance, of *N-* and *K-RAS* mutations in children with ALL treated with contemporary therapy. Leukemic blast DNA from 870 children was analyzed for the presence of activating mutations in the *N-* or *K-RAS* oncogenes using a sensitive mutation detection algorithm. *RAS* mutations were present in the blasts of 131 (15.1%) pediatric ALL patients. The spectrum of mutations included 81 (9.3%) mutations of codons 12/13 of *N-RAS*, 12 (1.4%) mutations of codon 61 of *N-RAS*, 39 (4.5%) mutations of codons 12/13 of *K-RAS*, and 2 (0.2%) mutations of codon 61 of *K-RAS*. The presence of *N-* or *K-RAS* mutations was not associated with white blood cell count at diagnosis, sex, race, extramedullary testicular involvement, central nervous system disease, or NCI/CTEP ALL Risk Group. Patients with an exon 1 *K-RAS* mutation (codons 12/13) were significantly younger at diagnosis ($P = 0.001$) and less frequently B-lineage phenotype ($P = 0.01$). *RAS* mutation status did not predict overall survival, event-free survival and disease-free survival. While *N-* and *K-RAS* mutations can be identified in 15% of children with newly diagnosed ALL, they do not represent a significant risk factor for outcome using contemporary chemotherapy regimens.

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Introduction

The human *RAS* proto-oncogene superfamily includes a series of related small proteins that have a central role in key cellular processes, including receptor-mediated signaling, proliferation, and survival (Figure 1).[1–3] The *RAS* subfamily involved in transmitting signals for essential cellular processes through growth factor and cytokine receptors consists of three genes (*N-RAS*, *K-RAS*, and *H-RAS*) that are located on separate chromosomes. *RAS* gene products are small proteins that are highly homologous to one another, and require association with the inner surface of the plasma membrane for activity.[4,5]

The *RAS* gene products, which bind the nucleotides guanosine triphosphate (GTP) or guanosine diphosphate (GDP), are GTPases. They are characterized by conserved structural motifs, and their ability to alternate between specific active or inactive conformations by binding either GTP or GDP, respectively. The process of converting between alternate conformations of the GTPases is normally tightly regulated by other components of the receptor-associated signal transduction system. This allows GTPases to function as 'molecular switches' in their specific activation of downstream cellular regulatory components. Mutations in the conserved GTP binding and hydrolysis domains can result in profound inhibition of the intrinsic GTPase activity, and consequent inappropriate high-level activation of downstream effectors. Most studies of human tumors have identified *RAS* codons 12, 13, and 61 as the most frequent site for these oncogenic mutations. RAS proteins containing these mutations bind GTP constitutively, and inappropriately activate targets that mediate proliferation and viability.

It is estimated that approximately 15% of all human malignancies possess mutations in the N-, K-, or H-RAS proteins.[6] In acute lymphoblastic leukemia (ALL), multiple studies have indicated that *RAS* mutations can be found in 6–20% of cases, with most mutations identified in *N-RAS*. Most studies have included too few patients to adequately analyze the impact of the presence of *RAS* mutations on the outcome of childhood ALL therapy. A study of 100 newly diagnosed pediatric patients with ALL suggested that *N-RAS* mutations were statistically insignificantly associated with an increased risk of relapse,[7] raising the possibility that the presence of *RAS* mutations in diagnostic ALL blast samples could be used as laboratory marker for risk-adapted therapies. However, a more recent analysis of 125 patients found no correlation of *RAS* mutations with outcome.[8] We sought to define the spectrum and clinical implications of *N-* and *K-RAS* mutations in a large series of 696 children with ALL who were treated on Children's Cancer Group (CCG) regimens.

Patients, materials, and methods

Population

The study population included 870 children with ALL who participated in a CCG epidemiologic study of ALL; a parent or guardian signed an institutional review board-approved informed consent document for participation.[9–14] In total, 696 cases also were enrolled on CCG therapeutic trials for untreated ALL and were available for analyses of treatment outcomes. All patients in this study were newly diagnosed with *de novo* ALL between 1 January 1989 and 15 June 1993. Clinical data collected prospectively included age, sex, white blood cell count at diagnosis, hepatomegaly, splenomegaly, and presence of testicular or central nervous system (CNS) disease. Classification of leukemia was conducted by morphologic review of bone marrow blast samples, immunophenotyping at the central

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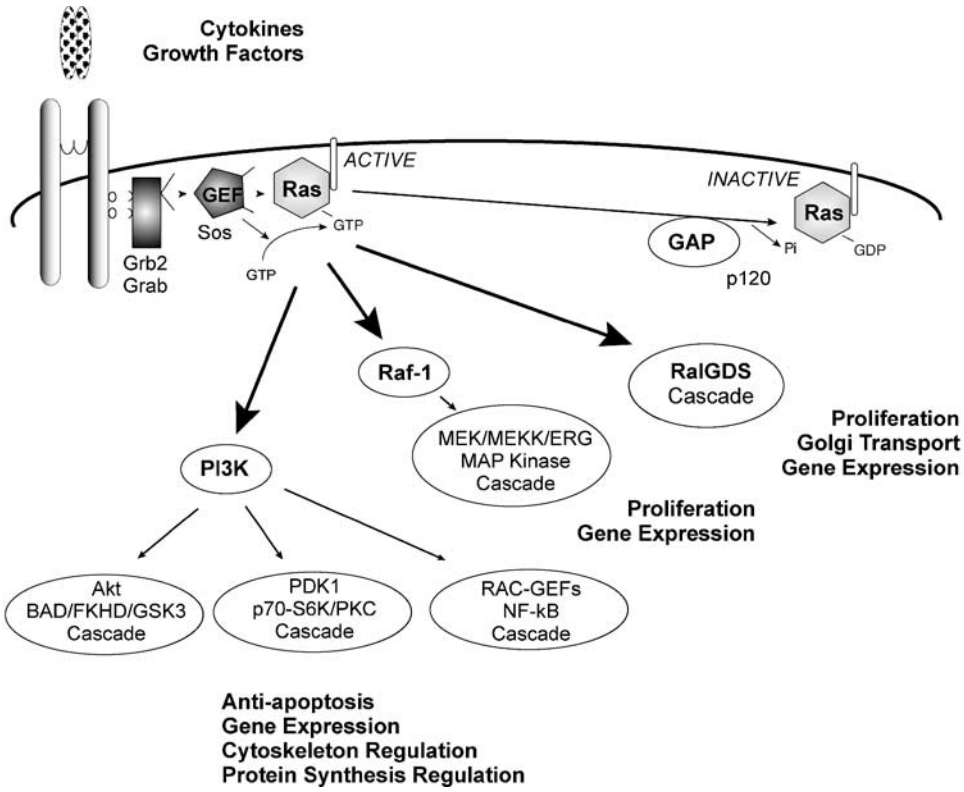


Figure 1 Overview of the RAS Signaling Pathway. RAS is active when it is in its GTP-bound state. The guanine nucleotide exchange factors (GEFs) catalyze the replacement of GDP with GTP bound to RAS resulting in RAS activation of its effector cascade. Activated signaling complexes recruit GEFs such as SOS, which in turn activate RAS. RAS is converted to an inactive state by the hydrolysis of GTP to GDP. The intrinsic ability of RAS to hydrolyze GTP is catalyzed by a large family of GTPase activating proteins (GAPs) including p120. There is a wide range of downstream RAS effectors that mediate key signals for cellular proliferation, antiapoptosis, gene expression, cytoskeleton regulation, Golgi transport, protein synthesis regulation, and other biological processes. Downstream or upstream mutations in elements in the RAS pathway also may lead to dysregulated cellular growth, and the neoplastic or malignant phenotype. MAP, mitogen-activated protein kinase; RALGDS, guanine nucleotide exchange factors for RAL; FKHD, Forkhead transcription factors; PKC, protein kinase C; ERK, extracellular regulated kinase; GSK3, glycogen synthase kinase3; MEK, mitogen-activated kinase/ERK kinase; p70S6K, p70 ribosomal protein S6 kinase; PDK1, phosphatidylinositol trisphosphate-dependent kinase 1.

Children's Cancer Group ALL Reference Laboratory, and cytogenetic analysis. Immunophenotyping included analysis with a standard panel of monoclonal antibodies that included the B-lineage markers CD19, 10, and 24, and the T-lineage markers CD2, 5, and 7. A subset of samples with blasts possessing B-lineage markers also was assayed for the presence of cytoplasmic immunoglobulin (Ig). Cases were classified as (1) early pre-B ALL (positive for B-lineage markers; negative for cytoplasmic Ig), (2) pre-B ALL (positive for B-lineage markers and for cytoplasmic Ig), (3) B-lineage ALL, not otherwise specified [NOS] (positive for B-lineage markers; cytoplasmic Ig not performed), (4) T-cell ALL (positive for T-lineage markers), and (5) unclassified. Patients studied for RAS mutation status did not differ in outcome (induction remission rate, overall survival, disease-free survival, and event-free survival) compared with those for whom RAS status was not determined.

Chemotherapy treatment regimens

Patients were treated on risk-adapted multidrug chemotherapy regimens. In general, risk was defined by initial white blood cell count, age, FAB-L3 morphology, and presence of lymphomatous disease. Patients in the 2–9 year age group with white blood cell counts less than 10 000/μl, and $<$25% marrow L3 blasts were classified as lower risk and treated according to the CCG-1881 protocol.[15] Patients in the 1–10 year age group, with white blood cell counts between 10 000 and 50 000/μl, but without lymphomatous features, were classified as intermediate risk and treated on the CCG-1891 protocol.[16] Patients with white blood cell counts greater than 50 000/μl and or age greater than 10 years (excluding those with lymphomatous features of L3 morphology) were classified as high risk and were treated according to the CCG-1882 protocol.[17] Patients with lymphomatous disease were treated on the CCG-1901 regimen.[18]

RAS mutation analyses

DNA was extracted from archival bone marrow slides or from cryopreserved marrow samples as described before.[19] DNA cells were scraped from the slide with a scalpel using sterile technique to prevent contamination. Cells were suspended in polymerase chain reaction (PCR) buffer (50 mM KCl, 10 mM Tris HCl (pH 9.0), 1% Triton X-100), boiled for 10 min, extracted twice with phenol, and precipitated with ethanol. DNA was washed once with 70% ethanol, resuspended in Tris-EDTA buffer, and amplified by PCR.

Polymerase chain reaction amplification of *N-* and *K-RAS* exon gene fragments was performed in a thermocycler (Perkin-Elmer Cetus) with approximately 200 ng DNA employ-

ing standard conditions in 100 μl total reaction volume. The PCR reaction buffer contained 50 mmol/l KCl, 10 mmol/l Tris Cl (pH 8.3), 1.5 mmol/l $MgCl_2$, 0.001% (wt/vol) gelatin (Sigma, St Louis, MO, USA), 200 μmol/l dNTPs (Boehringer Mannheim, Mannheim, Germany), and 2.5 U AmpliTaq (Perkin-Elmer Cetus, Norwalk, CT, USA). Sequences surrounding *N-* and *K-RAS* codons 12, 13, and 61 were amplified using the following protocol: cycle 1, 94°C for 5 min, 55°C for 1 min, and 72°C for 1 min; cycles 2–35, 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min. The oligonucleotide primers (Figure 2) were used at a concentration of 0.5 Φmol/l. Each PCR amplification of the *RAS* genes exon gene fragments from patient samples included positive and negative controls, and the efficiency and specificity of each amplification was assessed by analysis of the products after electrophoresis on a 3% NuSieve (FMC, Rockland, ME)/1% agarose gel and ethidium bromide staining.

Studies in our laboratory had demonstrated that direct DNA sequencing of amplified *RAS* exon fragments was sensitive for detection mutation in samples with greater than 50% blasts (data not shown). Samples with less than 50% blasts were screened by single-strand conformation polymorphism (SSCP) analysis at 4 and 22°C. Bands with abnormal migration on SSCP analysis were excised and subjected to PCR-amplification with nested primers, and analyzed with subsequent direct DNA sequencing.

In the confirmatory level of analysis, the DNA of *RAS* mutation-positive samples was subjected to an independent second PCR amplification and repeat DNA sequencing for *RAS* gene mutation sequence confirmation.

Statistical analysis

Differences in induction outcome, dichotomized into complete remission or no remission, were assessed with Pearson's χ^2 statistic. Survival estimates were based on the method of Kaplan and Meier,[20] with differences in overall survival, disease-free survival, and relapse-free survival evaluated using the log-rank global χ^2 analysis.[21] Disease-free survival and relapse-free survival were defined for those patients with a complete remission. Disease-free survival was defined as the time from the end of induction to relapse or death. Relapse-free survival was defined as the time from the end of induction to marrow relapse or death from progressive disease, censoring on deaths from other causes. Cox regression was used for multivariate models assessing differences between groups while adjusting for patient characteristics that have prognostic significance.[22]

N-RAS exon 1 (surrounding codons 12, 13)
5'-GTACTGTAGATGTGGCTCGC-3'
5'-CCTCACCTCTATGGTGGGAT-3'

N-RAS exon 2 (surrounding codon 61)
5'-ACCCCCAGGATTCTTACAGA-3'
5'-CTGTCCTCATGTATTGGTCT-3'

K-RAS exon 1 (surrounding codons 12, 13)
5'-GAAAATGACTGAATATAAACTTGTGGT-3'
5'-CATATTCGTCCACAAATGATTCT-3'

K-RAS exon 2 (surrounding codon 61)
5'-CTGTGTTTCTCCCTTCTCAGG-3'
5'-AGAAAGCCCTCCCCAGTCCT-3'

Figure 2 Oligonucleotide primers.

Results

RAS mutations and patient demographics, laboratory/ clinical characteristics

In total, 131 of 870 patients studied (15%) had leukemia with *RAS* mutations. The most frequent sites of mutation were in codons 12/13, located in exon 1, of the *N-* and *K-RAS* genes. Mutations at codon 61, located in exon 2, were infrequently present in either of the *N-* or *K-RAS* genes. The spectrum of mutations included 81 (9.3%) mutations of codons 12/13 of *N-RAS*, 12 (1.4%) mutations of codon 61 of *N-RAS*, 39 (4.5%) mutations of codons 12/13 of *K-RAS*, and 2 (0.2%) mutations of codon 61 of *K-RAS* (Table 1). Most mutations (78%) at codons 12/13 involved a G:C to A:T transition. In contrast, transitions accounted for only 43% of the mutations at codon 61. Three patients (0.3%) had leukemic blast populations with *RAS* mutations concurrently detected at two different sites. The spectrum of mutations in codons 12, 13, and 61 of the *N-* and *K*-RAS genes is shown in Figure 3. A glycine to aspartic acid (G to D; single letter amino-acid code) change was the most frequent amino-acid substitution, particularly in codon 13. The frequency of specific amino-acid substitutions is shown in Figure 4.

The presence of *RAS* mutation was not significantly associated with sex, race, initial white blood cell count, extramedullary disease (CNS or testicular), or prognostic risk group (Table 1). Patients with an exon 1 *K-RAS* mutation (codons 12/13) were significantly younger at diagnosis ($P = 0.001$) and less frequently B-lineage phenotype ($P = 0.01$).

RAS mutations and clinical outcome

Overall, 97.2% of patients achieved an initial remission. The proportion of induction failures was similar among those with and without any *RAS* mutation (1.9 and 2.9%, respectively, $P = 0.56$). No significant differences were found when induction outcome was analyzed according to specific *RAS* mutations. Analyses of overall survival ($P = 0.30$) (Figure 5), event-free survival ($P = 0.64$) (Figure 6), and disease-free survival ($P = 0.66$) found similar patterns of outcomes for patients who did not have a *RAS* mutation compared with those with any RAS mutation. Multivariate models including gender, race, age at diagnosis, initial white blood cell count, or NCI/CTEP risk group demonstrated that mutations in *N-RAS* exon 1 (codons 12/13), *N-RAS* exon 2 (codon 61), *K-RAS* exon 1 (codons 12/13), *K-RAS* exon 2 (codon 61) or any *RAS* mutation were not independent predictors of event-free survival (Table 2).

Discussion

To the best of our knowledge, this was the largest series of *N-* and *K-RAS* gene mutation analysis in ALL. We found a frequency of *RAS* mutations in our pediatric population that was similar to previous studies. In a series of 10 other studies, mutant *N-RAS* genes were identified in 4–18% of ALL samples.[7,8,23–30] The spectrum of *RAS* mutations in our study included a predominance of *N-* and *K-RAS* codon 12/13 mutations, with a low frequency of mutations identified at codon 61. In this respect, the mutation sites identified were similar to those described in other studies of ALL and AML (reviewed in references Beaupre and Kurzrock[1] and Yokota *et al*[8]).

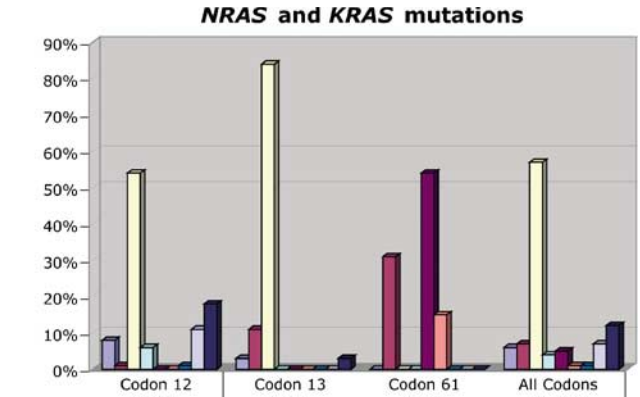
Table 1 Patient clinical characteristics and *RAS* mutations

Characteristic	*No Ras mutation # (%)*	*Any Ras mutation # (%)*	*K-Ras exon 1 (codons 12/13) # (%)*	*K-Ras exon 2 (codon 61) # (%)*	*N-Ras exon 1 (codons 12/13) # (%)*	*N-Ras exon 2 (codon 61) # (%)*
Total cases ($n=870$)	739 (85%)	131 (15%)	39 (4.5%)	2 (0.2%)	81 (9.3%)	12 (1.4%)
Clinically evaluable cases ($n=696$)	590 (85%)	106 (15%)	32 (4.6%)	1 (0.1%)	64 (9.2%)	12 (1.7%)
Age at Dx (year)	5.6	4.8	3.7	5.5	5.4	4.4
Mean	4.3	3.8	2.9	1.8	4.3	4.3
Median	0.1–14.9	(0.1–14.3)	(0.1–11.4)	0	(0.8–14.3)	(1.3–7.9)
Range						
<1	11 (2%)	4 (4%)	3 (9%)	1 (100%)	2 (3%)	0
1–2	49 (8%)	17 (16%)	7 (22%)	0	7 (11%)	3 (25%)
2–10	442 (75%)	73 (69%)	20 (63%)	0	45 (70%	9 (75%)
11+	88 (15%)	12 (13%)	2 (6%)		10 (16%)	0
Gender						
Male	322 (55%)	56 (53%)	16 (50%)	0	39 (61%)	4 (33%)
Female	268 (45%)	50 (47%)	16 (50%)	1 (100%)	25 (39%)	8 (67%)
Race						
Caucasian	509 (86%)	93 (88%)	27 (84%)	1 (100%)	56 (86%)	12 (100%)
African-American	30 (5%)	5 (5%)	2 (6%)	0	3 (5%)	0
Hispanic	29 (5%)	6 (6%)	1 (3%)	0	5 (8%)	0
Asian	5 (1%)	1 (1%)	1 (3%)	0	0	0
Other	17 (3%)	1 (1%)	1 (3%)	0	0	0
Initial WBC						
<10	264 (45%)	59 (56%)	16 (50%)	0	37 (58%)	7 (58%)
10–20	99 (17%)	16 (15%)	4 (13%)	1 (100%)	8 (13%)	3 (25%)
20–50	94 (16%)	14 (13%)	4 (13%)	0	11 (17%)	0
50–100	56 (10%)	8 (8%)	5 (15%)	0	4 (6%)	0
100+	77 (13%)	9 (9%)	3 (9%)	0	4 (6%)	2 (17%)
Risk group						
Standard	391 (66%)	76 (72%)	21 (66%)	1 (100%)	45 (70%)	10 (83%)
High	199 (34%)	30 (26%)	11 (34%)	0	19 (30%)	2 (17%)
Phenotype						
B lineage	383 (65%)	71 (67%)	18 (56%)	1 (100%)	46 (72%)	9 (75%)
T lineage	90 (157%)	4 (4%)	2 (6%)	0	2 (3%)	0
T/B lineage	55 (9%)	9 (8.5%)	1 (3%)	0	6 (9%)	2 (17%)
Null	1 (0.2%)	1 (0.9%)	1 (3%)	0	0	0
Unknown	61 (10%)	21 (3.5%)	10 (31%)	0	10 (16%)	1 (8%)
CNS disease						
Present	22 (4%)	3 (3%)	0	0	3 (5%)	0
Not present	562 (95%)	103 (97%)	32 (100%)	1 (100%)	61 (95%)	12 (100%)
Unknown	6 (1%)	0	0	0	0	0
Testicular disease						
Present	10 (2%)	3 (3%)	2 (6%)	0	2 (3%)	0
Not present	310 (53%)	53 (50%)	14 (44%)	0	37 (58%)	4 (33%)
Unknown	2 (0.3%)	0	0	0	0	0
Female	268 (45%)	50 (47%)	16 (53%)	1 (100%)	25 (39%)	8 (67%)

Most mutations identified in this study were G:C to A:T transitions, which was similar to findings of other reports of *RAS* mutations in ALL. Yokota *et al*[8] reviewed eight studies analyzing *N-RAS* mutations in 349 patients with ALL and found that 84% of the mutations at codons 12 and 13 were G:C to A:T transitions; our study found a frequency of 79% of G:C to A:T transitions at the same codons. In contrast, studies of AML have found that only about half of observed mutations at *N-RAS* codons 12 and 13 are G:C to A:T transitions.[6] The reasons for possible association of transition mutations with ALL merit further investigation. In experimental animal models, specific *N-*, *K-*, or *H-RAS* activation can be correlated with specific carcinogen exposures, premalignant states, and tumor histology.[31] In murine models for lymphohematopoietic neoplasia, exposure to methylnitrosourea (MNU) resulted in a predominance of *K-RAS*-activated mutations at codon 12 with a consistent G:C to A:T transition. Gamma radiation caused *K-* and *N-RAS* activation in a similar fashion. In contrast, a distinct and different spectrum of *K-* and *N-RAS* mutations was observed with neutron radiation, with infrequent transition and transversion mutations, suggesting the involvement of other pathways in carcinogenesis with this genotoxin.[31]

RAS is frequently mutated in adult epithelial malignancies with 80% of mutations occurring in *K-RAS*.[32] Only 31% of the

mutations in our study of pediatric ALL were found in *K-RAS*, suggesting a different mechanism of mutagenesis or repair. Studies of human lung adenocarcinoma have found mutagen induction of specific *K-RAS* mutations, with a frequency of 30% of patients with histories of smoking and less than 5% in nonsmokers.[33] Recent investigations have also demonstrated specific DNA adduct formation in bronchial cells exposed to the tobacco smoke metabolite benzo[α]pyrene diol epoxide, with



	Codon 12 N=83	Codon 13 N=38	Codon 61 N=13	All Codons N=134
Ala (A)	8%	3%	0%	6%
Arg (R)	1%	11%	31%	7%
Asp (D)	54%	84%	0%	57%
Cys (C)	6%	0%	0%	4%
His (H)	0%	0%	54%	5%
Leu (L)	0%	0%	15%	1%
Met (M)	1%	0%	0%	1%
Ser (S)	11%	0%	0%	7%
Val (V)	18%	3%	0%	12%

Figure 3 Frequency of specific amino-acid substitutions in N- and K-RAS.

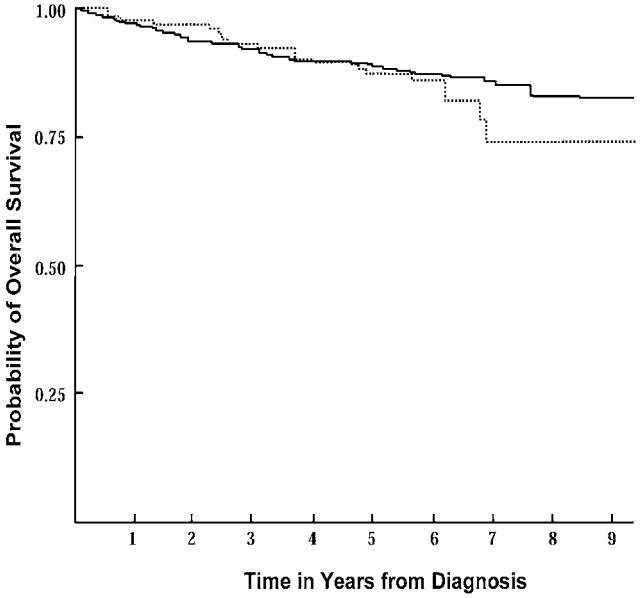


Figure 5 Kaplan–Meier estimates of overall survival for the 696 clinically evaluable cases according to the presence or absence of any *RAS* gene mutation (solid line = no mutation, broken line = *RAS* mutation).

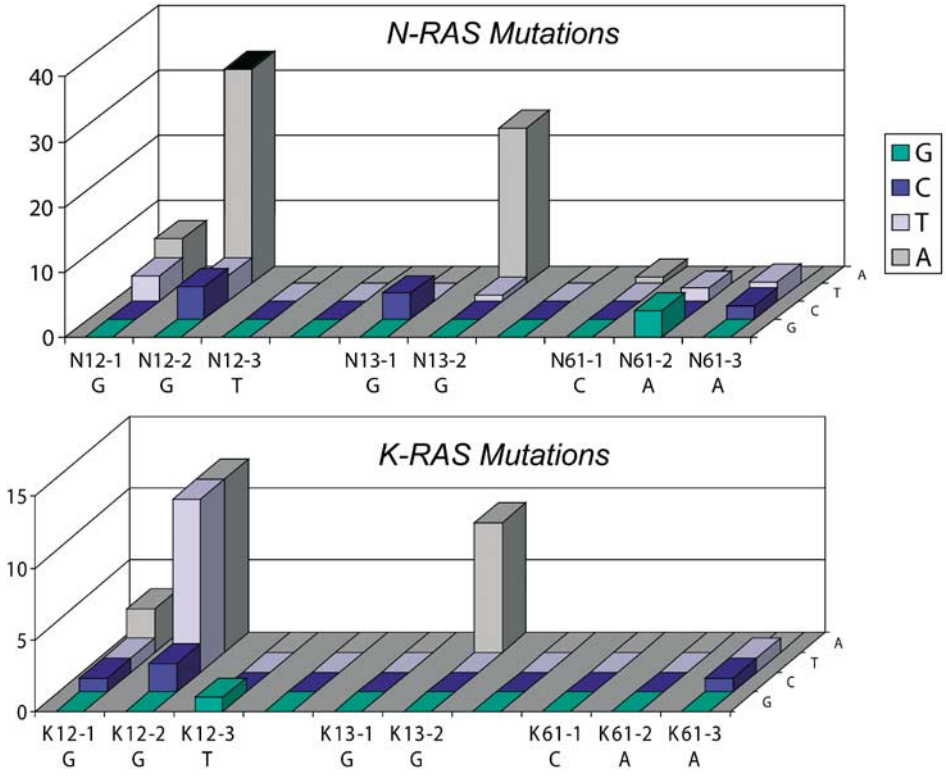


Figure 4 Mutation spectrum in codons 12, 13, and 61 of the *N-* and *K-RAS* genes. The *RAS* codon positions and wild-type sequence are identified, with the mutated bases and number of occurrences indicated. No mutations were identified in codon 13 position 3 of either of the *N-* and *K-RAS* genes.

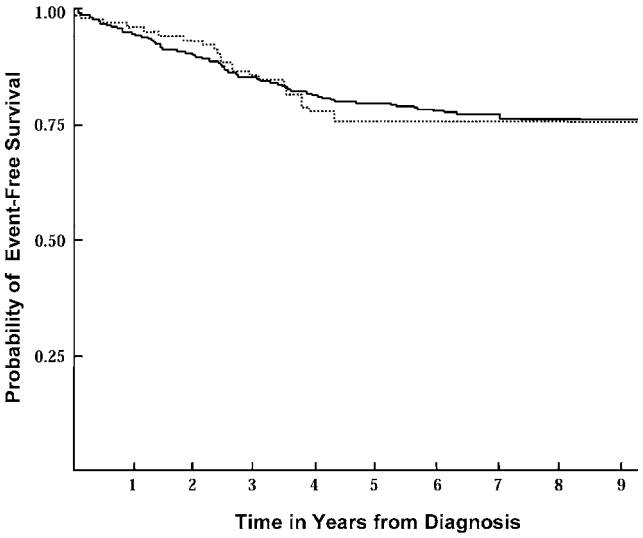


Figure 6 Kaplan–Meier estimates of event-free survival for the 696 clinically evaluable cases according to the presence or absence of any *RAS* gene mutation (solid line = no mutation, broken line = *RAS* mutation).

Table 2 Risk of an event (event-free survival) for patients with a RAS mutation[a]

RAS mutation	*Risk of event*[b] *(95% confidence interval)*	*P-value*
Any	1.1 (0.7–1.8)	0.5
K-RAS exon 1 (codons 12/13)	1.4 (0.7–2.7)	0.4
K-RAS exon 2 (codon 61)	6.5 (0.9–46.9)	0.06
N-RAS exon 1 (codons 12/13)	1.0 (0.6–1.8)	0.9
N-RAS exon 2 (codon 61)	0.4 (0.1–2.8)	0.3

[a]Multivariate model including age at diagnosis, initial WBC, sex, race and NCI risk group.
[b]Referent group includes those without the specified mutation.

three- to fourfold preferential adduct formation at *K-RAS* codon 12 when compared with adduct formation at codon 12 of *N-* or *H-RAS*.[34] Other studies also have suggested that the spectrum of *RAS* mutations in human thyroid cancer and melanomas may correlate with specific genotoxin exposures.[35–37] The spectrum of G:C to A:T transition mutations in our study of pediatric ALL may be consistent with endogenous oxidant-induced genetic damage and/or defective DNA repair mechanisms in early lymphoid cells.[38] A predominant spectrum of *RAS* mutations in ALL that is distinct from those found in adult epithelial malignancies supports the notion that the mutations are not the result of random genetic mutations acquired in tumor progression.

Few studies have had statistical power to closely examine the association between the presence of *RAS*-mutation positive or negative ALL and clinical features or outcome. In addition, the potential significance of *RAS*-mutations as an adverse risk factor in pediatric ALL may have lost significance in the face of contemporary intensive therapies. Early investigations with small samples suggested that *RAS* mutations were more frequent in blasts of pediatric ALL patients with unfavorable demographic features including older age at diagnosis and FAB-L2 morphology.[25] In a larger study of 100 patients treated in the mid-1980s on the total XI regimen at St Jude Children's Research Hospital, Lübbert *et al*[7] used univariate analysis to determine that presence of *RAS* mutations was not associated with several clinical or laboratory characteristics. However, they found that patients with *RAS* mutations had inferior outcomes, and significantly higher risk of relapse ($P = 0.01$) with a trend for lower continuous complete remission rate at 3 years after diagnosis ($P = 0.07$). A recent study of 125 pediatric patients with ALL who were treated on the Japanese Children's Cancer and Leukemia Study Group (CCLSG) regimens ALL874 and ALL9111 did not identify any correlation between *RAS* mutations in leukemic blasts and outcome.[8] It was hypothesized that the greater chemotherapy intensity of the CCLSG protocols may have reduced the relapse risk of the patients with poor prognoses, and effectively eliminated *RAS* as a risk factor for aggressive disease. In our study of 696 children treated on contemporary CCG intensive risk-adapted regimens, we also found no association of *RAS* mutations with adverse outcomes.

These results raise intriguing questions regarding the role of *N-* and *K-RAS* in the pathogenesis and biology of childhood ALL. Many studies suggest that the acquisition of *RAS* mutations is a late event in leukemia progression, as has shown in adult-type chronic myelogenous leukemia.[39] Terada *et al*[26] studied ALL patients in relapse, and identified leukemic blast *N-RAS* mutations in the relapse samples that were different compared with the mutation that was present at the time of diagnosis. They demonstrated that the primary leukemic clone at diagnosis and relapse was derived from the same precursor cell by confirming identical Ig heavy-chain and T-cell receptor gamma-chain gene rearrangements. In accord with this observation, several other studies found that some ALL patients possess only a subpopulation of leukemic blasts that carry *RAS* mutations.[28–30]

Our findings also might refute the suggestion that detection of *RAS* mutations in ALL blasts is associated with disease mass or biological aggressiveness. Other studies have shown that *RAS* mutations do not appear to be detected frequently at the time of ALL relapse,[26] which also contradicts the hypothesis that *RAS* mutations provide selective advantage for growth to a subpopulation. These observations suggest that there may be limitations for using mutant *RAS* as an immunotherapeutic target in ALL.[40]

In summary, we have completed the largest reported study of *N-* or *K-RAS* mutations in pediatric ALL. We found that *RAS* mutations are present in approximately 15% of pediatric cases, and are not associated with high-risk disease or adverse outcomes in children treated on contemporary risk-adapted intensive ALL therapy regimens. It is important to note that activation of the *RAS* pathway in leukemia can also occur through pathologic activation or alterations in the regulatory function of other proteins in the *RAS* signaling cascade (see Figure 1).[41–43] Future studies should define the role of these alterations in the pathogenesis of childhood ALL, and will assist in the design and implementation of *RAS* pathway-targeted therapies for high-risk disease.

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Appendix

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