(LSR sample=mean $fluorescence_{blast\ cells}$/mean $fluorescence_{lymphocytes}$). While the LSR for normal myeloid/monocytic cells was 1.82 + 0.19 (n=6), we observed LSR of 3.2, 2.7 and 2.6 from the leukemic cell lines K562, HL60 and ML2, respectively, and a LSR of 2.35±0.35 (n=7) in clinical AML blast cell samples. Thus leukemic cell lines as well as primary AML blasts have a high volume of lysosomal space per cell. In addition to drug extrusion by ABC transporters across the outer plasma membrane, subcellular drug sequestration in acidic organelles has repeatedly been described to occur in resistant cells.[5,6] In acidic vesicles weakly basic drugs are protonated, lose membrane permeability and are secreted by fusion of the vesicles with the plasma membrane (the so-called protonation, sequestration and secretion (PSS) model.[7] The microscopic data available for ABCA3 are consistent with it having a role in sequestration and secretion.[8,9] It remains to analyze whether ABCA3-involving secretory mechanisms are similar in different cell populations such as pneumocytes, hematopoietic cells and malignant leukemic cells. In this regard, with AML as a malignant disease of progenitor cells, it will be particularly important to elucidate whether the expression of ABCA3 in leukemic cells is a reflection of its expression in precursor cell subsets.

In summary, ABCA3 is overexpressed in adult and pediatric AML, may have a role in the turnover of acidic vesicles, and may participate in subcellular drug sequestration. Understanding ABCA3 biology may give novel insight into mechanisms of drug resistance and leukemic blast cell physiology.

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Acute Myeloid Leukemia

Prognostic significance of *N-RAS* and *K-RAS* mutations in 232 patients with acute myeloid leukemia

Reports on the prognostic impact of mutations in the *RAS* proto-oncogenes in patients with acute myeloid leukemia (AML) are conflicting. A peptide nucleic acid (PNA)-based technique was used on 232 AML samples to detect point mutations of the hotspots in *N-RAS* and *K-RAS*. No significant correlations between *RAS* mutations and clinical features, karyotype or FLT3 were found.

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The presence of defined karyotypes are among the most important prognostic factors in acute myeloid leukemia (AML). However, even within defined cytogenetic groups stability of remission and long-term survival may vary significantly. Therefore, additional recurrent aberrations may have a prognostic impact. The role of Ras proteins in human leukemias has been analyzed extensively,[1] but their prognostic role, if any, is still disputed. The uncontrolled activation of Ras proteins, which can be found in a variety of malignancies, contributes to several aspects of the malignant phenotype including the deregulation of tumor-cell growth and programmed cell death.[1] In this correlation study, we analyzed *RAS* mutations using a PNA-based detection method in 232 patients with centrally confirmed and classified AML. All patients were treated within a large prospective multicenter study exploring a risk-adapted treatment strategy (SHG AML 96). The study design has been published elsewhere.[2,3] Patients with acute promyelocytic leukemia (FAB M3) were excluded. Morphologic analysis and immunophenotyping were done centrally in designated reference laboratories in Dresden and Erlangen, respectively. The cytogenetic risk assessment was done as described previously.[2,3] Mutations of codons 12, 13, 61 of *N-RAS* and codons 12, 13 of *K-RAS* were detected using a polymerase chain reaction (PCR)-based technique with PNA-clamping as described.[2,4] The PNA molecules were designed to hybridize to the wild type sequences, thus inhibiting primer binding to wild type DNA. In the case of a mutated allele the PNA molecule does not bind thus allowing preferential amplification of the mutated DNA. Additionally, in 197 out of 232 samples FLT3-activating mutations either through internal

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Table 1. Clinical and laboratory data according to *RAS* mutation status.

	n	*Mutated RAS*	*Wild type RAS*	*p value*
All patients	*232*	*58*	*174*	
Age				
≤60	151	40	111	
>60	81	18	63	≤0.53*
			(60 vs. >60)	
Percentage of bone marrow blasts				
<50%	90	23	67	
>50%	127	30	97	0.75*
			(<50 vs. >50)	
Diagnosis				
de novo AML	183	46	137	1.0*
			(*de novo* vs. MDS-prephase)	
MDS-prephase	35	8	27	
MDS (RAEB-T)	3	1	2	
secondary treatment-related AML	11	3	8	
FAB classification (p value X vs. all others)				
M 0	8	1	7	
M 1	38	5	33	0.5*
M 2	100	21	79	0.1*
M 4	31	12	19	0.067*
M 4eo#	7	4	3	0.26*
M 5a	19	7	12	
M 5b	12	6	6	
M 6	10	0	10	
M 7	4	1	3	
RAEB-T	3	1	2	
Cytogenetic risk catagories				
low risk	14	5	9	0.52*
			(LR vs. IR)	
intermediate risk	149	38	111	
high risk	53	13	40	0.91*
			(HR vs. IR)	
no cytogenetics available		16	2	14
FLT3-activating mutations				
present	39	7	32	0.41*
			(present vs. absent)	
absent	158	41	117	
not analyzed	35	10	25	
Complete remission rate (%)	232	65	56	0.22*
Median overall survival (months)	232	11.7	12.4	0.88°
Median disease-free survival (months)	135	14.7	14.1	0.96°

*Presenting clinical and laboratory characteristics according to RAS mutation status. Univariate analysis using *Fisher's exact test showed no statistical significant differences (age, percentage of bone marrow blasts, diagnosis, FLT3, CR rate and risk catagories). °Overall (OS) and disease-free (DFS) survival were calculated using the Kaplan-Meier method and differences between survival rates were compared using the log-rank test. #Cytogenetics revealed inv(16) in all patients with AML M4eo.*

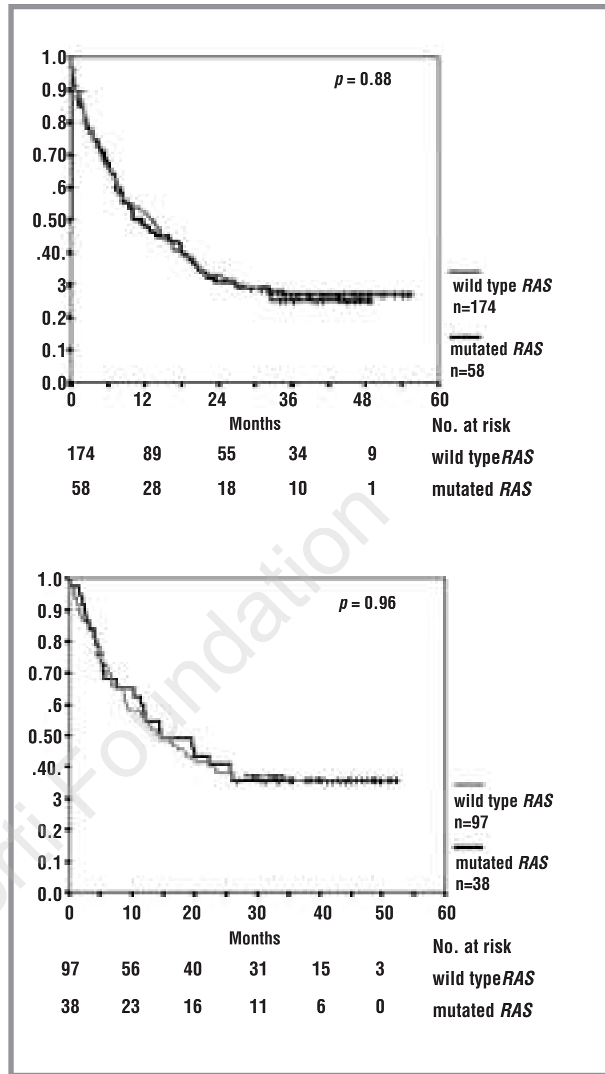

Figure 1. Overall (A) and disease-free (B) survival of 232 patients with AML with respect to *N*- and *K-RAS* mutation status. The numbers of patients at risk are shown below the X-axis.

tandem duplication (ITD) or point mutations in the second tyrosine kinase domain (TKD) were screened for as described previously.[3] Overall (OS) and disease-free (DFS) survival was calculated using the Kaplan-Meier method and differences between survival rates were compared using the log-rank test. Comparisons between different groups (e.g. mutated vs. wild type *RAS*) were made using Fisher´s exact-test. Multivariate analysis was performed by Cox regression analysis. Overall, 58 of 232 (28%) patients harbored 65 different mutations in codons 12 (n=27), 13 (n=10), and 61 (n=10) of *N-RAS* and codons 12 (n=12), 13 (n=6) of *K-RAS*. Five patients had two and one patient had three mutations.

The median follow-up of the 232 patients was 12.8 months (range 1-54 months) and their median age was 55 years (range 18-77 years). There were no significant differences between patients with mutated and wild type RAS with respect to age, sex, FAB type, bone marrow blast percentage,

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cytogenetic risk category, type (e.g. primary vs. secondary) and FLT3-status (Table 1). The overall complete remission rate (CR) was 58.2% and median OS was 12.4 months. The presence of *RAS* mutations had no influence on the rate of complete remissions. This was found throughout different cytogenetic risk and age groups. As expected, the CR rate was lower for patients >60 years and those in the high risk group. In the multivariate analysis including age, disease status, cytogenetics, *FLT3*- and *RAS*-status, the last had no independent influence on survival. Overall and disease-free survival was similar for patients with mutated and wild type RAS (Figure 1 A,B). Considering the specific risk stratification in our AML study (low risk=t(8;12)) we reanalyzed the data after regrouping all CBF leukemias (t(8;21)+ inv(16)) into the low risk group. Compared to the original data set no significant differences were found.

As far as we know, this report is the largest study on the prognostic significance of mutations of N- and *K-RAS* in this disease group. We used a previously published PNA-based PCR technique for the analysis of *K-RAS* and developed an analogous assay for *N-RAS*.[2,4] Since the samples were analyzed in a blinded fashion and randomly taken from a nationwide multicenter prospective trial our results should be representative of AML. The overall prevalence of *RAS* mutations was, albeit in the upper range, comparable to that in previous studies.[5] We could not confirm a correlation between *RAS* mutations with any clinical feature, e.g. blast percentage, as reported earlier.[6,7] Published reports addressing the clinical significance of RAS mutations in patients with acute myeloid leukemia are inconclusive. Whereas some studies demonstrated a beneficial clinical effect of RAS mutations,[7,8] others reached a different conclusion (e.g. lower CR).[9] Most studies did not that show that patients with *RAS* mutations had significantly different outcomes.[5,6]

In conclusion, despite the evidence that activation of the Ras-signaling cascade contributes to the molecular pathogenesis of myeloproliferative disorders,[10] the prognostic value of RAS-mutations seems to be of minor relevance compared to that of age or karyotype.

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Acute Lymphoblastic Leukemia

Overexpression of Her2/neu is observed in one third of adult acute lymphoblastic leukemia patients and is associated with chemoresistance in these patients

Among 100 patients with acute lymphoblastic leukemia (ALL), 15 B-ALL patients were found positive for surface Her2/neu expression. The incidence in children was only 3.4% compared to 31% in adults (*p*=0.001). Considering only adult B-ALL patients (n=38), surface Her2/neu expression was associated with chemoresistance (50% versus 11%, *p*=0.03) suggesting that it could be a prognostic marker of poor clinical outcome in ALL.

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The Her2/neu/c-erb-B2 protein is a transmembrane receptor tyrosine kinase related to the epidermal growth factor receptor. Her2/neu is overexpressed on several epithelial tumors and correlated with poor prognosis in some of them, especially breast and ovarian cancers.[1,2] Burhing *et al.*[3] reported that about 40% of patients with B-acute lymphoblastic leukemia (ALL) expressed Her2/neu on the surface of their leukemic blasts. More recently, Müller *et al.*[4] reported that the incidence of patients with Her2/neu-positive ALL was 16% (n=5/31) and demonstrated that Her2/neu-specific autologous cytotoxic T-lymphocytes could be generated *in vitro* using peptide-pulsed dendritic cells indicating that vaccination strategies targeting Her2/neu could be evaluated in a subset of patients with ALL.

Herceptin® (rhu-mAb-Her2, Trastuzumab, Genentech Inc., San Francisco, CA, USA) is the humanized equivalent of the murine 4D5 monoclonal antibody and is targeted against the