Acute Myeloid Leukemia

Effects of the protein tyrosine kinase inhibitor, SU5614, on leukemic and normal stem cells

FLT3 activating mutations are the most frequent single genetic abnormality in patients with acute myeloid leukemia. Thus targeting the FLT3 activated kinase is a promising treatment approach. We wanted to test whether the protein tyrosine kinase inhibitor SU5614 selectively eliminates leukemic stem cells while sparing their normal counterparts.

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The most common single genetic alterations in acute myeloid leukemia (AML) are activating mutations of *FLT3* such as the internal tandem duplication (*FLT3-LM*) or mutations in the second tyrosine kinase domain at codons 835, 836, 841, or 842 (*FLT3-TKD*).[1] These mutations constitutively activate protein tyrosine kinases, inducing factor-independent growth of hematopoietic cell lines or a myeloproliferative syndrome in mice.[2,3] Recently, a number of small molecule protein tyrosine kinase inhibitors have been developed which target constitutively activated FLT3. One member of the family of protein tyrosine kinase inhibitors is SU5614, which suppresses growth factor-independent growth of cells with constitutively activated FLT3 *in vitro* and has anti-leukemic activity in patients with AML or myelodysplastic syndrome.[4–6] However, like other protein tyrosine kinase inhibitors, SU5614 does not inhibit FLT3 selectively, but also inhibits stem cell factor receptor and vascular endothelial growth factor receptor. Activity against several protein tyrosine kinases has the advantage that the anti-leukemic potential of compounds such as SU5614 might not be restricted to FLT3-LM or FLT3-TKD, as demonstrated in the first clinical trials.[4,5] However, both receptors are pivotal for normal hematopoietic stem cell (HSC) development[7] and, therefore, might result in substantial toxicity against normal HSC. The aim of this study was to analyze and compare the effects of SU5614 on AML HSC and normal HSC.

Peripheral blood or bone marrow cells were obtained from 12 patients with newly-diagnosed AML after informed consent and with the approval of the Clinical Research Ethics Board of the LMU University of Munich. The diagnosis and classification of AML were based on the criteria of the French-American-British (FAB) group.[8] Cytogenetic analysis was performed on the bone marrow at initial diagnosis. Frozen normal $CD34^+$ bone marrow cells were obtained from CellSystems (St. Katharinen, Germany). Analysis of FLT3-TKD and FLT3-LM was performed as described previously.[1,9] AML bone marrow or peripheral blood as well as $CD34^+$ bone marrow cells from healthy donors were incubated at 1×10^6 cells/mL with 50 ng/mL granulocyte colony-stimulating factor with or without (control) 10 µmoL SU5614 (Calbiochem-Novabiochem, Bad Soden, Germany). This concentration was previously found to induce apoptosis in transduced myeloid leukemic cell lines.[6] After 24h incubation, equal fractions of the cells recovered from cultures with or without SU5614 were assayed without regard to change in cell numbers. Assays for colony-forming cells (CFC) for AML and normal bone marrow samples were performed as described previously,[10] plating $1\text{-}2\times10^5$ cells/mL in methylcellulose medium (CellSystems). Cultures were scored for the presence of clusters and colonies after 14 days.[10] Long-term culture-initiating cells (LTC-IC) were established from AML and normal bone marrow as previously described.[10] All experiments were done in triplicates. The mean values were used for our analyses. Statistical analysis comprised a Student's t-test (Microsoft

Table 1. Patients' characteristics and response to treatment.

Patient	Cytogenetics	Age	FAB	WBC ($x10^9$)	% blasts	FLT3	% killing CFC	% killing LTC-IC
1	46,XX, Nras+	68	M1	284	95	WT	0	100
2	47,XX,I(21),+i(21), c-KIT-, Nras-	67	M2	41	60	WT	0	100
3	46,XY, c-KIT-, Nras-	46	M4	105	90	WT	93	25
4	47,XY,+13, 48,XY,+13,+13, c-KIT-	71	M1	160	90	WT	26	59
5	46,XY, c-KIT-, MLL dupl-	47	M1	66.4		LM	98	100
6	46,XX, Nras-	55	M4	8.2	90	LM	77	100
7	46,XX, c-KIT-	73	M5b	206	90	LM	77	0
8	46,XX,t(11;16), c-KIT-, Nras-	42	M4	75	90	LM	n.g.	69
9	47,XX,+14, c-KIT-, Nras-	68	M5a	94	95	D835H	32	0
10	46,XX, c-KIT-, Nras-	85	M5b	268	95	D835H	100	100
11	46,XY, c-KIT-, Nras-	48	M2	9	80	Del835	n.g.	83
12	46,XX, c-KIT-, Nras-	38	M4	10,6	90	D835Y/ITD	n.g.	14

*n.s. not successful; n.g. no growth in control arm; WT: wild type; *% killing as compared to AML or normal BM CFC and LTC-IC respectively, after 24 hrs pre-incubation without SU5614 (control).*

Excel).

Three groups of patients (n=4 per group) were analyzed with non-mutated wild-type FLT3, FLT3-LM, and FLT3-TKD (D835H, D835H, D835Y, del835). The *in vitro* results from each group treated with SU5614 were compared to those of the respective untreated control cells. At the level of clonogenic progenitors (CFC) 2/4 patients with wild-type FLT3, 3/3 with FLT3-LM ($p<0.004$) and 2/2 with FLT3-TKD responded to therapy with as much as 100% reduction of the number of leukemic CFC as compared to the untreated AML cells (Table 1). At the level of HSC (LTC-IC), the compound achieved > 50% cell killing in 3/4 ($p<0.03$) patients with wild-type FLT3, 3/4 with FLT3-LM ($p<0.04$) and 2/4 with FLT3-TKD. The response of both CFC as well as leukemic HSC to SU5614 could not be predicted from the level of expression of FLT3 or the presence of activating mutations or surface expression of c-KIT, a protein tyrosine kinase also targeted by the SU5614 compound (*data not shown*). As a control, $CD34^+$ bone marrow stem cells from healthy donors were analyzed in the same way. At the level of CFC level SU5614 had considerable toxicity, killing a mean (range) of 67.5 % (30-100) of the cells (n=3). In addition, the compound eliminated normal HSC (n=3) with a range between 78 – 100% after 24h incubation. These data demonstrate the efficacy of tyrosine kinase inhibitors at eliminating leukemic stem cells in AML patients with mutated as well as non-mutated FLT3. However, the data also point to a considerable toxicity to normal HSC, which should be taken into account in the management of patients with compromised normal hematopoiesis.

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Key words: AML, FLT-3, leukemic stem cell, receptor tyrosine kinase inhibitor

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Malignant Lymphomas

Rituximab in patients with mucosal-associated lymphoid tissue-type lymphoma of the ocular adnexa

Eight patients with ocular adnexal mucosal-associated lymphpid tissue (MALT) lymphoma were treated with rituximab, at diagnosis (n=5) or relapse (n=3). All untreated patients achieved lymphoma regression, while relapsing patients had no benefit. Four responding patients experienced early relapse. The median time to progression was 5 months. The efficacy of rituximab in ocular adnexal lymphoma is lower than that reported for gastric MALT lymphomas.

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Any CD20-positive lymphoproliferative disorder is a potentially suitable candidate for treatment with rituximab. Significant rituximab activity has been reported in extranodal mucosal-associated lymphoid tissue (MALT) lymphomas.[2] However, the clinical activity of this drug in MALT lymphomas arising in different organs remains to be defined.[2] MALT-type ocular adnexal lymphoma is a very indolent malignancy that would appear to be a suitable candidate for treatment with a drug that has an excellent safety profile, such as rituximab. However, the use of rituximab in this setting has been only anecdotally investigated.[1,3-5]

We report a series of eight patients with MALT-type ocular adnexal lymphoma treated with rituximab, at diagnosis (patients #1 to 5) or relapse (patients #6 to 8) (Table 1). Patients were treated with rituximab 375 mg/m^2, weekly, for four weeks, according to the conventional administration schedule which includes pre-medication. Patients did not receive steroids or any other concomitant antineoplastic therapy. CD20-positivity and MALT lymphoma histotype were confirmed both at diagnosis and relapse in all