

LETTER TO THE EDITOR

SWR/J mice are susceptible to alkylator-induced myeloid leukemia

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Therapy-related acute myeloid leukemia (t-AML) is a late complication following chemotherapy and/or radiation therapy. Approximately 10% of AML cases are therapy related and the incidence is rising.¹ Alkylator-associated t-AML has a latency of 5–7 years, is often preceded by a myelodysplastic phase (t-MDS) and is frequently associated with loss of material from chromosomes 5 and/or 7 in humans.^{2,3} The survival of these patients is poor, motivating efforts to improve treatment and prevention strategies.

It is not yet clear whether t-AML risk is influenced by host factors, or is solely a stochastic process. Previous studies have shown that inherited polymorphisms in drug detoxification (for example, p450 enzymes, phase II conjugation enzymes and NAD(P)H:quinoneoxidoreductase) and DNA repair (including homologous recombination and nucleotide excision) pathways may contribute to t-MDS/AML susceptibility, but confer only modestly increased risk in humans.^{4–7}

Alkylators (for example, cyclophosphamide and ethyl-N-nitrosourea (ENU)) are often used to generate cooperating mutations in genetically engineered mouse models of leukemia.^{8,9} In standard laboratory strains (for example, C57BL/6J, 129Sv/J), alkylators promote thymic lymphomas efficiently, reducing the number of mice evaluable for myeloid neoplasms. We had previously demonstrated that susceptibility to alkylator-induced cancer has a genetic component in mice.¹⁰ For myeloid leukemia, 6 strains demonstrated variable degrees of susceptibility, whereas 14 were resistant (including C57 and 129 substrains). Of the 20 strains tested, SWR/J was the most susceptible. In the current study, we evaluated a large cohort of SWR/J mice and characterized the phenotype of tumors induced by the prototypical alkylator, ENU.

SWR/J mice received either no treatment ('N' cohort, $n = 22$), hydrocortisone (HC) alone ('HC' cohort, $n = 11$), ENU alone ('E' cohort, $n = 27$), ENU followed by HC ('EH' cohort, $n = 111$) or HC followed by ENU ('HE' cohort, $n = 107$; see Supplementary Information). A spectrum of diseases was observed, including hematopoietic tumors of both myeloid and lymphoid origins, and invasive lung carcinoma (Supplementary Table 1). Lymphomas were characterized by infiltrating CD4⁺CD8⁺ cells (>10%) in the bone marrow or spleen, large mediastinal masses and disruption of splenic architecture. Myeloid leukemias were identified by excess myeloblasts (>20% of myeloid precursors) in the bone marrow or fixed tissues. Lung cancers were grossly visible and were phenotyped by histologic examination.

Of the 245 mice treated with ENU, 205 were evaluable for cancer susceptibility (40 died immediately after ENU injection or were found postmortem). ENU treatment (with or without HC) resulted in 65 evaluable mice (31.7%) with lymphoma, 23 mice (11.2%) with myeloid leukemia and 192 mice (92.7%) with lung carcinoma (Supplementary Table 1). These diagnoses were not mutually exclusive; 5 mice had both lymphoma and myeloid leukemia, 20 had both myeloid leukemia and lung cancer,

and 61 had both lymphoma and lung cancer. The lung cancer, lymphoma and leukemia incidences in this strain differ from our previously published results (60%, 0%, and 80%, respectively), likely owing to small cohort size in the previous study ($n = 12$ ENU-treated mice).¹⁰ Control mice developed rare lung cancers (1/22 and 1/11 evaluable mice from the untreated and HC only groups, respectively), but no spontaneous hematologic cancers.

Previous studies in mice have shown that coadministration of steroids increased the frequency of radiation-induced leukemias.¹¹ To test whether steroids could increase the incidence of ENU-induced leukemias and reduce the competing incidence of thymic lymphoma, we treated mice with HC either before or after ENU treatment. Neither regimen decreased the proportion of lymphomas, nor increased the proportion of leukemias, compared with ENU alone (Supplementary Table 1). It is not clear why steroids increased the incidence of radiation-induced leukemia and had no impact on ENU-induced leukemia, but this may be attributable to differences in the dose, schedule of administration or type of steroid used.

The overall survival was significantly shorter in ENU-treated mice compared with control mice (N or HC, $P = 0.002$; Figure 1a). Two mice in the control groups died of lung cancer and the remainder were electively killed, whereas most of the ENU-treated mice were killed when moribund. For the mice treated with ENU, the median survival was longer in the EH cohort (331 days) compared with E or HE (290 and 291 days, respectively; $P < 0.0001$). The cumulative probability of developing lymphoma was higher than leukemia (48.4% vs 23.9%), but the latency for these diseases was identical (134 days) following ENU exposure (Figure 1b).

Lymphomas were characterized by splenomegaly, lymphadenopathy, leukocytosis, anemia and mediastinal enlargement (Supplementary Table 2 and data not shown). The bone marrow was infiltrated by CD4⁺CD8⁺ cells (53.4% of cases), or single positive CD8⁺ cells (1.7% of cases) or CD4⁺ cells (19% of cases). Myeloid and erythroid precursors were reduced in frequency (Supplementary Table 3). Histologically, the lymphoblasts were characterized by open chromatin, numerous nucleoli and vacuolization of the cytoplasm (Supplementary Figure 1).

Myeloid leukemias were characterized by splenomegaly, leukocytosis and accumulation of immature myeloid precursors in the bone marrow (Supplementary Tables 2 and 3). The leukemias were uniformly Gr1⁺CD11b⁺ (Supplementary Table 2). The myeloblasts were characterized by abundant cytoplasmic granulation and frequent mitotic figures (Supplementary Figure 1). The bone marrow of six ENU-treated mice had significant dysplasia in the myeloid and/or erythroid lineages, of which two had myeloid leukemia, and all had significant anemia (mean hemoglobin = 11.3; Supplementary Table 4). Twenty-seven additional mice (all with concurrent lung cancer) did not meet criteria for myeloid leukemia, but had splenomegaly and excess myeloblasts (10–15%) in the bone marrow. Although we favor that these bone marrow proliferations are clonal in origin, a reactive proliferation cannot be excluded because of the co-occurrence of a non-hematopoietic tumor.

Tumors from two donors with myeloid leukemia were transplanted into sublethally irradiated congenic recipients.

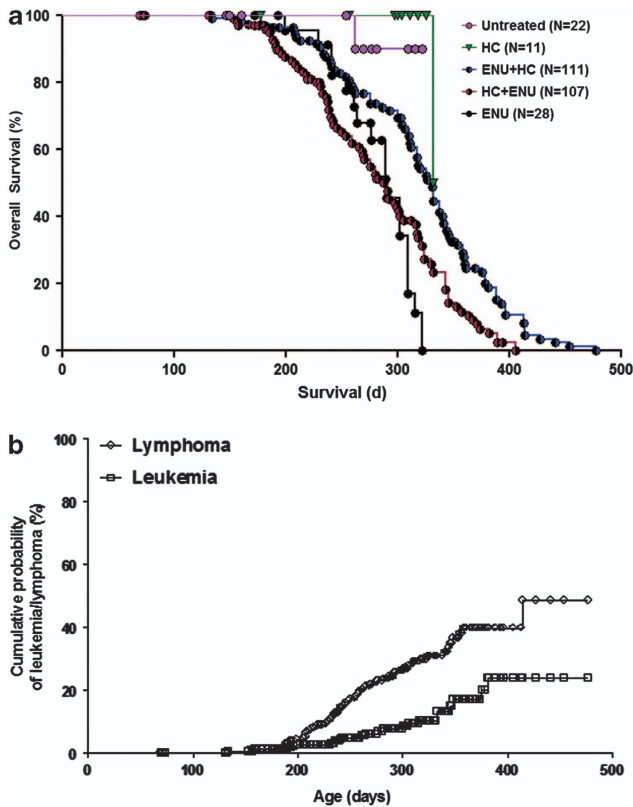


Figure 1. Kaplan–Meier analysis of overall survival in ENU-treated mice. (a) Overall survival was decreased in ENU-treated mice compared with controls ($P=0.004$; Tukey-adjusted log rank). Administration of HC before ENU extended median and overall survival slightly compared with ENU alone or HC after ENU ($P<0.0001$). (b) The cumulative probability of lymphoma was higher than that of myeloid leukemia (48.4% vs 23.9%), but occurred with identical latency (134 days after ENU exposure).

All of the recipients died rapidly (3–8 weeks) after adoptive transfer (Figure 2a). Phenotypic features of the primary mice (that is, blood counts, spleen weight and immunophenotype) were recapitulated in the recipients, suggesting that the leukemias were cell-intrinsic transplantable tumors (Figures 2b–d).

SWR/J mice developed rare spontaneous lung cancers, but hematopoietic malignancies were not observed in mice that did not receive ENU. A study conducted in 1973 reported a 37% incidence of lung tumors (both adenomas and adenocarcinomas) in untreated SWR/J mice.¹² Rare spontaneous lymphomas (3.6%) and myeloid leukemias (0.3%) were also observed in SWR/J mice followed up to 30 months in that study,¹² supporting the notion that this strain has intrinsic susceptibility to hematopoietic malignancies. The mechanistic basis of cancer susceptibility in SWR/J is not known, but it is noteworthy that a quantitative analysis of myeloid progenitor activity in 10 inbred strains demonstrated that SWR/J had the lowest frequency,¹³ suggesting that this cellular compartment may be under proliferative stress in this strain.

Genetically engineered mouse models containing a single lesion detected in humans with myeloid leukemia may develop leukemia only after long latency or not at all, suggesting that additional cooperating mutations are required. Alkylator exposure is one strategy frequently used to induce secondary mutations, but this is fraught by a high incidence of competing thymic lymphomas, particularly in the most commonly used inbred strains (C57 and 129 substrains). The incidence of ENU-induced myeloid leukemia in SWR/J mice, although still modest, is higher than what we and others have observed in C57 and 129 substrains, suggesting that polymorphisms in the SWR/J genetic background predispose mice to myeloid leukemia. Susceptibility loci for a variety of cancers have been mapped in mice, including a *Cdkn2a* allele in lymphoid malignancies and a *Ptch* allele in skin cancer.^{14,15} We have mapped loci associated with t-AML susceptibility in mice,¹⁰ however, the specific genes/polymorphisms responsible for this phenotype are not yet known.

t-AML is a lethal complication arising in patients treated for antecedent cancers or autoimmune disorders. Preventative

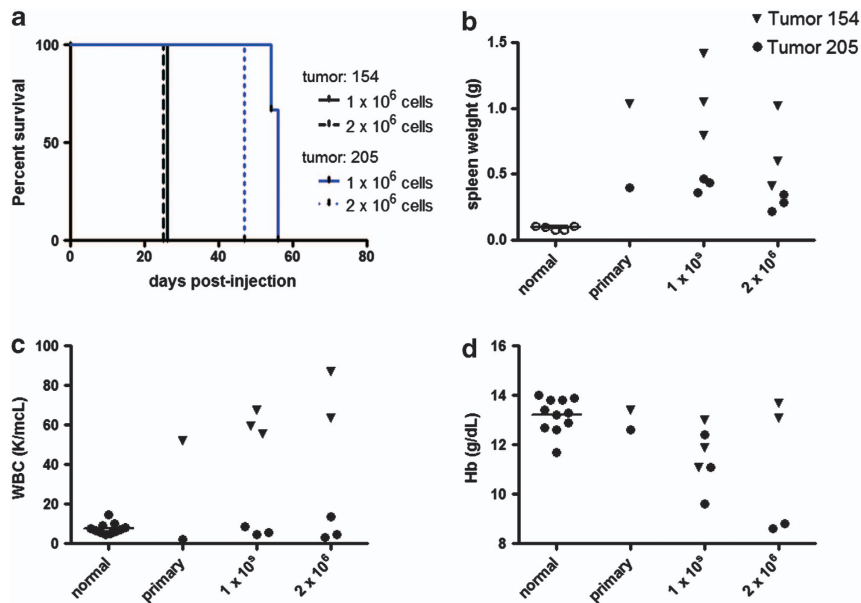


Figure 2. ENU-induced myeloid leukemias are transplantable. (a) Adoptive transfer of splenocytes from two donors with ENU-induced myeloid leukemia caused lethality with short latency. (b) The spleen weight, (c) white blood cell count and (d) hemoglobin were similar in donor and recipient mice.

strategies are needed but robust predictors of susceptibility are not yet available. Here, we show that SWR/J mice develop lethal, transplantable myeloid leukemias with dysplastic features after ENU exposure, recapitulating features of the human disease. The SWR/J strain provides a suitable model for investigation of germline and somatic events that cooperate with alkylator exposure to cause myeloid leukemia.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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