Initially disadvantaged, TEL-AML1 cells expand and initiate leukemia in response to irradiation and cooperating mutations

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Although TEL-AML1 is present in one-fourth of childhood precursor B-cell acute lymphoblastic leukemia (pre-B ALL), the expression of the ETV6-RUNX1 gene encoding this fusion protein has been a poor initiator of leukemia in multiple mouse models. To gain insight into why this common lesion does not readily initiate disease, we took advantage of a novel mouse model, in which TEL-AML1 expression was linked to a fluorescence marker (designated herein as ZEG mice), and subsequently generated an inducible TA1 transgene (Cre/TA1 mice). Mice carrying the inducible TA1 transgene but not all, mature hematopoietic cells of Cre/ZEG mice (Figures 1a and b). The fact that Tie2-Cre resulted in variegated EGFP expression enabled us to determine whether TEL-AML1 expression had a selective advantage or disadvantage on hematopoietic development. If TEL-AML1 provided a selective advantage in Cre/TAE1 mice, we expected the percentage of EGFP expressing cells to be increased relative to that observed in the Cre/ZEG mice. However, if TEL-AML1 provided a selective disadvantage, EGFP expressing cells would be decreased relative to the Cre/ZEG control. We initially surveyed 5-week-old mice for induction of EGFP in various hematopoietic tissues (Figure 1b). We did not observe any cell type in which TEL-AML1 provided a selective advantage. There was a clear selective disadvantage of TEL-AML1 in B-cells and in T-cells. TEL-AML1 had a modest negative impact on the development of bone marrow myeloid cells and, interestingly, was somewhat tolerated by peritoneal B-cells. We also took advantage of mice that were part of our survival study (see below) to assess how TEL-AML1 impacted hematopoiesis in aging mice. TEL-AML1 did not impact the fitness of hematopoietic stem and most progenitor cells, but was selected against in megakaryocyte-erythroid progenitors (MEP, Figure 1c). Results in these aged mice confirmed a selective disadvantage in maturing lymphocytes, as well as a sizeable disadvantage during granulopoiesis in older mice (Figure 1d). The substantially impaired contribution to granulopoiesis in older mice might reflect an interaction of TEL-AML1 with the shifting hematopoietic stem/early progenitor numbers and lineage output that occur in aged mice. However, as shown in Supplementary Figure S1, TEL-AML1 expression had no impact on survival. Low-dose radiation can cause lymphoid tumors in mice (Gross et al. and references therein). We irradiated Cre/ZEG and Cre/TA1 mice at 5 and 6 weeks of age with 1.7 Gy. We observed the impact of this irradiation on the percentage of cells in the peripheral blood that expressed EGFP and on survival. Cre/ZEG mice had a high percentage of EGFP expressing cells in their peripheral blood whether or not they were irradiated (Figure 1e). This high percentage of EGFP was seen in cells with low side scatter (primarily lymphocytes) and cells with higher side scatter (primarily granulocytes). In contrast, Cre/TA1 mice had low levels of EGFP in the blood before irradiation (greater expression of EGFP was observed in granulocytes than in lymphocytes), and the percentage of GFP increased greatly in the blood (in both the low and higher side scatter blood cells) when assessed 5–14 months after irradiation. Irradiation curtailed survival and induced tumors in both the Cre/ZEG and Cre/TA1
mice (Supplementary Figure S2), but no B-cell leukemias or lymphomas were observed. CDKN2A is lost in approximately one-third of ALL overall and in one-fourth of leukemias expressing TEL-AML1. We therefore sought to assess possible cooperation between TEL-AML1 and Cdkn2a null mice. The initial description of Cdkn2a null mice noted the presence of lymphomas, including B-cell lymphomas in these mice but the characterization of this disease was limited. We therefore assessed the incidence and some of the features of these lymphomas in Cdkn2a null mice. As shown in the right hand page.

Figure 1. Selective disadvantage to maturing hematopoietic cells that express TEL-AML1 is reversed after irradiation. (a) Representative flow cytometry plots of bone marrow from aged control, Cre/ZEG and Cre/TA1 mice showing variegated transgene expression. (b) Percentage of maturing cell populations that express the EGFP marker (indicating transgene expression) in 5-week-old Cre/ZEG and Cre/TA1 mice, mean ± s.d. n = 3 per group. Differences between groups are statistically significant for all populations (P < .05). Granulocytes: Gr1+ /Mac-1+ cells. B-cells: CD19+ cells. T-cells: CD3+ cells for spleen and lymph node, CD5+ cells for thymus. (c) Percentage of stem and progenitor cell populations that express the EGFP marker (indicating transgene expression) in aged Cre/ZEG and Cre/TA1 mice (19–21 months), mean ± s.d. n = 3 per group. The only statistically significant difference between the groups is for MEP (P < .05). HSC: Lin− , C-kit+, Sca-1+, Flk2+, CD48+, CD150+. MPP: Lin+, C-kit+, Sca-1+, Flk2+. CMP: Lin− , C-kit+, Sca-1− , CD34+, FcgR+. GM: Lin− , C-kit− , Sca-1− , CD34− , FcgR−. MEP: Lin− , C-kit+, Sca-1− , CD34−. MEP: Lin−, FcgR+. CLP: Lin−, Flk2+, IL7−R+, C-kitlo, Sca-1lo. (d) Percentage of maturing cell populations that express the EGFP marker (indicating transgene expression) in aged Cre/ZEG and Cre/TA1 mice (19–21 months), mean ± s.d. n = 3 per group. Differences between groups are statistically significant for MEP (P < .05), and trend towards statistical significance for CD3+ T-cells (P = .06). (e) Percentage of blood cells that expressed the EGFP marker (indicating transgene expression) in unirradiated or irradiated Cre/ZEG and Cre/TA1 mice, mean ± s.d. Percentages are shown for total blood cells, as well as for subpopulations: cells with low side-scatter (predominantly lymphocytes, Lymphs) and cells with higher side-scatter (granulocytes, Grans). Unirradiated mice were 2–16 months of age. Irradiated mice had received 1.7 Gy of gamma irradiation at 5 weeks and again at 6 weeks of age and were studied when 5–14 months of age. Data were derived from 10 unirradiated and 15 irradiated Cre/ZEG mice and from 14 unirradiated and 11 irradiated Cre/TA1 mice. The differences seen between Cre/ZEG and Cre/TA1 mice are statistically significant for all comparisons (P < .00001) for all comparisons excepting ‘postirradiation, all cells’ for which P = .009. There were no statistically significant differences when Cre/ZEG unirradiated mice were compared with Cre/ZEG irradiated mice. In contrast, irradiation led to an increase in EGFP expressing cells in the total, low SSC and high SSC cell populations (P < .00001).
Curves in Figure 2, Cdkn2a null mice died of malignancies with a median latency of 259 days (Figure 2a) and B-cell lymphoma/leukemia was observed in 9 of 34 mice followed as a cohort (Figure 2b). We performed flow cytometric immunophenotyping (Supplementary Table) of eight of these samples as well as an additional seven B-cell lymphomas that arose in Cdkn2a null mice and that we had collected from selected non-cohort animals (we had previously sporadically assessed ill mice in our Cdkn2a null background). As shown in the left hand curves of Figure 2, TEL-AML1 increased the incidence of B-cell lymphomas that arose in Cdkn2a null mice: without irradiation, 35% of bone marrow CD45R(B220)+, CD19+, CD43+, with 11 of 15 samples lacking surface immunoglobulin (lg) M (and hence compatible with pre-B LBL, precursor B-cell lymphoblastic lymphoma/leukemia) and CD43+ with 11 of 15 samples showing some degree of surface IgM expression (and hence compatible with BLL, Burkitt like lymphoma including mature B-cell lymphomas with lymphoblastic morphology).14

To assess the interaction of TEL-AML1 with Cdkn2a loss, the Tie2-Cre and inducible TEL-AML1 transgenes were crossed onto the Cdkn2a null background. As shown in the left hand curves of Figure 2, TEL-AML1 decreased the median cancer free survival of Cdkn2a null mice to 177 days (Figure 2a, \( P = .002 \)) for decreased cancer free survival, and increased the incidence of B-cell lymphoma from 26–50% (Figure 2b, 11 of 22 mice; \( P = .003 \) for decreased survival due to B-cell disease). Morphological examination of the lymphomas showed a lymphoblastic morphology and demonstrated infiltrates in the liver and spleen (Supplementary Figure S3). Flow cytometric immunophenotyping of these leukemias demonstrated disease similar to that observed with Cdkn2a alone, including 7 of 11 samples compatible with pre-B LBL and 4 of 11 compatible with BLL (Supplementary Figure S4A and Supplementary Table). All 11 samples expressed weak to moderate levels of EGFP, whereas a mast cell neoplasm and a histiocytic sarcoma that arose in this cohort were more strongly EGFP positive. As expected, TEL-AML1 transcripts could be detected in B-cell lymphoma from TEL-AML1 Cdkn2a null mice but not in lymphoma that arose in Cdkn2a null mice in the absence of TEL-AML1 (Supplementary Figure S4B). In addition, we were able to demonstrate expression of TEL-AML1 protein by western blot in a lymphoma with moderate EGFP expression (Supplementary Figure S4C).

We assessed the potential for radiation exposure to enhance leukemia development in these mice. We exposed 9-day-old pups to 1.88 Gy or did not irradiate them. At 5 weeks of age, bone marrows were harvested and analyzed by flow cytometry before transplantation into lethally irradiated congenic recipient animals. We observed that irradiation fostered outgrowth of TEL-AML1 expressing cells in Cdkn2a null mice: without irradiation, 35% of bone marrow CD45R+ /CD19+/IgM- immature B-cells expressed EGFP, whereas in irradiated mice 76% of these immature B-cells expressed EGFP (n = 5 per group, \( P = .0002 \)). Survival curves (Figure 2c) provided further confirmation of the leukemogenic effect of TEL-AML1 in Cdkn2a null mice: median survival for recipients of unirradiated Cre/TA1 Cdkn2a null marrow was 128 days vs 164 days for Cdkn2a null unirradiated marrow (\( P = .0003 \)); median survival for recipients of irradiated Cre/TA1 Cdkn2a null
marrow was 118 days vs 140 days for Cdkn2a null irradiated (P = .009). In all four groups, the majority of deaths were due to B-cell lymphoma/leukemia.

We had hypothesized that irradiation would accelerate the development of disease in at least some of the recipient animals. Although there was substantial overlap of the survival curves of genetically matched irradiated and unirradiated groups, the survival curves of the irradiated groups appeared shifted to the left as compared with the respective unirradiated groups. Using the shortest time to leukemia in the control Cdkn2a null unirradiated group for comparison (97 days) we observed that 9 of 25 mice in the Cre/TA1 Cdkn2a irradiated group developed disease prior to this time, whereas only 2 of 24 mice in the Cre/TA1 Cdkn2a unirradiated group showed early disease (P = .02; see Materials and Methods for statistical discussion). This finding suggests that some mice received cells for which low-dose radiation caused mutations that cooperated with TEL-AML1 activity. This finding provides support for a model of TEL-AML1 leukemogenesis, in which environmental exposures may both enable expansion of initially disadvantageous TEL-AML1 expressing cells and cause cooperative leukemogenic mutations.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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Landmark analysis of DNMT3A mutations in hematological malignancies

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Methylation of CpG islands is an epigenetic modification that is important for cellular development. Different members of the DNMT family are involved in de novo CpG methylation. The DNMT3A gene is located on chromosome 2p23.3 and codes for a DNA methyltransferase. Ley et al.1 and Yan et al.2 were able to unravel DNMT3A mutations (DNMT3Amut) in acute myeloid leukemia (AML), thus supporting a role for aberrant DNA methylation to contribute to the pathogenesis of this disease. Subsequent studies detected DNMT3Amut in 17–34% of AML with normal karyotype (CN-AML) (see Table 1). Depending on the respective patient cohort characteristics, occurrence of the mutations was associated with an inferior survival (Table 1). Subsequently, DNMT3Amut were studied and observed in other hematological malignancies (Table 1): mutation frequencies were

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