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Somatic inactivation of Tp53 in hematopoietic stem cells or thymocytes predisposes mice to thymic lymphomas with clonal translocations

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Abbreviations: DSBs, DNA double-stranded breaks; Tcr, T-cell receptor; Ig, immunoglobulin; HSCs, hematopoietic stem cells; VP, Vav-cre$^{+/-}$p53$^{flox/flox}$; LP, Lck-cre$^{+/-}$p53$^{flox/flox}$

TP53 protects cells from transformation by responding to stresses including aneuploidy and DNA double-strand breaks (DSBs). TP53 induces apoptosis of lymphocytes with persistent DSBs at antigen receptor loci and other genomic loci to prevent these lesions from generating oncogenic translocations. Despite this critical function of TP53, germline *Tp53*$^{-/-}$ mice succumb to immature T-cell (thymic) lymphomas that exhibit aneuploidy and lack clonal translocations. However, *Tp53*$^{-/-}$ mice occasionally develop B lineage lymphomas and *Tp53* deletion in pro-B cells causes lymphomas with oncogenic immunoglobulin (*Ig*) locus translocations. In addition, human lymphoid cancers with somatic TP53 inactivation often harbor oncogenic *IG* or T-cell receptor (*TCR*) locus translocations. To determine whether somatic *Tp53* inactivation unmasks translocations or alters the frequency of B lineage tumors in mice, we generated and analyzed mice with conditional *Tp53* deletion initiating in hematopoietic stem cells (HSCs) or in lineage-committed thymocytes. Median tumor-free survival of each strain was similar to the lifespan of *Tp53*$^{-/-}$ mice. Mice with HSC deletion of *Tp53* predominantly succumbed to thymic lymphomas with clonal translocations not involving *Tcr* loci; however, these mice occasionally developed mature B-cell lymphomas that harbored clonal Ig translocations. Deletion of Tp53 in thymocytes caused thymic lymphomas with aneuploidy and/or clonal translocations, including oncogenic *Tcr* locus translocations. Our data demonstrate that the developmental stage of *Tp53* inactivation affects karyotypes of lymphoid malignancies in mice where somatic deletion of *Tp53* initiating in thymocytes is sufficient to cause thymic lymphomas with oncogenic translocations.

Introduction

The TP53 tumor suppressor maintains cellular homeostasis in response to a wide range of stresses, including aneuploidy, DSBs, genomic instability, and oncogene activation.[1,2] These stresses stabilize and activate TP53, leading to changes in expression of target genes, including those involved in cell cycle control and apoptosis.[1,2] *TP53* is the most frequently inactivated tumor suppressor gene, with mutation or deletion occurring in over 60% of all human cancers,[1,3] indicating that TP53 prevents malignant transformation of multiple cell types. Although *TP53* inactivation occurs less frequently in lymphoid malignancies than in solid tumors, TP53 loss is more common in aggressive lymphoma subtypes and correlates with increased tumor grade, treatment resistance, and poor patient survival.[4-6]

Lymphocyte development involves cellular proliferation and antigen receptor gene assembly. Bone marrow HSCs differentiate into early progenitor B cells that remain in the bone marrow or into early thymic progenitors that migrate to the thymus. These cells proliferate and differentiate into pro-B or pro-T cells, respectively, which induce expression of the RAG1/RAG2 (RAG) endonuclease.[7,8] RAG catalyzes the assembly of TCR and Ig genes in G_1 phase cells through induction of DSBs at variable (V), diversity (D), and joining (J) gene segments.[9,10] Non-homologous end-joining (NHEJ) factors repair these DSBs to generate V(D)J coding joins that encode the first exons of TCR and Ig genes.[10,11] Assembly of TCRβ, TCRγ, and TCRδ genes occurs in CD4$^-$CD8$^-$ "double-negative" (DN) pro-T cells.[12,13] Expression of functional TCRγ and TCRδ genes signals differentiation into mature γδ T cells.[14] In contrast, expression of functional TCRβ genes triggers proliferation as cells differentiate into CD4$^+$CD8$^+$ "double positive" (DP) thymocytes.[15] In DP thymocytes, TCRα gene assembly followed by αβ TCR selection permit differentiation into CD4$^+$ or CD8$^+$ "single positive" (SP) thymocytes that

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exit the thymus as naive αβ T cells.[12,16] Assembly and expression of IgH genes in pro-B cells drives proliferation as cells differentiate into pre-B cells, which must recombine either Igκ or Igλ genes to differentiate into immature B cells that exit the bone marrow and migrate to the spleen as they mature.[13,17,18] In response to antigen, mature B cells proliferate and undergo IgH class switch recombination (CSR) through DSB intermediates.[19-22] In addition to programmed DSBs in antigen receptor loci, lymphocytes experience spontaneous DSBs that arise from errors in DNA replication during periods of proliferation.[23]

TP53 inactivation occurs in human B and T lineage lymphomas containing aneuploidy as well as in those exhibiting genomic instability,[3,24] suggesting that functions of TP53 in response to chromosome missegregation and DSBs are each important to suppress transformation of differentiating lymphocytes. Aberrant segregation of chromosomes during cellular division leads to TP53-dependent apoptosis of ensuing aneuploid daughter cells.[25] Induction of DSBs stabilizes and activates TP53, which promotes temporary cell cycle arrest to provide cells time to repair these lesions or induce apoptosis if they cannot be repaired.[2] Germline *Tp53* inactivation in mice leads to aneuploidy and genomic instability in differentiating and mature lymphocytes[26] and enables pro-T and pro-B cells with un-repaired RAG-induced Tcrδ and IgH locus DSBs to survive, progress into S phase, and generate translocations.[27-31] Despite roles of TP53 in response to both chromosome missegregation and DSBs, most $Tp53^{-/-}$ mice succumb to aneuploid $TCR\beta^{+}$ thymic lymphomas that lack *Tcr* translocations, though a small fraction succumb to B-cell lymphomas that have not been assayed for translocations.[32-37] However, mice with combined germline inactivation of Tp53 and NHEJ factors reproducibly succumb to pro-B cell lymphomas with RAG-dependent IgH translocations that amplify the c-Myc oncogene[28,38,39] and occasionally develop $TCR\beta^{-}$ thymic lymphomas with Tcrδ translocations.[40] In addition, $Tp53^{-/-}$ mice with germline inactivation of the H2ax DSB repair factor predominantly succumb to $TCR\beta^{-}$ thymic lymphomas with clonal translocations not involving *Tcr* loci, but occasionally develop $TCR\beta^{-}$ lymphomas with Tcrα/δ translocations or pro-B cell lymphomas with *Igh;c-myc* translocations.[21,36] Furthermore, on a genetic background with a block in αβ T-cell development at the DN stage, germline Tp53 deficiency causes $TCR\beta^{-}$ thymic lymphomas with RAG-dependent translocations that may involve Tcrδ or IgH loci.[41] Together, these studies showed that TP53 functions in response to chromosome missegregation and DSBs are critical to prevent transformation of differentiating lymphocytes. However, they concluded that Tp53 only suppresses oncogenic translocations in cells with DSB repair or differentiation impaired.

Cancers develop through somatic acquisition and selection of mutations such as *TP53* inactivation and other oncogenic lesions.[42] We previously showed that, while germline $H2ax^{-/-}$ $Tp53^{-/-}$ mice succumb to $TCR\beta^{-}$ thymic lymphomas with clonal translocations, conditional deletion of *H2ax* and *Tp53* in mouse DN thymocytes prolongs lifespan and leads to $TCR\beta^{+}$ thymic lymphomas.[21,36,43] That somatic inactivation of H2ax and Tp53 causes more mature thymic lymphomas with longer latency compared with germline inactivation indicates that oncogenic lesions prior to T cell commitment drive transformation of thymocytes. We recently showed that conditional deletion of *Tp53* in pro-B cells predisposes mice to B lineage lymphomas with oncogenic translocations, including *Igh;c-myc* and other *Ig* translocations.[44] The tumor-free survival of these mice is similar to that of $Tp53^{-/-}$ mice, suggesting that development of thymic lymphomas from aneuploidy prevents B-cell lymphomas from oncogenic *Ig* translocations in $Tp53^{-/-}$ mice. To determine whether somatic inactivation of *Tp53* unmasks translocations or alters the frequency of B lineage lymphomas in mice, we generated mice with conditional *Tp53* deletion initiating in HSCs or DN thymocytes. These strains each succumbed at similar ages to thymic lymphomas, although HSC deletion occasionally caused B lineage lymphomas. HSC deletion of Tp53 led to clonal translocations not involving *Tcr* loci in thymic lymphomas and *Igh* translocations in B lymphomas, while DN deletion of Tp53 caused thymic lymphomas with aneuploidy and/or clonal translocations including *Tcr*α/δ locus translocations. Our data demonstrate that the developmental stage of *Tp53* inactivation affects karyotypes of lymphoid cancers in mice, where somatic deletion of *Tp53* initiating in thymocytes is sufficient to cause thymic lymphomas with oncogenic translocations.

Results

Conditional deletion of *Tp53* in HSCs or thymocytes predisposes mice to thymic lymphomas

To determine whether *Tp53* inactivation initiating in HSCs or thymocytes predisposes mice to lymphoma, we established and characterized *Vav*–$cre^{+/-}p53^{flox/flox}$ (*VP*) and *Lck*–$cre^{+/-}$ $p53^{flox/flox}$ (*LP*) mice. *Vav*-cre and *Lck*-cre induce deletion of "floxed" genes in HSCs and DN thymocytes, respectively.[45,46] We detected nearly complete deletion of *Tp53* in bone marrow cells and thymocytes of *VP* mice and in thymocytes of *LP* mice, but no *Tp53* deletion in *LP* bone marrow cells (**Fig. S1**), confirming the expected developmental timing of *Tp53* inactivation in *VP* and *LP* mice. We also found grossly normal T and B cell development in *VP* and *LP* mice as compared with age-matched, wild-type controls (**Fig. S2**), consistent with the phenotype of $Tp53^{-/-}$ mice.[47] We generated and aged cohorts of *22 VP* and 20 *LP* mice to evaluate their spontaneous predisposition to cancer. We observed that cohort *VP* mice survived cancer-free between 91–365 d with median age of mortality of 144.5 d, whereas cohort *LP* mice survived cancer-free between 70–365 d with a median age of mortality of 119 d (**Fig. 1**). The median ages of cancer-free survival of cohort *VP* and *LP* mice were not statistically significant ($p = 0.134$) and were comparable to the survival of germline $Tp53^{-/-}$ mice.[32,33] All *VP* and *LP* mice succumbed to lymphoma, except for one *LP* mouse that was euthanized due to development of a sarcoma and one mouse of each genotype that died without lymphoma (**Tables 1 and 2**). Most *VP* mice and *LP* mice succumbed to lymphomas that were only visible in the thymus (**Tables 1 and 2**). However, 3 *VP* mice (nos. 202, 228, and 623) and 2 *LP* mice (nos. 119 and 983) had lymphoma cells in their spleens, while one *VP* mouse (no. 602) succumbed

Table 1. Summary of VP tumor cohort

Mouse	Lifespan, days	Gross phenotype	Karyotype[1]	Surface expression
820	108	thy lymph	t(2;17) (28/33)	CD3+ TCRβ+ CD4/8 var
202	159	thy lymph + spleen	–	–
105	106	thy lymph	–	CD3+ TCRβ+ DP
118	119	thy lymph	t(11;15) (16/26), t(12;19) (9/26)	CD3+ TCRβ+ DP
974	99	thy lymph	–	CD3+ TCRβ+ CD4/8 var
975	103	thy lymph	t(12;12) (12/36), t(11;11) (15/36)	CD3– TCRβ– DP
112	134	thy lymph	–	CD3– TCRβ– DP
979	102	thy lymph	–	CD3+ TCRβ+ DP
977	119	thy lymph	–	CD3+ TCRβ+ DN
124	176	thy lymph	–	CD3– TCRβ– DP
930	93	thy lymph	–	–
207	231	thy lymph	aneuploid	CD3+ TCRβ+ DP
228	123	thy lymph + spleen	–	CD3– TCRβ– DN
306	129	thy lymph	–	CD3+ TCRβ+ DP
919	155	thy lymph	–	CD3+ TCRβ+ DN
421	176	LN tumor	t(12;15) (24/25)	B220+ IgM+ Igκ+/–
426	305	LN tumor	t(6;4) (23/24)	B220+ IgM+ Igκ–
618	213	thy lymph	–	CD3– TCRβ– DN
602	217	widely disseminated	–	CD3– TCRβ– DN
623	260	thy lymph + spleen	–	CD3+ TCRβ+ CD4/8 var
773	216	thy lymph	t(4;4) (15/15), t(5;5) (14/15), t(15;17) (12/15)	CD3– TCRβ– DN
720	365	none apparent	–	–

[1]For each prevalent structural aberration, numbers in parentheses indicate proportion of metaphases analyzed carrying that aberration. –, dashes indicate parameters not assessed. Abbreviations: thy lymph, thymic lymphoma; CD4/8 var, variable levels of CD4 and/or CD8 expression; DP, CD4+CD8+ double positive; DN, CD4− CD8− double negative; LN, lymph node.

to a disseminated lymphoma found in the thymus, spleen, and multiple lymph nodes (**Tables 1 and 2**). In addition, 2 *VP* mice (nos. 421 and 426) succumbed to lymphomas that were located in the spleen and multiple lymph nodes but not visible in the thymus. We did not characterize cohorts of *Vav*–cre$^{+/-}$, *Lck*–cre$^{+/-}$, or *p53*$^{flox/flox}$ mice, since none of these mice exhibits increased tumor predisposition.[45,46,48] Consistent with this notion, none of the *Vav*–cre$^{+/-}$, *Lck*–cre$^{+/-}$, or *p53*$^{flox/flox}$ mice we used for breeding until 1 y of age developed cancer.

***Tp53* inactivation in HSCs or thymocytes causes clonal immature T-cell lymphomas**

To determine the lymphocyte lineages and developmental stages to which inactivation of *Tp53* in HSCs or DN thymocytes causes cellular transformation, we first analyzed *VP* and *LP* lymphomas by flow cytometry using antibodies that recognize cell surface markers of specific lymphocyte lineages and developmental stages. For lymphomas found within the thymus, we assessed cell surface expression of TCRδ, TCRβ, CD4, CD8, and the CD3ε molecule through which αβ TCRs signal.[15] For lymphomas found in lymph nodes, we assessed cell surface expression of the B220, IgM, Igκ, and Igλ molecules. Due to the variation in cell staining observed within some tumors, we simplified our classification by denoting a lymphoma as positive for an epitope if more than half of the cells fell within a positive gate established from flow cytometry of non-malignant lymphocytes. Of the 17 *VP* thymic lymphomas assayed, most were TCRβ$^+$CD3$^+$CD4$^+$CD8$^+$, but a substantial number were also TCRβ$^-$CD3$^-$ with or without CD4 and CD8 expression (**Fig. 1B**; **Table 1**; **Fig. S3**). Lymphoma cells in the spleens of the 2 *VP* mice (nos. 421 and 426) that lacked thymic lymphomas were B220$^+$IgM$^+$ (**Fig. 1B**; **Table 1**; **Fig. S3**). Lymphoma no. 421 contained Igκ$^+$ and Igκ$^-$ cells, while lymphoma no. 426 contained mostly Igκ$^-$ cells (**Fig. 1B**; **Table 1**; **Fig. S3**). Of the 15 *LP* thymic lymphomas assayed, 10 were TCRβ$^+$CD3$^+$CD4$^+$CD8$^+$; only one was TCRβ$^-$CD3$^-$ (**Fig. 1B**; **Table 2**; **Fig. S3**). Many *VP* and *LP* thymic lymphomas displayed subpopulations with different expression patterns of TCRβ, CD3, CD4, and/or CD8, indicating that they represent either oligoclonal lymphomas arising from distinct initiating cells or clonal lymphomas with subpopulations that have differentially silenced and/or re-expressed genes. *VP* thymic lymphomas more often showed TCRβ, CD4, CD8, and CD3 expression characteristic of immature T-cell developmental stages than *LP* thymic lymphomas. Collectively, our flow cytometry analysis of *VP* and *LP* lymphomas indicates that deletion of *Tp53* in mouse HSCs or DN thymocytes causes predominantly immature T-cell lymphomas, with HSC deletion leading to a

higher percentage of tumors from an earlier T-cell developmental stage and occasional mature B-cell lymphomas.

To determine the lymphocyte lineage of the 4 TCRβ$^-$CD3$^-$CD4$^-$CD8$^-$ *VP* thymic lymphomas and to distinguish between oligoclonal and clonal immature T cell lymphomas in *VP* and *LP* mice, we next analyzed *Tcr*β gene rearrangements in *VP* and *LP* lymphomas. For DN thymocytes to survive and differentiate, *Tcr*β rearrangements must occur on one allele.[49] To characterize *Tcr*β rearrangements, we conducted Southern blotting on *Hind*III-digested genomic DNA of *VP* and *LP* lymphomas with *3′Jβ1* and *3′Jβ2* probes (**Fig. 2A–C**). We isolated DNA from the tumor-containing organ without further purification, under assumption that most cells were malignant. This was confirmed by flow cytometry. Further, this approach does not detect unique rearrangements in single cells, such as those occurring in normal lymphocytes. Thus, only germline configurations in non-lymphoid cells will be detected as background. We detected *Tcr*β rearrangements in the TCRβ$^-$CD3$^-$CD4$^-$CD8$^-$ *VP* thymic lymphomas (nos. 228, 602, 618, and 773) (**Fig. 2B**), demonstrating that these malignancies are immature T cell lymphomas. We also found that 16 of 18 *VP* and all 15 *LP* thymic lymphomas analyzed contained one or two rearranged *Tcr*β alleles and therefore arose from the expansion of a single cancer-initiating cell (**Fig. 2B and C**). These data indicate that the diverse expression of surface epitopes observed within some *VP* and *LP* lymphomas represents tumor subpopulations that have differentially silenced and/or re-expressed these genes. The remaining 2 *VP* thymic lymphomas (nos. 124 and 618) contained 3 *Tcr*β rearrangements (**Fig. 2B**),

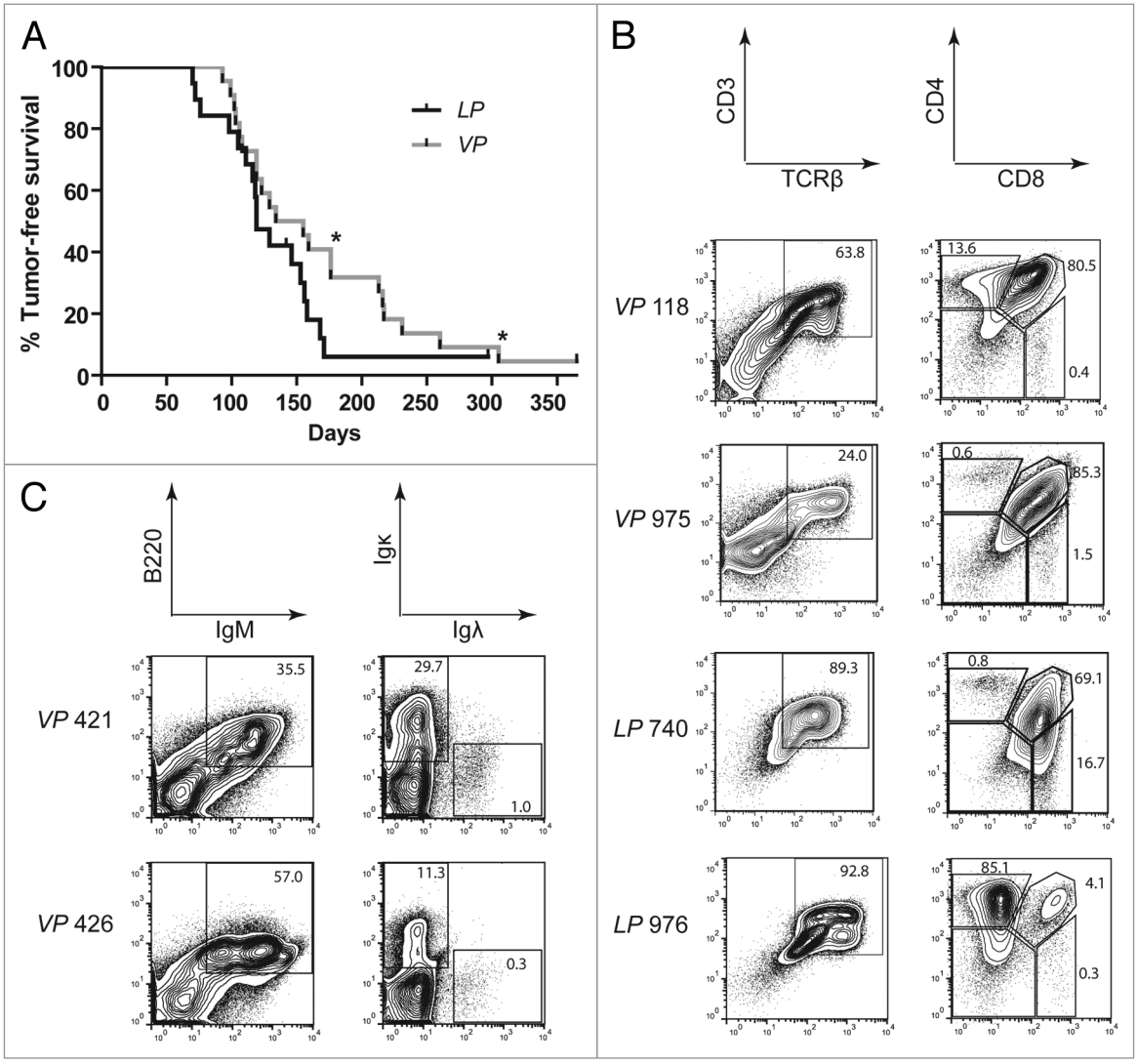

Figure 1. Mice with conditional inactivation of *Tp53* initiating in HSCs or DN thymocytes reproducibly succumb to thymic lymphomas. (**A**) Kaplan–Meier curves comparing tumor-free survival of 22 *VP* and *20 LP* mice. All tumors were thymic lymphomas other than 2 *VP* mice that succumbed to B lineage lymphomas (indicated by asterisks). Additionally, one LP mouse succumbed to a sarcoma, and one mouse of each cohort did not succumb to any tumors by 1 year of age. Curves were compared using the log-rank (Mantel–Cox) test, $P = 0.134$ (**B**) Flow cytometry analyses of *VP* lymphomas no 118 and 975 and of *LP* lymphomas no. 740 and 976 showing their surface expression of CD3 and TCRβ or CD4 and CD8. Gates were drawn on normal T cells analyzed in parallel with the lymphomas. The percentages of cells in each gate are indicated. (**C**) Flow cytometry analyses of *VP* lymphomas no. 421 and 426 showing their surface expression of B220 and IgM or Igκ and Igλ. Gates were drawn on normal B cells analyzed in parallel with lymphomas. The percentages of tumor cells in each gate are indicated.

indicating that these cancers either arose from the expansion of two cancer-initiating cells or one cancer-initiating cell that continued to assemble *Tcr*β genes after malignant transformation. We also performed Southern analysis of *Tcr*β rearrangements on *VP* lymphomas found in the spleen or lymph nodes of mice. We found that 3 of these 4 *VP* lymphomas analyzed contained the same *Tcr*β rearrangements as the thymic lymphomas from the same animals (**Fig. 2B**), demonstrating that these mice succumbed to a disseminated clonal immature T cell lymphoma. *VP* lymphoma (no. 202) displayed an additional *Tcr*β rearrangement that was not present in the thymic lymphoma of this mouse (**Fig. 2B**), suggesting that this splenic lymphoma developed from the thymic lymphoma in association with ongoing *Tcr*β rearrangement. Our Southern analysis of *Tcr*β rearrangements in *VP* and *LP* lymphomas indicates that inactivation of *Tp53* in mouse HSCs or DN thymocytes causes mainly clonal immature T-cell malignancies.

To determine whether the 2 B lineage lymphomas that arose in *VP* cohort mice were clonal and whether they arose from developing or mature B cells, we analyzed *Igh* and *Ig*κ rearrangements in these tumors. To characterize *Igh* rearrangements, we conducted Southern blot analysis of *Eco*RI-digested genomic DNA of *VP* lymphomas nos. 421 and 426 with $3'J_H$ and $3'S\mu$ probes (**Fig. 3A–C**). We found that these tumors contained one (no. 426) or two (no. 421) J_H rearrangements and therefore arose from the clonal expansion of a single cancer-initiating cell (**Fig. 3B**). We also detected Sμ recombination in lymphoma no. 421 (**Fig. 3C**), suggesting that this tumor may have developed from a B cell that had attempted CSR. Southern blot analysis of *BamH*I-digested genomic DNA from *VP* lymphomas nos. 421 and 426 with the 3′*J*κ probe showed that each of these tumors contained *J*κ rearrangements (**Fig. 3D and E**). This suggests that $IgM^+Ig\kappa^-$ *VP* lymphoma no. 426 developed from a B lymphocyte that had developed at least to the pre-B cell stage. Our Southern analysis of these 2 *VP* B lineage lymphomas indicates that deletion of *Tp53* in mouse HSCs can cause clonal B lineage lymphomas.

Conditional inactivation of *Tp53* in HSCs or thymocytes causes lymphomas with clonal translocations

To determine whether conditional deletion of *Tp53* initiating in HSCs or DN thymocytes causes lymphomas with chromosomal translocations, we conducted spectral karyotyping (SKY) on 7 *VP* and eight *LP* lymphomas. SKY is a molecular cytogenetic approach to visualize all chromosomes in a single metaphase spread to identify translocations and fusions between chromosomes.[50] We defined clonal translocations as those found in greater than half of the metaphases analyzed for a given tumor. SKY revealed that 2 of the 5 *VP* thymic lymphomas (nos. 118 and 820) analyzed had clonal translocations, with lymphoma no. 773 containing 3 different clonal chromosome fusions (**Fig. 4A**; **Table 1**; **Fig. S4**). One VP thymic lymphomas (no. 975) contained two non-clonal chromosome fusions (**Table 2**; **Fig. S4**). The remaining *VP* thymic lymphoma (no. 207) lacked translocations but exhibited aneuploidy (**Table 1**; **Fig. S4**). None of the clonal translocations in *VP* thymic lymphomas involved chromosomes on which *Tcr* or *Ig* loci reside. In contrast, SKY revealed

Table 2. Summary of LP tumor cohort

Mouse	Lifespan, days	Gross phenotype	Karyotype	Surface expression
722	72	thy lymph	–	CD3+ TCRβ+ CD4/8 var
734	76	thy lymph	aneuploid	CD3+TCRβ+ DP
740	70	thy lymph	aneuploid	CD3+ TCRβ+ DP
824	105	thy lymph	aneuploid	CD3+ TCRβ+ CD4/8 var
826	111	thy lymph	t(11;19) (12/20)	CD3− TCRβ− DP
983	156	thy lymph + spleen	–	–
646	116	thy lymph	aneuploid	CD3+ TCRβ+ DP
902	158	thy lymph	t(4;15;14) (9/13), t(2;2) (10/13)	CD3+ TCRβ+DP
654	118	thy lymph	–	CD3− TCRβ− CD4/8 var
907	119	thy lymph	–	CD3+ TCRβ+DP
975	119	thy lymph	–	–
976	98	thy lymph	t(16;14) (8/9)	CD3+ TCRβ+ CD4+
913	129	thy lymph	–	CD3+ TCRγδ+ TCRβ+ DP
915	171	thy lymph	–	–
930	146	thy lymph	–	CD3+ TCRβ+DP
767	142	none apparent	–	–
111	153	thy lymph	–	CD3+ TCRβ+DP
126	168	thy lymph	–	CD3+ TCRβ+DP
119	192	thy lymph + spleen	aneuploid	CD3+ TCRβ+DP
288	297	abdominal sarcoma	–	–

Abbreviations explained in **Table 1**.

that both *VP* B lymphomas contained clonal translocations involving chromosomes on which the *Igh* (chromosome 12 in no. 421) or *Igκ* (chromosome 6 in no. 426) locus resides (**Fig. 4B**; **Table 1**; **Fig. S4**). SKY also demonstrated that 3 of the 8 *LP* thymic lymphomas (nos. 826, 902, and 976) analyzed harbored clonal translocations, with lymphoma no. 902 also containing a clonal chromosome fusion (**Fig. 4A**; **Table 2**; **Fig. S4**). Notably, 2 of these tumors (no. 902 and 976) contained clonal translocations involving chromosome 14, which carries the *Tcrα/δ* locus. The other 5 *LP* thymic lymphomas lacked translocations but exhibited aneuploidy (**Table 2**). Our SKY analysis shows that conditional deletion of *Tp53* deletion in HSCs or thymocytes causes lymphomas with clonal translocations, with thymocyte deletion also causing aneuploid lymphomas.

To determine whether conditional deletion of *Tp53* initiating in HSCs or DN thymocytes causes lymphomas with clonal *Ig* or *Tcr* translocations, respectively, we conducted FISH on the 4 tumors with potential clonal *Igh*, *Igκ*, or *Tcrα/δ* translocations. For this purpose, we hybridized 5′ and 3′ *Ig* or *Tcr* locus probes and identified *Ig* or *Tcr* translocations by detection of probe signals on different chromosomes (**Fig. 4B and C**). Since the *c-myc* oncogene on chromosome 15 is activated by *Igh* or *Tcrα/δ* translocations in mouse lymphomas,[40,44] we also used a *c-myc* probe to identify potential *Igh;c-myc* and *Tcrα/δ;*c-myc translocations in *VP* lymphoma no. 421 and *LP* lymphoma no. 902. FISH revealed co-localization of 3′*Igh* and *c-myc* probe signals on one chromosome derivative in *VP* B lineage lymphoma no. 421 (**Fig. 4B**), indicating that the clonal t(12;15) translocation in this tumor is an *Igh;c-myc* translocation. Unfortunately, we were unable to determine potential involvement of the *Igκ* locus in the clonal t(6;4) translocation of *VP* B lineage lymphoma no. 426 due to insufficient numbers of metaphases from this tumor. FISH revealed splitting of 5′ and 3′ probes on the clonal t(16;14) translocation in metaphases from *LP* thymic lymphoma no. 976 (**Fig. S4** and not shown), indicating that this translocation tumor involves the *Tcrα/δ* locus. Finally, FISH showed co-localization of multiple copies each of 5′*Tcrα/δ* and *c-myc* probe signals on one chromosome derivative in metaphases from *LP* thymic lymphoma no. 902 (**Fig. 4C** and not shown), indicating that the clonal t(14;15;4) translocation in this tumor is a *Tcrα/δ;*c-myc translocation with amplification of the *c-myc* oncogene and *Tcrα* sequences. Our FISH analysis of metaphases from *VP* and *LP* lymphomas demonstrates that deletion of *Tp53* initiating in HSCs or DN thymocytes can cause, respectively, B lineage lymphomas with oncogenic *Igh* translocations or thymic lymphomas with oncogenic *Tcrα/δ* translocations.

Discussion

Our study demonstrates that the context of *Tp53* inactivation influences lymphoma predisposition, consistent with diverse roles of Tp53 throughout lymphocyte development and its interactions with known oncogenes. For example, Tp53 is important for termination of activated T-cell responses through induction of the pro-apoptotic SAP protein,[51] and inactivation of one or both copies of *Tp53* synergizes with *C-myc* and *MafB* oncogenes in mouse

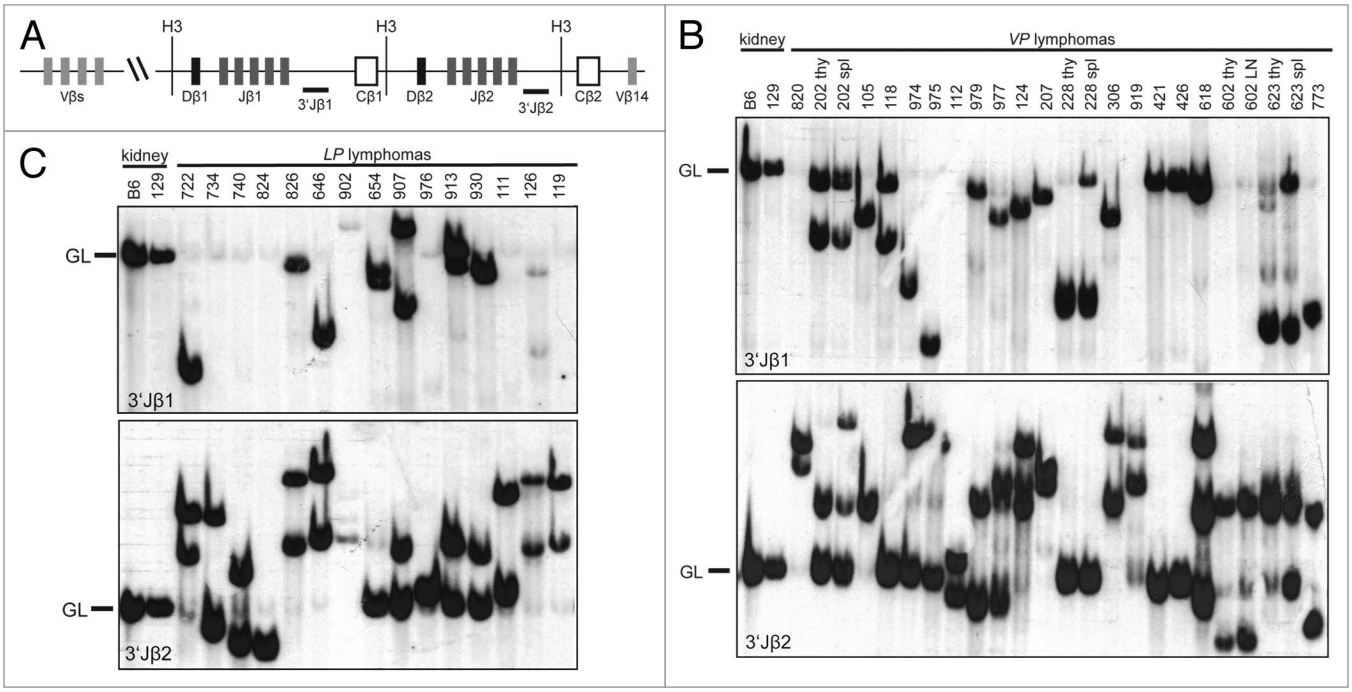

Figure 2. Mice with conditional inactivation of *Tp53* initiating in HSCs or DN thymocytes develop clonal thymic lymphomas. (**A**) Schematic of the mouse TCRβ locus showing relative locations of representative upstream Vβs, the 2 Dβ-Jβ-Cβ clusters, and the Vβ14 segment. The positions of the *Hind*III restriction sites (H3) and 3′Jβ1 and 3′Jβ2 probes used for Southern analyses are also shown. (**B and C**). Southern blot analysis of *Hind*III-digested DNA isolated from the indicated (**B**) *VP* or (**C**) *LP* lymphomas or from kidneys of C57BL/6 (B6) or 129/SvEv (129) control mice using the 3′Jβ1 or 3′Jβ2 probe. Germline (GL) bands for each probe are indicated. Membranes were hybridized with 3′Jβ1 probes, stripped, and then hybridized with 3′Jβ2 probes to reveal which lymphomas contained Vβ rearrangements to the Dβ2-Jβ2 cluster on both alleles.

models of mature B cell lymphomas.[52-54] However, our data adds to our understanding of how Tp53 suppresses lymphoid malignancies by demonstrating that developmental timing of *Tp53* inactivation in the absence of enforced oncogene expression can alter characteristics of resulting lymphomas. We generated mice with conditional deletion of *Tp53* initiating in HSCs or in DN thymocytes and compared their tumor predisposition to the well-characterized cancer phenotype of germline *Tp53*-deficient mice. We found that HSC initiation of *Tp53* inactivation predisposed mice to predominantly thymic lymphomas with clonal translocations not involving antigen receptor loci and occasionally to peripheral B cell lymphomas with clonal Ig translocations. Inactivation of *Tp53* in DN thymocytes predisposed mice to thymic lymphomas that exhibited aneuploidy or contained clonal translocations frequently involving *Tcr*α/δ loci. In contrast, germline inactivation of *Tp53* predisposes mice to aneuploid thymic lymphomas lacking clonal translocations.[32,33,36] Other than the timing of *Tp53* inactivation, the only difference between *VP* and *LP* mice and *Tp53*$^{-/-}$ mice is constitutive expression of Cre. Constitutive Cre expression causes genomic instability, at least in mouse embryonic cells cultured in vitro,[55,56] suggesting that the translocations found in *VP* and *LP* lymphomas could be Cre-induced lesions. Since the lymphoma predisposition of *Vav–cre:Tp53*$^{-/-}$ mice has not been reported, we cannot conclude whether the clonal translocations and chromosome fusions found in *VP* thymic lymphomas arise independently of *Vav–cre* expression. Yet, considering that *Vav–cre* mice are not predisposed to cancer,[46] our findings demonstrate that Tp53 serves important functions in HSCs and/or thymocytes to suppress malignant transformation in association with genomic instability. Notably, constitutive Cre expression from *Lck–cre* initiating in DN thymocytes of *Tp53*$^{-/-}$ mice does not alter onset or karyotype of thymic lymphomas that arise in these mice.[57] Therefore, we conclude from the cancer predisposition of *LP* mice that Tp53 serves critical functions in suppressing generation and/or oncogenic potential of *Tcr*α/δ translocations during αβ T cell development.

The objective of our study was to determine whether oncogenic lesions arising during embryogenesis and/or in HSCs preclude development of thymic lymphomas with clonal translocations including *Tcr* translocations. *VP* and *LP* mice succumb to tumors at similar ages as *Tp53*$^{-/-}$, *Lck–cre:Tp53*$^{-/-}$ mice, and *Mb1–cre:Tp53*$^{flox/flox}$ mice.[32,33,36,44] In contrast to the aneuploid thymic lymphomas that arise in *Tp53*$^{-/-}$ mice, we found that *VP* and *LP* mice developed lymphomas with aneuploidy or clonal translocations, including *Ig* or *Tcr*α/δ translocations. The distinct cancer phenotypes of these mice indicate that loss of Tp53 during embryogenesis, in cells before lymphocyte commitment, and/or in thymocytes masks development of lymphomas with oncogenic translocations in germline *Tp53*-deficient mice. In addition, the development of *LP* thymic lymphomas with aneuploidy or clonal translocations indicates that functions of Tp53 in response to both chromosome missegregation and DSBs are each critical for preventing malignant transformation of thymocytes.

T-cell acute lymphoblastic leukemia (T-ALL) remains a significant cause of cancer morbidity and mortality in both children and adults.[58,59] Advances have been made in treatment of patients with T-ALL; however, drug-resistance and relapse are

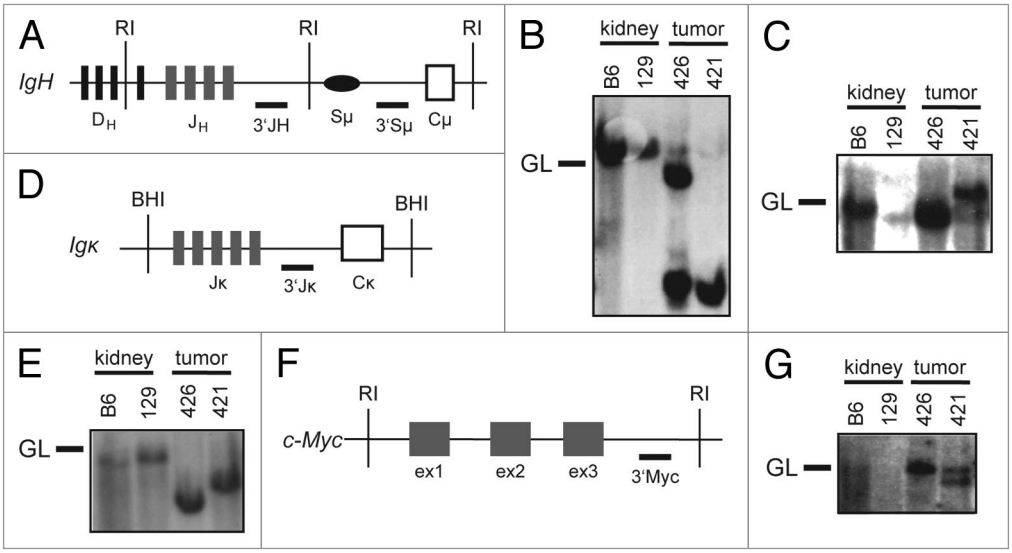

Figure 3. Mice with conditional inactivation of *Tp53* initiating in HSCs also develop clonal B lineage lymphomas. (**A**) Schematic of the mouse IgH locus showing relative locations of representative D_H segments, the 4 J_H segments, the Sμ region, and the first C_H exon, Cμ. The positions of the *Eco*RI restriction sites (RI) and 3′J_H and 3′Sμ probes used for Southern blots are also shown. (**B and C**) Southern blot analysis of *Eco*RI-digested DNA isolated from *VP* lymphomas no. 421 or 426 or from kidneys of C57BL/6 (B6) or 129/SvEv (129) control mice using the (**B**) 3′J_H and (**C**) 3′Sμ probes. Germline (GL) bands for each probe are indicated. (**D**) Schematic of the mouse Igκ locus showing relative locations of the 5 Jκ segments and the Cκ exon. The positions of the *Bam*HI restriction sites (BHI) and 3′Jκ probe used for Southern blots are also shown. (**E**) Southern blot analysis of *Bam*HI-digested DNA isolated from *VP* lymphomas no. 421 or 426 or from kidneys of C57BL/6 (B6) or 129/SvEv (129) control mice using the 3′Jκ probe. Germline (GL) band for the 3′Jκ probe is indicated. (**F**) Schematic of the mouse c-Myc locus showing relative locations of the three c-Myc exons, and of the *Eco*RI restriction sites (RI) and 3′Myc probe used for Southern blots. (**G**) Southern blot analysis of *Eco*RI-digested DNA isolated from *VP* lymphomas no. 421 or 426 or from kidneys of C57BL/6 (B6) or 129/SvEv (129) control mice using the 3′Myc probe. Germline (GL) band for the 3′Myc probe is indicated.

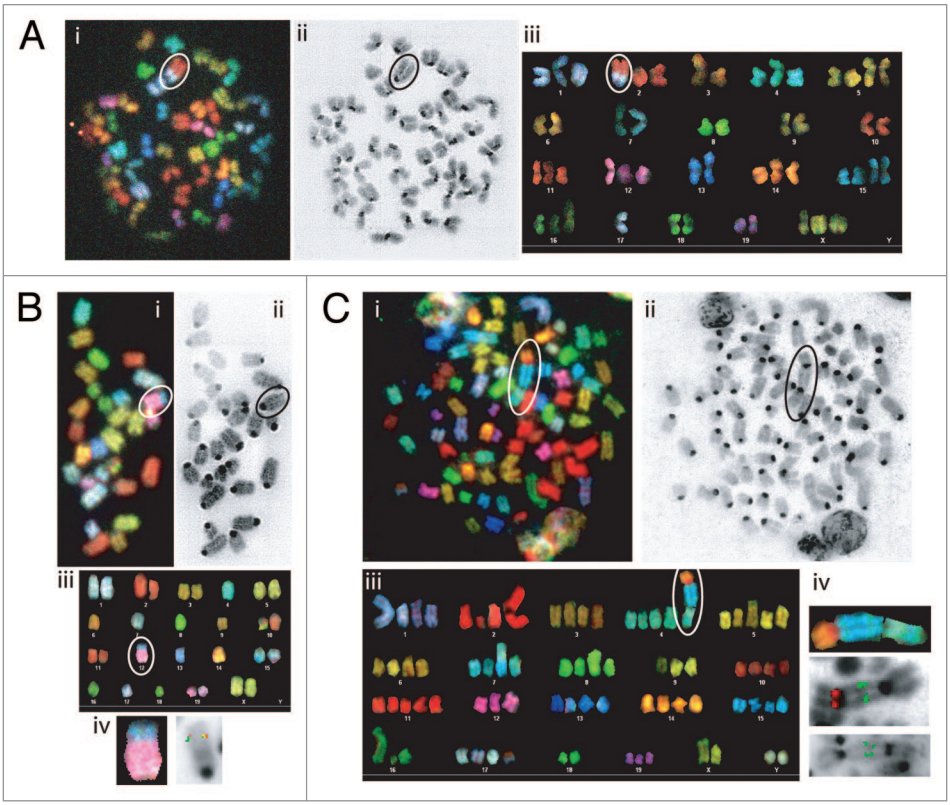

Figure 4. Mice with conditional inactivation of *Tp53* initiating in HSCs or DN thymocytes develop lymphomas with oncogenic antigen receptor locus translocations. (**A**) Cytogenetic analysis of a metaphase from *VP* lymphoma no. 820 with the clonal t(2;17) translocation circled or isolated. (**A**, i) Spectral image. (**A**, ii) DAPI image. (**A**, iii) Karyotype table. (**B**) Cytogenetic analyses of a metaphase or chromosome from *VP* lymphoma no. 421 with the clonal t(12;15) translocation circled. (**B**, i) SKY image. (**B**, ii) DAPI image. (**B**, iii) Karyotype table. (**B**, iv). SKY (left) or FISH image (right) of the t(12;15) translocation hybridized with 3′IgH (green) and c-Myc (red) probes. (**C**) Cytogenetic analyses of a metaphase or chromosome from *LP* lymphoma no. 902 with the clonal t(4;15;14) translocation circled or isolated. (**B**, i) SKY image. (**B**, ii) DAPI image. (**B**, iii) Karyotype table. (**B**, iv) SKY (top) or FISH images of the t(4;15;14) translocation. (middle) hybridized with TCRVδ3/Vα6 (green) and TCRCα (red) probes. (bottom) hybridized with c-Myc (green) probe.

common causes of treatment failure, and most patients with tumors that fail treatment do not survive.[58] T-ALL is typically treated using genotoxic drugs that can cause serious health issues through effects on normal cells, demonstrating a need to develop more specific and less toxic therapies.[60] T-ALLs have heterogeneous karyotypes, with about half being aneuploid and the remainder containing translocations including oncogenic Tcrα/δ translocations.[61-63] Although inactivating *TP53* mutations are not common in T-ALL, these genetic lesions are often associated with drug resistance, rapid disease progression, and poor survival.[3] Therefore, *LP* mice may provide a useful preclinical model to evaluate the potential efficacy of more specific and less toxic treatments for T-ALL with *TP53* inactivation.

Materials and Methods

Mice

Vav–cre,[46] *Lck*–cre,[45] and $p53^{flox/flox}$[48] mice were bred to generate the animals in this study. The genetic background strain of these mice was mixed 129SvEv and C57BL/6, with the 129SvEv strain predominant. We bred *Vav*–$cre^{+/-}$ and *Lck*–$cre^{+/-}$ males with $p53^{flox/flox}$ females to generate *Vav*–$cre^{+/-}p53^{flox/WT}$ and *Lck*–$cre^{+/-}p53^{flox/WT}$ males. These males were bred with $p53^{flox/flox}$ females to generate cohort *Vav*–$cre^{+/-}p53^{flox/flox}$ and *Lck*–$cre^{+/-}p53^{flox/flox}$ mice of either sex. Cohort mice were aged, monitored regularly, and euthanized upon showing visible signs of distress. This study was performed in accordance with national guidelines, and was approved by the Institutional Animal Care and Use Committee of the Children's Hospital of Philadelphia.

Kaplan–Meier analysis

Kaplan–Meier curves were generated in Graphpad Prism 5 (Graphpad Software Inc) and compared using the log-rank (Mantel-Cox) test.

Southern blotting

Genomic DNA (~20 μg) from lymphomas, non-transformed lymphocytes, or kidneys was digested with 100 units of indicated restriction enzymes (New England Biolabs), separated on 0.8% TAE agarose gels, transferred onto Zeta-probe membrane (BioRad), and hybridized with ^{32}P-labeled *Tcr*β, *Igh*, *Ig*κ, or *c-myc* DNA probes as described.[21,43,64]

Flow cytometry

Single-cell suspensions of mouse lymphomas or thymuses and spleens of 4–6-wk-old control mice were stained in PBS with 3% FBS using antibodies from BD Pharmingen. For analyses of thymic lymphomas and non-malignant T cells, APC-conjugated anti-TCRβ, FITC-conjugated anti-CD8, PE-conjugated

anti-CD4, PE-conjugated anti-TCRδ, and FITC-conjugated anti-CD3ε antibodies were used. For analyses of B lymphomas and non-malignant B cells, PE-Cy7-conjugated anti-B220, FITC-conjugated anti-CD43, APC-conjugated anti-IgM, PE-conjugated anti-Igκ, and FITC-conjugated anti-Igλ antibodies were used. Data were collected using a FACSCalibur (BD Biosciences) and CellQuest software (BD Biosciences) and analyzed using FlowJo software (Tree Star).

Cytogenetics

Metaphase spreads were prepared as previously described.[21] Spectral karyotyping and fluorescence in situ hybridization (FISH) were performed according to manufacturers' instructions (Applied Spectral Imaging). FISH probes were labeled with digoxigenin or biotin according to manufacturer's instructions (Roche). The TCRCα-232F19, TCRVδ3/Vα6–46G9, *Igh* C_H BAC199, and *c-myc*-454G15 FISH probes have been described previously.[43,44] Slides were examined at room temperature under a BX61 microscope (magnification: 600×) from Olympus, controlled by a LAMBDA 10-B Smart Shutter from Sutter Instrument (Novato). Images were captured using a LAMBDA LS light source from Sutter Instrument, and a COOL-1300QS camera ASI, then analyzed through Case Data Manager Version 5.5 configured by Applied Spectral Imaging.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Supplemental Materials

Supplemental materials may be found here:
www.landesbioscience.com/journals/cc/article/26299

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