Research Article

Notch1 deficiency alters the migratory behavior of developing T cells and calcium signaling in the thymus of medaka

Narges Aghaallaei1,2, Daigo Inoue2, Eva Hasel de Carvalho3, Advaita M. Dick1, Joachim Wittbrodt2, Maria Leptin3,4 and Baubak Bajoghli1,3

1 Department of Hematology, Oncology, Immunology, and Rheumatology, University Hospital of Tübingen, Tübingen, Germany
2 Centre for Organismal Studies (COS), Heidelberg University, Heidelberg, Germany
3 European Molecular Biology Laboratory (EMBL), Heidelberg, Germany
4 EMBO, Heidelberg, Germany

The differentiation of T cells from lymphoid progenitors in the thymus follows sequential developmental stages that constantly require interaction with thymic epithelial cells. Several distinct aspects of early T cell development depend on the activation of Notch receptors on thymocytes, while the selection of thymocytes at later stages are believed to be Notch independent. Using reverse genetic approaches and whole-thymus live imaging in an in vivo teleost model, the medaka, we report that Notch1 signals is required for proliferation and specification of developing T cells as well as involved in their selection in the thymus. We reveal that Notch1 controls the migratory behavior of thymocytes through controlling the chemokine receptor Ccr9b and thereby influence the T cell receptor (TCR) activation. Hence, we propose that, in lower vertebrates, the function of Notch signaling extends to all stages of T cell development, except when thymocytes undergo TCRβ rearrangement.

Keywords: Thymus · Cell migration · Notch1 · Medaka · Chemokine receptors

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Introduction

T cells originate from lymphoid progenitors that develop from HSCs and migrate to the thymus. Upon entry to the thymus, they traffic through specialized thymic microenvironments and interact with thymic epithelial cells (TECs) to receive signals essential for their specification and commitment. After recombination and expression of TCRs, developing T cells (thymocytes) undergo positive and negative selection, and only nonself-reactive T cells leave the thymus [1].

Members of the Notch protein family (Notch1, -2, -3, and -4) are highly conserved transmembrane receptors that regulate cell fate during the development of many cell lineages [2]. In the murine thymus, Notch1, Notch2, and Notch3, but not Notch4, display distinct and overlapping expression patterns in thymocytes [3–9]. Because Notch receptors are also involved in hematopoiesis, conditional KO mice and in vitro models have been used to study their role in T cell development. These studies
have shown that Notch1 activation triggers a genetic network that converts multipotent lymphoid progenitors to committed T cells. Notch1 upregulates the expression of transcription factors Gata3, Tcf7, and Bcl11b, which are required for T cell lineage commitment, survival, and subset diversification [1, 10–13]. In contrast to Notch1, the two other Notch paralogs appear to play minor roles in T cell development because their deficiencies have no significant effect on murine T cell development [5, 6, 14, 15]. However, recent in vitro studies have shown that Notch1 and Notch2 cooperatively control the expression of target genes during the early stages of T cell development [16]. At later stages, Notch1 signals influence the positive selection [17] as well as maturation of CD4+ and CD8+ T cell lineage [18, 19]; however, its impact is decreased before thymocytes undergo negative selection [1].

Although it is known that Notch1 activation influences the migration of T cell acute lymphoblastic leukemia cells [20–22], the impact of Notch1 deficiency on the migratory behavior of developing T cells within the thymus is poorly understood. One possible reason for the limited understanding is that in vivo studying intrathymic cell trafficking in murine models is technically challenging due to the anatomical position and size of the thymus [23]. In contrast, the thymus in medaka and zebrafish, two teleost model systems, is small and superficially located, making it possible to capture dynamics of T cell development, including thymus homing, intrathymic cell migration and interaction, and thymic egress at the cellular and subcellular levels in a noninvasive and quantitative manner [23, 24]. The genomes of teleosts have two Notch1 orthologs, notch1a and notch1b, and one ortholog each for Notch2 and Notch3 [25]. In zebrafish, notch1a, notch1b, and notch3, but not notch2, are required for HSC specification [26], which presents challenges in studying the role of Notch signaling during thymic T cell development. The use of medaka as a model system can overcome those complications because notch1b is the sole Notch receptor that is expressed in the embryonic thymus and is dispensable for HSC specification [27]. Therefore, we chose the medaka as a model system and show that notch1b is required for early stages of T cell development and also controls the migratory behavior of mature thymocytes during the thymic selection.

Results and discussion

Medaka notch1b is required for early stages of T cell development

Whole-mount in situ hybridization showed that the expression of notch1b is mainly restricted to the thymic outer zone (Supporting information Fig. S1) where lymphoid progenitors first seed the organ and early events of T cell development, such as T cell specification, proliferation, and lineage commitment take place.
Notch1b deficiency reduces $\text{rag}^2^+$ thymocytes but does not affect the TCRβ rearrangement

As proliferation slows in thymocytes, Notch signaling triggers a gene regulatory network required for the TCR recombination and assembly genes [1]. Indeed, the expression of recombination acti-

vating gene 2 ($\text{rag}2^+$) was strongly reduced, but not completely abolished, in the $\text{notch}1b^{-/-}$ thymus (Fig. 2A). To quantify $\text{rag}2^+$ thymocytes, we took advantage of the $\text{rag}2$-$\text{gfp}$-pest reporter, in which a destabilized GFP protein is under the control of $\text{rag}2$ promoter [28]. Heterozygous $\text{notch}1b^{-/-}$ fish carrying this reporter were used to generate homozygous mutants for further analysis. We then performed in toto imaging of the offspring (Fig. 2B) and counted the number of GFP$^+$ cells within the thymus. Compared to WT, the number of $\text{rag}2^+$ thymocytes was decreased sixfold in the $\text{notch}1b^{-/-}$ thymus (Fig. 2C). Next, we asked whether Notch1b
deficiency affects the TCR rearrangement because this process is Notch1 dependent in mouse [3]. To address this issue, we performed sequence analysis of tcrb cDNAs from notch1b+/− and notch1b−/− siblings (Fig. 2D and E). The majority of tcrb transcripts amplified from the mutant showed productive rearrangements, and there was no significant difference between siblings (Fig. 2F). However, the fraction of unique sequences among productive rearrangements (singlets), as a measure of sequence diversity, was reduced in the notch1b−/− embryos. This result strongly suggests that medaka Notch1b is dispensable for tcrb rearrangement.

Lack of Notch1b affects the intracellular calcium levels in thymocytes undergoing thymic selection

Productive TCRβ rearrangement and the detection of tcrb+ cells (Supporting information Fig. S2A) in the notch1b−/− thymus indicated that a small number of thymocytes could develop as naïve T cells and egress from the thymus. The presence of lck+ T cells in the extrathymic region of notch1b−/− larvae (Supporting information Fig. S2B) further supports this notion. Given that thymocytes must complete thymic selection before leaving the thymus, we investigated the extent to which intracellular calcium level was affected by Notch1b deficiency because a shift in basal Ca2+ level correlates with increased TCR signaling events in thymocytes during positive and negative selection [32, 33]. We, therefore, developed medaka transgenic reporters carrying the ccr9a:GCaMP6s construct, in which the ccr9a promoter drives the expression of the genetically encoded calcium indicator GCaMP6s [34]. To distinguish mature thymocytes, we out-crossed this reporter fish with the transgenic ccr9b:rfp fish [28] and generated double transgenic ccr9a:GCaMP6s;ccr9b:rfp reporter for confocal imaging. In medaka, the expression of chemokine receptor ccr9b in thymocytes begins at commitment stage and holds on until they undergo thymic selection and leave the thymus [28]. Therefore, this strategy allowed us to resolve the spatial distribution of Ca2+ levels simultaneously in all thymocytes as well as distinguish the ccr9b+ thymocytes. Time-lapse in toto recording revealed that thymocytes displaying highly dynamic Ca2+ influx were predominantly located in the innermost part of the thymus (Fig. 3A, Movie 2), that is, in the region where ccr9b+ thymocytes interact with DCs and undergo apoptosis because of thymic selection [28]. Furthermore, we noticed a strong interaction between thymocytes (Fig. 3B, Movie 3), a characteristic behavior that has been described for murine thymocytes undergoing positive [35] and negative [36] selection. In mammals, thymocytes display distinct patterns of TCR-induced Ca2+ signals during positive and negative selection [32]. In our model system, we could not find a difference in the duration of intracellular Ca2+ influx in interacting thymocytes, and the overall time lasts less than 2 min (Fig. 3B, right panel), which is much shorter than has been shown in murine thymocytes during positive and negative selection.
(15–30 min) in the thymic tissue explants [32]. To determine the effect of Notch deficiency on intracellular calcium level, we developed heterozygous notch1b/−/− fish carrying ccr9a:GCaMP6s;ccr9b:rfp and analyzed their progeny using live imaging. Surprisingly, we observed that the temporal Ca2+ influx was diminished in the notch1b/−/− thymus (Fig. 3C, Movie 4). By contrast, the distribution of intracellular Ca2+ level in ccr9a+ leukocytes located in the periphery was comparable between notch1b/−/− fish and their WT counterparts (Fig. 3D). The normal activity of ccr9a reporter (Fig. 1C) also excludes the possibility that the expression of GCaMP6s was reduced in the notch1b/−/− embryos.

To further confirm the effect of Notch signaling on thymic selection and exclude the possibility that altered thymic structure might indirectly influence the migratory behavior of notch1b/−/− thymocytes, we treated WT transgenic embryos carrying the ccr9a:GCaMP6s reporter with DAPT, a γ-secretase inhibitor that inhibits Notch signaling (Fig. 4A, top panel). Consistent with notch1b−/− thymus results, temporal Ca2+ influx was strongly decreased in thymocytes upon DAPT treatment (Fig. 4A, bottom panel; Movie 5), indicating that Notch signaling plays a role in controlling the migratory behavior of thymocytes and intracellular calcium influx. To further support this conclusion, we compared the migratory behavior of ccr9b+ thymocytes before and after DAPT treatment (Fig. 4B) and found that their directionality (Fig. 4C) and speed (Fig. 4D) were significantly reduced after Notch inhibition. Also, we observed a strong reduction of RFP signal (Fig. 4E) and Ca2+ influx (Fig. 4F, middle panel) in the DAPT-treated double transgenic ccr9a:GCaMP6s;ccr9b:rfp embryos (Movie 5). Next, we assessed the migratory behavior of notch1b−/− thymocytes carrying various reporter lines and found that the average cell speed and directionality of ccr9b+ or rag2− thymocytes were significantly decreased in notch1b−/− embryos compared to notch1b+/− siblings (Supporting information Fig. S3; Movie 6). These observations also reveal that Notch-deficient thymocytes displayed a “random walk” type movement indicating that the component of directed cell migration was lost, most probably through downregulation of ccr9b expression. We also counted the number of rag2+ and ccr9b+ cells in WT and notch1b−/− thymus because they represent thymocytes at pre- and postselection stages, respectively. Intriguingly, the \( \text{rag2}^+ / \text{ccr9b}^+ \) ratio was 0.55 and 0.49 in the WT and notch1b−/− thymus (data from more than three independent experiments), indicating that the preselection/postselection discrimination remains unchanged in Notch1b deficiency.

Ccr9b deficiency affects the migratory behavior of thymocytes during the thymic selection

Given that chemokine receptor-mediated cell migration is essential for thymic selection [37] and Notch1b, together with Bcl11b and Ccr9b, form a gene regulatory network required for αβ T-cell development [27], we next looked at the extent to which thymic selection depends on ccr9b expression. We performed time-lapse in vivo recording of ccr9b−/− larvae carrying the ccr9a:GCaMP6s;ccr9b:rfp reporters, and found a strong reduction of Ca2+ influx in ccr9b-deficient thymocytes (Fig. 4F, bottom panel). One possible explanation is that lack of Ccr9b may influence the interaction between mature thymocytes and TECs because the ccr9b ligand, ccl25a, is highly expressed in TECs that are located in innermost part of the medaka thymus [27], that is, in the region where thymic selection occurs [28]. Further supporting this notion, activation of murine Ccr9-Ccl25a axis influences the thymocyte-TEC interaction for the positive and negative selection [37]. Another possible explanation is that incorrect localization of ccr9b−/− thymocytes within the thymus [27] leads to failure of thymic selection, akin to Ccr7-deficient mice [38, 39]. Another similarity between medaka Ccr9b and mouse Ccr7 is that both are upregulated by Notch activation [27, 40]. Nonetheless, the impact of Notch1b on the migratory behavior of thymocytes might not be entirely mediated by Ccr9b because its deletion has a much stronger effect on migratory action than ccr9b−/− does.

In summary, our data presented here and in previous studies [25, 27] provide evidence that Notch1b is required for the early stages of T cell development in lower vertebrates, akin to mammalian Notch1 (Fig. 5). In mice, the role of Notch signals in the positive selection appears to be secondary due to its ability to sustain the effect of TCR signaling in CD4+CD8+ thymocytes [17, 19]. In lower vertebrates, the thymic selection is poorly characterized due to the lack of suitable T cell surface markers, and the use of fluorescent-based transgenic reporter lines partially circumvents this limitation. On the basis of strong changes in the intracellular Ca2+ dynamics and the migratory behavior of mature thymocytes, it is conceivable that Notch signal is involved during thymic selection in medaka fish. However, additional molecular and genetic tools will be needed to dissect the positive and negative selection in teleosts. Also, the role of Notch signals in the selection and maturation of CD4+ and CD8+ T cells in lower vertebrates remains to be elusive.

Materials and methods

Fish

Medaka (Oryzias latipes) husbandry was performed in accordance with the German animal welfare standards (Tierschutzgesetz §11, Abs. 1, Nr. 1, husbandry permit no. 35/9185.46/Uni TÜ. and no. 35-9185.64/BH Wittbrodt). The notch1b and ccr9b mutants used in this study were described previously [27]. Transgenic ccr9a:gfp, rag2:gfp-pest, lck:gfp, and ccr9b:rfp were described previously [28]. Note the same ccr9a promoter construct and vector [28] was used to generate the transgenic ccr9a:gfp; ccr9a:h2b-gfp, and ccr9a:GCaMP6s reporter lines. Generation of transgenic reporter lines and experimental protocols were approved by the EMBL Animal Care and Use Committee (IAUC no. 2019-03-19ML). All experiments have been performed in medaka embryos prior to the legal onset of animal life.

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Figure 4. DAPT treatment and ccr9b deficiency impairs the calcium influx in thymocytes. (A) Top, the experimental rationale. Embryos at hatching stage were imaged before and 24 h after treatment with 50 μM DAPT. Bottom, representative images of thymus before and after 50 μM DAPT treatment for 24 h. One frame from a Z-stack spanning the whole thymus is shown (Z = 1 μm). Scale bars, 20 μm. (B) Double transgenic ccr9a:GFP [green];ccr9b:RFP [white] embryo exposed to 50 μM DAPT for 24 h. Scale bars, 15 μm. Track plots (C) and average speed (D) of ccr9b⁺ thymocytes after DAPT treatment. (E) Thymus-scale RFP intensity in ccr9b⁺ thymocytes after DAPT treatment normalized to WT thymus. N indicates number of biological samples. (D and E) Data are shown as ±SD and are representative pooled from more than three independent experiments. (F) Still photographs from time-lapse in vivo recording of WT (top), DAPT treated (middle), and ccr9b⁻/⁻ thymus carrying the ccr9a:GCaMP6s reporter. Numbers indicate time in minutes. Scale bars, 10 μm. Images are representative of three independent experiments.

Survival of notch1b mutant

Fertilized eggs were bleached with 1:4000 diluted sodium hypochlorite (13% active chloride, Grüssing GmbH) in autoclaved 1× ERM for 2 min, and then rinsed twice with ERM for 2 min. Embryos were then transferred into the six-well plates (10 embryos per each plate) and incubated at 28°C. After hatching, larvae were fed, and medium was changed every day. The survival of the larvae was monitored daily, with dead larvae being immediately collected and genotyped. Fish were raised until 20 days posthatching, at which point all surviving larvae were genotyped. A Kaplan–Meier plot to
analyze the survival data was performed by the use of the Prism Software.

**DAPT treatment**

Dechorionated embryos were incubated in 50 μM 7N-[N-(3,5-difluorophenyl-L-alanyl)]-S-phenyl-glycine t-butyl ester (DAPT) diluted in ERM for 24 h.

**VDJβ rearrangement assay**

Total RNA isolation from notch1b siblings at hatching stage was performed using peqGOLD TriFast (Peqlab). RNA samples were treated with RNase-free DNase (Sigma) and then the cDNA synthesis was performed using random hexamer primers and SuperScript III (Invitrogen). After RNase H treatment, the single-strand cDNA was used as a template for PCR. cDNA from the tcrb gene was amplified by nested PCR. DNA fragments were cloned into the pCR2.1-TOPO vector (Invitrogen). Plasmids were sequenced by M13 forward and reverse primers. Only clones with mutations in the V-D-J-C junctions that caused frame shifts or nonsense mutations were considered as nonproductive. Primers were described previously [41].

**Whole-mount immunostaining**

Immunostaining was performed as described previously [28]. Mitotically active cells were detected using the rabbit anti-phosphohistone-3 antibody (Ser10, Millipore 06–570, 1:500 dilution).

**Whole-mount in situ hybridization**

RNA in situ hybridization and probes for notch1a, notch1b, notch2, notch3, and tcrb were described previously [25].

Live imaging of the thymus

Embryos at hatching stage were anesthetized with tricaine methansulonate (Pharmaq), mounted in 1.5% low melting agarose on glass-bottom culture dish, and then covered with ERM containing 0.001% tricaine. Time-lapse experiments were carried out on a PerkinElmer ERS Spinning disk confocal using a 40× water-immersion objective (LD C-Apochromat, 1.1 NA, Corr, Zeiss). The time interval for z-stacks of 30–55 μm spanning the whole thymus area (z-space = 1 μm) was less than 15 s. Live imaging of WT and notch1b−/− siblings was conducted under the same condition and on the same day.

**Image analysis**

Time-lapse 3D images of the transgenic reporters were analyzed with Imaris (Bitplane, version 9.2) software. The motility parameters average cell speed and straightness were calculated as described previously [28].

**Statistical analysis**

Wilcoxon–Mann–Whitney test was used to calculate significant differences where indicated. A p-value < 0.05 was considered statistically significant. The numbers of biological samples (N) for experiments are indicated in each figure. Data in bar graphs are shown as an absolute number with mean ± SD noted. All data were analyzed in GraphPad Prism software.

Acknowledgements: We thank Marleen Hanelt and Matteo Rauzi for technical help during the initial observations, and the Advanced Light Microscopy Facility at the EMBL-Heidelberg for support.

Open access funding enabled and organized by Projekt DEAL.

Conflict of interest: The authors declare that they have no financial and commercial conflict of interest.

Author contributions: NA and BB made initial observations and designed the work. NA, DI, EHC, and AMD performed analysis. ML and JW provided materials and fish lines. The manuscript was written by BB and NA with input from all authors.

Funding: This work was supported by the EMBO, German Research Foundation (DFG) [grant number: BA5766/3-1] and Madeleine Schickedanz Kinderkrebsstiftung.
Data availability statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Peer review: The peer review history for this article is available at https://publons.com/publon/10.1002/eji.202149512.

References

1. Yui, M. A. and Rothenberg, E. V. (2014). Developmental gene networks: a triathlon on the course to T cell identity. Nat. Rev. Immunol. 2014. 14: 529–545.

2. Artavanis-Tsakonas, S., Rand, M. D. and Lake, R. J. (1999). Notch signaling: cell fate control and signal integration in development. Science 1999. 284: 770–776.

3. Wolf, A., Wilson, A., Nemin, M., MacDonald, H. R. and Radtke, F. (2002). Activation of Notch1 impairs VDJbeta rearrangement and allows pre-TCR-independent survival of early alpha beta lineage thymocytes. Immunity 2002. 16: 869–879.

4. Koyanagi, A., Sekine, C. and Yagita, H. (2012). Expression of Notch receptors and ligands on immature and mature T cells. Biochem. Biophys. Res. Commun. 2012. 418: 799–805.

5. Saito, T., Chiba, S., Ichikawa, M., Kunisato, A., Assai, T., Shimizu, K., Yamaguchi, T. et al. (2003). Notch2 is preferentially expressed in mature B cells and indispensable for marginal zone B lineage development. Immunity 2003. 18: 675–685.

6. Kitamoto, T., Takahashi, K., Takimoto, H., Tomizuka, K., Hayasaka, M., Tabira, T., Hanaoka, Y. et al. (2005). Functional redundancy of the Notch gene family during mouse embryogenesis: analysis of Notch gene expression in Notch3-deficient mice. Biochem. Biophys. Res. Commun. 2005. 331: 1154–1162.

7. Fiorini, E., Merck, E., Wilson, A., Ferrero, I., Jiang, W., Koch, U., Auderset, F. et al. (2009). Dynamic regulation of notch 1 and notch 2 surface expression during T cell development and activation revealed by novel monoclonal antibodies. J. Immunol. 2009. 183: 7212–7222.

8. Radtke, F., Wilson, A., Stark, G., Bauer, M., van Meerwijk, J., MacDonald, H. R., Aguet, M. et al. (1999). Deficient T cell fate specification in mice with an induced inactivation of Notch1. Immunity 1999. 10: 547–558.

9. Garcia-Leon, M. J., Fuentes, P., de la Pompa, J. L. and Toribio, M. L. (2002). Dynamic regulation of NOTCH1 activation and Notch ligand expression in human thymus development. Development 2008. 145: dev165597.

10. Radtke, F., MacDonald, H. R. and Tachini-Cottier, F. (2013). Regulation of innate and adaptive immunity by Notch. Nat. Rev. Immunol. 2013. 13: 427–437.

11. Koch, U., Fiorini, E., Benedetto, R., Besseyrias, V., Schuster-Gossler, K., Pierres, M., Manley, N. R. et al. (2008). Delta-like 4 is the essential, nonredundant ligand for Notch1 during thymic T cell lineage commitment. J. Exp. Med. 2008. 205: 2515–2523.

12. Hozumi, K., Mailhos, C., Negishi, N., Hirano, K., Yahata, T., Ando, K., Zuklys, S. et al. (2008). Delta-like 4 is indispensable in thymic environment specific for T cell development. J. Exp. Med. 2008. 205: 2507–2513.

13. Ferrero, I., Koch, U., Claudinot, S., Favre, S., Radtke, F., Luther, S. A., MacDonald, H. R. et al. (2013). DL4-mediated Notch signaling is required for the development of fetal alphabeta and gammaddelta T cells. Eur. J. Immunol. 2013. 43: 2845–2853.

14. Suliman, S., Tan, J., Xu, K., Kousis, P. C., Kowalski, P. E., Chang, G., Egan, S. E. et al. (2011). Notch3 is dispensable for thymocyte beta-selection and Notch1-induced T cell leukemogenesis. PLoS One 2011. 6: e24937.
distinguished by intrathymic migration and T-cell receptor signaling patterns. Proc. Natl. Acad. Sci. USA 2014. 111: E2550–E2558.

34 Chen, T. W., Wardill, T. J., Sun, Y., Pulver, S. R., Renninger, S. L., Baohan, A., Schreiter, E. R. et al., Ultrasensitive fluorescent proteins for imaging neuronal activity. Nature 2013. 499: 295–300.

35 Choi, E. Y., Jung, K. C., Park, H. J., Chung, D. H., Song, J. S., Yang, S. D., Simpson, E. et al., Thymocyte-thymocyte interaction for efficient positive selection and maturation of CD4 T cells. Immunity 2005. 23: 387–396.

36 Melichar, H. J., Ross, J. O., Taylor, K. T. and Robey, E. A., Stable interactions and sustained TCR signaling characterize thymocyte-thymocyte interactions that support negative selection. J. Immunol. 2015. 194: 1057–1061.

37 Choi, Y. I., Duke-Cohan, J. S., Ahmed, W. B., Handley, M. A., Mann, F., Epstein, J. A., Clayton, L. K. et al., PlexinD1 glycoprotein controls migration of positively selected thymocytes into the medulla. Immunity 2008. 29: 888–898.

38 Kurobe, H., Liu, C., Ueno, T., Saito, F., Ohigashi, I., Seach, N., Arakaki, R. et al., CCR7-dependent cortex-to-medulla migration of positively selected thymocytes is essential for establishing central tolerance. Immunity 2006. 24: 165–177.

39 Nitta, T., Nitta, S., Lei, Y., Lipp, M. and Takahama, Y., CCR7-mediated migration of developing thymocytes to the medulla is essential for negative selection to tissue-restricted antigens. Proc. Natl. Acad. Sci. USA 2009. 106: 17129–17133.

40 Buonomici, S., Trimarchi, T., Ruocco, M. G., Reavie, L., Cathelin, S., Mar, B. G., Klinakis, A. et al., CCR7 signalling as an essential regulator of CNS infiltration in T-cell leukaemia. Nature 2009. 459: 1000–1004.

41 Swann, J. B., Weyn, A., Nagakubo, D., Bleul, C. C., Toyota, A., Happe, C. et al., Conversion of the thymus into a bipotent lymphoid organ by replacement of FOXN1 with its paralog, FOXN4. Cell Rep. 2014. 8: 1184–1197.

Abbreviation: rag2: recombination activating gene 2 · TEC: thymic epithelial cell

Full correspondence: Baubak Bajoghli, Department of Hematology, Oncology, Immunology, and Rheumatology, University Hospital of Tübingen, Tübingen, Germany e-mail: baubak.bajoghli@med.uni-tuebingen.de

Received: 19/7/2021
Revised: 13/10/2021
Accepted: 29/10/2021
Accepted article online: 3/11/2021