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Ulm University | International Graduate School in Molecular Medicine Ulm | Albert-Einstein-Allee 11 | 89081 Ulm

Project Proposal for a PhD position granted by the International Graduate School in Molecular Medicine Ulm

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2. Project Title:

Effects of MLL5-deficiency on leukemogenesis and pathogenic type I interferon responses

3. Keywords (max. five)

- Mll5-deficient mice
- cooperating events in leukemogenesis
- Dnmt3a, IDH2, histone mutations
- Atm-deficient mice
- cGAS/STING pathway of IFN-1 induction

4. Research Training Group

☐ Neurobiology

☐ Aging and Degeneration

X **Oncology and Endocrinology**

☐ Virology, Microbiology, Biotechnology and Systems Biology

☐ Development and Regeneration

☐ Trauma, Regeneration and Immune Modulation

☐ Pulmosens

5. Project description

5.1. Project background

Deletions on the long arm of human chromosome 7 or loss of the entire chromosome (7/7q-deletions) are recurrent cytogenetic alterations in myeloid malignancies, often indicating poor prognosis. Several years ago, Konstanze Döhner and her colleagues here in Ulm as well as researchers at UCSF/USA identified within a consensus deletion region on 7q a novel gene, termed *Mixed-Lineage-Leukemia-5* (*MLL5*), which seemed to fulfill typical criteria of a tumour suppressor. Comprehensive analysis of *Mll5*-deficient mice generated in my laboratory have revealed inefficient DNA double strand break (DSB) repair and associated severe functional defects in the hematopoietic stem and progenitor cell (HSPC) compartment, i.e. in exactly those cells known to be the target of leukemic transformation (Madan et al. 2009). However, neither we nor others have been able to detect any form of spontaneous leukemia in heterozygous or homozygous Mll5-deficient mice so far. Obviously, this does not exclude an important contribution of MLL5-deficiency to leukemogenesis. Clinical disease may depend on more than one genetic lesion and loss of MLL5 may require cooperation with other tumour-promoting mutations.

In an attempt to elucidate the molecular mechanisms underlying HSPC malfunction in the absence of MLL5, we recently identified a previously unknown molecular pathway involving an exaggerated innate immune response. In brief, we have been able to demonstrate that accrued DNA damage in *Mll5*-deficient mice results in a robust IFN-1 response, which triggers mitochondrial accumulation of the Bcl-2 family member BID and subsequent induction of toxic levels of reactive oxygen species (ROS), which are ultimately responsible for the observed defects in HSPC function (Tasdogan et al. 2016, Gross et al. 2017). Importantly, genetic abrogation of the exaggerated IFN-1 response in *Mll5*$^{-/-}$ x *Ifnar1*$^{-/-}$ double-mutant mice lacking the type I interferon receptor restored HSPC function, providing conclusive genetic evidence for the detrimental role of DNA-damage-induced IFN-1 responses with respect to HSPC function, at least in MLL5-deficient mice.

5.2. Scientific objectives

5.2.1. Identification of genetic lesions cooperating with MLL5-deficiency in leukemia induction: We wish to assess whether specific gene mutations repeatedly identified in patients with 7q-deletions can trigger leukemia in *Mll5*-mutant mice. A positive result would demonstrate for the first time a critical contribution of MLL5 to leukemogenesis and provide an entry point for studies into underlying molecular mechanisms. The mutations, which we wish to test on *Mll5*-deficient genetic background will be: (i) *DNA-methyltransferase 3a* (*Dnmt3a*) inactivation, (ii) Arg882His-mutated *Dnmt3a*, (ii) Arg172Lys-mutated isocitrate dehydrogenase 2 (IDH2), and (iii) a specifically mutated histone family member.

5.2.2. We wish to determine whether the newly discovered IFN1 > BID > ROS pathway is also operative in *Atm*-deficient mice, which exhibit well characterized defects in DNA DSB repair. Furthermore, we wish to assess to what extent the toxic IFN-1 response observed in *Mll5*$^{-/-}$ mice might be triggered by the cGAS-STING pathway of innate DNA recognition.

5.3. Preparatory work

5.3.1 In an attempt to identify patho-physiologically relevant gene mutations, which might cooperate with MLL5-deficiency in leukemic transformation, our collaborators K. Doehner and L. Bullinger exome-sequenced DNA from nine AML patients with non-complex 7q-deletions. Remarkably, six patients carried specific heterozygous amino acid changes in IDH2 (1 x Arg140Gln; 5x Arg172Lys), three patients inactivating mutations in the *DNMT3A* gene including the dominant negative mutation Arg882His and two patients had identical mutations in a specific histone variant. While the *DNMT3A* and *IDH2* mutations have been described previously in AML, the histone mutation has not been described previously in AML making it a promising leukemia-promoting candidate to be studied in the context of MLL5-deficiency. To this end, we have already generated MSC-based retroviral constructs encoding the wild-type and mutated histone forms for transduction of HSPCs from Mll5-deficient mice and wild-type controls.

5.3.2 We are in the process of intercrossing Atm-deficient mice with *Ifnar1*$^{-/-}$ and *Bid*$^{-/-}$ mice to assess to what extent abrogation of IFN-1 signaling and deletion of BID will restore HSPC function also in *Atm*$^{-/-}$ mice. We are also in the process of intercrossing *Mll5*- as well as *Atm*-

deficient mice with *Tmem173*$^{-/-}$ mice lacking the innate signal transducer STING, a key element in intracellular DNA sensing.

5.4. Working programme

5.4.1 We will use well-established bone marrow transplantation assays to assess potentially cooperating functions of the *IDH2-R172K*, the *DNMT3A-R882H* and the histone mutations. HSPCs from *Mll5*$^{+/+}$ (WT), *Mll5*$^{-/-}$ and *Mll5*$^{flox/flox}$ x *ROSA26*$^{CreERT2/+}$ mice will be transduced with MSC-based retroviruses encoding either the aforementioned mutated or the wild-type versions of IDH2, DNMT3A and the histone family member. Transduced HSPCs will be grafted into irradiated B6.SJL donor mice, which will then be monitored for leukemia development up to one year. Monitoring will include monthly blood analysis of all grafted mice. In addition, we will intercross *Dnmt3a*$^{flox/flox}$ mice, which we have already ordered from EMMA (European Mouse Mutant Archive) with *Mll5*$^{flox/flox}$ mice on ROSA26$^{CreERT2/+}$ background available in my mouse colony. The resulting mice will allow us to Tamoxifen-induce *Mll5* and *Dnmt3a* inactivation in adult mice with or without prior bone marrow grafting. Also Tamoxifen-treated mice will be monitored for leukemia development up to one year. In parallel, we will use the available retroviral constructs encoding mutated or wild-type IDH2, DNMT3A and histone genes to assess their ability to immortalize HSPCs from *Mll5*$^{-/-}$ or wild-type controls as simple read-out for oncogenic cooperativity.

5.4.2 To assess the effect of secondary mutations (*Sting, Ifnar1, Bid*) on HSPC function in *Mll5*$^{-/-}$, and *Atm*$^{-/-}$ mice, we will apply all assays which were used successfully to demonstrate the essential contribution of *Ifnar1* and *Bid* to the Mll5-mediated HSPC malfunction, as described in detail in our recent publication (Tasdogan et al. 2016) These assays, which are well established in my laboratory, will include multi-colour FACS analyses of HSPC subsets, comet assays, intracellular ROS measurements, bone marrow reconstitution experiments, sublethal irradiation of double deficient mouse mutants and appropriate controls, and quantitative measurements of surrogate markers for IFN-1 responses, like STAT-1 phosphorylation and Sca-1 upregulation, as well as qRT-PCR for known IFN-1 target genes.

5.5. Cooperation partners:

Konstanze Doehner, Lars Bullinger, Arefeh Rouhi, Florian Kuchenbauer

5.6. Funding of the project:

This project is funded in part by SFB1074-A2 and institutional funds (in-house budget).

The proposed project is a continuation of the work, which earned my former Ph.D. student Alpaslan Tasdogan this year's "Promotionspreis der Ulmer Universitätsgesellschaft".

Project-related publications:

Madan V, Madan B, Brykczynska U, Zilbermann F, Hogeveen K, Dohner K, Dohner H, Weber O, Blum C, Rodewald HR, Sassone-Corsi P, Peters AH, **Fehling HJ.** Impaired function of primitive hemato-poietic cells in mice lacking the Mixed-Lineage-Leukemia homolog MLL5. *Blood* **113** (2009), 1444-54.

Tasdogan, A., Kumar S, Allies G, Bausinger J, Beckel F, Hofemeister H, Mulaw M, Madan V, Scharffetter-Kochanek K, Feuring-Buske M, Doehner K, Speit G, Stewart AF, and **Fehling HJ:** DNA damage-induced HSPC failure depends on ROS accumulation downstream of IFN-1 signaling and Bid mobilization. *Cell Stem Cell* **19** (2016), 752-767.

Gross, A., Tasdogan A, **Fehling HJ:** The IFN-1 > BID > ROS pathway: Linking DNA damage with HSPC malfunction. *Cell Cycle* **16** (2017), 819-820.