suz12 inactivation in p53- and nf1-deficient zebrafish accelerates the onset of malignant peripheral nerve sheath tumors and expands the spectrum of tumor types

Felix Oppel1,*, Dong H. Ki1,*, Mark W. Zimmerman1, Kenneth N. Ross1, Ting Tao1, Hui Shi1, Shuning He1, Jon C. Aster2 and A. Thomas Look1,†

ABSTRACT
Polycrbomb repressive complex 2 (PRC2) is an epigenetic regulator of gene expression that possesses histone methyltransferase activity. PRC2 trimethylates lysine 27 of histone H3 proteins (H3K27me3) as a chromatin modification associated with repressed transcription of genes frequently involved in cell proliferation or self-renewal. Loss-of-function mutations in the PRC2 core subunit SUZ12 have been identified in a variety of tumors, including malignant peripheral nerve sheath tumors (MPNSTs). To determine the consequences of SUZ12 loss in the pathogenesis of MPNST and other cancers, we used CRISPR-Cas9 to disrupt the open reading frame of each of two orthologous suz12 genes in zebrafish: suz12a and suz12b. We generated these knockout alleles in the germline of our previously described suz12-deficient zebrafish model of MPNSTs. Loss of suz12 significantly accelerated the onset and increased the penetrance of MPNSTs compared to that in control zebrafish. Moreover, in suz12-deficient zebrafish, we detected additional types of tumors besides MPNSTs, including leukemia with histological characteristics of lymphoid malignancies, soft tissue sarcoma and pancreatic adenocarcinoma, which were not detected in lymphoid malignancies, soft tissue sarcoma and pancreatic MPNSTs, including leukemia with histological characteristics of deficient zebrafish, we detected additional types of tumors besides in the germline of our previously described MPNSTs compared to that in control zebrafish. Moreover, in suz12 deficient zebrafish, we detected additional types of tumors besides MPNSTs, including leukemia with histological characteristics of lymphoid malignancies, soft tissue sarcoma and pancreatic adenocarcinoma, which were not detected in p53/nf1-deficient control fish, and are also contained in the human spectrum of SUZ12-deficient malignancies identified in the AACR Genie database. The suz12knockout tumors displayed reduced or abolished H3K27me3 epigenetic marks and upregulation of gene sets reported to be targeted by PRC2. Thus, these zebrafish lines with inactivation of suz12 in combination with loss of p53/nf1 provide a model of human MPNSTs and multiple other tumor types, which will be useful for mechanistic studies of molecular pathogenesis and targeted therapy with small molecule inhibitors.

KEY WORDS: MPNST, Leukemia, SUZ12, p53, NF1, RAS signaling

INTRODUCTION
Alterations in genes encoding epigenetic regulators of gene expression have become increasingly important in cancer biology. Polycomb group (PcG) proteins are key epigenetic regulators that interact with each other to form chromatin-modifying complexes. The major PcG complexes include polycomb repressive complex 1 (PRC1) and polycomb repressive complex 2 (PRC2) (Margueron and Reinberg, 2011), with PRC2 identified as an especially promising drug target for cancer therapy (Shi et al., 2017). PRC2 core components include the proteins SUZ12, EED, the histone methyltransferases EZH1 or EZH2, and the histone binding protein RbAp48 (also known as RBBP4) (Margueron and Reinberg, 2011). The PRC2 complex silences the expression of its target genes by catalyzing the methylation of lysine 27 in the tail of histone H3 family proteins (the H3K27me3 mark). SUZ12 is required for the structural integrity of the PRC2 complex and to facilitate chromatin binding (Chen et al., 2018). Additionally, the detailed mechanisms of PRC2-mediated gene silencing depend heavily on cellular context, as PRC2 targets different sets of genes in different cell types (Squazzo et al., 2006).

The function of PcG proteins was first described in Drosophila development, where PcG multisubunit complexes repress Hox genes, which are conserved regulators of cell identity within the anterior-posterior axis (Comet et al., 2016; Lewis, 1978; Jones and Gelbart, 1990; Simon et al., 1992; Struhl and Akam, 1985). It has also been demonstrated that the PcG complexes are important to maintain cell identity by keeping previously silenced genes silent, rather than newly initiating transcriptional repression (reviewed in Comet et al., 2016). In particular, during differentiation of embryonic stem cells, PRC2 is not required for the establishment of repressive marks at target genes, but rather is required for maintenance of gene silencing (Rising et al., 2014; Yuan et al., 2012; Hosogane et al., 2013). Functional PRC2 is crucial for normal development and a complete loss of the core subunit Suz12 in mice is embryonically lethal (De Raedt et al., 2014). SUZ12 is reported to be a tumor suppressor gene in malignant peripheral nerve sheath tumors (MPNSTs) and high-grade gliomas (HGGs) (De Raedt et al., 2014; Lee et al., 2014), while in epithelial ovarian cancer, breast cancer and other malignancies (Li et al., 2012; Kirmizis et al., 2003), it might function to promote oncogenesis. Thus, the role of SUZ12 in tumorigenesis has remained unclear, which is also true for the PRC2 subunit EZH2 (Hock, 2012). In neurofibromatosis type 1, loss of PRC2 activity reduces the levels of H3K27me3 and leads to elevated RAS-dependent transcription that facilitates transformation of benign plexiform neurofibroma precursor lesions into MPNSTs (De Raedt et al., 2014; Baude et al., 2014). SUZ12 loss-of-function (LOF) has been shown to cooperate in tumorigenesis with combined loss of the RAS GTPase-activating protein (RASGAP) NF1 and the tumor suppressor p53 (also known as TP53) (De Raedt et al., 2014). Moreover, SUZ12 loss can elevate expression of Hox genes such as HOXCl3 (Marcinkiewicz and Gudas, 2014), which is implicated in metastatic dissemination in melanoma (Cantile et al., 2012), another tumor in which SUZ12 LOF...
often cooperates with NF1 loss (De Raedt et al., 2014). In addition to solid tumors, SUZ12 and EZH2 were previously identified as tumor suppressor genes in leukemia (Ntziachristos et al., 2012). In T cell acute lymphoblastic leukemia (leukemia), loss of PRC2 core subunits was reported to occur by mutation or deletion in about 25% of all cases, and in a NOTCH1-induced genetic mouse model of leukemia, NOTCH1 antagonizes PRC2 function, leading to a loss of H3K27me3 (Ntziachristos et al., 2012).

In order to function properly, PRC2 requires the additional binding of the α-thalassemia/mental retardation syndrome X-linked protein (ATRX), which is crucial for directing PRC2 to maintain the state of genes already silenced by PRC1. In the absence of ATRX, the deposition of H3K27me3 is misplaced to ectopic sites in the intergenic space and at non-canonical sites in the target genes, which impairs the maintenance of silenced genes (Sarma et al., 2014). In a previous atrx-knockout model in zebrafish, we observed the re-expression of PRC2 target genes upon Atrx depletion, despite initial H3K27me3 deposition (Oppel et al., 2019).

In this study, we report the consequences of loss of suz12 in a p53/nf1-deficient zebrafish tumor model that is suitable for drug testing (Ki et al., 2019; Shin et al., 2012; Ki et al., 2017). In our model, we have assessed suz12 LOF-mediated carcinogenesis in a dose-dependent manner and translated our results based on current studies of human cancer genetics. We dissect the consequences of suz12 depletion on oncogenic Ras-Mapk signaling and indicate MEK inhibition as an effective strategy in p53/nf1/suz12-deficient MPNSTs. Moreover, we present a model of suz12 LOF-induced leukemia, soft tissue sarcoma and pancreatic adenocarcinoma, which will aid in preclinical studies of these diseases.

RESULTS
Knockout of suz12a and suz12b in the zebrafish germline
To create knockout mutations in the suz12 tumor suppressor gene using CRISPR-Cas9, we designed sgRNAs to target exon 1 directly after the start of the coding sequence (Fig. 1A). Because zebrafish harbor two suz12 paralogs (suz12a and suz12b), sgRNAs targeting both genes, and Cas9 mRNA, were injected into one-cell embryos derived from a previously established p53/nf1-deficient line expressing a GFP marker gene under the control of the endogenous sox10 promoter, Tg(sox10:GFP) (Shin et al., 2012), or from

Fig. 1. Loss of suz12 in p53/nf1-deficient fish diversifies carcinogenesis. (A) CRISPR-Cas9-mediated targeting truncates Suz12a and Suz12b proteins before their functional domains, conferring a loss of function. VEFS, VRN2-EMF2-FIS2-SUZ12 domain; WDB, WD-40 binding domain; Zn, Zn-finger region. (B) p53/nf1/suz12-deficient fish are prone to tumors in various anatomical locations, e.g. the abdomen or head (arrows). (C) Histopathology analysis reveals a variety of cancer types in p53/nf1/suz12-deficient tumor-bearing fish, whereas p53/nf1-deficient control fish solely develop MPNSTs. Numbers underneath representative images indicate the frequency of the respective tumor type in nf1a−/− or nf1+/+ tumor-bearing fish analyzed by histology. Scale bars: 100 µm.
wild-type (AB strain) zebrafish. In the p53/nf1-deficient background, we employed two sets of sgRNAs targeting exon 1 of suz12a and suz12b sharing no sequence identity. In the AB background, sgRNA pairs were used to generate a suz12 knockout independently of p53/nf1 deficiency. This procedure efficiently resulted in germline mutations, which were passed from primary injected F0 zebrafish into the F1 generation (Table S1). In 13 tested F1 fish, both sgRNAs targeting suz12b exclusively induced small deletions (2 bp-8 bp) resulting in a frameshift mutation, whereas at the suz12a locus we observed both small deletions (1 bp-9 bp) and insertions (1 bp-18 bp). In the p53/nf1-deficient background, total loss of suz12ab was lethal in developing embryos, beginning between 8 and 15 days postfertilization (dpf), so at least one allele of either suz12a or suz12b was retained in adult fertile p53/nf1/suz12-mutant fish.

**Loss of suz12 diversifies carcinogenesis**

Our previously established model based on combinatorial loss of p53 and nf1 is prone to gliomas at low penetrance and MPNSTs developed tumors in abdomen, head, tail, and anal sites (Shin et al., 2012). As with suz12, the nf1 gene is duplicated in zebrafish, resulting in two nf1 paralogs termed nf1a and nf1b. Because a total loss of nf1 is lethal in developing fish, one allele of nf1a is preserved, which after inbreeding leads to a mixed population of p53m/m, nf1a+/+, suz12a+/+, suz12b+/+, and p53m/m, nf1b−/−, p53m/m, nf1a−/−, and p53m/m, nf1b−/− progeny. Zebrafish with an nf1a−/− genotype have a much faster tumor onset than nf1a−/+ siblings. To assess the biological impact of introducing a suz12 LOF mutation, we monitored tumor onset and penetrance in developing offspring. Zebrafish harboring suz12 mutations of both genotypes developed tumors in abdomen, head, tail, and anal sites (Fig. 1B) that were visually indistinguishable from the MPNSTs in p53m/m, nf1b−/−, p53-mutant population, 22 of the 24 tumor-bearing fish (91.7%) had MPNSTs, five displayed leukemia (20.8%), and a single fish displayed soft tissue sarcoma or adenosarcoma (4.2%) (Table S2).

Interestingly, six out of 52 analyzed fish simultaneously carried multiple distinct tumor lesions (11.5%). In the wild-type background, we did not detect MPNST or any of the additional tumors present in suz12-deficient zebrafish after at least 1 year of monitoring. Thus, the loss of suz12 alone was insufficient to drive tumorigenesis in our model within the time frame of our analysis.

**Loss of suz12 accelerates tumorigenesis**

Tumor onset was markedly accelerated overall in both the p53m/m, nf1b−/−, and p53m/m, nf1a−/− backgrounds (Fig. 2B) upon the depletion of suz12. Acceleration of tumor onset in suz12-mutant zebrafish compared to wild-type controls were significant for all suz12-mutant populations, independent of whether one (suz12a−/−/−) or two (suz12a−/−/−) or three (suz12a−/−/−) alleles in any combination were disrupted. Besides the faster onset, the proportion of tumor-bearing fish (penetrance) was increased in p53/nf1/suz12-knockout fish compared to that in controls (Fig. 2, Table 1).

A significant proportion of p53/nf1/suz12-mutant fish developed multiple tumor foci that were clearly distinguishable by the expression of the sox10:GFP marker gene (Fig. 3A). In all p53m/m, nf1b−/−, p53m/m, nf1a−/−, p53m/m, suz12a−/−/−, and p53m/m, suz12b−/−/− backgrounds, multiple tumor foci were observed in 70-100% of the tumor-bearing fish. In p53m/m, nf1b−/−, nf1a−/−, suz12a−/−/−, and suz12b−/−/− populations the incidence of multiple tumors was much lower, in the range of 0-35%. Again, the suz12a−/−, suz12b−/−, suz12a−/−, suz12b−/−, and suz12a−/−, suz12b−/− populations were not significantly different from each other. The tumor onset in the suz12a−/−, suz12b−/− and suz12a−/−, suz12b−/− populations of the nf1a−/+ cohort were both significantly different from the suz12a−/−, suz12b−/− fish (p<0.0005) (Fig. 3B). Because of the low incidence of multifocal tumors in the nf1a−/+ cohort, loss of suz12 did not significantly affect tumor onset. Importantly, although multifocal tumors were observed in all suz12-deficient populations, they never arose in susz12-deficient lines in the nf1b−/−, nf1a−/+ background. The suz12-deficient fish with multiple tumor foci either presented distinct malignancies (e.g. leukemia and MPNST; Table S2) or multiple malignancies (e.g. leukemia and pancreatic adenocarcinoma; 3.6%). In the p53m/m, nf1b−/−, nf1a−/−, suz12-mutant population, 22 of the 24 tumor-bearing fish (91.7%) had MPNSTs, five displayed leukemia (20.8%), and a single fish showed soft tissue sarcoma or adenosarcoma (4.2%) (Table S2). Interestingly, six out of 52 analyzed fish simultaneously carried multiple distinct tumor lesions (11.5%). In the wild-type background, we did not detect MPNST or any of the additional tumors present in suz12-deficient zebrafish after at least 1 year of monitoring. Thus, the loss of suz12 alone was insufficient to drive tumorigenesis in our model within the time frame of our analysis.

### Table 1. Average time of tumor onset and penetrance by genotype in 10 month duration tumor watch experiments

| Genotype | Mean (s.d.) tumor onset (days) | Number of fish (n) | Number of tumors detected | Penetrance (absolute) | Tumor types detected |
|----------|-------------------------------|--------------------|---------------------------|----------------------|---------------------|
| p53m/m, nf1b−/−, nf1a−/− controls | 275.8 (±51.5) | 26 | 4 | 16.00% | MPNST, soft tissue sarcoma |
| p53m/m, nf1b−/−, nf1a−/− cohort | 211.3 (±40.4) | 16 | 9 | 56.25% | MPNST, leukemia |
| p53m/m, nf1b−/−, nf1a−/− cohort | 211.3 (±40.4) | 16 | 9 | 56.25% | MPNST, adenosarcoma |
| p53m/m, nf1b−/−, nf1a−/− cohort | 211.3 (±40.4) | 16 | 9 | 56.25% | MPNST, leukemia |
| p53m/m, nf1b−/−, nf1a−/− cohort | 211.3 (±40.4) | 16 | 9 | 56.25% | MPNST, adenosarcoma |
| p53m/m, nf1b−/−, nf1a−/− cohort | 211.3 (±40.4) | 16 | 9 | 56.25% | MPNST, leukemia |
| p53m/m, nf1b−/−, nf1a−/− cohort | 211.3 (±40.4) | 16 | 9 | 56.25% | MPNST, adenosarcoma |
| p53m/m, nf1b−/−, nf1a−/− cohort | 211.3 (±40.4) | 16 | 9 | 56.25% | MPNST, leukemia |
| p53m/m, nf1b−/−, nf1a−/− cohort | 211.3 (±40.4) | 16 | 9 | 56.25% | MPNST, adenosarcoma |
| p53m/m, nf1b−/−, nf1a−/− cohort | 211.3 (±40.4) | 16 | 9 | 56.25% | MPNST, leukemia |
| p53m/m, nf1b−/−, nf1a−/− cohort | 211.3 (±40.4) | 16 | 9 | 56.25% | MPNST, adenosarcoma |
| p53m/m, nf1b−/−, nf1a−/− cohort | 211.3 (±40.4) | 16 | 9 | 56.25% | MPNST, leukemia |
| p53m/m, nf1b−/−, nf1a−/− cohort | 211.3 (±40.4) | 16 | 9 | 56.25% | MPNST, adenosarcoma |
| p53m/m, nf1b−/−, nf1a−/− cohort | 211.3 (±40.4) | 16 | 9 | 56.25% | MPNST, leukemia |
| p53m/m, nf1b−/−, nf1a−/− cohort | 211.3 (±40.4) | 16 | 9 | 56.25% | MPNST, leukemia |
| p53m/m, nf1b−/−, nf1a−/− cohort | 211.3 (±40.4) | 16 | 9 | 56.25% | MPNST, leukemia |
| p53m/m, nf1b−/−, nf1a−/− cohort | 211.3 (±40.4) | 16 | 9 | 56.25% | MPNST, leukemia |
| p53m/m, nf1b−/−, nf1a−/− cohort | 211.3 (±40.4) | 16 | 9 | 56.25% | MPNST, leukemia |
MPNST foci in distinct anatomic locations, so that a clear separation of these foci could be confirmed by histology (Fig. 3C).

The suz12-deficient tumor model in zebrafish is consistent with human tumor genetics

As emphasized above (Fig. 1C), the spectrum of tumorigenesis was diversified after disruption of suz12 in the p53/nf1-deficient background. To determine whether the additional tumor types were consistent with those observed in human patients, we examined the SUZ12 mutant sample cohort of the AACR Genie database (AACR Project GENIE Consortium, 2017). Mutations in SUZ12 are annotated in 35 tumor types in the AACR Genie database (v4.0), which include MPNST, pancreatic cancer, leukemia and soft tissue sarcoma (Table S3). The most frequently recorded SUZ12-mutated or SUZ12-deleted cancer type category is 'nerve sheath tumor', including MPNSTs (5.56% and 4.21%, respectively). Pancreatic cancer, leukemia and soft tissue sarcoma were also found within the sample cohort of the database for combined p53/SUZ12 mutations (AACR Project GENIE Consortium, 2017). Thus, the tumor types we identified in suz12-deficient fish are consistent with the malignancy spectrum in human patients that emerges from analysis of the AACR Genie database.

Tumors in suz12-deficient zebrafish display decreased H3K27me3 and elevated Ras-Mapk signaling

Because succ12 encodes a core subunit of PRC2 that maintains silencing of target genes through the deposition of H3K27me3 marks, we examined a detectable H3K27me3 signal in p53/nf1/suz12-deficient MPNSTs, which was reduced compared to p53/nf1-deficient MPNSTs with functional suz12 (Fig. 4, rows 1 and 2). The pancreatic adenocarcinoma, soft tissue sarcoma and leukemia cells each contained largely unstained nuclei (Fig. 4, rows 3, 5 and 6). By contrast, the mixed epithelial/mesenchymal adenosarcoma case displayed a heterogeneous H3K27me3 status with a strong signal in the epithelial glandular cytokeratin-positive compartment and a lack of H3K27me3 in the mesenchymal spindle-like cells (Fig. 4, row 4). The reduced H3K27me3 modification upon suz12-depletion would be expected to affect gene expression on a global scale. Thus, we performed RNA-seq on p53m/m,nf1b−/−,nf1a+/−,suz12−/− mutant MPNSTs (n=4) and p53m/m,nf1b−/−,nf1a+/−,suz12-wild-type control MPNSTs (n=3). The control samples were derived as part of a previous study (Oppel et al., 2019). The results demonstrated elevated expression of gene sets representing PRC2 targets and gene
sets related to oncogenic Ras signaling in suz12-deficient MPNSTs compared to suz12-wild-type MPNSTs in the p53m /n,f1b−/−, nf1a+/- background (Table 2, Tables S4 and S5). Similar results were obtained when we compared the gene expression profile of p53/nf1/suz12-deficient and p53/nf1/atrx-deficient tumor samples derived from a previous study (Table 2, Tables S6 and S7). To assess Ras-Mapk pathway signaling in suz12-mutant MPNSTs compared to wild-type MPNSTs, we performed immunohistochemistry staining to qualitatively detect phosphorylation levels of Erk, S6 (Rps6) and Akt (p-Erk, p-S6 and p-Akt). In this analysis, suz12-deficient MPNSTs showed much stronger phosphorylation levels of all three factors (Fig. 5A), indicating increased Ras-Mapk signaling. Thus, in our suz12-knockout zebrafish model, impaired PRC2-mediated gene silencing cooperates with loss of nf1 to increase signaling through the Ras-Mapk pathway.

Suz12-depletion sensitizes MPNST cells to pharmacological inhibition of MEK

Our results shown in Fig. 5A demonstrate increased Ras-Mapk signaling in MPNSTs arising in suz12/nf1/p53 combined mutant fish compared to nf1/p53 mutant fish harboring wild-type suz12 genes. This indicated a potentially increased vulnerability of p53m/nf1b−/−, nf1a+/-, suz12-mutant tumors towards pharmacological inhibition of this pathway. To test this hypothesis, we employed a previously described in vivo transplantation assay in living zebrafish embryos (Ki et al., 2019). For this assay, single cells were isolated from two groups of matched MPNST tumors: (1) p53m/nf1b−/−, nf1a+/-, suz12-mutant MPNSTs and (2) p53m/nf1b−/−, nf1a+/-, suz12-wild-type MPNSTs. These tumor cells were positive for a sox10:mCherry marker gene and injected into the pericardial cavity of transparent Casper recipient zebrafish embryos at 2 dpf. After 24 h, the injected cells had formed a fluorescent mass, and the embryos were treated with the MEK inhibitor PD-0325901 or with DMSO as a control. At 7 dpf, the tumor mass size was measured before and after treatment, and change in tumor area was compared in the MEK inhibitor-treated embryos and the DMSO-treated control embryos. We observed that MEK inhibition resulted in a decreased tumor size for both p53m/nf1b−/−, nf1a+/-, suz12-mutant MPNSTs and p53m/nf1b−/−, nf1a+/-, suz12-wild-type MPNSTs (Fig. 5B,C). However, when the responses of the MEK inhibitor-treated suz12-mutant tumors were compared to the suz12-wild-type tumors at 7 dpf, the suz12-mutant tumors were significantly smaller (Fig. 5B,C), indicating an increased dependency on high levels of Ras-Mapk pathway signaling in MPNSTs with loss of suz12 function.

DISCUSSION

Knockout of suz12 accelerates tumor development in cooperation with the p53/nf1-deficient background

Knockout of suz12a and suz12b in the zebrafish germline using CRISPR-Cas9 was highly efficient, and the vast majority of F1 fish
examined carried a target-locus mutation in both genes, consisting mainly of deletions spanning less than 10 bp. Using this strategy, we were able to establish two \( \text{suz12a} \)- and \( \text{suz12b} \)-knockout lines using distinct sgRNAs that shared no sequence similarity. Both \( \text{suz12} \)-deficient lines demonstrated a strongly accelerated overall tumor onset and penetrance, in both the \( \text{p53m/m, nf1b}^{-/-}, \text{nf1a}^{+/+} \) and the \( \text{p53m/m, nf1b}^{+/-}, \text{nf1a}^{-/-} \) backgrounds. These results correspond well with observations in a previous mouse model of combined \( \text{Tp53/Nf1/Suz12} \) LOF (De Raedt et al., 2014). In that study, \( \text{Suz12} \) LOF was found to cooperate closely with \( \text{nf1} \) in MPNST development. We observed the same relationship in the present study, evident by significantly accelerated tumor onset in all \( \text{suz12} \)-depleted populations and 90-100% incidence of MPNSTs in tumor-bearing fish examined using histology.

Because both \( \text{nf1} \) and \( \text{suz12} \) are duplicated in zebrafish and there are four alleles each gene, we were able to determine the extent of

---

**Table 2. Gene set enrichment analysis of \( \text{suz12} \)-knockout (KO) versus \( \text{suz12} \)-wild-type MPNSTs and \( \text{suz12} \) KO versus \( \text{atrx} \) KO MPNSTs in \( \text{p53m/m, nf1b}^{-/-}, \text{nf1a}^{+/-} \) background zebrafish**

| Name                                      | NOM | \( P \)-value | FDR value |
|-------------------------------------------|-----|---------------|-----------|
| Gene sets enriched in \( \text{suz12} \) KO MPNSTs compared to \( \text{suz12} \)-wild-type MPNSTs |     |               |           |
| PRC2-related                              |     | 0.0000        | 0.0023    |
| KAMMINGA\_EZH2\_TARGETS                   |     | 0.0186        | 0.1993    |
| Ras-related                               |     |               |           |
| SWEET\_KRAS\_TARGETS\_UP                  |     |               |           |
| Gene sets enriched in \( \text{suz12} \) KO MPNSTs compared to \( \text{atrx} \) KO MPNSTs |     |               |           |
| PRC2-related                              |     | 0.0000        | 0.0000    |
| KAMMINGA\_EZH2\_TARGETS                   |     | 0.0135        | 0.1679    |
| Ras-related                               |     | 0.0000        | 0.0035    |
| CHIARADONNA\_NEOPLASTIC\_TRANSFORMATION\_KRAS\_UP | 0.0000 | 0.0065 |
| SWEET\_KRAS\_TARGETS\_UP                  | 0.0000 | 0.0065 |

Selected significantly enriched gene sets related to PRC2 function or Ras signaling are shown. FDR, false discovery rate; NOM, nominal. The results for enrichment score (ES), normalized enrichment score (NES) and familywise error rate (FWER) \( P \)-values are presented in Tables S4-S7 for all gene sets with \( P < 0.05 \).
cooperation between these genes at 25%, 50% and 75% doses. Based on previous studies, it is clear that p53-deficient zebrafish are already prone to delayed-onset MPNSTs and that the additional loss of nf1 accelerates MPNST formation (Shin et al., 2012; Berghmans et al., 2005). However, the p53<sup>m/m</sup>, nf1<sup>b<sup>b</sup></sup>−/−, nf1<sup>a+/+</sup> genotype is only subtly more oncogenic than the p53<sup>m/m</sup> background. Only the loss of a third nf1 allele (nf1a<sup>+/-</sup>) switches this line to a high-penetrance MPNST model (Shin et al., 2012). Interestingly, we found that a reduction in suz12 gene dosage of only 25% is sufficient in zebrafish to cause a significantly accelerated onset and increased penetrance of tumors in the context of loss of both nf1 and p53, regardless of which of the two suz12 genes was inactivated on one allele (Fig. 2). Thus, mutating two or even three suz12 alleles (Fig. 2) had rather little additional effect over mutating just one allele on the time of tumor onset or tumor penetrance. Apparently, the optimal concentration of Suz12 proteins in the cell is rate limiting, such that a threshold concentration expressed from all four alleles of suz12 is critical for formation of the PRC2 complex, which contains Ezh2, Suz12, Eed and Rbap48. These tumor onset curves suggest that after one suz12 allele is lost, there will be little selection pressure in somatic cells to drive the outgrowth of clones that have lost additional alleles through somatic mutation or silencing. Seemingly, loss of one allele representing a quarter of the normal gene dosage is sufficient to deplete the PRC2 complex, relax repression of self-renewal and proliferation genes, and thus promote the onset of tumors in the nf1/p53-depleted background.

Immunostaining of H3K27me3 in paraffin-embedded sections of tumors arising in these zebrafish supports this hypothesis (Fig. 4). The soft tissue sarcoma shown in Fig. 4 (row 6) developed in a fish

---

**Fig. 5. Evaluation and inhibition of the Ras signaling pathway in suz12-mutant and suz12-wild-type MPNSTs.** (A) Immunohistochemistry analysis of signaling in three individual suz12-mutant (suz12<sup>+/-/+</sup>) and suz12-wild-type (suz12<sup>+/-/+</sup>) MPNSTs (n=3) each stained for phosphorylation of ERK, AKT, and S6 (p-ERK, p-AKT, and p-S6), indicating activation of Ras signaling. (B) The suz12<sup>a+/-</sup>, suz12b<sup>-/−</sup> (mut) or suz12-wild-type control MPNST (wt) tumor cell growth in the pericardial cavity of implanted embryos. These embryos were treated with DMSO vehicle control or PD-3025901 (PD901; 25 or 50 nM) (n=9 fish per treatment, doses based on the maximum tolerated dose of the individual drug). The fluorescent tumor area was determined for each embryo at 3 dpf (pretreatment) and 7 dpf (post-treatment), and was reported as the normalized ratio of the red fluorescent area at 3 dpf versus 7 dpf in individual embryos. Individual values with medians (black bars) are shown. *P<0.05, **P=0.0064, ***P<0.0001 (Student’s t-test). (C) Representative fish images at 3 dpf and 7 dpf after DMSO control or 50 nM PD0325901 treatment.
with loss of only one allele of suz12h, and immunostaining shows only very minimal staining for H3K27me3. The H3K27me3 levels were also very low in an MPNST (Fig. 4, row 2) as well as a pancreatic cancer (Fig. 4, row 3) with loss of two suz12 alleles, and a leukemia with loss of three suz12 alleles (Fig. 4, row 5). Among the tumors in suz12 mutant fish, only an adenosarcoma with loss of two alleles had high levels of staining for H3K27me3. Thus, there appears to be a tissue-specific influence, but in general loss of one allele of suz12 can have profound effects on the levels of the repressive mark H3K27me3 in the nucleus, as detected by immunostaining. We do not have an antibody that recognizes zebrafish suz12. In the future, after a specific antibody is available, it will be possible to directly assess the levels of suz12 expression in individual tumors and correlate these with levels of the H3K27me3 repressive mark.

Moreover, multiple tumor foci were observed only in zebrafish within the suz12-deficient cohort, and this result was only significant in p53^min/m, nef1^b+/−, nef1a^+/−, suz12-mutant populations. In our experiments, this phenomenon was not detected in p53/nef1-deficient, suz12-wild-type control fish. The presence of multiple tumor foci can be attributed to either simultaneous onset of distinct tumors or metastatic dissemination. Loss of SUZ12 has been linked directly to increased metastasis in gastric cancer and non-small cell lung cancer (Xia et al., 2015; Liu et al., 2014), suggesting that the multiple tumor foci with the same histology could in part be due to early dissemination from a single primary tumor.

**suz12 LOF broadens the tumor spectrum in p53/nef1-deficient zebrafish**

In the p53^min/m, nef1^b+/−, nef1a^+/− or nef1a^+/− backgrounds, we observed a strong diversification of tumorigenesis upon loss of the important epigenetic regulator tumor suppressor suz12. By contrast, loss of the Ras-inactivating tumor suppressor nef1 in suz12-wild-type fish mainly accelerated the onset of MPNSTs, while inducing none of the other neoplasms observed in our study (Shin et al., 2012). Notably, a recently described zebrafish model based solely on the full deletion of p53 (p53^del/del) was prone to generate a broad spectrum of tumors, including leukemias (Ignatius et al., 2018).

Loss of SUZ12 promotes the onset of a variety of malignancies, including blood cancer subtypes (De Raedt et al., 2014; Ntziachristos et al., 2012; AACR Project GENIE Consortium, 2017). Most previously described specific models of leukemia in zebrafish have been driven by Rag2-mediated overexpression of MYC, Akt2 and NOTCH1 (Chen et al., 2007; Harrison et al., 2016; Gutierrez et al., 2011; Feng et al., 2010). Moreover, the leukemia penetrance of 20% provides a workable model of human leukemia with these mutations for future studies and the potential to specify leukemia subtypes.

**Partial suz12 knockout decreases PRC2 activity and H3K27me3 deposition**

All of the viable progeny of our breeding protocols still had at least one functional suz12 allele, because a total knockout of each of the four genes was lethal in development. In the tumors, it is likely that additional suz12 alleles are inactivated either by somatically acquired mutations, deletions or silencing. Thus, we think that the loss of H3K27me3 detected by immunofluorescence staining, as shown in Fig. 4, reflects the strong selection against these suppressive epigenetic marks in the multistep clonal selection that occurred during transformation of these primary tumors. These observations indicate that even subtle disturbances in the relative abundance of single PRC2 subunits can affect the ability of PRC2 to maintain the silencing of key target genes. This is supported by previous studies indicating that epigenetic regulator complexes such as PRC2 or SWI/SNF are sensitive to the stoichiometry of single subunits (Kadoch and Crabtree, 2015; Kloe et al., 2016). NF1 functions as a suppressor of Ras signaling, whereas SUZ12 is essential for genome-wide gene silencing of PRC2 targets and thus might have broad influence on multiple cellular processes including proliferation and differentiation (Margueron and Reinberg, 2011). This might explain why, in contrast to nef1, single allele loss of suz12 in zebrafish is sufficient to promote the initiation of malignant tumors in a sensitized background.

As expected, zebrafish tumors with loss of suz12 exhibited decreased H3K27me3 and upregulation of PRC2 target gene sets. As described previously, MPNSTs characterized by H3K27me3 loss have worse survival rates than tumors retaining this epigenetic mark (Cleven et al., 2016; Prieto-Granada et al., 2016). This observation correlates well with the faster onset and higher penetrance of MPNSTs developing in our model upon suz12 inactivation. However, not all malignancies are promoted by a loss of PRC2 function. Certain types of breast cancer are known to harbor elevated PRC2 activity (Jang et al., 2016; Gao et al., 2016). Thus, our model appears to be consistent with a tumor spectrum that is driven by the upregulation of oncogene expression resulting from PRC2 loss, namely MPNSTs (De Raedt et al., 2014; Cleven et al., 2016) and leukemia (Ntziachristos et al., 2012). Our data further support the view that some types of leukemia are driven by the global loss of H3K27me3 (Ntziachristos et al., 2012). This mechanism extends to other low-penetrance cancer types observed in our model, such as pancreatic cancer. In human pancreatic cancer, lower H3K27me3 levels can predict a worse prognosis (Wei et al., 2008). In contrast, very little is known about the factors underlying the prognosis of soft tissue sarcomas. Our study suggests that loss of PRC2-mediated maintenance of gene expression might play a role in the multistep pathogenesis of human soft tissue sarcoma.

Interestingly, the adenosarcoma observed in our model displayed heterogeneous H3K27me3 staining, with intensely positive nuclei in the epithelial compartment and light to negative staining in the mesenchymal compartment. One hypothesis to explain this unusual finding in the epithelial compared to the mesenchymal components of this tumor is based on the presence of two functional alleles of suz12 in the zebrafish. Because this fish has a germline genotype of suz12a^+/−, suz12b^+/−, one possibility is that the cyto-keratin-positive epithelial cells express higher levels of suz12a in the nucleus and depend on suz12a for the formation of PRC2 complexes with Ezh2, Eed, and Rbap46. Thus, the epithelial component of the tumor contains abundant H3K27me3 histone modifications in the nucleus. According to this hypothesis, mesenchymal cells might naturally express much higher levels of suz12b, such that loss of suz12b in this fish would lead to the absence of detectable H3K27me3 histone modifications in these cells. Once specific antibodies for zebrafish Su12a and Su12b are available, we will be able to address this hypothesis.

**Loss of suz12 elevates Ras-Mapk signaling and sensitizes MPNSTs to MEK inhibition**

Upon loss of combinations of one to three alleles of suz12a or suz12b in the zebrafish germline, we observed significant acceleration of MPNST onset and penetrance accompanied by the onset of additional tumor types. It is known from the murine system that diminished PRC2 function caused by Su12 knockout leads to elevated RAS signaling, which promotes MPNST development by amplifying RAS-driven transcription due to modulation of the chromatin structure (De Raedt et al., 2014). In the zebrafish system, our studies show that partial loss of suz12 reduces silencing of PRC2
target genes and also activates the Ras-MAPK signaling cascade. It is reasonable to conclude that increased RAS-MAPK signaling initiated by the loss of PRC2-mediated transcriptional repression synergizes in tumorigenesis with loss of NF1, a potent deactivator of oncogenic RAS (Cawthon et al., 1990; Cichowski and Jacks, 2001; Viskochil et al., 1990; Wallace et al., 1990), because in this situation elevated RAS activation is combined with an impaired ability of cells to turn off RAS. This suggests that NF1-deficient MPNSTs carrying an additional loss of SUZ12 are more dependent on oncogenic RAS-MAPK signaling than SUZ12-wild-type MPNSTs. Indeed, in zebrafish we observed an increased sensitivity to MEK-inhibition in suz12-deficient MPNSTs in the p53/nf1-deficient background, which is in accordance with previous observations on the effects of MEK inhibition in MPNSTs (Ki et al., 2019; Jessen et al., 2013), especially in cooperation with the BRD4-inhibitor JQ1 (De Raedt et al., 2014). It has previously been described in the murine system that Suz12 acts as a tumor suppressor in NF1-deficient but not in NF1-wild-type tumors (De Raedt et al., 2014). In combination with our findings, this indicates that simultaneous loss-of-function mutations or deletions of the tumor suppressors SUZ12 and NF1 might be a marker for the clinical use of molecular targeted drugs against MPNSTs that inhibit the RAS-MAPK pathway, for example MEK inhibitors such as Trametinib, Cobimetinib, and Binimetinib. This could be investigated in future clinical trials.

In mice, it is known that combined deficiencies in Tp53 and NF1 synergize in the onset of MPNSTs and high-grade gliomas, and that the combined loss of Suz12 and NF1 cooperate in the onset of MPNSTs without loss of Tp53 (De Raedt et al., 2014). Our zebrafish p53/nf1/suz12-deficient model was created from the p53/nf1-knockout zebrafish line described previously (Shin et al., 2012). The mutational and deletional inactivation of all three of these genes occurs in at least 28% of human MPNST tumors (Lee et al., 2014), making this a very important genotype in MPNST biology. In future studies, it will also be important to determine whether nf1/suz12 loss in p53-wild-type zebrafish will also promote the onset of MPNSTs, as is the case in mice (De Raedt et al., 2014).

The impact of loss of p53 in this genetic context remains unclear. It is known that the combined deficiencies in p53 and nf1 synergize in the onset of MPNSTs and high-grade gliomas (Shin et al., 2012), and that the combined deficiencies in Suz12 and NF1 cooperate in widespread tumor development in mice (De Raedt et al., 2014). Thus, we believe that the loss of p53 further promotes, but is not essential for, the synergistic effects of deficiencies in nf1 and suz12. However, because our model does not allow the distinction between the p53/nf1/suz12 mutant and the nf1/suz12-mutant background, this has to be explored in future studies.

In summary, we show that the role of PRC2 in tumor suppression is very sensitive to the dosage of suz12 in multiple tissues and that complete loss of suz12 is not required to promote tumorigenesis. The inactivation of one or more alleles of suz12 in zebrafish with an nf1/p53 sensitized genetic background accelerates tumor onset and expands the spectrum of tumors in a fashion consistent with genetic abnormalities found in human cancers. Thus, the consequences of loss of H3K27me3 marks maintained by PRC2 during oncogenesis might be conserved between zebrafish and humans, raising the possibility that important strategies to counteract these epigenetic alterations can be investigated in zebrafish models, ultimately leading to the identification of specific molecules that antagonize the cancer-promoting effects of PRC2 deficiency. However, zebrafish is unlikely to be a good model of some tumors commonly associated with suz12 mutations, such as penile, endometrial, and bladder carcinomas.

**Materials and Methods**

**Zebrafish strains and maintenance**

All zebrafish (Danio rerio) strains used were either AB (wild type) background or p53/nf1a-deficient background (Shin et al., 2012). These fish carry a homozygous p53-M214K mutation (p53<sup>mm</sup>), as previously described (Berghmans et al., 2005). All zebrafish experiments and housing were performed according to Dana-Farber Cancer Institute IACUC-approved protocol #02-107.

**CRISPR-Cas9 genome editing**

Zebrafish strains with germline mutations in suz12 were generated by the CRISPR-Cas9 genome editing system (Hwang et al., 2013), using pCS2-nCas9n to transcribe Cas9<sup>in vitro</sup>. The plasmid constructs pDR274 (Addgene #42250) and pCS2-nCas9n (Addgene #47929) were purchased from Addgene. The following sgRNA sequences were employed to target exon 1 of suz12a or suz12b: suz12a-sgRNA 1, 5′-GGAGGAGGCACAAGCAGTTCTCTCC-3′; suz12a-sgRNA 2, 5′-AGCGGACACCAACTCTTC-3′; suz12b-sgRNA 1, 5′-GTGACCTACGCGCAAGATG-3′; suz12b-sgRNA 2, 5′-GGTGCCTGATACCATCCTTC-3′. All oligonucleotides were purchased from Eurofins Genomics (Louisville, KY, USA). To establish suz12-knockout line 1, we used sgRNAs 1 targeting exon 1 for suz12a and suz12b in combination (pair 11-11), and for suz12-knockout line 2 we used sgRNAs 2 targeting exon 1 for suz12a and suz12b in combination (pair 12-12).

**Injection in zebrafish embryos and genotyping**

pDR274-sgRNA plasmid DNA was linearized with Dnai (NEB, Ipswich, MA, USA), while sgRNA sequences were transcribed in vitro using the MAXIscript T7 Kit (Ambion Inc., Foster City, CA, USA). Cas9 mRNA was transcribed in vitro from pCS2-nCas9n plasmid DNA linearized with NotI (NEB), using the mMessage Machine SP6 Kit (Ambion Inc.). Oligonucleotides were mixed in ratios between 1:5 and 1:1 with a 0.5% Phenol Red solution (Sigma-Aldrich, Burlington, MA, USA) and set to a final concentration of 25 ng/µl sgRNA and 600 ng/µl Cas9 mRNA. To induce mutations in the genome of zebrafish, we injected one-cell-stage embryos with the oligonucleotide/Penol Red mix described above within 30 min after fertilization using a glass capillary mounted into an air pressure injector (Harvard Apparatus, Cambridge, MA, USA). The injection volume was 1 nl oligonucleotide/Penol Red mix per one-cell-stage embryo. Dead embryos were removed at 3 to 6 h after injection.

Injected fish were raised as mosaics and crossed to identify germline mutations inherited into the F1 generation. Next, fish mutants (suz12a and suz12b) were bred together to establish stable suz12-deficient lines. To genotype the suz12a/suz12b mutant line, genomic DNA was isolated with QuickExtract DNA Extraction Solution (Epicenter; Madison, WI, USA). PCR was performed using Taq-polymerase (NEB) according to the standard protocol, with T<sub>r</sub> of 60°C, and 40 cycles. Oligonucleotides used were: suz12a forward primer, 5′-AAACGTCTCGTTCGACCCC-3′; suz12a reverse primer, 5′-AGCCTCAAGCGAGGAGTG-3′; suz12b forward primer, 5′-CAAGGGCGACAGTTCTTC-3′; suz12b reverse primer, 5′-CAAGCGACAGTTCTGACAT-3′. The DNA products were sequenced with the forward or reverse primer.

**Tumor watch experiments**

The sox10:GFP zebrafish were genotyped for mutations in nf1a, suz12a and/or suz12b at age 2-3 months and sorted into separate tanks by genotype. Alleles of p53 and nf1b were maintained as homozygous knockouts. The fish were inspected every 1-2 weeks for visible tumors affecting any part of their bodies and also for abnormal behavior such as inactivity, abnormal or uncoordinated swimming, hovering near the bottom of the tank, or lack of aggressive feeding behavior at the daily feeding time. The fish in each tank were also counted to ensure fish were not unknowingly lost from the tanks. During the biweekly anesthetized examination of each fish under a Nikon C-DS2i15 fluorescence microscope, the fish were also inspected using brightfield microscopy for evidence of a tumor mass or a pallor reflecting anemia. If abnormalities were detected, the time of onset was established and the fish were observed for two more weeks to make sure the findings...
persisted. Then, the fish were humanely euthanized on ice using tricaine, fixed in formalin and analyzed by histology for tumors after sectioning the entire fish. Tumor onset is defined as the first visual recognition of tumor growth not regressing within a time frame of 2 weeks. GraphPad Prism 7 software was used to conduct survival analysis and to calculate P-values by t-test. Phototypes of tumor-bearing fish were taken using an iPhone 6. All fish that died before tumor onset were removed from the analysis.

**Histopathology analysis of zebrafish tissue**

Tumor-bearing fish were sacrificed and subsequently fixed for 1-3 days in 4% paraformaldehyde diluted in PBS at 4°C. After fixation, fish were washed in PBS and stored in 70% ethanol until embedded in paraffin. Paraffin sectioning (3 μm) and Hematoxylin/Eosin (HE) staining was performed at the Dana-Farber/Harvard Cancer Center Research Pathology Core using standard protocols. Individual tumor-bearing fish examined by histopathology were randomly selected. Immunohistochemistry staining was performed as described previously (He et al., 2016) using the following primary antibodies: phospho-p44/42 MAPK (ERK1/2) (Thr202/Tyr204; Cell Signaling Technology #4370; 1:150), phospho-AKT (Ser473; Cell Signaling Technology #4690; 1:100), phospho-S6 ribosomal protein (Ser240/244; Cell Signaling Technology #4838; 1:100). Between 24% to 31% of all tumor-bearing fish from the p53/ nf1/suz12-deficient population and 50% to 57% of the p53/ nf1-depleted, susz12-wild-type control fish were analyzed.

**Indirect immunofluorescence staining**

Indirect immunofluorescence staining was performed as described in previous studies (Oppel et al., 2019, 2011; Ball et al., 2017). Primary antibodies used were pan-cytokeratin (AE1/AE3; Novus Biologicals #NB2-29429; 1:200) and tri-methyl-Histone H3 (Lys27) (C36B11; Cell Signaling Technology #9733; 1:400). Secondary antibodies used were goat anti-rabbit-IgG and goat anti-mouse-IgG conjugated with Alexa 488 or 568 (Life Technologies; 1:400). RNA-seq data are available under GEO accession number GSE125040 (updated 12 August 2020).

**AACR Genie database analysis**

Data were extracted from the AACR Genie database (v4.0), September 2018, and further processed with Microsoft Excel.

**References**

AACR Project GENIE Consortium. (2017). AACR Project GENIE: powering precision medicine through an international consortium. Cancer Discov. 7, 818-831. doi:10.1158/2159-8290.CD-17-0151

Ball, C. R., Oppel, F., Ehrenberg, K. R., Dubash, T. D., Dieter, S. M., Hoffmann, C. M., Ahuja, U., Herbst, F., Koch, M., Werner, J., et al. (2017). Succession of transiently active tumor-initiating cell clones in human pancreatic cancer xenografts. EMBO Mol. Med. 9, 918-932. doi:10.15252/emmm.201607354

Baude, A., Lindroth, A. M. and Plass, C. (2014). PRC2 loss amplifies Ras signaling in cancer. Nat. Genet. 46, 1154-1155. doi:10.1038/ng.3124

Berghmans, S., Murphey, R. D., Wiendlows, E., Neuberg, D., Kutok, J. L., Fletcher, C. D., Morris, J. P., Liu, T. X., Schulte-Merker, S., Kaniki, J. P. et al. (2005). tp53 mutant zebrafish develop malignant peripheral nerve sheath tumors. Proc. Natl. Acad. Sci. USA. 102, 407-412. doi:10.1073/pnas.0406251102

Cantile, M., Scognaniglio, G., Anniello, A., Farina, M., Gentileco, G., Santostasi, G., Fulciniti, F., Gillo, G., Orbona, R. A., Asciento, P. et al. (2012). Increased HOX C13 expression in metastatic melanoma progression. J. Transl. Med. 10, 91. doi:10.1186/1479-5876-10-91

Cawthon, R. M., Weiss, R., Xu, G. F., Viskokich, D., Culver, M., Stevens, J., Robertson, M., Dunn, D., Gesteland, R., O’Connell, P. et al. (1990). A major segment of the neurofibromatosis type 1 gene: cDNA sequence, genomic structure, and point mutations. Cell 62, 193-201. doi:10.1016/0092-8674(90)90253-B

Chen, J., Jette, C., Kaniki, J. P., Aster, J. C., Look, A. T. and Griffin, J. D. (2007). NOTCH1-induced T-cell leukemia in transgenic zebrafish. Leukemia. 21, 462-471. doi:10.1038/sj.leu.2404546

Chen, S., Jiao, L., Shubbar, M., Yang, X. and Liu, X. (2018). Unique structural platforms of Suz12 dictate classical distinction for chromatin. Mol. Cell 69, 840-852.e5. doi:10.1016/j.molcel.2018.01.039

Cichowski, K. and Jacks, T. (2001). NF1 tumor suppressor gene: narrowing the gap. Cell 104, 583-584. doi:10.1016/S0092-8674(00)01245-8

Eleven, H., Sannaa, G. A., Briade-De Bruijn, I., Ingram, D. R., Van De Rijn, M., Rubin, B. P., De Vries, M. W., Watson, K. L., Torres, K. E., Wang, W.-L. et al. (2016). Loss of H3K27 tri-methylation is a diagnostic marker for malignant peripheral nerve sheath tumors and an indicator for an inferior survival. Mod. Pathol. 29, 582-590. doi:10.1038/modpathol.2016.45

Comet, I., Raising, E. M., Leblanc, B. and Helin, K. (2016). Maintaining cell identity: PRC2-mediated regulation of transcription and cancer. Nat. Rev. Cancer 16, 803-810. doi:10.1038/nrc.2016.83

De Raedt, T., Baert, E., Pasmin, L., Luscan, A., Brems, H., Ortonne, N., Helin, K., Hornick, J. L., Mautner, V., Kehrer-Sawatzki, H., et al. (2010). EZH2 mediates Myc oncogene dependence in a conditional zebrafish model of T cell acute lymphoblastic leukemia. J. Exp. Med. 208, 1595-1603. doi:10.1084/jem.20101691
