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Myeloproliferative Neoplasm Animal Models

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Synopsis

Myeloproliferative neoplasm (MPN) animal models accurately re-capitulate human disease in mice and have been an important tool for the study of MPN biology and therapy. Transplantation of *BCR-ABL* transduced bone marrow cells into irradiated syngeneic mice established the field of MPN animal modeling and the retroviral bone marrow transplantation (BMT) assay has been used extensively since. Genetically engineered MPN animal models have enabled detailed characterization of the effects of specific MPN associated genetic abnormalities on the hematopoietic stem and progenitor cell (HSPC) compartment and xenograft models have allowed the study of primary human MPN-propagating cells *in vivo.* All models have facilitated the pre-clinical development of MPN therapies. *JAK2V617F*, the most common molecular abnormality in *BCR-ABL* negative MPN, has been extensively studied using retroviral, transgenic, knock-in and xenograft models. MPN animal models have also been used to investigate additional genetic lesions found in human MPN and to evaluate the bone marrow microenvironment in these diseases. Finally, several genetic lesions, although not common, somatically mutated drivers of MPN in humans induce a MPN phenotype in mice. Future uses for MPN animal models will include modeling compound genetic lesions in MPN and studying myelofibrotic transformation.

Keywords

Myeloproliferative neoplasms; preclinical murine models; BCR-ABL; JAK2V617F; hematopoietic stem cells; bone marrow microenvironment; myelofibrosis; oncogenes

A. Introduction

Animal models have been used extensively in the study of myeloproliferative neoplasms (MPN) and have played a key role in advancing the biological understanding of these diseases. In general, these models have faithfully recapitulated human MPN in mice,

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enabled detailed characterization of the effects of specific MPN associated genetic abnormalities on the hematopoietic stem and progenitor cell (HSPC) compartment and provided excellent *in vivo* models for testing novel MPN therapeutic agents. In this review, we focus primarily on murine models of the *JAK2V617F* mutation and on the insights these have provided, and also briefly outline the central role *BCR-ABL* models played in establishing and developing the field. We discuss models of additional genetic lesions found in human MPN and outline genetic models that induce a MPN phenotype in mice. We describe the use of *JAK2V617F* models in the pre-clinical development of JAK2 kinase inhibitors and other MPN therapies. We summarize the studies of the bone marrow microenvironment that have been performed using MPN models and conclude with some thoughts as to how MPN animal models might be used in the future.

B. MPN Murine Models

BCR-ABL

The faithful modeling of human MPN in mice began in 1990 with the demonstration that retroviral transduction of *BCR-ABL* into murine bone marrow cells, followed by transplantation into irradiated syngeneic mice, recapitulated human chronic myelogenous leukemia (CML) in mice[5,6]. These groundbreaking experiments established the retroviral bone marrow transplantation (BMT) assay and with it the ability to accurately model human hematologic malignancies in mice.

1) Retroviral—The retroviral *BCR-ABL* model has provided a number of important insights into the molecular pathophysiology of CML. These include the demonstration that BCR-ABL expression in vivo is sufficient to induce CML[5,6], the identification of key *BCR-ABL* activated downstream signaling pathways in CML stem cells[7] and the provision of an accurate pre-clinical model that continues to be used in the evaluation of novel CML therapeutics[8].

2) Transgenic—Several *BCR-ABL* transgenic models have been developed and used primarily to investigate the kinetics and therapeutic susceptibilities of CML-propagating stem cells. Here, we discuss two models that have been particularly informative in this regard. Perez-Caro et al. generated transgenic mice that expressed BCR-ABL under the control of the *Sca-1* promoter, thus restricting transgene expression to the Sca-1 positive cell population, which includes HSC[9]. The mice developed a CML-like disease characterized by neutrophilia and hepatosplenomegaly as a result of extra-medullary hematopoiesis, with the majority of animals progressing to blast crisis. A subset of mice developed additional solid tumors, indicating that *Sca-1* driven BCR-ABL expression is not restricted to hematopoietic cells. The course of the CML-like disease was not modified by treatment with imatinib, suggesting that the HSC compartment in CML is insensitive to ABL kinase inhibition.

Koschmieder et al. developed an inducible *BCR-ABL* transgenic model in which the transactivator protein, tTA, was placed under the control of the murine stem cell leukemia (*SCL*) gene 3' enhancer, and double transgenic *SCL*tTA/*BCR-ABL* mice were generated[10]. With tetracycline withdrawal, BCR-ABL expression was induced in the HSPC compartment. These mice developed a CML-like disease that is reversible and re-inducible, suggesting that sustained BCR-ABL expression is required for the development of a CML disease phenotype[10]. The CML-initiating cell population was found to be present in the lineage^{low}Sca1^{+}Kit^{high} (LSK) compartment[11] and more recently this population has been further refined and shown to be present solely in the most immature LT-HSC compartment[12,13]. *BCR-ABL* positive LT-HSCs out-compete normal LT-HSC and aberrantly mobilize to the spleen in this model[12,13]. Finally, CML-propagating stem cells have been found to be resistant to treatment with imatinib alone[11], while the combination of

imatinib with histone deacetylase inhibitors (HDACi) appears to mitigate some of this resistance, in this *BCR-ABL* inducible murine model[14].

3) Xenograft—Xenograft models of chronic phase CML have been problematic in that the majority of SCID-repopulating cells are *BCR-ABL* negative[15], a finding thought to relate, at least in part, to the presence of a reservoir of normal HSC in chronic phase CML. These findings suggest that *BCR-ABL* positive SCID-repopulating cells (SRC) from chronic phase CML do not have a proliferative advantage over wild-type SRC. A major caveat of these experiments is the incompatibilities between cytokine receptors on human cells and cytokines produced by a murine microenvironment. Transplanting *BCR-ABL* positive cells taken from long-term culture-initiating cell (LT-CIC) assays results in more durable leukemic cell engraftment in NOD/SCID mice[16], as does the transplantation of cells from CML patients in accelerated or blast phase[17].

JAK2V617F

JAK2V617F is the most common molecular abnormality in *BCR-ABL* negative MPN and it has been extensively studied *in vivo* using retroviral, transgenic, knock-in and xenograft murine models. In general, these models reliably recapitulate the clinical features of human MPN in mice (see Figure 1, Figure 2 and Table 1) and they have helped advance the biological understanding of *JAK2V617F*-mediated MPN in humans.

1) Retroviral—Immediately following the discovery of the *JAK2V617F* mutation, retroviral models were generated to assess the phenotypic effects of JAK2V167F *in vivo*[18,19,20,21,22]. Mice transplanted with JAK2V617F expressing donor bone marrow developed striking polycythemia after a short latency with a high degree of penetrance. Leukocytosis, splenomegaly due to extramedullary hematopoiesis and the development of myelofibrosis were also seen. All groups observed constitutive activation of Jak2 kinase signaling and Stat5 phosphorylation in addition to cytokine-independent colony formation. These models demonstrated that JAK2V617F expression *in vivo* was sufficient to induce a MPN phenotype and indicated that genetic modifiers of *JAK2V617F*-mediated fibrosis exist (more prominent fibrosis was seen in the Balb/c strain as compared to C57Bl/6 strain[19]).

2) Transgenic—Three groups generated transgenic *JAK2V617F* models in 2008, with the main goal of correlating disease phenotype with differences in *JAK2V617F* gene dosage[23,24,25]. Tiedt et al. conditionally expressed human JAK2V617F from the endogenous human *JAK2* promoter and crossed with Vav-Cre or Mx1-Cre transgenic mice to induce hematopoietic specific JAK2V617F expression. The ratio of human JAK2V617F to murine wild-type Jak2 expression was quantified in mRNA[23]. Xing et al. expressed human JAK2V617F from the Vav promoter[24] while Shide et al. expressed murine Jak2V617F from the H-2Kb promoter[25].

In the model of Tiedt et al., JAK2V617F Vav-Cre mice had constitutive and sustained Cre expression, low transgene copy number and a *JAK2* mutant to wild-type ratio of approximately 0.5. In JAK2V617F MxCre mice, Cre expression was induced following treatment with pI:pC to activate the interferon response, transgene copy number was higher than in JAK2V617F Vav-Cre animals and the *JAK2* mutant to wild-type mRNA was approximately 1.0. The lower copy number in Vav-Cre compared to MxCre was attributed to sustained Cre expression in the Vav-Cre model, leading to more efficient deletion of additional transgene copies. The phenotype of *JAK2V617F* Vav-Cre mice was more consistent with essential thrombocythemia (ET) (high platelets, normal WBC count and hemoglobin), while the *JAK2V617F* MxCre animals more closely recapitulated polycythemia vera (PV) (elevated hemoglobin with suppressed erythropoietin (Epo), high

WBC count and platelets)[23]. In the model of Xing et al., the level of mutant JAK2 expression was extremely low and the phenotype most consistent with ET[24]. In the model of Shide et al., the MPN was incompletely penetrant in the founder line in which mutant Jak2 was expressed at a lower level than wild-type Jak2. In a second founder line in which mutant Jak2 expression was higher than wildtype Jak2, a MPN with myelofibrotic transformation developed[25].

The major conclusion to be drawn from the transgenic models is that the level of JAK2V617F expression influences the MPN phenotype, with the suggestion that lower JAK2V617F expression results in an ET phenotype and higher JAK2V617F expression give rises to a PV phenotype[23,24]. However, some of the MPN phenotypic differences observed by Tiedt et al. could be related to tissue specific Cre effects (i.e. Vav-Cre versus Mx1-Cre)[23]. JAK2V617F expression is driven by exogenous promoters in some of these transgenic models[24, 25], which could also influence the MPN phenotype.

3) Knock-in—Four *JAK2V617F* knock-in models were published in 2010[26,27,28,29]. These were generated with the major goals of: (i) recapitulating human MPN through physiological Jak2V617F expression, (ii) assessing the impact of *Jak2V617F* gene dosage on disease phenotype and (iii) evaluating the effects of *Jak2V617F* on the HSPC compartment.

The models of Akada et al., Marty et al. and Mullally et al. all expressed murine Jak2V617F from the endogenous murine *Jak2* promoter, but each in a slightly different manner. Akada et al. and Mullally et al. both generated conditional *Jak2V617F* knock-in models while Jak2V617F expression was constitutive in the model of Marty et al. Human JAK2V617F was conditionally expressed from the endogenous murine *Jak2* promoter in the model of Li et al[29]. In all of the models, the ratio of mutant to wild-type Jak2 expression was 1:1 or less. The model of Akada et al. was the only one in which homozygous *Jak2* mutant mice were generated, but since the Jak2 mutant allele expressed at approximately 50% of the wild-type allele the level of Jak2V617F expression in homozygous mice was relatively low[26]. A summary of the *Jak2V617F* knock-in models is outlined in Table 1 and a schematic representation of the targeting strategies for each of the models can be found in a recent review by Li et al[30].

The phenotype of the three models that expressed murine Jak2V617F was broadly similar[26,27,28]. In each of these models, mice heterozygous for the Jak2V617F mutation had profound erythrocytosis, leukocytosis, splenomegaly due to extramedullary hematopoiesis and myelofibrosis developed over time (although this was only observed in transplant recipients by Mullally et al.[31]). Compared with the other models, the hematologic phenotype was relatively mild in the model of Li. et al[29]. Only a modest increase in platelets and hemoglobin, more reminiscent of ET than PV, was observed (Epo levels were not suppressed), while approximately 10% mice developed marked erythrocytosis with prolonged follow up. In this model, human JAK2V617F was expressed from the endogenous murine *Jak2* promoter, and whether human JAK2V617F signals differently in murine cells remains undetermined.

In all of the models, the MPN was transplantable into secondary recipients indicating that the MPN is cell autonomous. The ability to transplant the disease enabled a functional analysis of the stem cell properties of Jak2V617F-expressing HSC. Recent reports on this analysis suggest that *Jak2V617F* mutant HSC have a clonal advantage over wild-type HSC[31,32,33]. Mullally et al. found that *Jak2V617F* mutant HSC have a subtle competitive repopulating advantage over normal HSC[28,31], while Marty et al.[32] and Lundberg et al.[33] recently described a stronger competitive advantage for *Jak2V617F* mutant HSC in a knock-

in model and a transgenic model respectively. The HSC phenotype in the knock-in model of Li. et al. differs from that of the others in that LSK cell numbers were reduced in JAK2V617F mice and demonstrated decreased cell cycle and increased senescence. In competitive repopulation experiments, *JAK2V617F* mutant HSC were out-competed by wild type cells in primary bone marrow transplant recipients as early as 5 weeks post transplantation, preventing transplantation of the MPN[29]. It remains unclear whether this finding is related to expression of human JAK2V617F from the endogenous murine *Jak2* promoter, or due to another cause such as increased replicative stress or immunological rejection. Finally, by transplanting sorted populations of progenitor cells, Mullally et al. found that expanded Jak2V617F progenitor cell populations such as megakaryocytic erythroid progenitor (MEP) cells are incapable of reconstituting MPN in a transplanted animal[28]. These results are consistent with previously published reports indicating that oncogenic kinase alleles are incapable of transforming progenitor cells[34,35].

The main conclusions from the Jak2V617F knock in models are that: (i) physiologic heterozygote expression of murine Jak2V617F in mice causes a polycythemia phenotype[26,27,28], (ii) the *Jak2V617F* mutation appears to provide a clonal advantage to HSC[31,32,33], and (iii) Jak2V617F does not confer self-renewal properties to committed myeloid progenitor cells[28]. While these models closely recapitulate human MPN, some differences remain. These include the fact that although Jak2V617F expression is driven by the endogenous *Jak2* promoter in these knock-in models, the mutant allele is expressed in all hematopoietic cells, rather than in a MPN clone as occurs in the human disease[36]. It is also important to note that reconstitution in competitive repopulation experiments is polyclonal and occurs in a bone marrow niche that has been perturbed by irradiation.

4) Xenograft—To study *BCR-ABL* negative MPN cells *in vivo*, xenograft models have been generated. These models enable a functional assessment of the repopulating capacity of primary human MPN CD34+ cells, which possess the full germline and somatic genotype of human MPN.

Peripheral blood CD34+ cells from patients with myelofibrosis engraft NOD/SCID mice and show clonal hematopoiesis with myeloid skewing[1]. Two independent groups have found relatively poor engraftment of *JAK2V617F* mutant CD34+ cells from patients with PV and ET[2,3] and the ratio of *JAK2V617F* to *JAK2* wild-type SRCs was found to be higher in myelofibrosis than in PV[3]. Functionally, *JAK2V617F* SRCs did not gain a proliferative advantage over wild-type SRCs over time[3].

The major conclusions from these studies are that: (i) *JAK2V617F* is found in a functionally competent LT-HSC population[1,2,3], (ii) the functional *JAK2V617F* LT-HSC compartment is expanded in myelofibrosis as compared with PV[3] and (iii) poor *JAK2V617F* CD34+ engraftment in xenografts may be related to downregulation of CXCR4[37]. Its important to note that incompatibilities between human cytokine receptors and murine cytokines in xenograft models, may be particularly relevant in assessing the *in vivo* functional effects of the *JAK2V617F* allele, which activates cytokine receptor signaling. In general, reliable xenotransplantation across a wide spectrum of genetically diverse MPN requires further optimization.

MPLW515L

A somatic activating mutation in *MPL* (*MPLW515L*) was originally identified in *JAK2V617F* negative myelofibrosis patients[38] and *MPLW515L* or *MPLW515K* mutations were subsequently found in approximately 5% and 1% of myelofibrosis and ET patients respectively[39]. In a retroviral BMT assay, *MPLW515L* induced a MPN in mice, characterized by leukocytosis, thrombocytosis, splenomegaly and reticulin fibrosis[38]. This

model has subsequently been used to provide preclinical evidence in support of the use of JAK2 kinase inhibitors in MPN patients who carry the *MPLW515L* mutation[40,41].

TET2

TET2 deletions and loss-of-function mutations occur in up to 12% of MPN patients[42,43]. Initial studies by Delhommeau et al. indicated that *TET2-JAK2V617F* co-mutated CD34+ cells from MPN patients show an increased capacity over *JAK2V617F*-mutated CD34+ cells to repopulate NOD-SCID mice, suggesting that loss of *TET2* enhances HSC self-renewal[42]. These findings have been validated with the publication by several groups of *Tet2* conditional knock-out mice, which demonstrate that *Tet2* null HSC have a competitive repopulating advantage over wild-type HSC[44,45,46,47]. Future studies will likely focus on murine modeling of compound mutants (e.g. *Jak2V617F/Tet2*) to evaluate the impact of loss of *Tet2* on the MPN phenotype, on MPN-propagating stem cell function and on the therapeutic susceptibilities of *Jak2V617F/Tet2* compound mutant hematopoietic cells.

LNK

Heterozygote loss-of-function mutations in the inhibitory adaptor protein, *LNK*, were originally reported in *JAK2* wild-type MPN patients[48]. Overall the frequency of *LNK* mutations in MPN is low and although mutations in *LNK* have been reported in *JAK2V617F* positive MPN, it is not known if both mutations co-occur in the same clone[49]. Knock-out mice for *Lnk* were generated prior to the identification of *LNK* mutations in MPN; *Lnk* null mice exhibit an MPN phenotype (CML-like disease), while heterozygous *Lnk* mice have an intermediate disease phenotype[50,51]. Lnk negatively regulates Mpl through its SH2 domain. Consistent with this, *Lnk* null mice exhibit potentiation of Jak2 signaling in response to thrombopoietin (Thpo), increased HSC quiescence and decelerated HSC cell cycle kinetics[52]. Lnk retains the ability to bind and inhibit Jak2V617F and consistent with this loss of Lnk exacerbates JAK2V617F-mediated MPN and accelerates the development of JAK2V617F-induced myelofibrosis, through the potentiation of JAK2V617F signaling[51]. These studies in *Lnk* null mice have provided important insights into the role of the Thpo/Mpl/Jak2/Lnk pathway in regulating HSC self-renewal and quiescence and informed the understanding of MPN stem cell kinetics.

c-CBL

Mutations in *c-CBL* were identified in myelofibrosis patients in a region of acquired uniparental disomy (aUPD) on 11q[53]. Most variants were missense substitutions in the RING or linker domains that abrogated CBL ubiquitin ligase activity[53]. These mutations were modeled in mice using retroviral over-expression in *c-Cbl* null cells, and were demonstrated to further augment the enhanced cytokine sensitivity of *c-Cbl* null HSPC, indicating a gain-of-function activity above that seen in *c-Cbl* null cells alone[54]. Subsequent *c-Cbl* mutant knock-in models, have demonstrated that mutations in the *c-Cbl* RING finger domain (but not germline deletion of *c-Cbl*) cause loss of E3 ubiquitin ligase activity and an inability of c-Cbl to interact with target receptor tyrosine kinases, such as Flt3. This in turn, causes activation of downstream signaling pathways and the development of a MPN *in vivo*, which is dependent on Flt3 signaling for the maintenance of disease[55].

Other genetic lesions in MPN

Other genetic lesions have been described in the epigenetic machinery in MPN such as in *ASXL1*[56], *EZH2*[56,57] and *DNMT3A*[58,59]. *IDH1* and *IDH2* mutations have been identified at low frequency in MPN[60]. Genetic murine models exist for some of these genetic abnormalities[61,62] while others are in development.

Other MPN animal models

Additional murine models have an MPN phenotype, though they are driven by alleles that are not common, somatically mutated drivers of MPN in humans. These include the K-*Ras*^{G12D} knock-in model, in which oncogenic K-*Ras* was conditionally expressed from its endogenous murine promoter[63,64] and the *FLT3* internal tandem duplication (ITD) knock-in model, in which a *FLT3*-ITD mutation was engineered into the *Flt3* locus and expressed from the endogenous murine *Flt3* promoter[65,66]. *RAS* and *FLT3* are both recurrently mutated in human AML, but K-*Ras*^{G12D} and *Flt3*^{ITD} mice both develop MPN, indicating that these genetic lesions alone are insufficient to induce AML. K-*Ras*^{G12D} mice develop a MPN characterized by leukocytosis, extra-medullary hematopoiesis and growth factor hypersensitivity, while *Flt3*^{ITD} mice develop MPN reminiscent of human chronic myelomonocytic leukemia (CMML), with prominent monocytosis and splenomegaly.

The AP-1 transcription factor, JunB, is a transcriptional regulator of myelopoiesis and its expression is downregulated in AML[67]. Inactivation of *JunB* in post-natal mice results in LT-HSC expansion and a MPN resembling early CML[68]. Murine models of some of the genetic lesions found in human juvenile myelomonocytic leukemia (JMML) have been developed and these also result in a MPN phenotype in mice. Conditional deletion of *Nf1* and conditional activation of *Ptpn11*^{D61Y} both induce a MPN characterized by leukocytosis and hypersensitivity to granulocyte-macrophage colony-stimulating factor (GM-CSF)[69,70].

Each of these *in vivo* models has advanced the biological understanding of these genetic abnormalities, particularly with respect to their effects on the HSPC compartment, and they continue to serve as useful preclinical models for testing novel pharmacological agents[71].

C. The use of *JAK2V617F* models in the preclinical development of MPN therapies

JAK2V617F and *MPLW515L* models have been employed extensively in the pre-clinical development of MPN therapies. These studies have provided the following insights:

- **i.** Provision of a “proof of concept” that JAK2 inhibitors could be safe and efficacious in the treatment of *JAK2V617F*[72,73] and *MPLW515L* mediated MPN[40,41]
- **ii.** Demonstration that MPN-propagating stem cells are not effectively targeted with JAK2 inhibitor monotherapy[28]
- **iii.** Identification of heat shock protein 90 (HSP90) as a promising therapeutic target in *JAK2* driven neoplasms[74,75]
- **iv.** Demonstration of the efficacy of the HDACi, vorinostat for the treatment of *JAK2V617F*-mediated MPN[76]
- **v.** Indication that pharmacological inhibition of STAT5 (if possible in MPN patients) has the potential to be efficacious in treating *JAK2V617F*-mediated MPN[77]

These insights have facilitated the clinical advancement of JAK2 kinase inhibitors, provided a rationale for clinical trials of HSP90 inhibitors in *JAK2* driven cancers and offered supportive evidence for the use of HDACi in MPN patients. No doubt, these models will continue to be employed in the evaluation of future MPN therapies.

D. The bone marrow microenvironment in MPN

The bone marrow microenvironment is perturbed in MPN (at least in the late stages of the disease), sometimes to such an extent that myelofibrosis and bone marrow failure develop. Murine models in this area have helped advance understanding in three main areas:

(i) Demonstration that genetic alterations in bone marrow stroma are sufficient to induce MPN

In 2007, Walkley et al. showed in two separate genetic models that MPN could be induced as a result of perturbation of the bone marrow microenvironment. In the first model, germline deletion of the nuclear hormone receptor, retinoic acid receptor γ (*RARγ*) resulted in an MPN that was TNFα mediated[78], while in the second model an MPN developed following conditional inactivation of the negative cell cycle regulator, *Rb* in hematopoiesis using Mx1-Cre[79]. In both scenarios, the MPN could not be propagated into secondary recipients and could not be rescued by transplantation with wild-type HSC indicating that the disease phenotypes were cell extrinsic.

(ii) Investigation of the biological mechanisms underlying myelofibrosis development

Prior to the advent of the *JAK2V617F* murine models, the main genetic models of myelofibrosis were *GATA1*^{low}[80], thrombopoietin (*THPO*) transgenic[81] and Tri21 mice[82]. These models are all characterized by marked megakaryocytic hyperplasia, underpinning the central role of the megakaryocytic lineage in the development of a fibrosis phenotype. A retroviral *THPO* over-expression murine model of myelofibrosis[83] has facilitated the demonstration that transforming growth factor beta (Tgfβ1) is required[84], while thrombospondin (Tsp) is redundant[85], for THPO-induced myelofibrosis, and that osteoprotegerin (Opg) is required for osteosclerotic transformation[86]. While much data exists on the abnormal production of pro-inflammatory cytokines in patients with myelofibrosis it is unclear which cytokines are the real drivers of fibrotic transformation in MPN. Cytokine profiling has been performed in the retroviral *JAK2V617F* and *MPLW515L* models[73,40] and further investigation of the specific contributions of individual cytokines to fibrotic transformation in *JAK2V617F*-mediated MPN will likely follow. There has been some heterogeneity in the fibrosis phenotype between the *Jak2V617F* knock-in models, which may be related to the level of Jak2V617F expression.

(iii) Investigation of cell non-autonomous contributions to clonal expansion in MPN

A positive paracrine loop involving interleukin-6 (IL-6) was recently identified in a genetic model of CML, where IL-6 produced by expanded CML myeloid cells was shown to act on leukemic multi-potent progenitors (MPPs) and drive further myeloid expansion[12]. Using the same inducible transgenic *BCR-ABL* model, Zhang et al. also recently demonstrated that altered cytokine expression (including of IL-1, IL-6 and TNFα) in CML bone marrow was associated with selective impairment of normal LT-HSC growth and a growth advantage to CML LT-HSC[13]. In a retroviral *JAK2V617F* model, TNFα has been demonstrated to facilitate the preferential expansion of *JAK2V617F* mutant cells[87]. In aggregate, these studies indicate that differential microenvironmental regulation of HSPC populations in MPN and normal bone marrow contributes to clonal expansion in MPN.

F. Future Directions

As the genetic landscape of MPN has been defined, corresponding genetically engineered murine models have been developed. Going forward, mutations that co-occur in human patients will be modeled in the mouse to investigate the functional effects and therapeutic susceptibilities of compound genetic lesions in MPN. These models will also be useful in

understanding the differential molecular dependencies and niche interactions of MPN-propagating stem cells and in studying fibrotic transformation in MPN. Given the recent identification of a nuclear role for JAK2[88], evaluating the effects of non-canonical JAK2V617F signaling *in vivo* will be another important focus. These models will continue to be employed in pre-clinical studies, particularly for the evaluation of combinatorial therapeutic strategies, aimed at targeting MPN-propagating stem cells and/or circumventing JAK2 kinase inhibitor resistance.

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Key Points

- Retroviral transduction of *BCR-ABL* into murine bone marrow cells followed by transplantation into irradiated syngeneic mice established the field of myeloproliferative neoplasm (MPN) animal modeling
- The effects of the *JAK2V617F* mutation in hematopoietic cells has been extensively modeled *in vivo* using retroviral, transgenic, knock-in and xenograft murine models
- The considerable phenotypic differences observed between broadly similar *JAK2V617F* murine models highlights the inherent variability in murine models that can occur as a result of multiple factors, e.g. promoter, oncogene expression level, murine versus human protein, mouse strain
- Mutant oncogenes found in human acute myelogenous leukemia (AML), e.g. *RAS, FLT3*, induce MPN in mice, indicating that these genetic lesions are insufficient to cause AML, and suggesting that additional co-operating genetic events are required for AML development
- As the increasing genetic complexity of MPN has become apparent, additional genetic models have been developed to investigate the functional effects and therapeutic susceptibilities of compound genetic lesions in MPN

Box 1 – Retroviral Bone Marrow Transplant Murine MPN Models

In the retroviral bone marrow transplantation (BMT) assay, bone marrow is harvested from donor mice that have been stimulated with 5-fluorouracil (5-FU). The 5-FU stimulated bone marrow cells are then transduced *ex vivo* with a retroviral construct expressing the gene of interest. This results in stable but random integration of the transgene into the host cell genome. The transduced cells are then transplanted into irradiated syngeneic mice, where hematopoietic reconstitution is polyclonal. Transgene expression is generally high (non-physiologic), and differences in the site of retroviral integration may result in variation in transgene expression level. Since retroviruses preferentially transduce mitotically active cells, quiescent long-term hematopoietic stem cells (LT-HSC) are relatively resistant to retroviral integration.

Box 2 – Genetically Engineered Murine MPN Models

Genetically engineered murine models can be classified as transgenic or endogenous. Transgenic mice express the gene of interest under the control of ectopic promoter and enhancer elements. They are generated by pronuclear injection of the transgene into a single cell of a mouse embryo, where it randomly integrates into the mouse genome. Knock-in mice express the gene of interest from their native promoters and thus represent endogenous genetically engineered mice. They are generated through the modification of embryonic stem (ES) cells using a DNA construct that contains sequences homologous to the target gene. The relevant mutation is thus introduced to the gene of interest under the control of its endogenous promoter via homologous recombination in ES cells. Conditional knock-in mice use site-specific recombinases, such as Cre, to control the timing and tissue-specificity of gene expression. Inducible transgenic and knock-in models use exogenous ligands (e.g. doxycycline or interferon) to reversibly control the timing of target-gene expression. In general, transgenic models result in over-expression of the gene of interest through the use of exogenous promoters, whereas expression is at physiological levels in knock-in models, where the gene of interest is expressed from its endogenous promoter. Knock-out mice are genetically engineered mice, in which the gene of interest is inactivated by replacing it or disrupting it with an artificial piece of DNA. This is achieved in ES cells via homologous recombination. Since germline homozygous gene deletions can be embryonically lethal, conditional knock-out mice are often generated to circumvent this problem.

Box 3 – Xenograft Murine MPN Models

In xenograft murine MPN models, human MPN CD34+ cells are transplanted and propagated in immunodeficient mice. To achieve engraftment, investigators have used NOD/SCID (non-obese diabetic, severe combined immunodeficiency) mice absolutely deficient in B and T cells and having impaired NK cell function[1,2]. Further impairment of the murine immune system has been achieved by depletion of NK cells using CD122 antibodies[3] or by crossing with a mouse strain deficient for the common gamma chain of the IL-2 receptor[4] (NSG mice). While these models allow the study of primary human MPN cells *in vivo*, some aspects of the bone marrow microenvironment are not recapitulated due to the absence of immune cells and species incompatibility for some cytokines and cytokine receptors.

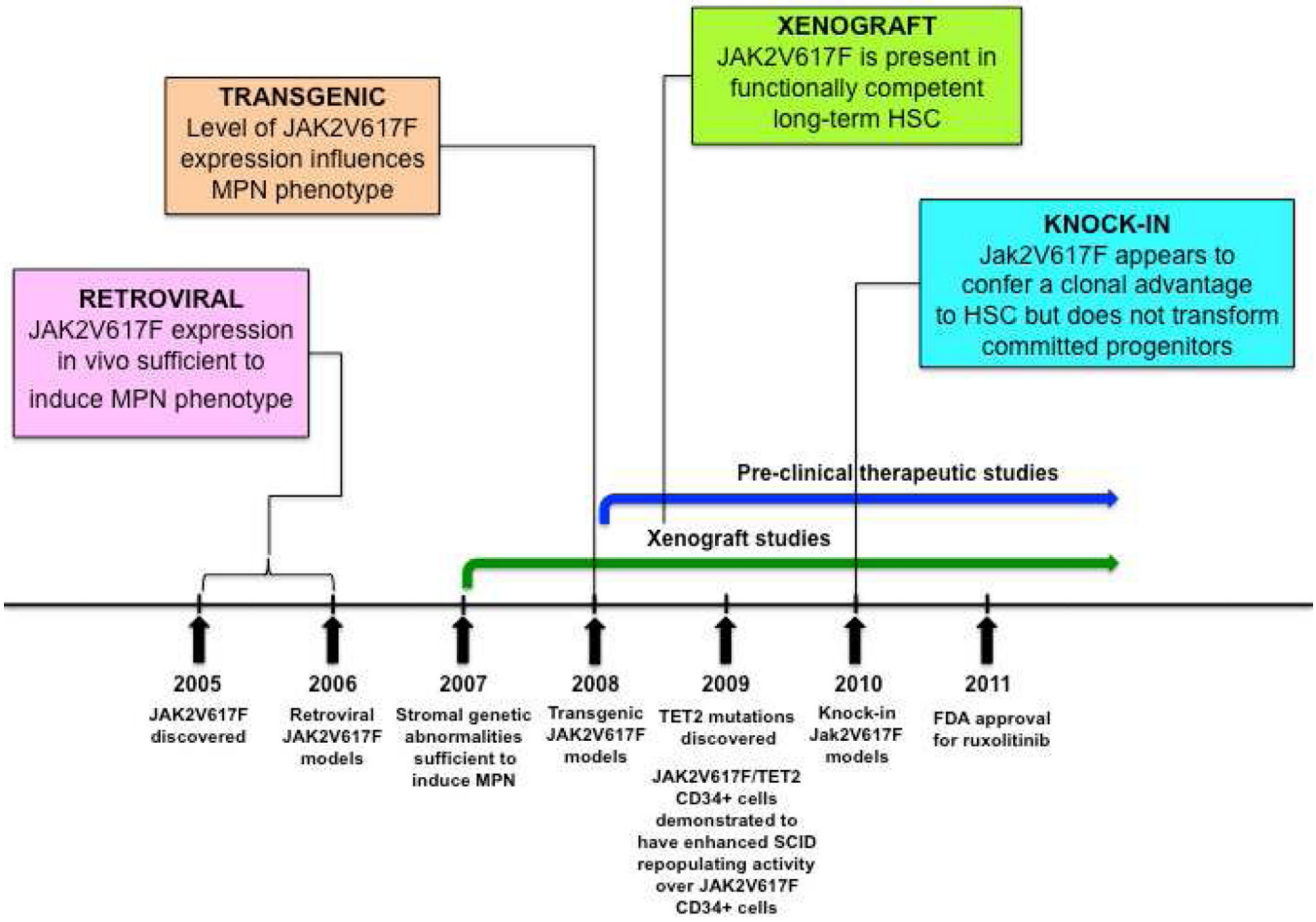

Figure 1. Timeline for development of *JAK2V617F* murine models

MPN = myeloproliferative neoplasm; HSC = hematopoietic stem cell; SCID = severe combined immunodeficiency; FDA = Food and Drug Administration

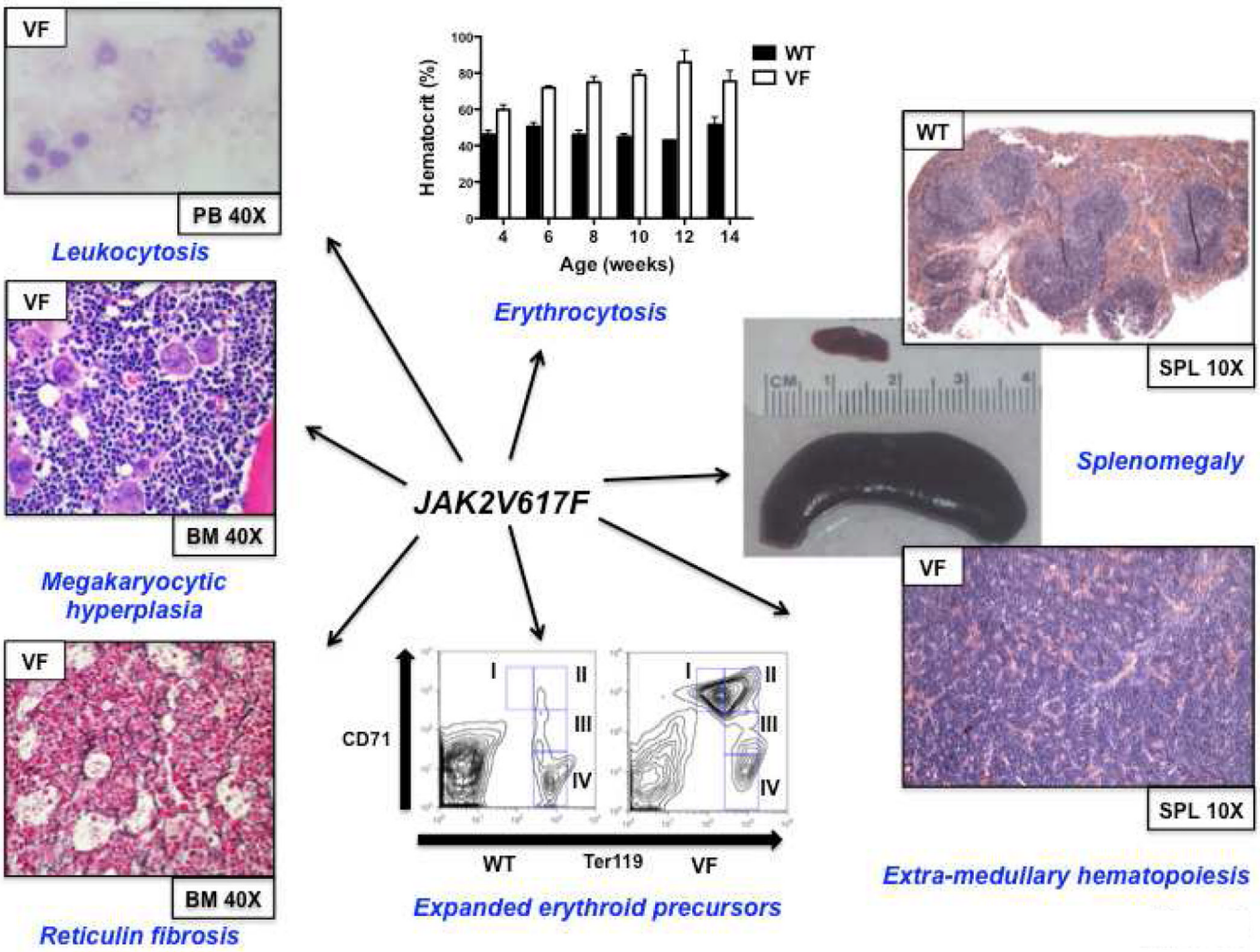

Figure 2. *JAK2V617F* murine models faithfully recapitulate features of human MPN

MPN features common to the majority of retroviral and genetic *JAK2V617F* models (data taken from knock-in model of Mullally et al.[28])

WT = wild-type; VF = JAK2V617F; PB = peripheral blood; BM = bone marrow; SPL = spleen

Table 1

Jak2V617F knock-in murine MPN models

First Author	JAK2 Origin	Strain	Type	VF:WT Expression	Homozygotes	Phenotype	Myelofibrosis
Akada	Mouse	129Sv/C57Bl/6	Conditional	< 1:1	Yes	PV-like	Yes
Marty	Mouse	129Sv/C57Bl/6	Constitutive	~ 1:1	No	PV-like	Yes
Mullally	Mouse	129Sv/C57Bl/6	Conditional	< 1:1	No	PV-like	Yes, with BMT
Li	Human	129Sv/C57Bl/6	Conditional	~ 1:1	No	ET-like	No

PV = polycythemia vera; ET = essential thrombocythemia; BMT = bone marrow transplantaton