Myeloproliferative neoplasms (MPN), are clonal hematopoietic stem cell (HSC) disorders driven by gain-of-function mutations in \( \text{JAK2} \) (\( \text{JAK2-V617F} \)), \( \text{CALR} \) or \( \text{MPL} \) genes. MPN treatment options currently mainly consist of cytoreductive therapy with hydroxyurea and \( \text{JAK2} \) inhibitors such as ruxolitinib and fedratinib. Pegylated interferon-alpha can induce complete molecular remission (CMR) in some MPN patients when applied at early stages of disease. The ultimate goal of modern MPN treatment is to develop novel therapies that specifically target mutant HSCs in MPN and consistently induce CMR. Basic research has identified a growing number of candidate drugs with promising effects \textit{in vitro}. A first step on the way to developing these compounds into drugs approved for treatment of MPN patients often consists of examining the effects \textit{in vivo} using pre-clinical mouse models of MPN. Here we review the current state of mouse models and the experimental setup for their optimal use in drug testing. In addition to novel compounds, combinatorial therapeutic approaches are often considered for the treatment of MPN. Optimized and validated mouse models can provide an efficient way to rapidly assess and select the most promising combinations and thereby contribute to accelerating the development of novel therapies of MPN.

**Key words:** MPN, myeloproliferative neoplasms, \( \text{JAK2} \), mouse model, pre-clinical models, novel therapies

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**INTRODUCTION**

Myeloproliferative neoplasms (MPNs) were proposed in 1951 by William Dameshek to represent “somewhat variable manifestations of proliferative activity of bone marrow cells” and to constitute a group of closely interrelated diseases. Today, three disease entities are summarized under the term “BCR/ABL-negative MPN” (ref.\(^2\)). Polycythemia vera (PV) is characterized by increased erythropoiesis and is frequently accompanied by thrombocytosis and leukocytosis, while essential thrombocytopenia (ET) manifests as augmented megakaryopoiesis with thrombocytosis, but with normal red cell parameters. Primary myelofibrosis (PMF) requires histological proof of reticulin fibrosis and is frequently accompanied by anemia and extramedullary hematopoiesis. The currently used diagnostic criteria for MPN are defined by the World Health Organization (WHO) (ref.\(^3\)). As proposed by Dameshek, MPN subentities are interrelated, as ET can sometimes convert to PV and both ET and PV can progress to myelofibrosis (post-ET-MF or post-PV-MF). MPNs have been demonstrated to be clonal diseases of the hematopoietic stem cell (HSCs) driven by acquired gain-of-function mutations in \( \text{Janus kinase 2} \) (\( \text{JAK2} \)), \( \text{Calreticulin} \) (\( \text{CALR} \)) and \( \text{Thrombopoietin receptor} \) (\( \text{MPL} \)) genes\(^4-10\). These driver gene mutations in most patients are mutually exclusive. Only a few patients with more than one driver gene mutation have been studied in detail and in these cases each driver gene mutation represented an independent clone\(^11,12\).

\( \text{JAK2-V617F} \), the most frequent MPN driver mutation, is found in \textgreater95% of PV patients, and also in approximately one-half of patients with ET or PMF (ref.\(^13\)). MPN progenitors and stem cells are hypersensitive to cytokines due to augmented intracellular signaling by the mutant \( \text{JAK2-V617F} \) (ref.\(^13\)). Mutations in \( \text{CALR} \) and \( \text{MPL} \) also increase the JAK2/STAT signaling by activating cytokine receptors independently of their cognate ligands\(^14\). In some MPN patients, driver gene mutations are complemented by additional somatic mutations, most frequently affecting genes involved in epigenetic regulation inflammation, DNA damage response and Ras signaling, that can modify the disease course and contribute to disease initiation and/or progression\(^15\).

A large number of mouse models have been developed to study MPN pathogenesis and to advance our current understanding of MPN disease initiation and progression\(^16-20\). These mouse models have been also used to examine the effects of therapeutic agents on MPN disease manifestations. Identification and validation of novel therapeutic agents in pre-clinical models of MPN is an extremely important and valuable step in the development of novel drugs for MPN patients. In this review we discuss the use of different types of mouse models and experimental setups to study candidate drugs for MPN treatment. We also provide examples of modern therapies that have been first tested in MPN mouse models.
1. Mouse models of MPN

Retroviral transduction models were first used to characterize the JAK2V617F signaling and to confirm its effects on MPN phenotype in vivo (summarized in Table 1) (ref.4,21-24). Bone marrow cells were transduced with retroviral vectors carrying cDNA encoding JAK2V617F and transplanted into syngeneic recipient mice, resulting in PV phenotype that in most cases rapidly progressed to myelofibrosis. Since retroviral transduction required HSCs to divide in culture, some of the retroviral models exhausted prematurely after a few months and were unable to engraft in secondary transplantations25.

Transgenic models of MPN (summarized in Table 1) provide a more robust and reproducible system for preclinical drug testing in vivo. The first transgenic models used a cDNA encoding mouse or human JAK2V617F that was placed under the control of a ubiquitous or tissue specific promoter and injected into mouse oocyte26,27. A more advanced transgenic model used a bacterial artificial chromosome (BAC) containing a large part of the human JAK2 gene (exons 1-12). The V617F mutation was introduced as a cDNA covering JAK2 exons 13-25 that were placed in reverse orientation and flanked by antiparallel loxP sites to make expression of JAK2V617F conditional27. When activated by Cre-recombinase, these BAC transgenic mice express the human JAK2V617F under the control of the human JAK2 promoter and manifested with ET or PV phenotypes, with late transformation to post-MPN myelofibrosis recapitulating the full MPN pathophysiology observed in patients. When constitutive VavCre was used activate the JAK2V617F transgene, ET phenotype was observed and was associated with lower JAK2 expression levels in bone marrow, whereas PV phenotype resulted when conditional MxCre or ScICre was used activate the transgene, leading to higher JAK2V617F expression27,28. Recently, transgenic mice expressing Jak2V617F under the tetracyclin dependent SCL-4TA-2S tet-off system were generated29. Using this system, the authors showed that turning on Jak2V617F expression resulted in MPN phenotype and turning off Jak2V617F reversed the phenotypic features, indicating that mutant selective JAK2 inhibitors should profoundly disable the Jak2V617F MPN clone.

Later several transgenic models were generated that introduced the V617F mutation into the endogenous mouse Jak2 locus as a conditional knock-in30,32. These mice express the mouse Jak2V617F and display a strong PV phenotype with moderate thrombocytosis and leukocytosis. In contrast, a knockin model where exon 2 of mouse Jak2 was replaced with a cDNA for the human JAK2V617F presented with a very mild ET-like phenotype, but when the knock-in allele was intercrossed to homozygosity, the mice displayed a strong PV phenotype33,34.

Recently, also mouse models for MPN driven by CALR mutations have been generated35,36. Most of the models used the CALRdel52 mutation, but recently also mice expressing the CALRins5 mutation have been described39. Both CALRdel52 and CALRins5 mice developed ET phenotype with thrombocytosis, leukocytosis and variable degree of fibrosis similar to MPN patients with CALR mutations. So far only a retroviral mouse model has been established for the MPL-W515L mutation found in patients with ET or PMF (ref.41-43). These mice develop a very rapid MPN-like disease with severe myelofibrosis, and early lethality. The phenotype is likely due to the increased and in part ectopic expression of MPL-W515L driven by the retroviral LTR.

Overall, these mouse mutants proved that expression of the Jak2, CALR or MPL mutants can alone cause MPN phenotype in mice and established them as driver gene mutations. Since about half of MPN patients carry additional somatic mutations that can modify the course of the disease44, the MPN mouse models have then been crossed with mice carrying mutations in such modifier genes. The double mutant mice drive expansion of LT-HSC compartment by increasing their self-renewal, stemness and repopulation capacity, leading to clonal dominance. In most cases these models faithfully recapitulated the acceleration and modifications of the phenotypes that were also observed in patients carrying the same mutations. This was observed in particular with double mutant mouse models with mutations in epigenetic regulator genes, such as Ezh2 (ref.45-47), Tet2 (ref.48,49), Dnmt3a (ref.50), and Asxl1 (ref.51), which are also among the most frequently found additional mutations in patients with MPN. As an alternative to intercrossing genetically

Table 1. MPN mouse models with JAK2V617F.

<table>
<thead>
<tr>
<th>JAK2-origin</th>
<th>Type of model</th>
<th>Promoter</th>
<th>Phenotype</th>
<th>Background</th>
<th>Ref.</th>
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<tbody>
<tr>
<td>Mouse</td>
<td>retroviral</td>
<td>viral LTR</td>
<td>PV</td>
<td>C57BL/6</td>
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<tr>
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<td>BALB/c and C57BL/6</td>
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<tr>
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<td>C57BL/6</td>
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<tr>
<td>Human</td>
<td>oocyte injection</td>
<td>Vav</td>
<td>PV</td>
<td>C57BL/6/DBA/2</td>
<td>25</td>
</tr>
<tr>
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<td>oocyte injection</td>
<td>H-2Kb</td>
<td>ET/PV</td>
<td>C57BL/6/DBA/2</td>
<td>26</td>
</tr>
<tr>
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<td>ET/PV</td>
<td>C57BL/6</td>
<td>27</td>
</tr>
<tr>
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<td>ET/ PV</td>
<td>C57BL/6</td>
<td>29</td>
</tr>
<tr>
<td>Mouse</td>
<td>knock-in</td>
<td>mJak2</td>
<td>PV</td>
<td>129Sv/C57BL/6</td>
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<tr>
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<td>ET</td>
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<tr>
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<td>PV</td>
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<td>Mouse</td>
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<td>PV</td>
<td>129Sv/C57BL/6</td>
<td>32</td>
</tr>
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</table>

engineered mouse models, recently techniques using clustered regularly interspaced short palindromic repeats (CRISPR) with CRISPR-associated protein 9 (Cas9) were applied to target specific genomic loci for loss of function mutations in vivo. CRISPR/Cas9 techniques are especially useful in modeling complex genotype combinations, exemplified by a study in which mutations in up to five genes (Dnmt3a, Runx1, Nf1, Tet2, and Ezh2) were introduced in a single HSC (ref.33). CRISPR/Cas9 in vivo system was also used to generate MPN mouse models39,50,51 that may replace complicated breeding’s for studies examining the contribution of multiple loci.

Finally, xenotransplantation models using patient-derived xenografts (PDX) into immuno-compromised recipient mice represent an attractive complement to genetic mouse models. PDX models allow studying human primary cancer and leukemia cells in vivo and they have been used for pre-clinical drug testing e.g. in leukemia34 and solid cancers55. They provide the advantage that primary human cells can be studied. However, PDX models for MPN so far showed limited multilineage engraftment and self-renewal capacity, with large patient to patient variability making PDX difficult to use for drug testing compared to standard MPN mouse models56,57. Injection of patient-derived CD34+ HSCs into mice that were implanted with ossicles derived from human mesenchymal stem cells showed only transient improvement in engraftment58. More recently, humanized Rag2+/Il2rg−/immuno-deficient mice that are engineered to express a number of hematopoietic stem cells showed non-cross-reactive human cytokines and growth factors became available59. One of these strains, the “MISTRG” mice, express human macrophage colony-stimulating factor (M-CSF), interleukin-3 (IL-3), granulocyte-macrophage colony-stimulating factor (GM-CSF), signal regulatory protein a (SIRPα). The interaction between human CD47 on the PDX graft and human SIRPα on mouse macrophages provides a “don’t eat me” signal that favors the expansion of human hematopoiesis in mice60.

**2. MPN mouse models for pre-clinical testing of new therapeutic agents**

Original studies to test the compounds imatinib, dasatinib, AG490, and TG101348 used retroviral models expressing JAK2V617F (ref.22,62). These studies provided first in vivo proof-of-principle that JAK2 inhibitors have therapeutic effects on MPN (Fig. 1). However, the retroviral models are not optimal for drug studies. The main disadvantages include the unphysiologically high expression levels of the mutant JAK2V617F driven by the retroviral LTR promoter (>10-fold higher that endogenous mouse JAK2), variable severity of the phenotype dependent on the retroviral titer and absence of thrombocytosis. However, the MPL-W515L retroviral model is still frequently used for drug studies because these mice rapidly develop severe myelofibrosis63,64.

Early studies in primary transgenic mice monitored normalization of blood counts and splenomegaly, but did not follow changes in the mutant allele burden16,32. To expand the size of the cohort that allowed testing of multiple drugs, bone marrow transplantations into irradiated recipient mice were introduced72. At the same time competitive transplantations of mixtures of JAK2V617F and wildtype bone marrow cells into recipient mice were performed to mimic the situation in MPN patients, where JAK2-mutant and wildtype hematopoietic cells co-exist. Furthermore, to follow the JAK2-mutant cells, a polymorphism in the Ptprc gene that encodes CD45, also called leukocyte antigen 5 (Ly5) was used65. C57BL/6 mice, including most JAK2V617F transgenic models express the CD45.2 variant. Congenic C57BL/6 mice that express CD45.1 were generated by backcrossing and used as donors of the wildtype bone marrow competitor cells and also as recipients (Fig. 2). The first such competitive transplantations were used to determine the effects of

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**Fig. 1.** Experimental setup for candidate drug testing in retroviral MPN mouse models. Wildtype (WT) bone marrow cells are transduced with murine retrovirus harboring the mutant JAK2V617F allele. Transduced bone marrow cells are selected based on expression of GFP reporter and transplanted via tail vein injection into the lethally irradiated syngeneic recipient mice. After engraftment, the recipient mice are allowed to develop MPN phenotype and are randomized into treatment or vehicle control groups. Complete blood counts and GFP chimerism are determined in peripheral blood at defined timepoints. At the end of the treatment the mice are sacrificed and terminal analysis of cells and organs is performed.
vorinostat, a small-molecule inhibitor of histone deacetylation, on Jak2-V617F allele burden. For this purpose, bone marrow (BM) cells expressing JAK2-V617F and CD45.2 (red) are mixed with wildtype (WT) competitor cells expressing CD45.1 (yellow) and injected into lethally irradiated CD45.1 recipient mice (blue). After engraftment, the recipient mice are allowed to develop MPN phenotype and are randomized into treatment or vehicle control groups. Complete blood counts and CD45.1/2 chimerism are determined in peripheral blood at defined timepoints. At the end of the treatment the mice are sacrificed, and terminal analysis of cells and organs is performed.

A disadvantage of the CD45.1/2 reporter lies in the absence of CD45 antigen on a surface of erythrocytes and platelets, two major lineages that define MPN disease phenotype. Therefore, CD45.1/2 does not allow following the JAK2-mutant chimerism in peripheral blood Ter119+ red cells and CD61+ platelets. In addition, recent studies show that despite backcrossing into C57BL/6, a small amount of the original SJL mouse background, from which CD45.1 was derived, is retained in C57BL/6 CD45.1 mice from some vendor sources. A new congenic CD45.1WT knock-in...
line that carries a single point mutation in Ptprc was generated directly in C57BL/6 background\(^5\). These CD45.1\(^{\text{STEM}}\) knock-in mice should now overcome this problem.

A more complete labeling of all JAK2-mutant cells was achieved using mice that express the green fluorescent protein (GFP) reporter driven by a ubiquitous UBC-promoter in all hematopoietic cells\(^8\). Crossing UBC-GFP mice with JAK2\(^{-}\) mice resulted in double transgenic mice that co-express JAK2\(^{-}\) and GFP in all hematopoietic cells, including peripheral blood leukocytes, erythrocytes and platelets and represent an improved system for drug studies (Fig. 3). Competitive transplantations with bone marrow cells from such double transgenic mice were first used to compare the efficacy of hydroxyurea and ruxolitinib\(^9\). The GFP reporter system combined with transgenic models of JAK2\(^{-}\) is reliable and can be applied also for testing combinations of drug treatment. Recently, this approach was used to show synergism between interferon-\(\alpha\) and arsenic\(^7\).

Since 1x10\(^6\) bone marrow cells from JAK2-mutant mice contain about 60-100 HSCs, competitive transplantations at 1:1 ratio with wildtype competitor cells results in MPN that is essentially polyclonal. Drugs that decrease JAK2-mutant allele burden may have to overcome potential escape mechanisms though selection of JAK2-mutant HSCs that are resistant to the treatment. Therefore, competitive transplantations at higher dilutions of the JAK2-mutant cells, e.g. 1:20, could be a better system to test the ability of drugs to selectively target the mutant JAK2 and decrease the mutant allele burden. In principle, recipient mice reconstituted from a single JAK2\(^{-}\) mutant HSC can be generated by transplantation of single HSCs or by limiting dilution of transplanted bone marrow cells\(^8\). However, such an approach would require transplanting a large number of recipients, since only about 30% of recipients at limiting dilution engraft and display MPN phenotype and would therefore not be practical for routine use.

CONCLUSIONS

Transgenic MPN mouse models have been developed for JAK2\(^{-}\), JAK2 exon 12 mutation and CALR mutations. The optimal experimental setup for candidate drug testing in pre-clinical MPN mouse models should include reporters expressed in all blood cells, e.g. UBC-GFP, that allows tracking the MPN cells in all organs including peripheral blood. Competitive bone marrow transplantations allow expanding the cohort for testing multiple compounds and combinations of drugs. Presence of wildtype HSCs, derived from the wildtype competitor bone marrow, can maintain hematopoiesis when MPN HSCs are effectively targeted by new therapeutic approaches. Transgenic mouse models with reversible on-off JAK2\(^{-}\) expression\(^8\), could serve as the ideal positive controls for drugs that are selective for the mutant JAK2 proteins.

Mice with more complex mutation genotypes, e.g. combinations of JAK2\(^{-}\) with mutations in Tet2, Ezh2, Dnmt3a or Axl\(^{\text{I1}}\) that are found in patients with MPN can also be used as bone marrow donors, which will allow testing drugs in these more complex MPN models. The CRISPR/Cas9 technology could further facilitate and speed up the generation of complex genotypes for such studies. Retroviral models, such as MPL-W515L can also remain useful in specific setting, e.g. to study rapid progression to myelofibrosis without the need to wait several months for myelofibrosis to develop, as is the case with the transgenic models for JAK2\(^{-}\).

The PDX models using improved recipient mice such as MISTREG (ref.\(^{3}\)) could become the next step in pre-clinical MPN mouse models for drug testing, if some of the main hurdles can be overcome. These include the difficulties obtaining a large cohort of recipient mice reconstituted from the same MPN patient donor, variability of phenotypes between mice engrafted with cells from the same MPN donor and inter-individual differences in MPN phenotypes between different MPN donors. Overall, the MPN field already now has excellent pre-clinical in vivo models for drug testing, which can contribute to accelerating the development of novel therapies of MPN.

Search strategy and selection criteria

Our research strategy was aimed at evaluating studies on the mouse models of myeloproliferative neoplasms used for pre-clinical testing of novel therapeutic agents. Data for this article were identified by searches of PubMed and Google Scholar databases and references from relevant articles using the terms “MPN”, “myeloproliferative neoplasms”, “JAK2”, “mouse models”, “transplantation”, “novel therapies”. All searches were up to date as of December 2020. Only articles published in English were included, abstracts and reports from meetings were not included. We gave preference to publications from the past 10 years.

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