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The mouse resource at National Resource Center for Mutant Mice

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Abstract
Mouse models are essential for dissecting disease mechanisms and defining potential drug targets. There are more than 18,500 mouse strains available for research communities in National Resource Center for Mutant Mice (NRCMM) of China, affiliated with Model Animal Research Center of Nanjing University and Gempharmatech Company. In 2019, Gempharmatech launched the Knockout All Project (KOAP) aiming to generate null mutants and gene floxed strains for all protein-coding genes in mouse genome within 5 years. So far, KOAP has generated 8,004 floxed strains and 9,769 KO (knockout) strains (updated to Oct, 2021). NRCMM also created hundreds of Cre transgenic lines, mutant knock-in models, immuno-deficient models, and humanized mouse models. As a member of the international mouse phenotyping consortium (IMPC), NRCMM provides comprehensive phenotyping services for mouse models. In summary, NRCMM will continue to support biomedical community with new mouse models as well as related services.

Keywords Mouse resource · Knockout · Transgenic mice · Humanized mice

Establishment of NRCMM

House mouse (*Mus musculus*) has been widely used as laboratory animal in biomedical research since early 1900. Studies found that 99% protein-coding genes display high homology by comparing the mouse and human genome sequences (Lander et al. 2001; Mouse Genome Sequencing et al. 2002). This provides a genetic explanation for mouse and human sharing many common features in embryonic development process, physiology homeostasis regulation, and disease etiology. Genetically engineering mouse models (GEMMs), therefore, have been a crucial tool to understand the function for human genes, probe the pathogenesis of human diseases and test the efficacy of potential drugs.

To support academic and pharmaceutical communities on the use of valuable mouse resources, NRCMM was established at Nanjing, China in 2001. NRCMM is the national mouse resource center established by Ministry of Science and Technology of China, and now are operated jointly by Model Animal Research Center of Nanjing (MARC) and GemPharmatech (GPT). The mouse strains from both parties are enlisted as the resources of NRCMM (shown in Online Resource 1), and MARC and GPT hold independent legal ownerships for their own mouse strains. NRCMM also accepts donations of mouse strains from different institutes/PIs. All mouse strains enlisted in NRCMM repository are available to both non-profit and for-profit organizations. A generic MTA, attached with modified items from respective owners, is required before shipment of these strains. Headquarters of NRCMM also keeps the frozen sperms for all mouse strains. Information of the NRCMM mice can be found at www.nrcmm.cn (the database is undergoing reconstruction at this moment). Because the majority mouse strains belong to GPT, GPT created an independent database for people searching for these GPT mice at www.gempharmatech.com.

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In the earlier years of the NRCMM, we created the tamoxifen-induced smooth muscle–specific myosin light chain kinase (MLCK) knockout mice. This model, the first cKO (conditional knockout) mouse developed in China, provided the direct evidence in vivo that MLCK play central role in phasic contractions in smooth muscle (He et al. 2008). In neurological research fields, NRCMM also helped to define the role of FSTL1 expression in dorsal root ganglion for pain sensing and role of FGF13 expression in the neocortex and the hippocampus for learning and memory (Li et al. 2011; Wu et al. 2012). Soon after the discovery of CRISPR/Cas9 gene editing technology, we managed to generate knockout mouse models in China in April 2013 (Shen et al. 2013). NRCMM also helped scientists in China to create the first CRISPR/Cas9 based knockout cynomolgus monkey and dog in the world (Ma et al. 2014; Niu et al. 2014).

In total, NRCMM, with the effort of all the co-operators, have developed over 18,500 (updated to Oct, 2021) genetically engineered mouse strains, including knockout/conditional knockout mice, Cre transgenic lines, mutant knockin models, immuno-deficient models, and humanized mouse models. Many of these strains may serve as models for metabolic diseases, development defects, cardiovascular diseases and neurodegenerative diseases (Table 1).

NRCMM is the founding member of Asia Mouse Mutagenesis and Resource Association (AMMRA), a pan-Asian organization for promoting and coordinating the development, archiving/distribution, phenotyping, and informatics of mutant mice, and for facilitating access to mice resources in Asia. NRCMM also joined International Mouse Phenotyping Consortium (IMPC) in 2011, which aims to create a comprehensive catalogue of mouse gene function.

Table 1 Mouse resource at NRCMM

Classification	Models
Disease models	Metabolic disorder
	Neurodegenerative disease
	Autoimmune disease
	Spontaneous tumor
	Rare disease
	Osteoporosis
Drug target humanized models	Immune checkpoint humanized
	Cytokine humanized
	Tumor antigen humanized
	Fc receptor humanized
	CD3 complex humanized
Organ humanized model	Liver humanized
Immune deficient models	Immuno-compromised model
	Next generation of NCG
Human immune system (HIS) model	HSC/PBMC reconstitution
Wild mice consomic strains	Chromosome 1 substitution model
Germ free mouse	Inbred strains
	GEMMs strains
Inbred	
Please refer to the website below based on classification search www.gempharmatech.com	

CKO/KO mice in Knockout All Project

With the support of sophisticated genetic manipulating technique, interruption or knockout of a specific gene in ES cells and mice have been developed for almost two decades (Goldstein 2001), and knockout mice showed excellent track record as models for elucidation of human gene function and disease mechanism. The systematic targeting of all mouse protein encoding genes would provide a comprehensive and standardized resource, thus benefit the academic and industrial community. Since its complexity and huge expense, such an undertaking usually required the deployment and coordination of resources on a global scale, and three global knockout projects have been launched in the US, Canada and European countries (Austin et al. 2004; Collins et al. 2007). This project is continued by International Mouse Phenotyping consortium (IMPC), an collaborative effort by 21 research institutions to identify the function of every protein-coding gene in the mouse genome. To this end, the IMPC has systematically switched off or 'knocked out' 10,125 mouse genes (updated to Oct, 2021), and systematical analysis of these mutant strains are providing transformational insights into the function of these genes (Brown 2021; Cacheiro et al. 2020). The information of IMPC targeted gene can be accessible through GenTar (https://www.gentar.org/tracker/#/).

Although the global knockout mouse projects have achieved great accomplishment, many genes still remain to be knocked out for comprehensively studying gene function (Peterson and Murray 2021). In Gempharmatech, we launched our Knockout All Project (KOAP) in 2018 with ambition to mutagenize all protein-coding gene in mouse genome within 5 years. KOAP creates mouse models via a high throughput CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) based gene editing strategy. Specifically, the CRISPR editing system, including the enzyme and guide RNAs designed to cleave the target genomic loci, and the repair donors carrying loxP sites, were injected into mouse zygotes. Two guide RNAs and two separate donors (each carrying a single loxP site) are used to target each specific gene without incorporating extra cassettes such as selection markers. Typically, generation of both the KO allele (fragment deletion product) and the cKO allele (expected recombination product) could be achieved in one single targeting event.

The schematics for flox sites insertion and QC approaches are presented in Online Resource 2. Briefly, exon(s) encoding the critical functional domain(s) are floxed or deleted in the KOAP mouse strains. The inserting sites of the loxP were carefully selected to avoid disruption the normal expression and function of the targeted gene(s) prior to Cre/loxP excision in the floxed strains. To reduce the risk of disruption of gene expression regulation or initiation of the de novo translation start site when the canonical start codon is deleted, the subsequent 5' end coding exon(s) right after the 1st coding exon harboring ATG is preferably selected to be floxed. If the critical functional domain(s) are difficult to be floxed, we will introduce a frame-shift into the mRNA transcript after Cre mediated excision to ensure the disruption of the target protein. The size of the floxed region is preferably smaller than 5 kb. In most cases, premature termination codon (PTC) will be produced by frame-shift and the resultant transcripts are predicted to be degraded by the Nonsene-Mediated Decay (NMD) pathway. In certain circumstances, if predicted in-frame start codon that would potentially restart the translation or the main functional domain is included in the first exon, a flox region larger than 5 kb is acceptable in our designs.The Statistics of flox interval was plotted in Online resource 3. However, in some cases, there is only one exon, or the functional domain is encoded by the 1st exon, the whole gene will be floxed. Moreover, according to the published cases using flox mouse strains from our repository, Cre/loxP excision of fragments around 30 kb in size is applicable (Wang et al. 2021; Qu et al. 2021). Finally, we prefer introns larger than 600 bp for inserting of loxP. The predicted functional motifs, such as the splicing branch site (YNCTRAY), will remain intact in our designs. For KOAP project, C57BL/6JGpt (C57BL/6J substrain) was selected as the background strain to facilitate future breeding of the cKO models with established Cre lines. The strategy for each KOAP line could be found in our repository's website www.gempharmatech.com.

The whole project is estimated to be accomplished in 2023. With the highly efficient gene editing technique and straightforward targeting strategies, we have established the high throughput gene targeting pipeline with capacity of creating around 5000 cKO strains each year. Till now, there were in total 9769 KO strains and 8004 cKO strains completed by KOAP (updated to Oct, 2021). Production timeline of KOAP is presented in Fig. 1A.

We further compared the overlap alleles between IMPC and KOAP null mutant mice. For the IMPC project, there are currently 10,125 genes from either ES cell or CRISPR projects in different states of 'Attempt in Progress', 'Chimeras/Founder Obtained' (if ES cells) or 'Embryo Obtained' (if CRISPR), and 'Genotype Confirmed'. For our KOAP project, there are 11,063 targeted genes for either KO, cKO strains or both (KOAP strains are all in F0 or F1 stage). Therefore, total 15,050 protein-coding genes have been targeted by these two projects. IMPC and KOAP have targeted only 6,138 overlapping genes, indicating both repositories are useful to support the biomedical research community (Fig. 1B).

Transgenic mice that express recombinase in a specific manner are necessary for generation of conditional knockout mouse. Thus, we generated 199 mouse strains expressing Cre recombinase in specific tissues, such as immune system, nervous system, bone, muscle and adipocytes, gastrointestinal tract, cardiovascular system, reproductive system and so on. In order to ensure the appropriate expression of the Cre, we developed comprehensive strategies to generate the Cre lines. When the promoter of the driver gene is well defined, the defined promoter will be selected to direct the specific expression of Cre. Specifically, to avoid affecting other genes, the transgenic element harboring the driver promoter, the Cre coding sequence and a polyA will be introduced into safe harbor sites such as Gt(ROSA)26 (Friedrich and Soriano 1991) or Hipp11 (Hippenmeyer et al. 2010) locus to establish the Cre expressing strains. However, when the promoter and regulatory elements of the driver gene are unknown, we tend to introduce the Cre coding sequence into the endogenous gene to achieve the Cre expressing under the control of inherent regulators, i.e., inserting the Cre coding sequence after the ATG code if the driver gene works well in a heterozygote. Moreover, putting the Cre coding before the stop condon to avoid inactivating the driver gene. As presented in Fig. 1C, 123 strains are created using the in situ insertion strategies; 49 Cre strains are generated via recombinase cassette insertion into ROSA26 or H11 loci, and 27 Cre lines are transgenic strains with random integration of recombinase cassette into mouse genome. Although some of Cre driven genes are not first reported by our work, the strategies we used here (ROSA26/H11 and in situ insertion) to reproduce the Cre lines could provide the information of recombinase location, which will facilitate to make the breeding strategies to generate cKO models. Moreover, all the Cre lines are created in C57BL/6 strains, making them feasible to breed with flox strains created in KOAP to produce cKO mice. Codon-Improved Cre Recombinase (iCre), demonstrating to express at a higher level in mouse, was employed in most GPT Cre lines to allow high efficacy excision (Shimshek et al. 2002). The Cre mouse strains with the driving promoters and the mutation type have been listed in the Online Resource 4. So far, 39 of 199 Cre lines have been bred with flox strains to generate cKO mice or validated with Cre reporters as indicated in Online Resource 4 (updated to Oct, 2021).

Scientists could access the interested mouse strains through our repository's website www.gempharmatech.com. To better illustrate the interaction network of multiple genes and facilitate scientists to choose the connected

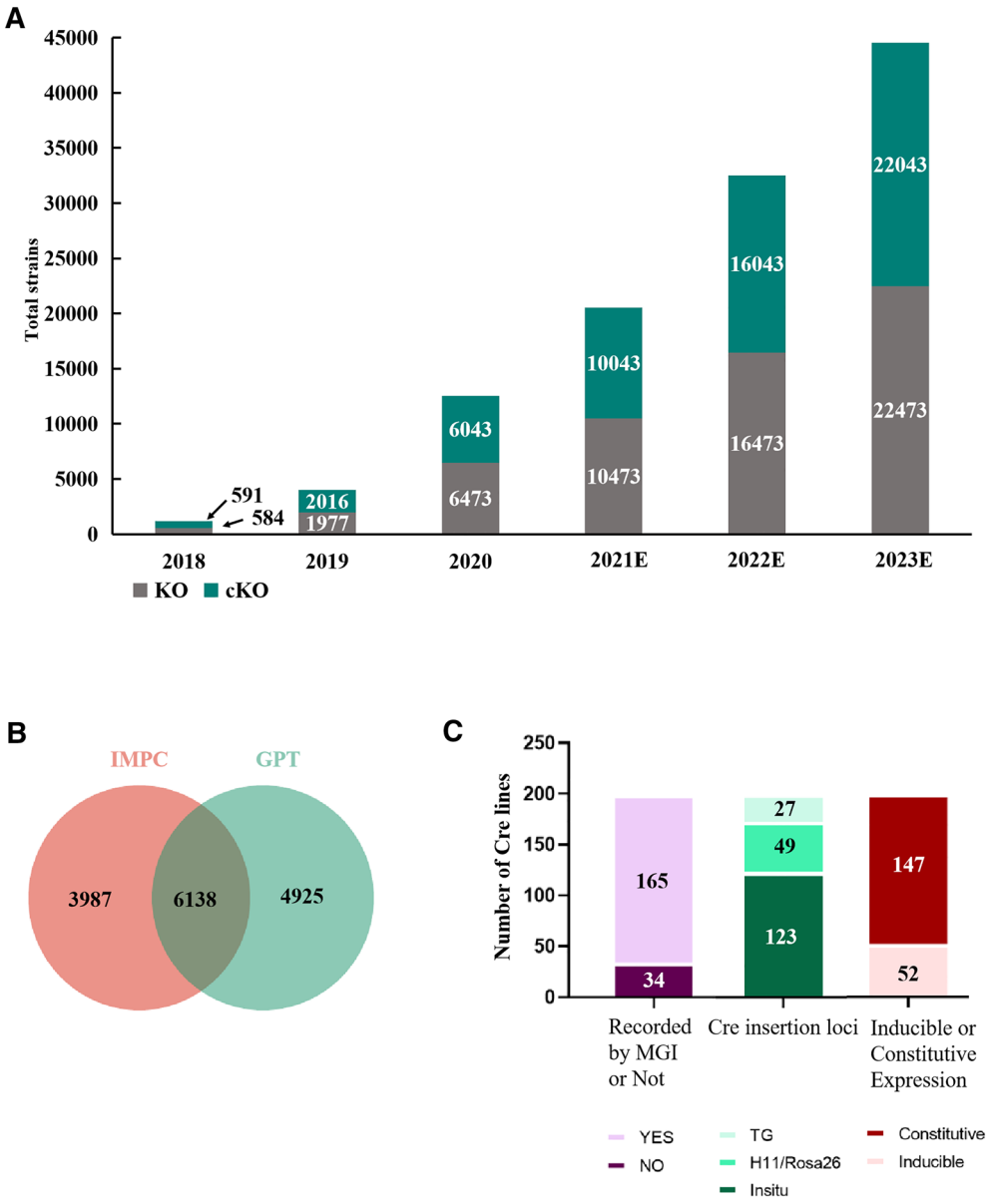

Fig. 1 Overview of KOAP (updated to Oct, 2021). **A** Production timeline of KOAP. **B** The comparison of alleles between IMPC and KOAP. **C** The statistics of Cre lines. 165 of 199 Cre strains have been recorded in MGI and 34 strains are novel. CreER versus constitutive, 52 versus 147; H11/R26 KI versus 3' endogenous KI alleles, 49 versus 123

genes, we developed the pathway searching option. Pathway searching is a graphic online tool that incorporated most of our KOAP strains into several classic signaling pathway networks. People interested to a specific pathway related genes can easily find the available CKO/KO mouse strains linking with target genes. As exemplified by Akt signaling pathway schematic in Fig. 2, the genes labeled by orange circles are directly linked to the available target strains in our repository. We highly recommend scientists to use the pathway searching to find all the connected genes.

Humanized mouse strains

Tremendous progress was made in understanding human diseases such as cancer, autoimmune disease, and infectious diseases in the last decades. Consequently, great success have been achieved in drug development. However, the species barrier between mouse and human severely limited accurate prediction of drug efficacy and safety study in mouse models. Humanized mice, the chimeric mice with human cells, tissues, organs, or carrying human genes, are pivotal models to fill the translational gap in the preclinical

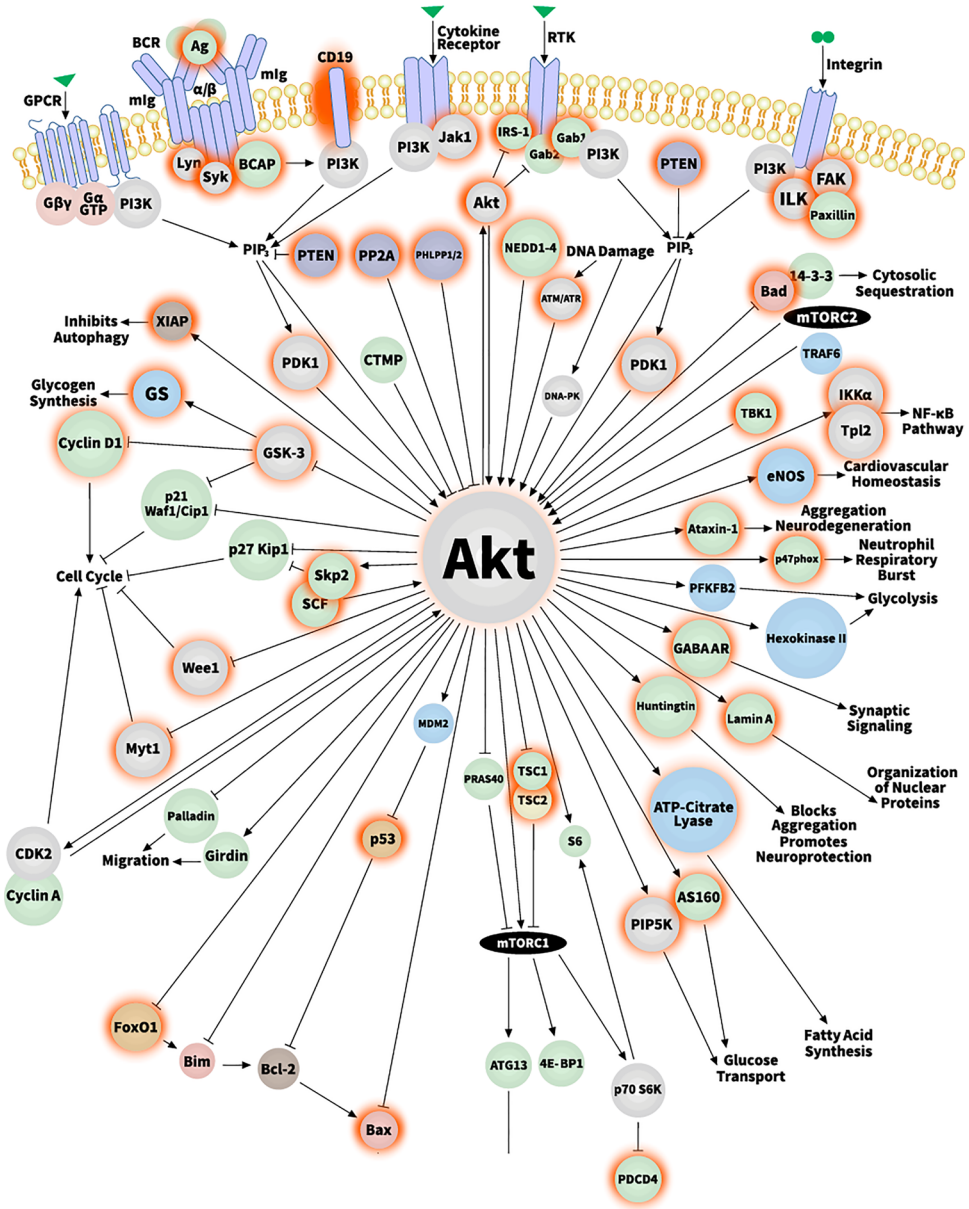

Fig. 2 Example of signaling pathway schematic for cKO/KO strains searching. Target gene could be searched through signaling pathways in our website www.gempharmatech.com. The genes highlighted by orange circles link to the available cKO/KO strains

studies (Aartsma-Rus and van Putten 2019; De La Rochere et al. 2018).

Human immune system (HIS) models

Because functional immune system would reject xenograft in mice, various immune-compromised strains were established to ensure the engraftment of human cells, tissues and organs. IL2Rγ is a shared subunit of several interleukin receptors that transduce signals required for differentiation and function of hematopoietic cells. Inactivation of Il2rg gene blocks NK cell development and eventually leads to NK cell defects (Cao et al. 1995). *Prkdc* is required for the V(D)J recombination process during the B cell and T cell development, and loss-of-function mutation of *Prkdc* leads to T and B lymphocytes defect (Blunt et al. 1995). The *scid* mice carrying *Prkdc* mutation, which led to elimination of mature T and B cells, were broadly used to engraft human tumors and facilitated the drug studies over 50 years (Bosma and Carroll 1991; Pelleitier and Montplaisir 1975). Additionally, NOD strain exhibited reduction of innate immunity, with defective macrophage activity, reduced dendritic cell function, and lack of the hemolytic complement system (Shultz et al. 1995). Thus, we disrupted the *Prdkc* and *Il2rg* genes directly in NOD mice and developed the NCG model with enhanced engraftment of human immune cells and tissues. The immune system of NCG was severely impaired due to the defects of functional T, B, NK cells, the complement system, the dendritic cells and macrophages (data not shown), showing quite similar features to that of the

previously developed NSG (Ishikawa et al. 2005; Shultz et al. 2005) and NOG (Ito et al. 2002; Yahata et al. 2003) strains. Besides broadly used to constitute cell-derived xenograft (CDX)and patient-derived xenograft (PDX)models (Yang et al. 2019; Zheng et al. 2020), NCG is also an ideal model for generation of human immune system (HIS), as sustainable long-term hematopoiesis of the engrafted human hematopoiesis stem cell (HSC) could be maintained in NCG. This model has been widely distributed over the world and applied in cancer research, regeneration medicine and infectious disease research (Cao et al. 2017; He et al. 2019; Lee et al. 2017; Ludwik et al. 2018; Poggio et al. 2019; Wen et al. 2018; Xia et al. 2020), as well as drug efficacy evaluation in preclinical studies. Although the long-term engraftment of human immune cells, mainly the T cells, was achieved in NCG, other populations of immune cells were challenging to be reconstituted, largely due to the lack of essential cytokines or environment for proliferation, differentiation, and maturation of the human cells. Previous works have reported that expression of human cytokines in immune-compromised mice could improve engraftment of human immune cells. For example, knock-in of human *IL2* or *IL15* gene in severely immune deficient mice implanted with human HSC cells could enhance human NK cells differentiation as reported (Herndler-Brandstetter et al. 2017; Huntington et al. 2009; Katano et al. 2015; Matsuda et al. 2019); knockout of mouse *Flt3* could improve human DC and ILC cells development (Douam et al. 2018; Li et al. 2016; Lopez-Lastra et al. 2017); overexpression of mouse *tslp* could recover murine lymphatic tissues in BRSG, thus benefit the maturation of reconstituted human T/B cells (Li et al. 2018). Thus, we generate the next generation of NCG, with certain intensive traits such as human cytokines and human HLA expression along with enhanced human cells engraftment, to create more suitable models for immune cells differentiation which are accessible for academic community (Table 2). The enhanced engraftment of human immune cells has been validated in our lab and data could be download in our website when search interested strains.

Immune checkpoint humanized mice

The promising therapeutic effects of immune checkpoint antibodies such as CTLA4 antibody *Ipilimumab* (Leach et al. 1996; Weber et al. 2009) and PD1 antibody *Pembrolizumab* (Syn et al. 2017) have extensively promoted the investment and development of macromolecular drugs in recent years. However, species barriers made the mouse not a suitable model in the preclinical evaluation of antibodies that recognize unique human targets. The human immune system (HIS) models engrafted with human tumors were applicable for the preclinical study of the checkpoint inhibitors (Wang et al. 2018). However, failure of reconstitution of different immune cell populations made such HIS models lack a fully functional immune system. Immune checkpoint gene humanized mouse strains with the competent immune system were developed to address the species obstacles between mouse and human. Over 60 immune checkpoint humanized models, e.g., *PD1*, *CTLA4, SIRPα*, *TIGIT,* and *LAG-3,* have been established at NRCMM.

Mono- and Combo- therapies against immune checkpoints are under active development in recent years. A clinical study announced in the 2020 ASCO meeting showed meaningful improvement in overall response rates (ORR) and progression-free survival (PFS) in NSCLC with combo-treatment by *Tiragolumab* (TIGIT antibody) and *Atezolizumab* (PDL1 antibody) (Rodriguez-Abreu et al. 2020). The outstanding clinical performance of the TIGIT antibody, which is a novel inhibitory immune checkpoint mainly expressed on activated T cells and NK cells in the tumor microenvironment, attracted a great deal of attention (Chauvin and Zarour 2020; Dietze et al. 2013; Solomon and Garrido-Laguna 2018). Treg cells with high TIGIT expression were found to be immune-suppressive in the tumor environment (Joller et al. 2014; Kurtulus et al. 2015). As presented in the 2018 AACR meeting, the anti-TIGIT antibody EOS884448 was proven to kill Tregs in vitro when carrying hIgG1, but not other isotypes. Similarly, the surrogate antibody of EOS884448 with the mIgG2a isotype (the homolog of human IgG1), rather than mIgG1 (the homolog of human IgG4), inhibited the CT26 tumor growth in vivo significantly (Dekkers et al. 2017; LEROY et al. 2018). These results indicated that Fc-mediated Tregs depletion might be a critical mechanism of action (MOA) for therapeutic anti-TIGIT antibodies. To enable the direct in vivo evaluation of human TIGIT antibodies in preclinical animal models, we generated TIGIT mono-humanized and TIGIT/PD1 double-humanized mice in both C57BL/6 and BALB/c background which are prone to Th1 and Th2 predominant immune response respectively (Sellers et al. 2012). Interestingly, the antitumor effect of human TIGIT antibody (*Tiragolumab analog,* hIgG1 isotype) was recapitulated in humanized mice in BALB/c engrafted with CT26 tumor but not in humanized C57BL/6 mice with MC38 tumor. Furthermore, noticeable Tregs depletion and robust NK cells increase were observed in tumor-infiltrating lymphocytes (TILs) analysis in the BALB/c background models, while no significant change was identified in C57BL/6 background models. Additionally, the potent antitumor effect of *Tiragolumab* analog was eliminated while using the DANA variant (FcγR-mediated ADCC blockage) to treat tumor bearing mice (data not shown). Together, these results indicated that the antitumor activity of TIGIT antibody was attributed to the Fc-mediated Tregs depletion through NK cells, which was consistent with previous reports (LEROY et al. 2018; Yang et al. 2020). Thus, TIGIT humanized

Table 2 Next generation NCG mice with association phenotypes

Strain code	Models	Phenotype	Genetic background	Status
T003802	NCG-X (With *Kit*^{W41} mutation in NCG)	Engraftment with HSC without radiation, and improved human erythropoiesis and platelet formation (Bao et al. 2021; McIntosh et al. 2015; Rahmig et al. 2016; Waskow et al. 2009)	NCG	Live mice
T004886	NCG-*hIL15*	Enhanced human NK cells reconstitution and function as reported (Herndler-Brandstetter et al. 2017; Huntington et al. 2009; Matsuda et al. 2019)	NCG	Live mice
T017543	NCG-*hIL2*	Improved development of mature and functional human reconstituted NK cells and T cells as reported (Katano et al. 2015)	NCG	Live mice
T005667	NCG-*Tslp*	Has been reported with accelerated recovery of murine lymphatic tissues, and improved antigen-specific immune responses(Li et al. 2018)	NCG	Live mice
T006831	NCG-*hIL6*	Has been reported with improved T/B cell differentiation and antigen-specific immune responses (Yu et al. 2017)	NCG	Live mice
T006833	NCG-*hIL7*	Has been reported with enhanced T cells engagement and development (van Lent et al. 2009)	NCG	Live mice
T006835	NCG-*hBAFF*	Has been reported with improved B cells survival in human peripheral blood lymphocytes(Hu-PBL) model (Schmidt et al. 2008)	NCG	Live mice
T050104	NCG-*Flt3* KO	Has been reported with enhanced human DCs, NK cells and ILCs differentiation (Douam et al. 2018; Li et al. 2016; Lopez-Lastra et al. 2017)	NCG	Under development
T036669	NCG-*SGM3*	Has been reported with enhanced hematopoietic activity and stem cell colonization, and myeloid cell frequencies and function (Billerbeck et al. 2011)	NCG	Live mice
T003278	NCG-*HLA-A2.1*	Has been reported with enhanced functional T cells percentage and immune responses (Masse-Ranson et al. 2019)	NCG	Cryopreserved
T004670	NCG-*B2m* KO	Has been reported with decreased GvHD in the Hu-PBL model (Brehm et al. 2010, 2019)	NCG	Live mice
T038060	NCG-*H2d1-H2k1*-KO	Has been reported with decreased GvHD in the Hu-PBL model(Brehm et al. 2019)	NCG	Under development
T050886	NCG-*H2k1-H2d1-H2ab1*-KO	Has been reported with decreased GvHD in the Hu-PBL model, (Ashizawa et al. 2017; Madsen et al. 1999)	NCG	Under development

mouse model in BALB/c background not only recapitulated the antitumor efficacy, but also the mechanism of action (MOA), of the therapeutic TIGIT antibodies. Also, we found obviously different responsiveness of PD1 antibodies when tested in PD1 humanized models in BALB/c and C57BL/6 background (data not shown), which might cause by the distinct immune profile, such as proportion of Treg (Chen et al. 2005) and M2 macrophages (Martinez 2011; Sellers et al. 2012), and as well as Th2 cytokines production (Trunova et al. 2011), between BALB/c and C57BL/6 background.

Given the importance of the genetic background of mouse models on the immune responsiveness to cancer treatment, we developed immune checkpoint humanized mouse models on C57BL/6 and BALB/c, respectively. These models, especially humanized models in BALB/c background, would provide tools for better understanding of disease and investigation of therapies for the academic and industrial communities.

ACE2-humanized mouse models

The COVID-19 pandemic outbreak at the end of 2019 is caused by severe acute respiratory syndrome coronavirus 2(SARS-CoV-2) (Ou et al. 2020). SARS-CoV-2 enter host cells via the angiotensin-converting enzyme 2 (ACE2) receptor, which has been identified as common receptor for various viruses, such as SARS-CoV (Kuhn et al. 2004). Since SARS-CoV or SARS-CoV-2 does not bind efficiently to the mouse ACE2 (Qiu et al. 2020), *ACE2*-humanized mice are indispensable animal models for studying the mechanism of *ACE2* related to the COVID-19 outbreak and developing specific drugs, antibodies, and vaccines. We have generated several *hACE2* strains based on different strategies and mouse genetic background as listed in Table 3 since COVID-19 outbreak. The strain Hu*ACE2*-FL(T037659), with full length human *ACE2* CDS in situ replacement of mouse *Ace2*, showed moderate alveolar septal thickening and inflammatory cell infiltration following SARS-CoV-2 challenge in the work presented by Qiao et al. (2021). In this study, they designed 32 protease inhibitors targeting the main protease (M^{pro}) of SARS-CoV-2, the one plays a central role in viral replication, and two of these showed strong antiviral activity with significantly reduced lung viral loads and lung lesions in Hu*ACE2*-FL(T037659) mice (Qiao et al. 2021). The strain K18-Hu*ACE2* (T037657) was developed by inserting *hACE2* CDS driven by the human cytokeratin 18 (*K18*) promoter targeting epithelial cells. Human *ACE2* mRNA was detected with the highest expression level in lung and also in other tissues, including the heart, kidney and intestine of the transgenic mice (data not shown). More severe lung pathologic changes with massive infiltrating cells and obvious alveolar necrosis following influenza A virus (IAV) and SARS-CoV-2 co-infection were presented in this mouse model (Bai et al. 2021). Another strain Hu*ACE2*-Chimera (T037630) was generated by in situ replacement of mouse *Ace2* with a chimeric *ACE2* containing a murine signal peptide, a human extracellular domain, a murine transmembrane domain and intracellular domain. Ma et al. evaluated nanoparticle vaccines efficacy in this transgenic mouse, and reported that high titer of Receptor Binding Domain (RBD) specific IgG after RBD and RBD-HR vaccines immunized were produced, followed by the challenge of authentic SARS-CoV-2

Table 3 Humanized ACE2 mouse models with association phenotypes

Strain code	Models	Phenotype	Genetic background	Strategy
T037657	K18-Hu*ACE2*	Accelerated thrombus formation following SARS-CoV-2 spike protein-treated (Zhang et al. 2020), and more severe lung pathologic changes with massive infiltrating cells and obvious alveolar necrosis following IAV and SARS-CoV-2 co-infection (Bai et al. 2021)	C57BL/6JGpt	Human *ACE2* CDS under control of *K18* promoter
T037630	Hu*ACE2*-Chimera	Severe inflammation and swelling of the whole lung after infected with SARS-CoV-2S-RBD (Alitongbieke et al. 2020) and production of high titer of RBD-specific IgG after RBD and RBD-HR vaccines immunized, followed by the challenge of authentic SARS-CoV-2 (Ma et al. 2020)	C57BL/6JGpt	Chimeric human *ACE2* CDS in suit insertion
T037659	Hu*ACE2*-FL	Moderate alveolar septal thickening and inflammatory cell infiltration following SARS-CoV-2 challenge (Qiao et al. 2021)	C57BL/6JGpt	Full length of human *ACE2* CDS in suit insertion
T037915	BALB/c-h*ACE2*	N/A	BALB/cJGpt	Refer to T037630
T037766	NCG-Hu*ACE2*-FL	N/A	NCG	Refer to T037659

(Ma et al. 2020). Severe inflammation and swelling of the whole lung after infected with SARS-CoV-2S-RBD were also displayed in this strain (Alitongbieke et al. 2020). The information including model strategies could be found in our website www.gempharmatech.com. The users can also access to the ACE2 information as well as the strategies in MGI-Find Mice (IMSR, repository-GPT).

Healthy and genetic quality control

Healthy monitoring is important for providing high quality mouse models. We preformed the strict SPF monitoring program including necropsy, histopathological analysis and serologic, bacteriologic and parasitic tests. Sentinel mice exposed to dirty bedding in each rack are used for periodic pathogens monitoring, and pathogens need to be tested and excluded are listed in Table 4.

Genetic monitoring is another important quality control to confirm strain identity and generate congenic lines. We use single nucleotide polymorphisms (SNPs), the most abundant class of markers in genome, to distinguish genetically close strains (Mekada et al. 2009). Genome-wide SNP scanning panels were developed for 13 inbred strains including a total of 794 SNP loci, and performed every year for genetic monitoring of inbred and genetically engineering strains (Table 4).

Genetic modifications of these mouse strains were confirmed by PCR-based genotyping or southern blot. More validation studies, such as tissue specific expression/knockout analysis and pathological assessment were also performed based on the model types and their application. All validation data, model design strategies and genotyping protocol could be downloaded from website for individual model.

Future perspective

The GEMMs for rare disease research initiative

Rare diseases (RDs), the one do not yet have universal definition, refer to any disease with low prevalence, which comprise more than 7000 different conditions (Montserrat Moliner and Waligora 2013). They are usually life-threatening, chronic and clinically complex, affecting 3.5–5.9% of the world's population (Murillo-Cuesta et al. 2020). With the lacking of larger enough market and economic gains, they are also known as orphan diseases, meaning difficulty to gain support and resources for discovering treatment. Although greater attention being paid to RDs worldwide, leading to increasing number of studies of RDs and new drug development, more than 95% of the RDs currently have no effective treatment (Kaufmann et al. 2018). Accordingly, RDs are considered a public health priority, and specific research programs like the International Rare

Table 4 Healthy and genetic monitor panels

Classification	Pathogens
Virus	Ectromelia virus (Ect), Mouse hepatitis virus (MHV), Sendai virus (SV), Pneumonia virus of mice (PVM), Reovirus 3 (Reo-3), Minute virus of mice (MVM), Lymphocytic choriomeningitis (LCMV), Hantavirus (HV), Theiler's Mouse Encephalomyelitis Virus (TMEV), Mouse Adenovirus (FL)(MAD 1), Mouse Adenovirus (K87)(MAD 2), Polyoma Virus (POLY), Mouse Parvovirus (MPV), Mouse Cytomegalovirus (MCMV), Murine Norovirus (MNV), Epizootic Diarrhea of Infant Mice (EDIM), K virus LDH elevating virus (LDV), Mouse Kidney Parvovirus (MKPV)
Bacteria and mycoplasma	Salmonella spp, Mycoplasma pulmonis, Corynebacterium kutscheri, Tyzzer's organism, Pasteurella pneumotropica, Klebsiella pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa, Pathogenic dermal fungi, Yersinia pseudotuberculosis, Yesinia enterocolitica, Streptobacillus moniliformis, Escherichia coli O115 a,C,K(B), Streptococcus pneumoniae, β-hemolytic streptococcus, Helicobacter, Cilia-Associated Respiratory Bacillus (CARB), Corynebacterium bovis, Klebsiella oxytoca, Pasteurella multocida, Citrobacter rodentium, Proteus mirabilis
Parasites and protozoa	Ectoparasites (e.g. fleas, mites and lice), Toxoplasma gondii, Helminth (e.g. pinworm), Flagellata, Ciliata, Encephalitozoon cuniculi, Pneumocystis spp
Inbred SNP panels	
C57BL/6NGpt, C57BL/6JGpt, CBA/CaJGPt, DBA/1JGpt, DBA/2JGPt, A/JGpt, BALB/cJGpt, C57BL/10JGpt, FVB/NJGpt, C57BLKS/JGpt, NOD/ShiLtJGpt, B6(Cg)-Tyrc-2J/N, 129S1/SvImJGpt	

Diseases Research Consortium (IRDiRC) have been established, with the goal of accelerating diagnosis and approving 1000 new therapies for RDs by 2027 (Gahl et al. 2016; Murillo-Cuesta et al. 2020). NRCMM joined China Alliance for Rare Disease in 2020. The China Alliance for Rare Disease (CHARD, https://www.chard.org.cn/) is a national, non-profit, cooperative exchange platform for rare diseases, approved by the national health authority, and supported by Peking Union Medical College Hospital, PhiRDA, the Chinese Medical Association, the Chinese Hospital Association and the Chinese Research Hospital Association, as well as multiple universities, scientific research institutes and enterprises. Alliance aims to improve standardized rare disease diagnosis, treatment and education for patients and the medical community in China.

RDs are excellent candidates for generation of GEMMs, as most RDs involve mutations in a single gene. GEMMs are indispensable to identify the genetic bases and molecular mechanisms of RDs, as well as to understand their physiopathology, clinical heterogeneity and genotype–phenotype correlations, and ultimately, to develop intervention or treatment of RDs (Boat and Field 2011). Usually, medical translation moves from bench to clinic, but in the study of RDs, we need change direction to work from clinic to bench. To this end, NRCMM launched another large scale mission, "The GEMMs for Rare Disease Research Initiative" in 2020. The initiative aims to provide high quality and cost effective genetically engineered mouse models (GEMMs) for previously underfunded biomedical research areas. With high throughput pipeline successfully practicing in KOAP and abundant of clinical RDs findings, the initiative is with ambitious to cover all human rare diseases models caused by point mutation, which will serve to illustrate the phenotypic variability of RDs to fill the knowledge gaps in this area. We hope to provide a set of models for clinicians and scientists to create an optimal pathway for translating clinic to bench and then back to clinic, and contribute to drug discovery and development process. We are enthusiastic to collaborate with clinicians and scientists to develop the point mutation models. So far, "The GEMMs for Rare Disease Research Initiative" has generated 87 GEMMs, which are of great value in supporting biomedical research and novel drug research and development in this area. In 2021, we continue to encourage applications for "The GEMMs for Rare Disease Research Initiative" to promote the research and novel therapeutic development for the treatment of rare diseases. The introduction of "The GEMMs for Rare Disease Research Initiative" is linked below

https://www.gempharmatech.us/pages/gem_funding_opportunities.php

We are dedicated to keep expanding our repository by developing various new GEMMs models, e.g. knockout/conditional knockout mouse models, drug target gene humanized models, human disease mouse models. With the high throughput pipelines for mouse phenotyping, we contributed to the phenotyping project of knockout mice initiated by IMPC followed standardized phenotyping protocols from international mouse phenotyping resource of standardized screens (IMPRess). The integration of massive GEMMs resource with standardized phenotyping platform in our center will help academic and industrial community to harnessing the power of the genome to drive biomedical discovery. We are willing to keep contributing to the basic research and drug preclinical studies. By creating a publicly available resource of GEMMs and phenotyping platform, NRCMM will continue to make effort to knock down barriers for biologists to use mouse models in their research.

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Data availability Addition datasets used or analyzed for the current study are available from the corresponding authors on reasonable request.

Code availability Not applicable.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures involving animals were approved by Institutional Animal Care and Use Committee (IACUC) of GemPharmatech Co.Ltd.

Consent to participate Informed consent was obtained from all individual participants included in this work.

Consent for publication Consent for publication was obtained from all authors.

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