Genetically Engineered Mouse Models of K-Ras-Driven Lung and Pancreatic Tumors: Validation of Therapeutic Targets

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K-RAS signaling has been intensely studied for over 40 years. Yet, as of today, no drugs have been approved to treat *K-RAS* mutant cancers. Since the turn of the century, scientists have used genetically engineered mouse (GEM) models to reproduce *K-RAS* mutant cancers in a laboratory setting to elucidate those molecular events responsible for the onset and progression of these tumors and to identify suitable therapies. In this review, we outline a brief description of available GEM models for two tumor types known to be driven by *K-RAS* mutations: lung adenocarcinoma and pancreatic ductal adenocarcinoma. In addition, we summarize a series of studies that have used these GEM tumor models to validate, either by genetic or pharmacological approaches, the therapeutic potential of a variety of targets, with the ultimate goal of translating these results to the clinical setting.

Saturation analysis of more than 4700 human tumors has identified more than 200 bona fide cancer genes (Lawrence et al. 2014). Among the five cancer genes most frequently mutated appears to be *K-RAS*, an oncogene mutated in more than a quarter of all human tumors. *K-RAS* mutations are not equally distributed. They appear in pancreatic ductal adenocarcinoma (PDAC) (>90%), colorectal carcinoma (45%), lung adenocarcinoma (LAC) (25%), and endometrial carcinoma (15%) with lower incidence rates in several other tumor types (Pylayeva-Gupta et al. 2011; Eser et al. 2014). U.S. estimates for 2016 alone indicate that there will be more than 330,000 new cases and close to 120,000 deaths caused by *K-RAS* mutant pancreatic and lung tumors, two tumor types

in which experimental evidence indicates that these mutations are the initiating events of the neoplastic process.

K-RAS signaling has been intensely studied for over 40 years. These studies have revealed that the main signaling pathways that convey *K-RAS* oncogenic activity are kinase cascades that could be amenable to interference with selective inhibitors. Yet, as of today, not a single targeted drug has been approved to treat *K-RAS* mutant cancers. Thus, it is essential to get a better understanding about *K-RAS* signaling and to develop experimental systems in which scientists can test novel therapeutic strategies that can be eventually translated to the clinic.

Since the turn of the century, scientists have focused on the generation of genetically engi-

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neered mouse (GEM) models of *K-RAS* mutant cancer that faithfully reproduce the natural history of the human disease. Most of these models carry endogenous mutations in those loci known to be the key drivers in human cancer such as *K-Ras*, *p53*, *Ink4a/Arf*, *Lkb1*, *Pten*, etc. In this review, we summarize current information regarding available GEM models for two tumor types known to be driven by *K-RAS* mutations, LAC, and PDAC. In addition, we summarize those studies that have used these GEM tumor models to validate, either by genetic or pharmacological approaches, the therapeutic potential of selected targets with the ultimate goal of translating these results to the clinical setting.

MOUSE MODELS OF *K-RAS* MUTANT LUNG ADENOCARCINOMA

Several laboratories have developed GEM models that faithfully reproduce human LAC by introducing oncogenic mutations into resident *K-Ras* alleles. These GEM models allow systematic and reproducible interrogation of the therapeutic properties of suspected targets using sophisticated genetic strategies. However, although these GEM models often recapitulate the natural history and histologic properties of human tumors, in general, they display a more limited mutational complexity than those human tumors they are intended to model (McFadden et al. 2016).

The first GEM model of *K-RAS* mutant LAC was developed by Jacks, Tuveson, and colleagues by generating mutant *K-Ras* alleles, designated as *K-Ras*^{LA1} and *K-Ras*^{LA2}, which after spontaneous somatic recombination encoded a functional *K-Ras*^{G12D} oncoprotein (Johnson et al. 2001). These mice developed LACs with 100% incidence as well as thymic lymphomas and papillomas with reduced prevalence. Soon thereafter, these investigators developed a more sophisticated model that expressed an endogenous *K-Ras*^{G12D} allele (*K-Ras*^{LSLG12D}) in a temporally and spatially controllable manner by Cre-mediated recombination (Jackson et al. 2001). Selective activation of *K-Ras*^{G12D} expression in lung tissue can be easily accomplished by intranasal instillation or intratracheal in-

fection with adenoviral particles expressing the Cre recombinase. A similar strain carrying a G12V mutation was generated in our laboratory (Guerra et al. 2003). This strain, *K-Ras*^{+/LSLG12Vgeo}, also carries sequences that encode a bacterial protein (geo) that provides β-galactosidase activity that serves as a color marker (Guerra et al. 2003). Intratracheal infection with adenoviral particles yields results very similar to those reported by Tuveson, Jacks, and coworkers. More recently, Jacks and colleagues have generated a variant of their *K-Ras*^{+/LSLG12D} strain in which the loxP sites flanking the transcriptional termination element were replaced by frr sequences (Young et al. 2011). This strain, *K-Ras*^{+/FSFG12D}, allows uncoupling of oncogene expression (mediated by the yeast FLP recombinase) from target inactivation or any other genetic manipulation that relies on the use of the Cre recombinase. It is worth mentioning that Varmus and colleagues developed another transgenic model by generating a strain that expressed the *K-Ras4b*^{G12D} isoform in alveolar type II cells under the control of a doxycycline response element (*Spc-rtTA-tetO-K-RAS4b*^{G12D}) (Fisher et al. 2001). This model gives rise to LACs similar to the GEM strains described above, and has also been used to show that these tumors require continuous expression of the *K-Ras4b*^{G12D} oncoprotein for maintenance.

Although all strains described above develop LACs that are highly reminiscent of human tumors, human LACs carry additional oncogenic insults that result in more aggressive neoplasias that are likely to alter therapeutic responses (Skoulidis et al. 2015). Some of these cooperating oncogenic insults have also been modeled in GEM models of *K-Ras* LAC by concomitantly ablating tumor suppressor genes such as *p53* (Jackson et al. 2005), *Ink4a/Arf* (Ji et al. 2007), or *Lkb1* (Ji et al. 2007). Loss of *p53* or expression of mutant *p53* alleles causes lung tumors to progress much more rapidly and eventually yield metastatic lesions (Jackson et al. 2005). Similar results were obtained by deletion of the *Ink4a/Arf* locus. Ablation of *Lkb1* caused tumors to progress even faster and to metastasize with higher incidence (Ji et al. 2007).

TARGET VALIDATION: TUMOR INITIATION MODELS

GEM tumor models were first used to genetically validate targets of potential therapeutic interest by knocking-out candidate genes in the germline (providing they did not significantly affect viability) or by using conditional floxed alleles that were ablated concomitantly with the induction of *K-Ras* oncogene expression (Kwon and Berns 2013; Drosten and Barbacid 2016). Thus, the observed therapeutic responses may have limited clinical applicability, because the requisites to block tumor initiation are likely to be far less stringent than those necessary to induce tumor regression (Drosten and Barbacid 2016). The majority of these studies have focused on targets that are thought to play a role as K-Ras downstream effector molecules such as the members of the mitogen-activated protein kinase (MAPK) pathway (Raf, Mek, and Erk kinases, Etv5, Jnk, Cdk4, Smyd3), other Ras-like molecules (Ral, Rac) or metabolic enzymes (Acsl, Siva, Hk2, Cox2). Other studies have validated targets known to be involved in the Notch (Notch1, Psen1, Psen2, Rbpj), NF- κ B (I κ B α , p65/RelA, I κ B β), or PI3K (p110 α , p85 α , p85 β , mTRAIL-R) pathways. Genes encoding targets involved in other cellular processes such as ribosomal RNA (rRNA) synthesis (Ect2), telomere elongation (Trf1), senescence prevention (Wt1), and control of cell proliferation (Fadd, Csn1k1, Foxm1) have also been validated (Table 1). Altogether, these studies have helped to unravel mechanisms implicated in the early steps of tumor development. Because of space limitations, they will not be discussed in this review but they are listed in Table 1.

TARGET VALIDATION: THERAPEUTIC MODELS

K-Ras^{LA2} Model

This model develops spontaneous tumors. Hence, target validation can be performed using floxed alleles that can be inactivated on activation of a Cre recombinase in tumor-bearing animals (Table 2). Downward and col-

leagues used a conditional *Pik3ca*^{RBDlox} allele that on Cre-mediated recombination expresses a p110 α PI3K subunit defective in Ras binding (Castellano et al. 2013). Acute expression of this p110 α mutant isoform in established *K-Ras*^{G12D} lung tumors caused long-term tumor stasis and, in some cases, partial regression (Castellano et al. 2013). The same laboratory also identified a requirement for the Gata2 transcriptional network. Conditional elimination of Gata2 from established lung tumors led to near-complete tumor regression rates (Kumar et al. 2012). The same model was also used to ablate Hexokinase 2 from established tumors causing reduced tumor progression rates (Patra et al. 2013).

K-Ras^{LSLG12D} and K-Ras^{LSLG12Vgeo} Models

Since these models rely on the Cre recombinase for tumor induction, they are not particularly well suited to validate therapeutic targets in preexisting tumors because target ablation often relies on the Cre/loxP system. However, there are some examples in which target inactivation was performed by different experimental approaches. For instance, the *K-Ras*^{+ / LSLG12D} strain was used to validate Myc by expressing a dominant-negative peptide (Omomyc) using a doxycycline-dependent expression system (Soucek et al. 2008). Induction of Omomyc expression led to overt tumor regression even in aggressive tumors driven by mutant K-Ras expression and p53 ablation without significant toxicity under conditions of controlled Omomyc expression (Soucek et al. 2013). This model was also used to block the NF- κ B pathway by infecting *K-Ras*^{+ / LSLG12D}; *p53*^{lox / lox} mice with a lentivirus that expressed a dominant negative I κ B α super repressor under the control of a doxycycline-inducible promoter. Tumors still grew but at a lower rate than in control mice (Meylan et al. 2009). Finally, Tran et al. (2012) observed tumor stasis when they reduced Twist1 expression in *K-Ras*^{+ / LSLG12D} mice carrying advanced lung tumors using a doxycycline-dependent transgene. Finally, the related *K-Ras*^{+ / LSLG12Vgeo} model has been used to validate the therapeutic effect of Cdk4 by using con-

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Table 1. Target validation in tumor initiation models driven by K-Ras oncogenes

Model	Target(s)	Tumor phenotype	References
<i>K-Ras</i> ^{+/LA2}	<i>p110α</i> ^{RBDmut/RBDmut}	No tumor development	Castellano et al. 2013
<i>K-Ras</i> ^{+/LSLG12D}	<i>c-Raf</i> ^{lox/lox}	No tumor development	Karreth et al. 2011
<i>K-Ras</i> ^{+/LSLG12D}	<i>RalA</i> ^{lox/lox} ; <i>RalB</i> ^{-/-}	No tumor development	Peschard et al. 2012
<i>K-Ras</i> ^{+/LSLG12D}	<i>Rac1</i> ^{lox/lox}	No tumor development	Kissil et al. 2007
<i>K-Ras</i> ^{+/LSLG12D}	<i>Jnk1</i> ^{lox/lox} ; <i>Jnk2</i> ^{-/-}	No tumor development	Cellurale et al. 2011
<i>K-Ras</i> ^{+/LSLG12D}	<i>Etv5</i> ^{lox/lox}	No tumor development	Zhang et al. 2017
<i>K-Ras</i> ^{+/LSLG12D}	<i>Pik3r1</i> ^{lox/lox} ; <i>Pik3r2</i> ^{-/-}	Decreased tumor development	Engelman et al. 2008
<i>K-Ras</i> ^{+/LSLG12D}	<i>Notch1</i> ^{lox/lox}	Decreased tumor development	Licciulli et al. 2013
<i>K-Ras</i> ^{+/LSLG12D}	<i>Wt1</i> ^{lox/lox}	Decreased tumor development	Vicent et al. 2010
<i>K-Ras</i> ^{+/LSLG12D}	<i>Fadd</i> ^{lox/lox}	Decreased tumor development	Bowman et al. 2015
<i>K-Ras</i> ^{+/LSLG12D}	<i>Csn1k1</i> ^{lox/lox}	Decreased tumor development	Bowman et al. 2015
<i>K-Ras</i> ^{+/LSLG12D}	<i>Siva</i> ^{lox/lox}	Decreased tumor development	Van Nostrand et al. 2015
<i>K-Ras</i> ^{+/LSLG12D}	<i>Smyd3</i> ^{-/-}	Decreased tumor development	Mazur et al. 2014
<i>K-Ras</i> ^{+/LSLG12D}	<i>Acs13</i> ^{-/-}	Decreased tumor development	Padanad et al. 2016
<i>K-Ras</i> ^{+/LSLG12D}	<i>Cox2</i> ^{-/-}	Decreased tumor development	Pan et al. 2015
<i>K-Ras</i> ^{+/LSLG12D}	<i>Hk2</i> ^{lox/lox}	Decreased tumor development	Patra et al. 2013
<i>K-Ras</i> ^{+/LSLG12D} ; <i>p53</i> ^{lox/lox}	DN-IκBα (Tet-On)	Decreased tumor development	Meylan et al. 2009
<i>K-Ras</i> ^{+/LSLG12D} ; <i>p53</i> ^{lox/lox}	<i>p65</i> ^{lox/lox}	Decreased tumor development	Bassères et al. 2010
<i>K-Ras</i> ^{+/LSLG12D} ; <i>p53</i> ^{lox/lox}	<i>Ect2</i> ^{lox/lox}	Decreased tumor development	Justilien et al. 2017
<i>K-Ras</i> ^{+/LSLG12D} ; <i>p53</i> ^{lox/lox}	<i>Trkb</i> ^{lox/lox}	Reduced metastasis	Sinkevicius et al. 2014
<i>K-Ras</i> ^{+/LSLG12D} ; <i>p53</i> ^{+/LSLR172H}	<i>mTRAIL-R</i> ^{lox/lox}	Decreased tumor development	von Karstedt et al. 2015
<i>K-Ras</i> ^{+/LSLG12D} ; <i>sh-p53</i>	<i>Ikkβ</i> ^{lox/lox}	Decreased tumor development	Xia et al. 2012
<i>K-Ras</i> ^{+/LSLG12Vgeo}	<i>c-Raf</i> ^{lox/lox}	No tumor development	Blasco et al. 2011
<i>K-Ras</i> ^{+/LSLG12Vgeo}	<i>Mek1</i> ^{lox/lox} ; <i>Mek2</i> ^{-/-}	No tumor development	Blasco et al. 2011
<i>K-Ras</i> ^{+/LSLG12Vgeo}	<i>Erk1</i> ^{-/-} ; <i>Erk2</i> ^{lox/lox}	No tumor development	Blasco et al. 2011
<i>K-Ras</i> ^{+/LSLG12Vgeo}	<i>Cdk4</i> ^{-/-}	No tumor development	Puyol et al. 2010
<i>K-Ras</i> ^{+/LSLG12Vgeo}	<i>Psen1</i> ^{lox/lox} ; <i>Psen2</i> ^{-/-}	Decreased tumor development	Maraver et al. 2012
<i>K-Ras</i> ^{+/LSLG12Vgeo}	<i>Rbpj</i> ^{lox/lox}	Decreased tumor development	Maraver et al. 2012
<i>K-Ras</i> ^{+/LSLG12Vgeo} ; <i>p53</i> ^{-/-}	<i>Trf1</i> ^{lox/lox}	Decreased tumor development	García-Beccaria et al. 2015
<i>Tet-op-K-Ras4b</i> ^{G12D}	<i>Foxm1</i> ^{lox/lox}	Decreased tumor development	Wang et al. 2014

ditional *Cdk4*^{flr} alleles (Puyol et al. 2010). However, this approach is not recommended because the Flp recombinase is less efficient than the bacterial Cre recombinase, resulting in limited target ablation.

Spc-rtTA-tetO-K-RAS4b^{G12D} Model

In this model, the K-Ras4b protein is selectively expressed in lung tissue (mainly SP-C⁺ AT2 cells) on treatment with doxycycline (Fisher et al. 2001). Once the tumors are formed, targets to be validated can be ablated by incorporating their corresponding floxed alleles

along with an inducible CreERT2 recombinase followed by exposure to tamoxifen (Tam). This model has been used to show that ablation of the metabolic enzyme lactate dehydrogenase A caused limited regression of K-Ras^{G12D}-induced tumors (Xie et al. 2014).

K-Ras^{+/FSFG12D} Model

This tumor model allows tumor induction on expression of the Flp recombinase in lung tissue, most frequently via intranasal instillation or intratracheal infection with adenoviral particles. Once tumors are formed, targets can be

Table 2. Target validation in established *K-Ras*-driven lung adenocarcinomas

Model	Target	Tumor phenotype	Systemic toxicity	References
<i>K-Ras</i> ^{+/LA2}	<i>p110α</i> ^{RBDlox/-}	Tumor stasis and partial regression	No significant toxicity	Castellano et al. 2013
<i>K-Ras</i> ^{+/LA2}	<i>Gata2</i> ^{lox/lox}	Regression of established tumors	No significant toxicity	Kumar et al. 2012
<i>K-Ras</i> ^{+/LSLG12D}	Omomyc (c-Myc)	Long-term tumor eradication	Reversible toxicity	Soucek et al. 2013
<i>K-Ras</i> ^{+/LSLG12D}	Twist1 (Tet-On)	Tumor stasis, senescence	ND ^a	Tran et al. 2012
<i>K-Ras</i> ^{+/LSLG12D} ; <i>p53</i> ^{ER/ER}	Omomyc (c-Myc)	Long-term tumor eradication	Reversible toxicity	Soucek et al. 2013
<i>K-Ras</i> ^{+/LSLG12D} ; <i>p53</i> ^{lox/lox}	DN-IκBα (Tet-On)	Inhibition of tumor progression, apoptosis	ND	Meylan et al. 2009
<i>K-Ras</i> ^{+/LSLG12Vgeo}	<i>Cdk4</i> ^{frt/frt}	Inhibition of tumor progression	No significant toxicity	Puyol et al. 2010
<i>Tet-op-K-Ras4b</i> ^{G12D}	<i>Ldha</i> ^{lox/lox}	Inhibition of tumor progression	Nonlethal anemia	Xie et al. 2014
<i>K-Ras</i> ^{+/FSFG12D} ; <i>p53</i> ^{frt/frt}	<i>Atg7</i> ^{lox/lox}	Inhibition of tumor progression	Long-term toxicity	Karsli-Uzunbas et al. 2014

^aND, Not determined.

validated by using conditional floxed alleles along with an inducible CreERT2 recombinase activated by exposure to Tam or by using inducible Cre transgenes. White and coworkers have used this model, including conditional *p53*^{frt} alleles (Lee et al. 2012), to show that the autophagy-related protein Atg7 was required for progression of *K-Ras*/*p53*-driven LAC tumors (Karsli-Uzunbas et al. 2014).

PHARMACOLOGICAL STUDIES

GEM tumor models, if properly designed, closely recapitulate the natural history of those human tumors they try to model. The fact that GEM tumors are initiated by the same endogenous mutations responsible for their human counterparts also serve as an excellent tool to validate pharmacological approaches before they are translated to the clinic. Indeed, GEM tumor models have proven their value in mouse coclinical trial platforms, where mice are subjected to experimental protocols that mirror clinical trials in human patients (Clohessy and Pandolfi 2015).

Johnson and colleagues have treated *K-Ras*^{+/LSLG12D}; *p53*^{frt/frt} mice infected with adenoviruses expressing Flp and Cre recombinases

to generate aggressive LACs, which were subsequently treated with an antibody against vascular endothelial growth factor (VEGF) in combination with standard chemotherapy under experimental conditions that mirrored prior clinical trials (Singh et al. 2010). The similarity between the results obtained in the mouse tumor models with those previously observed in the clinic proved the value of these models to predict clinical outcomes (Singh et al. 2010). Another coclinical trial showed that treatment with the MEK inhibitor Selumetinib in combination with chemotherapy was beneficial only in *K-Ras*^{+/LSLG12D} or in compound *K-Ras*^{+/LSLG12D}; *p53*^{lox/lox} mice, but not in *K-Ras*^{+/LSLG12D}; *Lkb1*^{lox/lox} animals, thus indicating that loss of Lkb1 modified the therapeutic response (Chen et al. 2012). The same group has further shown that blocking two of the most relevant K-Ras downstream pathways with MEK and PI3K inhibitors resulted in synergistic shrinkage of lung tumors in *K-Ras*^{+/LSLG12D} mice (Engelman et al. 2008). Another study has shown that combining the MEK inhibitor Trametinib with inhibition of FGFR1 was also effective in *K-Ras*^{+/LSLG12D}; *p53*^{-/-} tumors (Manchado et al. 2016). Unfortunately, the differential tolerance of rodents and humans

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to MEK inhibitors have limited the clinical applicability of these studies.

Other investigators have reported that metastases from $K-Ras^{+/LSLG12D};Lkb1^{lox/lox}$ mice showed increased Src signaling and that combination of MEK, PI3K, and SRC inhibitors was able to cause regression of these metastasis (Carretero et al. 2010). In another study, Scaglioni and colleagues observed that loss of *Ink4a/Arf* caused aberrant signaling through RhoA and FAK in $K-Ras^{+/LSLG12D}$ mice (Konstantinidou et al. 2013). Treatment with the FAK inhibitor PF562271 caused regression of lung tumors in $K-Ras^{+/LSLG12D};Ink4a/Arf^{-/-}$ mice, but not of those initiated in $K-Ras^{+/LSLG12D}$ mice. Work from our laboratory has identified DDR1 as a promising target by focusing on genes induced during the earliest stages of tumor development (Ambrogio et al. 2016). Pharmacological treatment of established $K-Ras/p53$ -driven lung tumors with a DDR1 inhibitor in combination with a Notch inhibitor, a pathway activated by DDR1, resulted in significant tumor regression with less toxic effects than standard chemotherapy.

More recently, the particular metabolic requirements of $K-Ras$ mutant LAC were also exploited pharmacologically. Shaw and colleagues have shown that blocking fatty acid oxidation by pharmacologically blocking the enzyme Acetyl-CoA carboxylase with an allosteric inhibitor suppressed growth of tumors derived from both $K-Ras^{+/LSLG12D};p53^{lox/lox}$ and $K-Ras^{+/LSLG12D};Lkb1^{lox/lox}$ mice (Svensson et al. 2016). Similarly, Vander Heiden and coworkers have revealed increased glucose contribution to the tricarboxylic acid (TCA) cycle in $K-Ras$ mutant lung tumors (Davidson et al. 2016). This property made inhibition of glutaminase ineffective, but suggested that inhibition of mitochondrial metabolism of pyruvate could be a beneficial strategy.

MOUSE MODELS OF PDAC

PDAC is one of the most lethal types of cancer for which there are no effective therapies available (Hidalgo 2010). Current experimental and pathological evidence suggests that PDAC stems

from $K-RAS$ mutations in acinar cells. When these cells undergo acinar-ductal cell metaplasia, a physiological and reversible process, they become blocked in a ductal-like phenotype that eventually progresses to form preneoplastic lesions known as pancreatic intraepithelial neoplasias (PanINs). Indeed, these benign lesions are present in many healthy pancreata (Maitra and Hruban 2008). Unfortunately, low-grade PanINs, such as PanIN1A, can progress to more aggressive lesions such as PanIN1B and PanIN2, possibly caused by additional mutations such as loss or inactivation of the *INK4a* tumor suppressor (Kanda et al. 2012). These lesions further progress to yield high-grade PanIN3, considered already as a form of in situ carcinoma, as well as invasive PDAC on accumulation of additional genetic insults, mainly inactivation of other tumor suppressors such as *P53*, *SMAD4*, or *BRCA2* (Maitra and Hruban 2008). During the last decade or so, several experimental models of human PDAC have been generated, most of them by engineering within the mouse genome those mutations responsible for the development of the human tumors. A brief description of the models most frequently used is described below and listed in Table 3.

$K-Ras^{LSLG12D};Pdx1-Cre/P48-Cre$ Model

The first experimental model that faithfully reproduced the natural history of human PDAC in mice involved expression of an endogenous $K-Ras^{G12D}$ oncogene in all epithelial pancreatic lineages during early embryonic development by crossing the conditional $K-Ras^{+/LSLG12D}$ strain described above with transgenic mice expressing the Cre recombinase under the control of the *Pdx1*- or the *Ptf1a/P48*-specific pancreatic promoters (Hingorani et al. 2003). Both compound strains, $K-Ras^{+/LSLG12D};Pdx1-Cre$ or $K-Ras^{+/LSLG12D};Ptf1a/P48-Cre$, generically known as KC mice, develop the full spectrum of PanIN lesions and PDAC tumors histologically indistinguishable from those present in human patients (Hingorani et al. 2003). A variant of the KC mice in which the Cre recombinase is driven by a *Nestin-Cre* transgene has also

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Table 3. Genetically engineered mouse pancreatic ductal adenocarcinoma (PDAC) models

Driver genes(s)	Recombinase	References
<i>K-Ras</i> ^{+/LSLG12D}	<i>Pdx1-Cre</i>	Hingorani et al. 2003
<i>K-Ras</i> ^{+/LSLG12D}	<i>Ptf1a/P48-Cre</i>	Hingorani et al. 2003
<i>K-Ras</i> ^{+/LSLG12D}	<i>Nestin-Cre</i>	Carriere et al. 2007
<i>K-Ras</i> ^{+/LSLG12D}	<i>Pdx1-CreERT</i>	Gidekel-Friedlander et al. 2009
<i>K-Ras</i> ^{+/LSLG12D}	<i>Ela-CreER</i>	De La O et al. 2008
<i>K-Ras</i> ^{+/LSLG12D}	<i>Ela-CreERT2</i>	Habbe et al. 2008
<i>K-Ras</i> ^{+/LSLG12D}	<i>Mist1-CreERT2</i>	Habbe et al. 2008
<i>K-Ras</i> ^{+/LSLG12D}	<i>proCPA1-CreERT2</i>	Gidekel-Friedlander et al. 2009
<i>K-Ras</i> ^{+/LSLG12D} ; <i>p53</i> ^{+/LSLR172H}	<i>Pdx1-Cre</i> and <i>Ptf1a/P48-Cre</i>	Hingorani et al. 2005
<i>K-Ras</i> ^{+/LSLG12D} ; <i>p53</i> ^{lox/lox}	<i>Pdx1-Cre</i> and <i>Ptf1a/P48-Cre</i>	Bardeesy et al. 2006
<i>K-Ras</i> ^{+/LSLG12D} ; <i>Ink4a/Arf</i> ^{lox/lox}	<i>Pdx1-Cre</i>	Aguirre et al. 2003
<i>K-Ras</i> ^{+/LSLG12D} ; <i>Brca2</i> ^{lox/lox}	<i>Pdx1-Cre</i>	Skoulidis et al. 2010
<i>K-Ras</i> ^{+/LSLG12D} ; <i>Lkb1</i> ^{+/lox}	<i>Pdx1-Cre</i>	Morton et al. 2010
<i>K-Ras</i> ^{+/LSLG12D} ; <i>p21Cip1</i> ^{+/−}	<i>Pdx1-Cre</i>	Morton et al. 2010
<i>K-Ras</i> ^{+/LSLG12D} ; <i>Smad4</i> ^{lox/lox}	<i>Pdx1-Cre</i> and <i>Ptf1a/P48-Cre</i>	Bardeesy et al. 2006
<i>K-Ras</i> ^{+/LSLG12D} ; <i>Smad4</i> ^{lox/lox} ; <i>p53</i> ^{+/LSLR172H}	<i>Ptf1a/P48-Cre</i>	Whittle et al. 2015
<i>K-Ras</i> ^{+/LSLG12D} ; <i>Smad4</i> ^{lox/lox} ; <i>Ink4a/Arf</i> ^{lox/lox}	<i>Pdx1-Cre</i> and <i>Ptf1a/P48-Cre</i>	Bardeesy et al. 2006
<i>K-Ras</i> ^{+/LSLG12D} ; <i>TGFBR1</i> ^{lox/lox}	<i>Ptf1a/P48-Cre</i>	Ijichi et al. 2006
<i>K-Ras</i> ^{+/LSLG12D} ; <i>Pten</i> ^{lox/lox}	<i>Pdx1-Cre</i>	Ying et al. 2011
<i>K-Ras</i> ^{+/LSLG12D} ; <i>p53</i> ^{lox/lox}	<i>Pdx1-CreERT</i>	Gidekel-Friedlander et al. 2009
<i>K-Ras</i> ^{+/LSLG12D} ; <i>p53</i> ^{lox/lox}	<i>proCPA1-CreERT2</i>	Gidekel-Friedlander et al. 2009
<i>K-Ras</i> ^{+/LSLG12D} ; <i>Ink4a/Arf</i> ^{lox/lox}	<i>Pdx1-CreERT</i>	Gidekel-Friedlander et al. 2009
<i>K-Ras</i> ^{+/LSLG12D} ; <i>Ink4a/Arf</i> ^{lox/lox}	<i>proCPA1-CreERT2</i>	Gidekel-Friedlander et al. 2009
<i>K-Ras</i> ^{+/LSLG12V_{geo}}	<i>Elas-tTA;Tet-O-Cre</i>	Guerra et al. 2007
<i>K-Ras</i> ^{+/LSLG12V_{geo}} ; <i>p53</i> ^{lox/lox}	<i>Elas-tTA;Tet-O-Cre</i>	Guerra et al. 2011
<i>K-Ras</i> ^{+/LSLG12V_{geo}} ; <i>Ink4a/Arf</i> ^{lox/lox}	<i>Elas-tTA;Tet-O-Cre</i>	Guerra et al. 2011
<i>TetO-K-Ras4b</i> ^{G12D}	<i>Ptf1a/P48-Cre;R26-LSLrtTA-IRES-EGFP</i>	Collins et al. 2012
<i>TetO-LSL-K-Ras4b</i> ^{G12D}	<i>Ptf1a/P48-Cre;R26-LSLrtTA-IRES-EGFP</i>	Ying et al. 2012
<i>K-Ras</i> ^{+/FSFG12D}	<i>Pdx1-Flp</i>	Schönhuber et al. 2014
<i>K-Ras</i> ^{+/FSFG12D}	<i>Pdx1-Flp;R26-CAG-FSF-CreERT2</i>	Schönhuber et al. 2014
<i>K-Ras</i> ^{+/FSFG12D} ; <i>p53</i> ^{lox/lox}	<i>Pdx1-Flp;R26-CAG-FSF-CreERT2</i>	Schönhuber et al. 2014
<i>K-Ras</i> ^{+/FSFG12V}	<i>Elas-tTA;Tet-O-Flp</i>	MT Blasco, C Guerra, and M Barbacid, Unpubl.
<i>K-Ras</i> ^{+/FSFG12V}	<i>Elas-tTA;Tet-O-Flp;hUBC-CreERT2</i>	MT Blasco, C Guerra, and M Barbacid, Unpubl.
<i>K-Ras</i> ^{+/FSFG12V} ; <i>p53</i> ^{frt/frt}	<i>Elas-tTA;Tet-O-Flp</i>	MT Blasco, C Guerra, and M Barbacid, Unpubl.
<i>K-Ras</i> ^{+/FSFG12V} ; <i>p53</i> ^{frt/frt}	<i>Elas-tTA;Tet-O-Flp;hUBC-CreERT2</i>	MT Blasco, C Guerra, and M Barbacid, Unpubl.

been generated (Carriere et al. 2007). Yet, the incidence of PDAC tumors in these strains is rather low, at least at 1 year of age. Additional mutations present in human tumors, including inactivation or deletion of tumor suppressors

such as *p53* (KPC strain), *Ink4a/Arf*, *Brca2*, *Lkb1*, *p21Cip1*, or *Smad4* significantly accelerate progression of PanIN lesions to invasive PDAC and induce metastasis in a significant percentage of cases (Table 3). Similar results have

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been obtained on elimination of the genes encoding the transforming growth factor β (TGF- β) receptor II (*Tgfb2*) and the Pten phosphatase (*Pten*) (Ijichi et al. 2006; Ying et al. 2011) (Table 3).

K-Ras^{LSLG12V^{geo}};Ela-tTA;TetO-Cre Model

An independent GEM tumor model that also recapitulates human PDAC was generated by expressing an endogenous *K-Ras*^{G12V} oncogene in pancreatic acinar cells during late embryonic development (Guerra et al. 2007). This model, *K-Ras*^{+/LSLG12V^{geo}};Ela-tTA;TetO-Cre, uses an inducible Tet-Off strategy to express the Cre recombinase under the control of the elastase promoter (Guerra et al. 2007). This strain develops PanIN lesions as well as full-blown PDAC tumors with similar latencies and penetrance as the KC mice (Hingorani et al. 2003; Guerra et al. 2007). *K-Ras*^{+/LSLG12V^{geo}};Ela-tTA;TetO-Cre mice also show complete tumor penetrance and reduced survival on inactivation of the *p53* or *Ink4a/Arf* tumor suppressors (Guerra et al. 2011). This model has the advantage that Cre expression can be inhibited by doxycycline. Hence, continuous exposure of pregnant mothers and their offspring to this antibiotic prevents activation of these genetic insults. Doxycycline withdrawal at any time during postnatal development or in adult mice allows activation of *K-Ras* oncogene expression as well as ablation of the *p53* and/or *Ink4a/Arf* tumor suppressors. This strategy has been used to show that adult pancreatic acinar cells are completely resistant to transformation by the *K-Ras* oncogene even in the absence of *p53* (Guerra et al. 2011). These studies have also served to show the importance of pancreatic injury in the development of PDACs in adult mice (Guerra et al. 2007, 2011).

Inducible CreERT2-Based Models

Other investigators have developed GEM models of PDAC that allow expression of *K-Ras*^{G12D} oncogenes in adult mice by using the inducible CreERT2 recombinase driven by specific promoters. They include *Ela-CreER* (De La O

et al. 2008), *Ela-CreERT2* (Habbe et al. 2008), *Mist1-CreERT2* (Habbe et al. 2008), *Pdx1-CreERT* (Gidekel-Friedlander et al. 2009), and *proCPA1-CreERT2* (Gidekel-Friedlander et al. 2009). Although these strains display differences in the incidence of PanIN development, it is clear that the susceptibility of acinar cells to *K-Ras* oncogene-induced transformation is progressively lost during postnatal development. The “age limit” for adult acinar cells to become resistant to *K-Ras*-induced transformation may depend on the genetic background, the expression pattern of the Cre recombinase, and the different conditional strategies.

Inducible K-Ras4b^{G12D} Transgenic Models

Some PDAC models allow to turn on and off the *K-Ras* driver oncogene at will (Collins et al. 2012). These models consist of a transgene that encodes the K-Ras4B^{G12D} oncoprotein by using a *TetO-K-Ras4b*^{G12D} complementary DNA (cDNA) (Fisher et al. 2001) or the variant construct *TetO-LSL-K-Ras4b*^{G12D} (Ying et al. 2012). Expression of K-Ras4B^{G12D} requires a reverse tetracycline-controlled transactivator (rtTA), which in these models was knocked in within the *Rosa26* locus (*R26-LSL-rtTA-IRES-EGFP* allele) (Belteki et al. 2005). Expression of the rtTA is restricted to pancreatic cells by means of the *Ptf1a/P48-Cre* transgene. The resulting strains, *TetO-K-Ras4b*^{G12D};R26-LSL-rtTA-IRES-EGFP;Ptf1a/P48-Cre and *TetO-LSL-K-Ras4b*^{G12D};R26-LSL-rtTA-IRES-EGFP;Ptf1a/P48-Cre express the K-Ras4B^{G12D} oncoprotein on exposure to doxycycline leading to the frequent generation of PanIN lesions but not PDAC tumors. Addition of a *p53* null (Collins et al. 2012) or floxed (Ying et al. 2012) alleles to these mice resulted in the induction of PDAC with full penetrance. These models have the disadvantage that the levels of *K-Ras* oncogene expression are dictated by the integration site of the *TetO-K-Ras4b*^{G12D} and *TetO-LSL-K-Ras4b*^{G12D} transgenes rather than by the normal *K-Ras* regulatory sequences. However, they allow the possibility to turn on and off the expression of the K-Ras^{G12D} oncoprotein at will.

Flp/frt-Based Models

More recently, a new generation of PDAC models has been developed by shifting their dependency from the bacterial Cre/loxP to the yeast Flp/frt recombinase system. As in the case of the lung tumor models, the use of two independent recombinase systems allows the spatial and temporal separation of tumor development from target ablation/inactivation, an experimental approach essential to evaluate the therapeutic potential of selected targets in preexisting tumors. Saur and colleagues used this dual recombinant system by expressing the Flp recombinase under the control of the *Pdx1* promoter (Schönhuber et al. 2014). The *Pdx1-Flp* transgene was incorporated to the $K-Ras^{+/FSFG12D}$ and $K-Ras^{+/FSFG12D};p53^{lox/lox}$ strains to recapitulate the KC and KPC mice, respectively, originally developed by Tuveson and colleagues (Hingorani et al. 2003, 2005). These strains can be combined with any conditional Cre-based strains such as those that make use of the Tam-dependent CreERT2 or those that express Cre under an inducible promoter to ablate/inactivate those targets whose therapeutic activity needs to be evaluated. In the original model described by Saur and colleagues, the CreERT2 recombinase was part of a *CAG-FSF-CreERT2* transgene inserted within the *Rosa26* locus (*Rosa26*^{CAGFSFCreERT2} allele). A second *Rosa26* allele carrying a *CAG-LSL-Tomato* transgene was added to the model to mark those cells that express an active CreERT2 recombinase on exposure to Tam. Saur and colleagues also developed a variant model in which they incorporate $p53^{lox}$ alleles to increase the tumorigenic properties of the $K-Ras^{G12D}$ expressing cells. These models, however, have a limitation because the CreERT2 recombinase can only be expressed in those cells derived from the pancreatic precursors that expressed the *Pdx1-Flp* transgene. Thus, preventing the analysis of potential toxicities caused by the ablation/inactivation of the target in nonpancreatic tissues. This limitation could be easily overcome by using a *Rosa26* allele that allows the systemic expression of the Cre-ERT2 recombinase.

We have also developed a variant PDAC model that uses a two-recombinase strategy by replacing the *TetO-Cre* transgene present in the $K-Ras^{+/LSLG12Vgeo};Elas-tTA;TetO-Cre$ mice by a *TetO-Flp* insert. Likewise, the $K-Ras^{LSLG12Vgeo}$ allele was replaced with a $K-Ras^{FSFG12V}$ allele (MT Blasco, C Guerra, and M Barbacid, unpubl.). The resulting strain, $K-Ras^{+/FSFG12V};Elas-tTA;TetO-Flp$, develops PanIN lesions and PDAC with similar incidence and latencies to mice carrying the Cre recombinase. Exposure of pregnant mothers and their offspring to doxycycline can also prevent expression of the oncogene. Finally, addition of $p53^{frt}$ alleles to these mice (Lee et al. 2012) allows the rapid development of PDAC tumors with complete penetrance and kinetics similar to those observed in $K-Ras^{+/LSLG12Vgeo};Elas-tTA;TetO-Cre;p53^{lox/lox}$ animals. Finally, a ubiquitously expressed hUBC-CreERT2 transgene was added to these mice to allow the ablation/inactivation of targets encoded by their corresponding floxed alleles on Tam exposure.

PDAC TUMORS: TARGET VALIDATION

The above tumor models have been instrumental in identifying targets with therapeutic properties in PDAC tumors (reviewed in Guerra and Barbacid 2013 and Gopinathan et al. 2015). Unfortunately, most of these studies have used strategies in which the target was ablated or inactivated before or at the time of tumor initiation, hence limiting the translational value of these results to a clinical scenario. Yet, targets whose inhibition can prevent or delay tumor development should be taken into consideration for testing in second generation models that allow their inactivation in advanced tumors. A summary of targets known to block or inhibit PanIN/PDAC development when ablated or inactivated at the time of *K-Ras* oncogene expression is shown in Table 4. They include molecules involved in epidermal growth factor receptor (EGFR) signaling (EGFR, ADAM17, Stat3), Notch signaling (Notch2), NF- κ B pathway (Ikk β), PI3K/mTOR pathway (p110 α , Pdk1, Rictor, mTRAIL-R), actin reorganization (Rac1), lysosomal proteases

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Table 4. Target validation in tumor initiation models of pancreatic ductal adenocarcinoma (PDAC) driven by K-Ras oncogenes

Model	Target	Tumor phenotype	References
<i>K-Ras</i> ^{+/LSLG12D} ; <i>Pdx1-Cre</i>	<i>Bmi1</i> ^{lox/lox}	No PanIN/PDAC	Bednar et al. 2015
<i>K-Ras</i> ^{+/LSLG12D} ; <i>Pdx1-Cre</i>	<i>CathepsinB</i> ^{lox/lox}	Reduced PanIN burden	Gopinathan et al. 2012
<i>K-Ras</i> ^{+/LSLG12D} ; <i>Pdx1-Cre</i>	<i>eNOS</i> ^{lox/lox}	Reduced PanIN/PDAC formation	Lampson et al. 2012
<i>K-Ras</i> ^{+/LSLG12D} ; <i>Pdx1-Cre</i>	<i>Ikkβ</i> ^{lox/lox}	Reduced PanIN/PDAC formation	Maniati et al. 2011
<i>K-Ras</i> ^{+/LSLG12D} ; <i>Pdx1-Cre</i>	<i>Ikkβ</i> ^{lox/lox}	Reduced PanIN/PDAC formation	Ling et al. 2012
<i>K-Ras</i> ^{+/LSLG12D} ; <i>Pdx1-Cre</i>	<i>Stat3</i> ^{lox/lox}	Reduced PanIN/PDAC formation	Corcoran et al. 2011
<i>K-Ras</i> ^{+/LSLG12D} ; <i>Pdx1-Cre</i>	<i>mTRAIL-R</i> ^{lox/lox}	Reduced PanIN/PDAC formation	von Karstedt et al. 2015
<i>K-Ras</i> ^{+/LSLG12D} ; <i>Ptf1a/P48-Cre</i>	<i>EGFR</i> ^{lox/lox}	No PanIN/PDAC	Ardito et al. 2012
<i>K-Ras</i> ^{+/LSLG12D} ; <i>Ptf1a/P48-Cre</i>	<i>ADAM17</i> ^{lox/lox}	No PanIN/PDAC	Ardito et al. 2012
<i>K-Ras</i> ^{+/LSLG12D} ; <i>Ptf1a/P48-Cre</i>	<i>Notch2</i> ^{lox/lox}	No PanIN/PDAC	Mazur et al. 2010
<i>K-Ras</i> ^{+/LSLG12D} ; <i>Ptf1a/P48-Cre</i>	<i>Stat3</i> ^{lox/lox}	Reduced PanIN/PDAC formation	Fukuda et al. 2011
<i>K-Ras</i> ^{+/LSLG12D} ; <i>Ptf1a/P48-Cre</i>	<i>Stat3</i> ^{lox/lox}	Reduced PanIN/PDAC formation	Lesina et al. 2011
<i>K-Ras</i> ^{+/LSLG12D} ; <i>Ptf1a/P48-Cre</i>	<i>Rictor</i> ^{lox/lox}	Reduced PanIN/PDAC formation	Driscoll et al. 2016
<i>K-Ras</i> ^{+/LSLG12D} ; <i>Ptf1a/P48-Cre</i>	<i>Pdk1</i> ^{lox/lox}	No PanIN/PDAC	Eser et al. 2013
<i>K-Ras</i> ^{+/LSLG12D} ; <i>Elas-CreERT</i>	<i>Ikkβ</i> ^{lox/lox}	Reduced PanIN/PDAC formation	Daniluk et al. 2012
<i>K-Ras</i> ^{+/LSLG12D} ; <i>p53</i> ^{+/LSLR172H} ; <i>Pdx1-Cre</i>	<i>Rictor</i> ^{lox/lox}	Increased survival	Driscoll et al. 2016
<i>K-Ras</i> ^{+/LSLG12D} ; <i>p53</i> ^{+/LSLR172H} ; <i>Pdx1-Cre</i>	<i>Pik3ca</i> ^{loxKD/loxKD}	No tumor development	Baer et al. 2014
<i>K-Ras</i> ^{+/LSLG12D} ; <i>p53</i> ^{+/LSLR172H} ; <i>Pdx1-Cre</i>	<i>CathepsinB</i> ^{lox/lox}	Increased survival	Gopinathan et al. 2012
<i>K-Ras</i> ^{+/LSLG12D} ; <i>p53</i> ^{+/LSLR172H} ; <i>Pdx1-Cre</i>	<i>eNOS</i> ^{lox/lox}	Increased survival	Lampson et al. 2012
<i>K-Ras</i> ^{+/LSLG12D} ; <i>p53</i> ^{+/LSLR172H} ; <i>Pdx1-Cre</i>	<i>mTRAIL-R</i> ^{lox/lox}	Increased survival	von Karstedt et al. 2015
<i>K-Ras</i> ^{+/LSLG12D} ; <i>p53</i> ^{+/LSLR172H} ; <i>Ptf1a/P48-Cre</i>	<i>Rac1</i> ^{lox/lox}	No tumor development	Wu et al. 2014
<i>Ras</i> ^{+/LSLG12D} ; <i>p53</i> ^{+/LSLR172H} ; <i>Ptf1a/P48-Cre</i>	<i>Pik3ca</i> ^{lox/lox}	No tumor development	Wu et al. 2014
<i>K-Ras</i> ^{+/LSLG12D} ; <i>p53</i> ^{lox/lox} ; <i>Ptf1a/P48-Cre</i>	<i>EGFR</i> ^{lox/lox}	Delayed tumor development	Ardito et al. 2012
<i>K-Ras</i> ^{+/LSLG12D} ; <i>Ink4a/Arf</i> ^{-/-} ; <i>Pdx1-Cre</i>	<i>Bmi1</i> ^{lox/lox}	Lack of PDAC rescue	Bednar et al. 2015
<i>K-Ras</i> ^{+/LSLG12D} ; <i>Ink4a/Arf</i> ^{lox/lox} ; <i>Pdx1-Cre</i>	<i>Ikkβ</i> ^{lox/lox}	Reduced PanIN/PDAC formation	Ling et al. 2014
<i>K-Ras</i> ^{+/LSLG12Vgeo} ; <i>Elas-tTA</i> ; <i>TetO-Cre</i>	<i>EGFR</i> ^{lox/lox}	No PanIN or PDAC	Navas et al. 2012
<i>K-Ras</i> ^{+/LSLG12Vgeo} ; <i>p53</i> ^{lox/lox} ; <i>Elas-tTA</i> ; <i>TetO-Cre</i>	<i>EGFR</i> ^{lox/lox}	Delayed tumor development	Navas et al. 2012

(CathepsinB), ROS-related molecules (eNOS), and epigenetic regulators (Bmi1) (Table 4). So far, there are no studies in which targets have been validated in advanced established tumors possibly because of the recent generation of the two-recombinase models described above. Schönhuber et al. (2014) ablated expression of the Pdk1 kinase in PanIN containing young mice. Examinations of these animals 6 months later revealed the almost complete disappearance of these lesions. Whether Pdk1 ablation or inactivation will have a similar therapeutic effect in advanced PDAC remains to be determined.

K-RAS is considered to be a “nondruggable target” with the possible exception of the K-RAS^{G12C} isoform (Ostrem et al. 2013; Patricelli et al. 2016). Yet, it is of significant interest to determine whether direct targeting of this oncoprotein will result in complete tumor regression. Removal of doxycycline exposure results in significant tumor regression in both *TetO-K-Ras4b^{G12D};R26-LSL-rtTA-IRES-EGFP;Ptf1a/P48-Cre* and *TetO-LSL-K-Ras4b^{G12D};R26-LSL-rtTA-IRES-EGFP; Ptf1a/P48-Cre* mice (Collins et al. 2012; Ying et al. 2012). However, prolonged observation of these tumors revealed that as many as 70% of them relapsed between 2 and 10 months. Whereas half of these relapsed tumors reexpressed the *K-Ras4b^{G12D}* oncogene, the other half harbors other oncogenic insults, including *Yap1* amplification (Kapoor et al. 2014). Thus, indicating that even effective inhibition of K-RAS, the initial event in PDAC development, may not be sufficient to induce the complete and permanent regression of these tumors.

PHARMACOLOGICAL STUDIES

GEM PDAC models have also been instrumental in pharmacological studies. The fact that these tumors display the same histological features as human tumors, including the formation of an abundant desmoplastic stroma, makes them more informative than classical xenograft or even PDX models. Some of these studies include those involving the γ -secretase inhibitor, MRK-003, which blocked PanIN formation in KPC mice treated at very early stages

(Plentz et al. 2009). In PDAC-bearing mice, this inhibitor, in combination with gemcitabine, induced increased survival (nine vs. 26 days). In a more recent study, Tuveson and colleagues (Chio et al. 2016) treated KPC mice with an AKT inhibitor (MK2206) and an inhibitor of glutathione biosynthesis (BSO), a combination that mimics Nfr2 down-regulation. In a week-long treatment, the combination slowed down tumor growth but did not cause tumor regression. Whether longer treatments will have a more profound therapeutic effect remains to be determined. Treatment of advanced PDAC-bearing KPC mice with JQ1, a BET inhibitor, in combination with the HDAC inhibitor SAHA, led to a significant increase in survival (15–47 days) (Mazur et al. 2015). More importantly, none of the treated mice died of PDAC. Indeed, histological analysis only revealed residual tumor clusters. Similar results were obtained in a related PDAC tumor model in which the *p53* conditional alleles were replaced by those of the *Ink4a/Arf* locus (Mazur et al. 2015).

TARGETING THE STROMA

GEM tumor models have also been used to ascertain the role of the desmoplastic stroma in PDAC. Although it has been generally accepted that the stroma enhances PDAC growth, two recent studies performed in KPC mice suggest that inhibiting the stroma may result in more aggressive pancreatic cancer (Ozdemir et al. 2014; Rhim et al. 2014). As expected, ablation of *Shh* in pancreatic cells (*K-Ras^{+/LSLG12D};p53^{+/-lox};Pdx1-Cre* mice) resulted in a significant decrease in desmoplastic stroma. Yet, the PDAC tumors were more aggressive and significantly decreased survival of these mice. These results have been interpreted as evidence that inhibition of the desmoplastic stroma contributes rather than inhibits tumor development. However, it is also possible that loss of *Shh* may alter the tumorigenic properties of the tumor cells in a cell-autonomous manner. In the second study, Ozdemir et al. (2014) use the *K-Ras^{+/LSLG12D};Tgfb β 2^{lox/lox};Ptf1a-Cre* model to ablate cancer-associated fibroblasts (CAFs), the main cellular component of the

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desmoplastic stroma, using a TK⁺/ganciclovir strategy. Ablation of CAFs resulted in the death of the mice caused by pulmonary emphysema, thus precluding from establishing a direct correlation between the reduction in CAFs and PDAC-dependent survival. Yet, analysis of the tumors appeared to indicate a more aggressive phenotype. In support of these studies, pharmacological inhibition of stromal cells using the smoothened inhibitor IPI-926 favored tumor progression and reduced survival (Rhim et al. 2014). These results, however, are at variance with a previous study in which treatment of KPC mice with the same compound allowed improved gemcitabine delivery and increased therapeutic efficacy (Olive et al. 2009).

In a different set of studies, exposure of KPC mice to a new paclitaxel formulation (nanoparticle albumin-bound [nab]-paclitaxel), in combination with gemcitabine, slowed tumor growth and in some cases induced tumor regression (Frese et al. 2012; Alvarez et al. 2013). Interestingly, the advantage of this formulation appears to be primarily caused by a decrease in the main gemcitabine-metabolizing enzyme, cytidine deaminase, rather than an effect on the stroma (Frese et al. 2012).

The desmoplastic stroma of PDAC tumors is rich in glycosaminoglycan hyaluronan (HA), a property that may prevent drugs from reaching the tumor cells (Toole et al. 2008). Treatment of KPC mice with PEGPH20, a PEGylated human recombinant PH20 hyaluronidase, in combination with gemcitabine, led to inhibition of PDAC tumor growth and prolonged survival (Provenzano et al. 2012; Jacobetz et al. 2013). Based on these studies, several clinical trials combining PEGPH20 with gemcitabine have already been initiated.

Regardless of whether the desmoplastic stroma has pro- or antitumoral effects, this tissue clearly serves as a barrier for T cells, thus preventing the potential use of checkpoint inhibitors, such as anti-CTLA4 or anti-PD1 antibodies, to treat PDAC patients. In a recent study, Feig and colleagues (2013) observed that CAF depletion allowed checkpoint antagonists to control tumor growth. The inhibitory activity of CAFs was mediated by the chemokine

CXCL12, because treatment of KPC mice with a CXCL12 receptor inhibitor, AMD3100, induced the rapid accumulation of T cells among the tumor cells and acted synergistically with an antibody against the PD1 ligand, PD1-L1 (Feig et al. 2013). Whether these strategies can be successfully translated to the clinic remains to be determined.

CONCLUDING REMARKS

Several challenges need to be resolved before the results obtained with GEM tumor models, such as those summarized here, can smoothly and steadily reach the clinic. First of all, target or drug validation studies have to be routinely performed in tumor-bearing mice, ideally harboring advanced or metastatic tumors. In addition, target ablation/inactivation should take place systemically and not only in the tumor cells or in selected tissues to mimic future studies with selected inhibitors. In pharmacological studies, it is essential to take into consideration the differential susceptibility to secondary toxic effects of humans versus rodents (e.g., MEK inhibitors) as well as their distinct pharmacodynamic and pharmacokinetic properties.

Another challenge involves the need to carry out combination studies. The mutational load of most human tumors strongly suggests that the successful use of precision medicine will require interference with several targets, either at the same time or sequentially. Use of single targeted therapies (erlotinib, vemurafenib, etc.) has already highlighted their weaknesses: mainly incomplete responses and appearance of resistance mechanisms. Combination studies in GEM tumor models will represent an important challenge for genetic strategies caused by the already significant complexity of the GEM strains. Along the same lines, future target validation experiments should make use of conditional knocked-in alleles in which targets are inactivated (e.g., by expressing a kinase dead isoform) rather than ablated as with standard conditional floxed alleles, because, as of today, protein ablation cannot be achieved by standard medicinal chemistry. It will also be important that future target validation studies, either ge-

netic or pharmacological, give more attention to the ultimate outcome of their results. A 50% or even an 80% decrease in tumor size in a GEM tumor model may have little value in the clinic, where these responses can be often obtained with classical chemotherapy treatments. Likewise, results that yield increased survival by a few days or weeks may not represent significant benefits if translated to human patients.

Finally, we should always keep in mind that no matter how similar GEM tumor models are to the human disease, mouse tumors are considerably smaller, they take less time to develop, and have a more limited mutational load. In any case, we hope that studies using GEM tumor models such as those summarized here will become an indispensable tool in preclinical studies and serve to streamline the translation of novel therapeutic strategies into future clinical trials.

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