**Name of Journal:** *World Journal of Gastroenterology*

**Manuscript NO:** 53411

**Manuscript Type:** REVIEW

**Mouse models of colorectal cancer: Past, present and future perspectives**

Bürtin F *et al*. Mouse models of CRC

Florian Bürtin, Christina S Mullins, Michael Linnebacher

**Florian Bürtin**, Department of General, Visceral, Vascular and Transplantation Surgery, University Medical Center Rostock, University of Rostock, Rostock 18057, Germany

**Christina S Mullins,** Department of Thoracic Surgery, University Medical Center Rostock, University of Rostock, Rostock 18057, Germany

**Michael Linnebacher,** Molecular Oncology and Immunotherapy, Department of General, Visceral, Vascular and Transplantation Surgery, University Medical Center Rostock, Rostock 18057, Germany

**Author contributions:** Linnebacher M was the main author involved in conception of the review including topics and angles addressed; Bürtin F performed the extensive PubMed search and drafted a first version; Mullins CS was the main author involved in manuscript editing including language editing; all authors participated in drafting the article and revising it critically for important intellectual content; and all authors gave their final approval of the submitted and revised version.

**Supported by** the State Mecklenburg-Vorpommern, No. TBI-V-1-241-VBW-084.

**Corresponding author:** **Michael Linnebacher,** **PhD, Academic Fellow, Research Fellow, Research Scientist, Senior Researcher, Senior Scientist,** Molecular Oncology and Immunotherapy, Department of General, Visceral, Vascular and Transplantation Surgery, University Medical Center Rostock, Schillingallee 69, Rostock 18057, Germany. michael.linnebacher@med.uni-rostock.de

**Received:** December 18, 2019

**Revised:** March 5, 2020

**Accepted:** March 10, 2020

**Published online:**

**Abstract**

Colorectal cancer (CRC) is the third most common diagnosed malignancy among both sexes in the United States as well as in the European Union. While the incidence and mortality rates in western, high developed countries are declining, reflecting the success of screening programs and improved treatment regimen, a rise of the overall global CRC burden can be observed due to lifestyle changes paralleling an increasing human development index. Despite a growing insight into the biology of CRC and many therapeutic improvements in the recent decades, preclinical *in vivo* models are still indispensable for the development of new treatment approaches. Since the development of carcinogen-induced rodent models for CRC more than 80 years ago, a plethora of animal models has been established to study colon cancer biology. Despite tenuous invasiveness and metastatic behavior, these models are useful for chemoprevention studies and to evaluate colitis-related carcinogenesis. Genetically engineered mouse models (GEMM) mirror the pathogenesis of sporadic as well as inherited CRC depending on the specific molecular pathways activated or inhibited. Although the vast majority of CRC GEMM lack invasiveness, metastasis and tumor heterogeneity, they still have proven useful for examination of the tumor microenvironment as well as systemic immune responses; thus, supporting development of new therapeutic avenues. Induction of metastatic disease by orthotopic injection of CRC cell lines is possible, but the so generated models lack genetic diversity and the number of suited cell lines is very limited. Patient-derived xenografts, in contrast, maintain the pathological and molecular characteristics of the individual patient’s CRC after subcutaneous implantation into immunodeficient mice and are therefore most reliable for preclinical drug development – even in comparison to GEMM or cell line-based analyses. However, subcutaneous patient-derived xenograft models are less suitable for studying most aspects of the tumor microenvironment and anti-tumoral immune responses. The authors review the distinct mouse models of CRC with an emphasis on their clinical relevance and shed light on the latest developments in the field of preclinical CRC models.

**Key words:** Colorectal cancer; Mouse models; Patient-derived xenografts; Carcinogen-induced models; Genetically engineered mouse models; Preclinical drug development

Bürtin F, Mullins CS, Linnebacher M. Mouse models of colorectal cancer: Past, present and future perspectives. *World J Gastroenterol* 2020; In press

**Core tip:** This review highlights the different approaches to model colorectal cancer in the mouse. Carcinogen-induced rodent models, genetically engineered mouse models, heterotopic and orthotopic models as well as patient-derived xenografts are discussed with an emphasis on their specific advantages and disadvantages. Moreover, the historical background of animal models for cancer research and the future perspectives of colorectal cancer research are reviewed as well.

**INTRODUCTION**

Colorectal cancer (CRC) is the third most common diagnosed malignancy among both sexes in the United States as well as in the European Union[1,2]. A decrease in the incidence and overall mortality of CRC in countries with a very high development index over the last decades can be attributed to an interplay of screening programs with detection of pre-cancerous lesions or early stage cancers on the one hand[3,4], and improved therapeutic concepts leading to an increased stage adjusted survival for all stages of CRC on the other hand[5]. This development is in sharp contrast to countries with a rapidly growing high development index. Together with an increased CRC incidence and mortality this reflects an adoption of the so-called “western lifestyle” including the risk factors for CRC. While obesity, smoking and red meat consumption are significantly associated with an elevated CRC risk[6], physical activity, high vegetable, fruit and fiber intake as well as metronomic aspirin therapy, have shown to decrease CRC risk[7,8]. Besides these modifiable risk factors, a variety of genetic factors influences CRC risk. About 5% of CRC cases are caused by hereditary, highly penetrant cancer syndromes, like familial adenomatous polyposis (FAP) and Lynch syndrome (LS); whereas up to 20%-30% of cases are considered as “familial” due to different germline mutations of varying penetrance[9]. Since the discovery of the link between APC germline mutations and FAP[10], followed by the genetic pathology of LS in the early 1990s[11,12], a myriad of mutations contributing to CRC genesis has been identified, constantly reshaping the genomic landscape of the disease[13]. The ideal model of CRC should recapitulate the progress from a precancerous adenoma to an invasive carcinoma with metastatic potential and at the same time it has to reflect the inter-individual molecular diversity of the disease. Consequently, a single (mouse) model of CRC simply cannot match all of these requirements. In this review, we discuss the different mouse models of CRC with their distinct advantages and disadvantages with a focus on their translational and clinical relevance.

**CARCINOGEN-INDUCED MODELS**

Carcinogen-induced models (CIM) in rodents look back on a long tradition but maintained their usefulness for certain applications to the present day. They provide a platform for dietary studies and give insights into the pathways of food-borne carcinogens and colitis-associated carcinogenesis. Administration of the chemical compounds is possible *via* ad libitum feeding, oral gavage, intraperitoneal/subcutaneous (s.c.) or intramuscular injection, or enema.

In 1915, Yamagiwa *et al*[14] proofed the carcinogenic properties of coal tar by its repetitive application on the ears of rabbits. At about the same time, first researchers worked on colon carcinogenesis by applying chemical or radioactive substances[15-17]. In the 1960s, cycasin and its metabolite, methylazoxymethanol, have shown to induce cancers in rodents[18-20]. In the following years, the more chemically stable substances, azoxymethane (AOM) and its precursor molecule, 1,2-dimethylhydrazine as well as methylazoxymethyl acetate, were extensively used to induce colon carcinogenesis in mice and rats. All three compounds are metabolized to methylazoxyformaldehyde, which is able to alkylate the DNA bases guanine and thymine[21]. After being processed by Phase-II-reaction, it is secreted to the bile and exceeds its carcinogenic effect to the intestinal mucosa[22]. Interestingly, these compounds show different carcinogenic potential depending on the mouse strain, housing conditions and the way of administration[23-25]. Although most authors claim a certain organotropism for AOM and dimethylhydrazine, tumor formation happens mostly in the small intestine and relevant amounts of alkylated DNA adducts can be observed in the liver and the kidneys[26]. Moreover, intestinal carcinogenesis can be achieved by the oral or rectal application of the direct alkylating topic agents N-methyl-N-nitrosourea (MNU), 3,2’-dimethyl-4-aminobiphenyl and N-Methyl-N’-nitro-N-nitrosoguanidine of which the latter two are traditionally used in rats[27-29].

Other carcinogens gained attention in connection with the association between meat consumption and increased CRC risk[30]. Heterocyclic aromatic amines (HAA) form from the reaction between free amino acids, sugars and creatine at high temperatures during the cooking process of meat and fish[31], whereby 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) and 2-Amino-9H-pyrido[2,3-b]indole are the most abundant HAA in various foods[32]. PhIP is metabolized by the liver enzyme CYP1A2 to N2-Hydroxy-PhIP, which then, after sulfation or acetylation, forms activated esters capable of DNA adduct formation[33]. Detoxification of PhIP and its metabolites is driven by glutathione conjugation and glucuronidation[34,35]. Glucuronide conjugates are excreted through urine and bile[36]. In case of the latter, hydrolyzation by bacterial beta-glucuronidases in the intestines liberate reactive PhIP metabolites, which not only affect the intestinal mucosa, but undergo enterohepatic circulation[37]. Important to consider is, that the metabolism of PhIP in rodents results in less reactive metabolites than in humans, and its carcinogenic potential measured in animal studies might be even higher in humans[38]. Nakagama *et al*[39], by combining a high fat diet with PhIP intake, showed the tumor enhancing properties of this food borne agent simulating the carcinogenic effects of the s.c. “western diet”. Moreover, PhIP led to the formation of high-grade dysplasia and adenocarcinomas in a mouse model of chemical induced colitis[40]. Although other common foodborne HAAs have shown to induce dysplasia and carcinomas in rodents[41], they are rarely used for modelling colon carcinogenesis. Polycyclic aromatic hydrocarbons, as benzo[a]pyrene, may be used for chemoprevention studies but are insignificant for CRC modeling in general[42,43]. Dextran sodium sulfate (DSS) must also be mentioned when discussing chemical-induced CRC mouse models. Since the first report of an DSS-induced colitis model nearly 30 years ago[44], countless studies used DSS to simulate chronic inflammatory bowel diseases and we would recommend the reader to refer to excellent reviews discussing inflammatory bowel diseases and DSS[45]. As a sulfated polysaccharide, DSS directly damages the colonic epithelium resulting in an impairment of the mucosal barrier with consecutive entry of luminal bacteria and associated antigens into the mucosa, triggering inflammation[46]. Depending on the animal strain, DSS dosage and administration regimen, mice can develop acute and chronic colitis or even colitis-induced dysplastic lesions[47-50]. DSS in combination with carcinogenic compounds, primarily AOM, has been proven useful for the research of colitis-induced cancer[51]. By use of mice with germline *Apc* mutation (APCMin mice) for DSS treatment, the rate of dysplasia and carcinoma formation was further enhanced[52]. Cooper *et al*[53] reported an increased CRC incidence of 40% in APCMin mice after two cycles of 4% DSS treatment compared to untreated control animals and identified the loss of heterozygosity (LOH) of *Apc* as underlying cause.

Chemical-induced mouse models are not homogenous and possess specific advantages and disadvantages. They are by far the oldest method inducing CRC in animals and a multitude of studies has been traditionally carried out in rats and other rodents diminishing the comparability of older data with more recent results. Most models reflect very well the progression from aberrant crypt foci to adenomas to carcinomas of the human adenoma-carcinoma sequence[54]. Therefore, they are still useful to evaluate the influence of diet[55], dietary supplements[56,57], chemopreventive interventions[58,59] and the gut microbiome[60]. Especially the combination of DSS with carcinogenic agents provided many insights in the link between CRC and inflammation[61]. However, chemical-induced carcinomas rarely show invasive properties and local or distant metastases are usually absent. Albeit Yang *et al*[62] reported lymph node metastases in an intrarectal MNU-model, the use of shrews (phylogenetic unrelated to rodents) as test animals interdicts the comparison to rodent animal models. A further exception is the work of Derry *et al*[63], who could observe a relevant number of lung metastases in an AOM-induced mouse model, which is to our knowledge the only report of metastatic spread in a chemical-induced mouse model. Besides the lack of invasiveness, a lot of CIM show a high latency from the first application to tumor development. Depending on the carcinogen, dosing protocol and mouse strain, latencies from 24 to 50 wk were reported[64-66]. By combining the carcinogen with DSS, the time to tumor development can be notably shortened to 10-18 wk[23,67]. Although the minority of sporadic CRC patients show synchronous adenomas[68,69], nearly all CIM show a “carpeting” of the colonic mucosa with polyps[70]. Tumor formation is not restricted to the colon, but can be commonly observed in the whole gastrointestinal tract[71,72]. Moreover, MNU additionally induces leukemia and lung adenomas[73] and PhIP leads to formation of mammary and prostate neoplasia[74]. Another complexing aspect of CIM concerns the genetic aberrations associated with the adenoma-carcinoma sequence, *i.e.*, the accumulation of mutations, predominantly affecting *APC,* *KRAS* and *P53*[75]*.* While the AOM model shows frequent *Kras* and *β-catenin* mutations, *Apc* and *P53* are rarely affected[76-79]. In contrast PhIP, IQ and MNU lead to *Apc* mutations, but show no *P53* or *Kras* mutations[21]. In general, the vast quantity of different dosing protocols, application forms and animal strains, makes direct comparisons and the selection of the right CIM difficult[54]. Another aspect, not to be neglected, is the agenda of animal welfare. Quite a few protocols lead to significant weight loss and diarrhea, which is, in combination with often long study durations, detrimental for the animal wellbeing[80].

**GENETICALLY ENGINEERED MOUSE MODELS OF COLORECTAL CANCER**

With the knowledge explosion concerning the genetic pathways altered in CRC at the end of the 20th century, the scientific community demanded specific genetic mouse models to focus on certain molecular mechanisms of colorectal carcinogenesis.

In the 1980s, the first genetically engineered mouse models (GEMM) of brain tumors, lymphoma, pancreatic cancer, breast cancer and osteosarcoma emerged[81-87]. Based on the groundbreaking work of Evans, Smithies and Capecchi[88] on gene targeting, the first tumor suppressor knock-out mouse models emerged in the early 1990s[89,90]. To circumvent the obstacle of frequent embryonic lethality caused by germline knock-outs of tumor suppressors, *Cre-loxP* mediated mouse models were designed to allow the tissue specific and conditional knock-out of tumor suppressor genes or activation of oncogenes, respectively[91-93]. Interestingly, the very first GEMM of CRC, the APCMin mouse, was created without sophisticated methods. Moser *et al*[94] showed, that the application of N-ethyl-N-nitrosourea leads to nonsense mutations in codon 380 of the *Apc* gene and subsequent breeding of these animals established the first model for multiple intestinal neoplasia. APCMin mice develop a large number of adenomas in the small intestine after 120-140 d due to LOH and show a high mortality with increasing age as a result of intestinal obstruction and anemia without progression to invasive carcinoma[95]. While these models contributed to the understanding of the early stages of FAP, they do not reflect the majority of spontaneous CRC[96]. Since a homozygous *Apc* mutation is lethal during embryonic development, breeding of homozygous APCMin mice is impossible[97]. However, additional treatment of APCMin mice with AOM or other carcinogenic compounds increases malignancy of the resulting tumors and simultaneously shortens the time to tumor development[98-100]. Till the present day, these models are in use for chemoprevention studies[101,102] and have enormously contributed to the understanding of the early tumor initiating events[103]. Interestingly, a change from C57BL/6 to a hybrid genetic background can extend the lifespan of APCMin mice beyond one year, resulting in a high proportion of invasive adenocarcinoma[104]. Sødring *et al*[105] changed the APCMin genetic background from C57BL/6 to A/J mice resulting in increased tumor formation in the colon, with a reasonable number of tumors progressing to carcinomas. Transgenic mice with alternative *Apc* mutations, like the APC+/1638 mouse[106,107], the APCΔ716 mouse[108] and the APC*Δ242*/+ mouse[109] vary in tumor count and histopathology. Tumor formation predominantly in the small intestine instead of the colon is the major flaw of most *Apc*-based mouse models. Colnot *et al*[110] designed the APCΔ14/+ mouse, which shows a more severe phenotype with invasion of the muscularis, an increased lethality and a higher colonic tumor burden compared to APCMin mice but unfavorably still shows relevant tumor development in the small intestine. Early attempts of combining *Apc* mutations with homozygous *P53* knockouts yielded conflicting results, with either no increase[111,112], or a small increase of gastrointestinal malignancy[113]. The most likely explanation is that in human cancers *P53* missense mutations frequently act in a dominant negative fashion, overruling the tumor-suppressive function of the wildtype allele[114,115]. In contrast, targeting *Kras* without tissue specific promoters leads either to embryogenic lethality[116] or few viable animals succumbing to rapidly developing lung tumors[117]. To avoid abundant distribution of mutations in the whole organism, transgene expression controlled by a tissue specific gene promotor, most commonly by application of the *Cre-loxP*-system[91], has proven to be extraordinarily useful. Many workgroups used *Villin-Cre* transgenes to restrict recombination of *floxP*-flanked genes to the epithelial cells of the small and large intestines either with a constitutive expression (Vil-Cre) or with a tamoxifen-inducible expression (Vil-Cre-ERT2)[118]. Another option of site-specific *Cre* expression is the fatty acid binding protein liver Cre transgene (*Fabpl Cre*), which can be combined with a tetracycline-inducible tet-on system[119]. Yet, both transgenes’ expression is not limited to the large intestine: While *Villin-Cre* is expressed in the epithelial cells of the whole intestines, *Fabpl-Cre* expression can be detected in the distal small intestine, cecum and colon[119,120]. Also, the AhCre strain, carrying Cre under control of the Cyp1A promotor, is commonly used for colon cancer models[121,122]. Here, *Cre*-expression is induced by β-naphthoflavone in the liver and intestines, but constitutive recombination can be observed in other tissues like the renal epithelium[123]. To achieve a more colon specific expression of *Cre*-recombinase, Hinoi *et al*[124] constructed a transgene of *Cre* and the promotor region of the *CDX2* homeobox gene. By inserting a guanine repeat tract to this transgene (*CDX2P9.5-G22Cre*), stochastic activation of *Cre-*expression by means of spontaneous frameshift mutations leads to a mosaic-like recombination in the mucosa of the terminal ileum, cecum, and colon[125]. At last, carbonic anhydrase 1 promoter/enhancer-Cre recombinase transgene (*CAC*) facilitates recombination strictly limited to the large intestine[126]. Paralleling *Cre*-transgene implementation, others achieved spatiotemporal oncogene expression in the large intestine by delivering Cre by viral transfection *via* transanal injection, surgery or colonoscopy[127-129] leading to exquisite models of CRC with metastatic spread[130]. Supplied with thiscomprehensive genetic toolbox, a plethora of CRC mouse models were generated and used to evaluate the role of different mutations and their interplay. Among the non-hypermutated tumors, the most frequently mutated genes are *APC*, *P53*, *KRAS*, *PIK3CA*, *FBXW7*, *SMAD4*, and *TCF7L2*[13]. The role of *Apc* LOH as a driver mutation is highlighted in several mouse models. While generalized deletion of both *Apc* alleles (*Apc*flox/flox) leads to rapid death by disorder of cell differentiation[131], mice with a mosaic–like deletion of both *Apc* genes die rapidly from florid polyposis[125]. Depending on the type and modality of *Apc* mutation, heterozygotes develop adenomas or invasive adenocarcinomas[120,132,133]. Whilst mutated *Kras* alone is insufficient to induce colorectal tumorigenesis, it increases the susceptibility of the intestinal mucosa to chemical carcinogenesis[134] and leads to accelerated tumor formation in combination with *Apc* loss[122,135]. *Nras* mutation, in contrast, does not alter the effect of *Apc* loss[136]. In humans, *P53* mutations are often associated with vascular and lymphatic invasion and advanced cancer stages[137,138]. In fact, the combination of *Apc* mutation with a dominant-negative *P53* mutation leads to increased invasiveness of intestinal tumors with signs of epithelial to mesenchymal transition[139,140]. Also, loss of *P53* in a constitutively active Notch signaling background leads to intestinal tumor formation and metastasis[141], whereas Notch signaling does not cooperate with the Wnt-pathway[142]. Nevertheless, *Apc* deficiency seems to represent a key prerequisite of cancer progression, since *Apc* restoration leads to spontaneous tumor regression of *Kras*-mutated, *P53*-deficient adenocarcinomas[143]. *FBXW7* codes for the F-box/WD repeat-containing protein 7, the substrate receptor of a ubiquitin ligase, which degrades several proto-oncogenes like *MYC, CCNE1, NOTCH1* and *JUN* and acts synergistically with *P53* as haploinsufficient tumor suppressor[144-146]. Intestinal *Fbxw7* deletion enhances tumor development in an *Ap*cMin/+background[147] and a combined deletion of *Fbxw7* and *P53* results in highly aggressive intestinal cancers with metastatic spread to the lymph nodes and liver[148]. The PI3K/AKT- pathway is well known for its pro-oncogenic and anti-apoptotic signaling and *PI3K* mutations are common in CRC and many other human cancers[149]. As demonstrated by Leystra *et al*[150], the intestinal expression of a constitutively active *Pi3k* (PIK3ca) is a sufficient driver mutation to induce rapid tumorigenesis with invasion of adjacent organs and addition of PIK3ca to a homozygous loss of *Apc*, drives adenoma-to-carcinoma progression with metastatic spread[129]. Although loss of *Pten*, the counterpart of *Pik3*, does not affect intestinal cell proliferation; in the context of *Apc* deficiency or other mutations, it promotes cancer progression[151-153]. *SMAD4* is considered as a tumor suppressor, similar to other constitutes of the TGFβ-pathway[154,155]. Since the genes *Apc*, *Smad2* and *Smad4* are all located on chromosome 18 in the mouse, they are suited to generate distinct cis- and trans compound heterozygotes by meiotic recombination. Compared to their single mutation littermates, mice with combined *Apc* and *Smad4* mutations, show accelerated tumor development[156] and increased malignancy[157,158]. In contrast, compound heterozygotes of *Apc* and *Smad2* mutations show no increased tumor development compared to littermates with a single *Apc* mutation[159]. Notably, homozygous *Smad3* mutation leads to aggressive CRC with lymphatic spread and, upon *Apc* deficiency, drastically reduced life span[160,161]; but *SMAD3* is rarely mutated in human CRC[162]. Findings from several CRC-GEMM highlight the role of TGFβ-signaling as a strong tumor suppressor, since *Tgfβ*[163-165], as well as *Tgfβ*-receptor 2[140,166,167] knockout, induce local invasion and metastatic spread. Regarding the role of *TCF7L2* mutations in CRC, so far, no GEMM of CRC addressing this topic have been published. Besides the conventional adenoma-carcinoma sequence, the serrated pathway represents an alternative route of CRC development with distinct molecular and clinical features. The underlying *BRAFV600E* mutation occurs in 15%-20% of sporadic CRC, causes a constitutive activation of the MAPK/ERK pathway and is strongly associated with the CpG Island methylator phenotype and microsatellite instability (MSI) due to *MLH-1* methylation[168-170]. *BrafV600E* causes crypt hyperplasia and combined with *Apc* or *P53* mutations, as well as mutations affecting *Ink4A/Arf*, gives rise to invasive carcinomas[171-174]. Although not common in human CRC, mutation of the GSK3-β phosphorylation site causes degradation-resistant β-catenin[175,176], and has been remodeled in the mouse. GSK3β-resistant β-catenin increases proliferation of the intestinal epithelium and causes adenoma formation, but does not mediate malignant progression[177-179]. Paralleling the research on canonical cancer pathways, there has been reasonable effort to reproduce MSI, a hypermutable phenotype caused by malfunction of DNA mismatch repair (MMR) enzymes[180,181]. MSI-CRC can occur in the context of hereditary MMR gene mutations (LS) or can be detected in up to 15% of spontaneous CRC, caused by hypermethylation of MMR genes (spMSI)[182,183]. The constitutes of the MMR machinery have been extensively studied by somatic knockout models. Since the knockout of the MMR genes *MLH1*[184], *MSH2*[185,186], *MSH6*[187] and *PMS2*[188] predominantly cause hematopoietic malignancies with consecutive reduced lifespan in the mouse and consequently only a minor fraction of homozygotes develop intestinal neoplasia[189,190], these knockouts were frequently put in an APC deficient setting, to increase intestinal carcinogenesis[184,191,192]. Kucherlapati *et al*[193,194] demonstrated, that mutations of *Fen1* and/ or *Exo1*, both cooperation partners of MMR enzymes, lead to similar patterns of MSI tumor development. However, MSI-high tumors rarely show activation of the Wnt-pathway and are typically chromosomal stable, whereas *APC* mutations are typically associated with a chromosomal instable phenotype[195,196]. Therefore, these models do not adequately recapitulate Lynch-type or spMSI tumors. To overcome the aforementioned obstacles, a floxed *Msh2* allele was combined with either a LS related missense mutation (*Msh2*G674D) or an *Msh*2Δ7 null mutation in mice carrying a *Villin-Cre* transgene, leading to intestinal carcinogenesis and chemoresistance typical for MSI-high tumors[197]. An overview of the above addressed mutations can be found in Figure 1 and are summarized in Table 1.

In summary, GEMM have contributed enormously to the understanding of the molecular processes of CRC initiation, progression and crosstalk of common cancer-associated pathways. Besides the models for spontaneous CRC and common cancer syndromes, like Familiar Adenomatius Polyposis and LS, several models recapitulate metastatic disease, either as “classic” GEMM[141,148,166,198] or upon viral *Cre* delivery[129,130]. Yet, these models have several limitations. First of all, cancer development is a step-wise process with an initial driver mutation and subsequent acquisition of further mutations[199], and thus can only be partly reflected in tumor mouse models by the combination of a constitutively active with an inducible mutation[172]. Roper *et al*[128] recently demonstrated *in vivo* genome editing of *Apc* and *P53* by viral delivery of the correspondent sgRNA in mice expressing CRISPR-Cas9 under the control of a *Villin-Cre* transgene. Second, the number of combined mutations is limited in the mouse, since the resulting phenotype shows often a drastically reduced lifespan[152]. Triple mutant (*Car1*CreER/+; *Apc*fl/f*l*; *Kras*LSL–G12D/+) and quadruple mutant mice (*Car1*CreER/+; *Apc*fl/fl; *Kras*LSL–G12D/+; *P53*KO*; Smad4*fl/fl) showed a reduced lifespan of merely one month[158]. Moreover, GEMM are time consuming and expensive, since breeding of the transgenic mice often takes multiple generations and requires careful interbreeding to yield the desired alterations. In terms of animal welfare, it should be noted that the breeding process yields many “reject” mice, which are neither used for further breeding nor for research. Also, the construct of the transgene, or the viral vector, respectively, is complicated. While GEMM represent a valuable tool for basic research, their use for preclinical studies is limited due to a lack of genetic heterogeneity on the one hand, and discrepancies to the human tumor development on the other hand. On a final note, it should be added, that GEMM of CRC can be applied the other way round: mice harboring mutagenic *SB* transposons were crossed to mice expressing SB transposase under control of a *Villin-Cre-*transgene, to generate mice, that develop intestinal lesions due to random insertional mutagenesis and can be screened for unknown CRC driver mutations[200]. Moreover, by combining this approach with well-known driver mutations, new pathway-associated mutations could be identified[201,202].

**TRANSPLANT MODELS FOR COLORECTAL CANCER**

Transplant mouse models can be classified in various ways: Syngeneic tumor transplantation is characterized by tumor tissue or cancer cell line engraftment within the same mouse strain; whereas xenogeneic grafts are derived from a different mouse strain or human donors. Additionally, it can be distinguished between heterotopic and orthotopic models. As cancer grafts tumor cells, organoids and tumor tissue pieces can be employed. In 1876, Novinsky successfully transferred tumors (likely canine venereal sarcomas) from one dog to another in two independent trials[203]. Shortly thereafter, Hanau[204] and Triolo[205]reported independently the successful passaging of epithelial rodent tumors. Around the turn-of-the-century, several transplantable rodent tumors, mostly of a sarcomatoid phenotype, were established[206]. Later, the discovery of the human leukocyte antigens and oncogenic viruses elucidated the results of the earlier transplantation studies[207,208]. Toolan pioneered in the xenograft field by attenuating graft rejection through X-radiation and cortisone treatment[209,210].

***Animals for transplant models***

A fundamental prerequisite for the successful engraftment of xenogeneic tissue in mice is the impairment of the host immune system. A detailed explanation of the development and sophistication of immunocompromised mice would fill several pages and is exquisitely reviewed elsewhere[211]. In short, with the discovery of “nude” mice (recessive mutation of FOXN1 leading to hairlessness and athymia)[212] and the subsequent breeding of NMRInu/nu mice, xenotransplantation was possible for the first time without additional immunoablative treatment[213]. A more immunodeficient animal strain, C:B:-17 scid, carrying a homozygous mutation of the *Prkdc* gene resulting in a lack of functional B and T lymphocytes, was established by Bosma *et al*[214] in 1983. To overcome NK cell function, SCID mice were crossed with non-obese diabetic mice (NOD)[215] by several workgroups generating NOD/LtSz‐scid[216], NOD/LtSz-scid β2mnull[217] and NOD/Shi-SCID mice[218]. However, the life span of NOD/SCID mice is limited by the development of thymic lymphomas[219] and they show relevant “immune leakiness” caused by spontaneous rearrangement of T and B cell receptors[220,221]. Lastly, to abolish NK cell activity completely, deficiency of the IL-2 receptor subunit gamma (IL2Rγ) and the Janus kinase 3 (Jak3) were introduced to NOD/SCID mice generating the commonly used strains NOG (NOD/SCID/IL2Rγtm1Sug)[222], NSG (NOD/SCID/IL2Rγtm1Wjl)[223] and NOJ (NOD/SCID/Jak3null)[224]. Since the DNA-dependent protein kinase catalytic subunit, encoded by the *Prkdc* gene, is also responsible for DNA repair, SCID mice are very sensitive for radiation and DNA-damaging agents. Therefore, Shultz *et al*[225] developed the more robust, but equal immunodeficient NOD/LtSz-Rag1nullPfpnull strain. Since then, many more immunodeficient strains, with in part different genetic backgrounds, have been developed[211,226]. In general, it can be noted, that the more severe immunodeficient the host, the higher are the engraftment rates. Nevertheless the NMRInu/nu strain is still of high relevance for xenografting: The strain is less prone to opportunistic infections[227], more tolerant for chemotherapeutic agents and shows reasonable engraftment rates for primary patient-derived xenografts (PDX) and good engraftment rates for subsequent mouse to mouse passaging[228,229]. An economical plus is that this mouse strain is the least expensive immunodeficient one. It is mandatory to house immunodeficient mice in a specific pathogen free environment, using sterile techniques and microisolator caging. For syngeneic mouse models, viz. transplantation of cell or organoids from mice, exact strain conformity of donor and recipient mouse must be guaranteed, since even closely related substrains can differ genetically[230].

***Heterotopic tumor models***

The advantages of s.c. tumor engraftments are glaring: They require nominal surgical skills, allow high throughput of samples due to time efficacy and tumor growth can be monitored by the naked eye. Early s.c. models of solid tumors were mostly carried out by injection of a tumor cell suspension into the mouse flank. Although some of these models correctly predicted clinical response for specific cancer entities and therapeutics[231] large drug screens revealed that these models are of rather modest value for the prediction of clinical response in humans[232-235]. Moreover, the resulting tumors from a homogenous cell suspension do not reflect the intratumor-heterogeneity and an adequate tumor microenvironment is also absent[236]. Especially established CRC cell lines show low genetic diversity due to high passage and selective pressure[237]. On a site note, one of the most cited CRC cell lines, HCT116, was established almost 40 years ago; enough time for mislabeling, cross-contaminations and high passage selection[238,239]. While tumor cell lines in general remain a cornerstone of cancer research[240,241], their heterotopic *in vivo* application creates no scientific added value. The creation of a s.c. tumor graft as intermediate step for subsequent orthotopic transplantation can be considered as an exception. Even though these cell-derived grafts are frequently referred to as PDX in the literature, we believe that this term should be avoided and “cell line-derived xenograft - CDX” is more reasonable. The implantation of tumor cells or tumor pieces under the renal capsule follows the rational that the high vascularized environment propagates better engraftment[242,243] and was historically used for the subrenal capsule assay[244]. While this model might be advantageous for some cancers[245], we see no advantage for CRC engraftment over the s.c. PDX model, a fortiori comparing practical effort, monitoring of tumor growth and animal welfare. Intravenous injection of cell suspension is often used to simulate hematogenous dissemination of tumor cells[246,247], but circumvents crucial steps of metastasis, namely degradation of the surrounding tissue and lymphatic and/or vascular invasion[248]. Thus, the same applies to the splenic injection of tumor cells to generate liver metastasis[249,250] or the intraperitoneal injection to simulate peritoneal carcinosis[251].

***PDX models***

PDX differ greatly from the aforementioned heterotopic tumor models. They are established by the s.c. implantation of a tumor piece from a surgical resection or biopsy into the flanks of immunocompromised mice; with lower tumor take rates for biopsy samples[237]. Tissue can either be implanted directly after resection or cryopreserved in fetal calf serum containing 10% DMSO for implantation at a later time[229]. Incubation of the tissue in Matrigel®prior to tumor implantation, significantly increases engraftment rates[228]. In order to obtain sufficient tumor tissue for larger scale studies, the resulting tumor can be fragmented and re-grafted subsequently. In recent years, our workgroup focused on the build-up of a large CRC biobank consisting of more than 140 PDX-models (general procedure is presented in Figure 2). This PDX panel reflects adequately the clinical and molecular heterogeneity of the patient population undergoing surgical resection of primary or metastatic CRC[252]. It is well accepted that PDX closely recapitulate the histology of the original “donor” tumor over several passages[253-255] and are also genetically stable[256,257]. However, Ben-David *et al*[258] demonstrated recently that changes in copy number alterations occurred in early passages of PDX tumors compared with both the P0 PDX and the donor tumor. A common criticism in heterotopic mouse models is related to the absence of tumor-stroma interaction or the “tumor microenvironment”[259]. While this is true for immune cells, the stromal component remains intact in PDX[255]. Although the human stromal compounds (fibroblasts, blood vessels *etc*.) are quickly and steadily replaced by their murine counterparts, the overall architecture of the tumor remains unaffected in the majority of cases[260,261]. Moreover, these murine stroma cells adopt and maintain a human-like metabolic phenotype[262]. These features indicate that PDX are indeed good and valuable models for preclinical testing of conventional and novel anticancer agents in the era of personalized cancer therapy[263,264]. A proof of concept study with advanced refractory cancers demonstrated that drug responses measured in a PDX model, can be used for successful clinical decision making[265]. A PDX model of CRC metastases closely resembled the efficacy of cetuximab and identified druggable targets in resistant tumors[266]. Moreover, a PDX clinical trial approach (one animal per model per treatment) reflects well the heterogeneity of the patient population and allows testing of new drugs and combinatorial regimen[267]. A large study with over 1000 PDX models confirmed the consistency between clinical and PDX clinical trial drug response[268]. The ultimate goal of precision medicine would comprise of the profound genetic and functional characterization of a given tumor to identify relevant drug targets and subsequent validation of potential therapies with aid of a PDX bearing “avatar” mouse to provide the most efficient treatment for the individual patient[264,269]. Currently, several clinical trials following this approach for colorectal[270,271] and pancreatic cancer are recruiting[272]. Yet, a median duration of 12.2 mo until PDX model establishment[237] remains an unsolved issue for patients urgently in need for treatment. When it comes to drug testing, it should be considered, that immunotherapy approaches can only be restrictedly evaluated in immunodeficient host mice. A further disadvantage of PDX models is a potential selection for more aggressive tumors. The data concerning the association between successful PDX engraftment and clinical or molecular features is in part conflicting. While we and others could not find significant associations between tumor grading and PDX engraftment[237,252], a Korean study observed significant correlations with tumor staging and grading[273]. A smaller study with a high PDX establishment rate found a significant correlation of PDX success with positive nodal status and grading[274], while Julien *et al*[275] only found significances for the combination of a positive nodal status with an elevated carcinoembryonic antigen level. Moreover, we observed a significant correlation between PDX engraftment and molecular features like *KRAS* and *BRAF* mutations as well as MSI[252]. Apart from the tumor biology, choice of the host mouse strain, repeated attempts of engraftment, quantity and quality of the resected tissue as well as previous treatment of the patient are additional factors influencing the success of model establishment. Collins *et al*[276] recently reported PDX engraftment rates varying from 14 to 100% with a median PDX establishment rate of 68% reviewing 14 CRC-PDX studies. Compared to establishment rates for primary CRC cell lines of about 10% the PDX is clearly superior[252,277]. A selection bias relating to cancer biology can be diminished by increasing the number of enrolled patients and molecular characterization of the individual PDX. In fact, a very recent PDX study found an underrepresentation of the consensus molecular subtype number 2, due to worse engraftment[278]. Another crucial pitfall of the PDX model is the development of EBV-associated lymphomas at the implantation site, which can mimic successful engraftment[279]. Depending on the mouse strain and cancer entity, between 2.3% (colorectal) and 75% (prostate cancer) of primary engrafted bona fide xenografts turn out to be human de-novo lymphomas[280-284]. Since this condition is more frequently reported in NSG and comparable strains, development of de-novo lymphoma in NMRInu/nu mice might be hindered by high NK cell activity[285]. Thus, after successful engraftment of a PDX in NSG mice, we conduct the subsequent passaging in NMRInu/nu mice and xenograft histology is frequently evaluated by an experienced pathologist. Interestingly, Butler *et al*[286] significantly reduced the frequency of lymphomas in an ovaria cancer PDX model by a unique dosage of rituximab during implantation. Additionally, PDX serve not only in the field of therapy development; they are also a vital tool for the maintenance of a healthy biobank, allowing the establishment of secondary cell lines and supply sufficient tumor samples for the exchange with other work groups[252].

In summary, we consider the PDX model as keystone of cancer research, holding great potential in the developing field of precision medicine for CRC.

***Orthotopic models***

Orthotopic CRC models are implemented to overcome the drawbacks of heterotopic models, videlicet lack of an adequate tumor microenvironment and metastatic behavior.

At the beginning of the 1980s, the first orthotopic engraftments of CRC cell lines was reported by Snipes[287], demonstrating the feasibility of an intramural cell injection causing locally invasive cancer growth. A few years later, Bresalier *et al*[288] demonstrated that the orthotopic injection of human CRC cells causes metastases in the liver. In 1991, Fu *et al*[289] successfully engrafted 13 patient-derived tumors by removing the serosa and sewing the tumor pieces to the cecal wall. Beside the local tumor outgrowth, lymph node and liver metastases were reported for a few mice. Since the murine cecum is relatively large compared to its human counterpart and readily accessible by laparotomy, it rapidly became the favored site for orthotopical engraftment by different approaches. Many authors generated a s.c. cell graft in a donor mouse, of which a small piece was sewed to the cecum after damaging the serosa[290-292]. Several varieties of this technique can be found in the literature: While some authors removed the mucosa and sewed the tumor pieces onto the cecal wall[293,294], others formed a subserosal tunnel for tumor inoculation, which was afterwards closed by a suture[295] or surrounded the tumor piece with a “pouch” formed by a serosal duplicature[296]. Others reported the technically more challenging subserosal injection of a cell suspension into the cecal wall[297]. The cell injection method is often referred to as subserosal[298], while other work groups describe a submucosal injection[299]; both frequently with Matrigel® addition to the cell suspension to avoid cell spillage[300]. Considering the gauntness of the cecal wall, we will here refer to both techniques as “intracecal” injection. The possibility of a “preconditioning” *via* s.c. PDX has also been described for the cell injection approach[301]. Beside the cecum, other colonic sites like the descending colon can be accessed for cell injection[302,303]. Despite frequent metastases, some aspects of these techniques may be viewed critically. First, given the fact that CRC arise from the mucosa, these models cannot be considered as genuine orthotopic and also the injection approach might mimic an advanced CRC. Second, they allow the contact of cancerous tissue with the abdominal cavity, hence it cannot be ruled out that some of the metastases are the result of intraabdominal cell spillage. Moreover, these approaches require the opening of the abdomen and can cause inflammation and morbidity. Nevertheless, the cecal orthotopic model is frequently applied, especially for basic research to identify the underlying mechanisms of metastatic progression, since it can render metastatic, end stage disease within a few weeks[304]. The upregulation of genes associated with advanced CRC, could also be observed in liver metastasis from an orthotopic model[290,305]. The orthotopic approach allows to elucidate the role of certain molecular pathways by direct comparison of the metastatic properties of a given cell with their genetically engineered counterpart[306-310]. The additional transfection of these cells with a reporter, like GFP, DsRed or luciferase, allows monitoring of tumor progress by *in vivo* imaging[311-313]. The orthotopic approach can be used in transgenic mice to clarify the role of distinct molecules[314] or certain cell types[315]. To further stress the functionality of stromal components, a co-injection of tumor cells and stromal cells is feasible[316-318]. By using NSG mice, the efficiency of an immune cell-based therapy can be tested in the context of such an orthotopic model[319]. Furthermore, circulating tumor cells (CTC) can be isolated from murine blood[320]. In addition to the above discussed surgical approaches, there are less invasive concepts that do not require surgery and diminish the risk of intraabdominal cell spillage. Kashtan *et al*[321] demonstrated the successful engraftment of murine tumor cells by submucosal injection in the distal rectum in 1992 and this approach was adapted by several work groups[322-324]. To reach more proximal parts of the rectum or the descending colon, submucosal cell application can be performed *via* small endoscopic instruments[325,326]. Depending on the cell line, liver metastases have been observed[327]. Lastly, tumor cells can be inoculated in the colon mucosa by the acid enema approach described by Kishimoto *et al*[328]; a true orthotopic model with intramucosal tumor development and liver metastases. In brief, the mucosa is damaged by 4% acetic acid enema and after neutralization with PBS, a tumor cell suspension is instilled and the anus temporaryly sealed. Hite *et al*[329] subjected all three orthotopic models (intracecal injection, transanal submucosal injection and acid enema) to a direct comparison. They found the submucosal injection to be the most efficient in tumor formation and metastatic behavior and at the same time well tolerated by the animals. In contrast, the acid enema approach showed the lowest tumor formation frequency but a considerable mortality of 15%. Enquist *et al*[312] pursued a different concept by sewing a tumor piece directly to the mucosa by creating an artificial rectal prolapse. They were able to engraft pieces from transgenic A*pcMin/+;KrasLSLG12D/+;Villin-Cre* adenomas in the colon of syngeneic animals and a small subset of these tumors progressed to carcinomas. More strikingly, they managed also to transfer s.c. human PDX to the colons of NSG mice reflecting stage-dependent biological behavior as lymph node metastases could be observed for stage III PDX[312].

Today, orthotopic models are copiously used for the *in vivo* validation of new therapeutic compounds and as a proof-of-principle approach[330-333]. However, their clinical relevance is limited by the common use of similar, poorly differentiated cell lines. The orthotopic PDX model is promising, but its tumor take rate is not higher as in the s.c. PDX model. A very recently published, “crossover” concept comprising the s.c. engraftment of a PDX, followed by enzymatic disintegration of the PDX to a cell suspension and subsequent orthotopic injection into the rectal submucosa yielded an engraftment rate of 70% for s.c. PDX and 46% for the orthotopic model. Moreover, a metastatic spread was observed for 60% of the tumors successfully engrafted orthotopically[334].

**RECENT DEVELOPMENTS AND FUTURE PERSPECTIVES**

The increasing field of precision medicine has a growing need for highly translational cancer models. Conversely, the increasingly negative public perception of animal studies constrains the scientific community to further stress the 3R-principle (replacement, reduction and refinement) in cancer research[335]. Aside from the improvement of *in vivo* models, this implies the refinement of *in vitro* methods as well.

Humanized mice are severely immunocompromised mice, which can be reconstituted with various types of human bone marrow-derived cells or CD34+ hematopoietic stem cells[336,337]. Since human stem and progenitor cells can be attained from umbilical cord blood or from peripheral drawn blood samples after GM-CSF treatment and cultured *in vitro*[338], these cells can be transferred to sub-lethally irradiated NSG mice. Morton *et al*[339] observed that PDX of head and neck cancer engrafted into these, so called “Xact mice”, are infiltrated with human B and T lymphocytes. Many transgenic mice further support engraftment with CD34+ stem cells by overexpression of human interleukins and signaling molecules. Thus NBSGW[340], hIL2-NOG[341], NSG-SGM3[342] and SRG-15 mice[343] have been introduced recently and antitumor effects against PDX of different cancers could be observed[341,343]. These models hold great promise for the research of CRC immunotherapy, especially for highly immunogenic hypermutated CRC. Capasso *et al*[344] showed very recently that check point inhibition with nivolumab leads to growth inhibition of human MSI-H PDX thereby accurately reflecting the clinical response of this CRC subtype. In contrast, no sustainable growth inhibition was observed in MSS tumors or MSI-H tumors in “standard” NSG mice[344,345].

Although patient-derived cell cultures are a valuable tool for high-throughput drug screenings, they exhibit considerable shortcomings[346]. First, the establishment rate of primary patient-derived CRC cells with conventional 2D culturing methods approximates some 10%[252,275], although higher success rates of 40% and more can be found in the literature[347,348]. Second, conventional 2D cultures change the biological properties of cells, possibly altering drug response *in vitro*[349]. Cell polarization, lack of stroma and abundance of growth factors, nutrients and oxygen are factors that might change the behavior of tumor cells[350]. In recent years, more complex cell culturing methods have emerged. CRC cells, cultured in an extracellular matrix form three dimensional spheres, so called spheroids, that differ in their biological properties from 2D cultured cells[351]. In contrast, organoids are three dimensional structures, derived from intact tumor pieces or tumor stem cells cultured in an extracellular matrix scaffold[352]. Patient-derived organoids (PDO), quite similar to PDX, recapitulate closely the histological and genetic properties of their parental tumors. Moreover, high rates of successful establishment have been reported[353] and reliable drug response prediction seems possible[354-356]. PDOs can be implanted s.c. or orthotopically into mice, resulting in PDO xenografts (PDOX)[264,354,357]. Furthermore, the Clevers group pioneered in the inauguration of non-malignant intestinal organoids exploiting the stem cell niche *in vitro*[358]. These organoids can be modified *in vitro* to exhibit malignant properties and used to enlighten the role of cancer-driving pathways by *in vivo* engraftment[359,360]. The available data strongly suggests, that PDOs reflect more faithfully the biological virtue and drug response of the parental tumor compared to conventional 2D cell cultures[352]. The circumstance, that organoids can be derived from CTC, could render them an excellent tool for the preclinical testing of patients with advanced stage cancer that do not undergo surgery[361]. Additionally, CTC reflect genetic changes associated with acquired drug resistance during chemotherapy[362]. Further steps to a reduction of animal experiments imply the faithful remodeling of the host organism *in vitro*. Several research groups created a “cancer on a chip” model that combines the advances of 3D cell culture connected with artificial organs that resemble the most common organs of metastatic spread[363]. Miller and Shuler introduced a “body on a chip model” with 14 artificial organs, which could be modified for cancer research[364]. At last, the widely acknowledged work of Guinney *et al*[365] regarding the consensus molecular subtypes of CRC draw great attention to the value of computed models in cancer science and the capabilities of bioinformatic research. Retrospective analysis of clinical trial samples partly demonstrated the association of drug response with molecular subtypes[366]. The constantly growing knowledge of cancer pathways and their crosstalk on the one hand, and the increased inter-individual complexity of tumors on the other hand, call for a method to integrate and interpret the overwhelming amount of data[367]. In silico methods, like data mining, pattern recognition, machine learning and network approaches, are able to predict the behavior of “virtual” HCT116 cells[368], can reveal genetic patterns associated with survival[369], can be used to detect new biomarkers[370], allow the identification of unknown driver mutations[371] and potential preclinical compounds[372]. Yet, in silico models often lack explanatory power and need careful interpretation by bioinformaticians. Their ability to correctly predict treatment response for an individual patient to a new compound is still a long way off[373].

**ACKNOWLEDGEMENTS**

We kindly thank Jenny Burmeister, graphical assistant, for the supply with excellent pictures.

**REFERENCES**

1 **Siegel RL**, Miller KD, Jemal A. Cancer statistics, 2019. *CA Cancer J Clin* 2019; **69**: 7-34 [PMID: 30620402 DOI: 10.3322/caac.21551]

2 **Ferlay J**, Colombet M, Soerjomataram I, Dyba T, Randi G, Bettio M, Gavin A, Visser O, Bray F. Cancer incidence and mortality patterns in Europe: Estimates for 40 countries and 25 major cancers in 2018. *Eur J Cancer* 2018; **103**: 356-387 [PMID: 30100160 DOI: 10.1016/j.ejca.2018.07.005]

3 **Mengual-Ballester M**, Pellicer-Franco E, Valero-Navarro G, Soria-Aledo V, García-Marín JA, Aguayo-Albasini JL. Increased survival and decreased recurrence in colorectal cancer patients diagnosed in a screening programme. *Cancer Epidemiol* 2016; **43**: 70-75 [PMID: 27399311 DOI: 10.1016/j.canep.2016.06.003]

4 **Hübner J**, Lewin P, Pritzkuleit R, Eisemann N, Maier W, Katalinic A. Colorectal cancer screening by colonoscopy and trends in disease-specific mortality: a population-based ecological study of 358 German districts. *Int J Colorectal Dis* 2019; **34**: 599-605 [PMID: 30627848 DOI: 10.1007/s00384-018-03226-6]

5 **Kawabata-Shoda E**, Charvat H, Ikeda A, Inoue M, Sawada N, Iwasaki M, Sasazuki S, Shimazu T, Yamaji T, Kimura H, Masuda S, Tsugane S. Trends in cancer prognosis in a population-based cohort survey: can recent advances in cancer therapy affect the prognosis? *Cancer Epidemiol* 2015; **39**: 97-103 [PMID: 25541411 DOI: 10.1016/j.canep.2014.11.008]

6 **Johnson CM**, Wei C, Ensor JE, Smolenski DJ, Amos CI, Levin B, Berry DA. Meta-analyses of colorectal cancer risk factors. *Cancer Causes Control* 2013; **24**: 1207-1222 [PMID: 23563998 DOI: 10.1007/s10552-013-0201-5]

7 **Waluga M**, Zorniak M, Fichna J, Kukla M, Hartleb M. Pharmacological and dietary factors in prevention of colorectal cancer. *J Physiol Pharmacol* 2018; **69**: [PMID: 30149368 DOI: 10.26402/jpp.2018.3.02]

8 **Cole BF**, Logan RF, Halabi S, Benamouzig R, Sandler RS, Grainge MJ, Chaussade S, Baron JA. Aspirin for the chemoprevention of colorectal adenomas: meta-analysis of the randomized trials. *J Natl Cancer Inst* 2009; **101**: 256-266 [PMID: 19211452 DOI: 10.1093/jnci/djn485]

9 **Jasperson KW**, Tuohy TM, Neklason DW, Burt RW. Hereditary and familial colon cancer. *Gastroenterology* 2010; **138**: 2044-2058 [PMID: 20420945 DOI: 10.1053/j.gastro.2010.01.054]

10 **Bodmer WF**, Bailey CJ, Bodmer J, Bussey HJ, Ellis A, Gorman P, Lucibello FC, Murday VA, Rider SH, Scambler P. Localization of the gene for familial adenomatous polyposis on chromosome 5. *Nature* 1987; **328**: 614-616 [PMID: 3039373 DOI: 10.1038/328614a0]

11 **Peltomäki P**, Aaltonen LA, Sistonen P, Pylkkänen L, Mecklin JP, Järvinen H, Green JS, Jass JR, Weber JL, Leach FS. Genetic mapping of a locus predisposing to human colorectal cancer. *Science* 1993; **260**: 810-812 [PMID: 8484120 DOI: 10.1126/science.8484120]

12 **Lindblom A**, Tannergård P, Werelius B, Nordenskjöld M. Genetic mapping of a second locus predisposing to hereditary non-polyposis colon cancer. *Nat Genet* 1993; **5**: 279-282 [PMID: 7903889 DOI: 10.1038/ng1193-279]

13 **Cancer Genome Atlas Network**. Comprehensive molecular characterization of human colon and rectal cancer. *Nature* 2012; **487**: 330-337 [PMID: 22810696 DOI: 10.1038/nature11252]

14 **Yamagiwa K**, Ichikawa K. Experimental study of the pathogenesis of carcinoma. *CA Cancer J Clin* 1977; **27**: 174-181 [PMID: 406018 DOI: 10.3322/canjclin.27.3.174]

15 **Lorenz E,** Steward HL. Intestinal Carcinoma and Other Lesions in Mice Following Oral Administration of 1,2,5,6-Dibenzanthracene and 20-Methylcholanthrene. *J Natl Cancer Inst* 1940; **1**: 17-41 [DOI: 10.1093/jnci/1.1.17]

16 **Druckrey H,** Küpfmüller K. Quantitative Analyse der Krebsentstehung. *Zeitschrift für Naturforschung B* 1948; **3**: 254–266 [DOI: 10.1515/znb-1948-7-806]

17 **Lisco H**, Finkel MP, Brues AM. Carcinogenic properties of radioactive fission products and of plutonium. *Radiology* 1947; **49**: 361-363 [PMID: 20266010 DOI: 10.1148/49.3.361]

18 **Laqueur GL**. Carcinogenic Effects of Cycad Meal and Cycasin, Methylazoxymethanol Glycoside, in Rats and Effects of Cycasin in Germfree Rats. *Fed Proc* 1964; **23**: 1386-1388 [PMID: 14236160]

19 **Morgan RW**, Hoffmann GR. Cycasin and its mutagenic metabolites. *Mutat Res* 1983; **114**: 19-58 [PMID: 6338356 DOI: 10.1016/0165-1110(83)90018-0]

20 **Druckrey H**, Preussmann R, Ivanković S, Schmidt CH, So BT, Thomas C. [Carcinogenic effect of azoethane and azoxyethane on rats]. *Z Krebsforsch* 1965; **67**: 31-45 [PMID: 4221131]

21 **Rosenberg DW**, Giardina C, Tanaka T. Mouse models for the study of colon carcinogenesis. *Carcinogenesis* 2009; **30**: 183-196 [PMID: 19037092 DOI: 10.1093/carcin/bgn267]

22 **Fiala ES**, Stathopoulos C. Metabolism of methylazoxymethanol acetate in the F344 rat and strain-2 guinea pig and its inhibition by pyrazole and disulfiram. *J Cancer Res Clin Oncol* 1984; **108**: 129-134 [PMID: 6430908 DOI: 10.1007/bf00390984]

23 **Suzuki R**, Kohno H, Sugie S, Nakagama H, Tanaka T. Strain differences in the susceptibility to azoxymethane and dextran sodium sulfate-induced colon carcinogenesis in mice. *Carcinogenesis* 2006; **27**: 162-169 [PMID: 16081511 DOI: 10.1093/carcin/bgi205]

24 **Turusov VS**, Lanko NS, Krutovskikh VA, Parfenov YD. Strain differences in susceptibility of female mice to 1,2-dimethylhydrazine. *Carcinogenesis* 1982; **3**: 603-608 [PMID: 7116553 DOI: 10.1093/carcin/3.6.603]

25 **Izumi K**, Otsuka H, Furuya K, Akagi A. Carcinogenicity of 1,2-dimethylhydrazine dihydrochloride in BALB/c mice. Influence of the route of administration and dosage. *Virchows Arch A Pathol Anat Histol* 1979; **384**: 263-267 [PMID: 160118 DOI: 10.1007/bf00428228]

26 **Sohn OS**, Fiala ES, Requeijo SP, Weisburger JH, Gonzalez FJ. Differential effects of CYP2E1 status on the metabolic activation of the colon carcinogens azoxymethane and methylazoxymethanol. *Cancer Res* 2001; **61**: 8435-8440 [PMID: 11731424]

27 **Narisawa T**, Wong CQ, Maronpot RR, Weisburger JH. Large bowel carcinogenesis in mice and rats by several intrarectal doses of methylnitrosourea and negative effect of nitrite plus methylurea. *Cancer Res* 1976; **36**: 505-510 [PMID: 1260748]

28 **Reddy BS**, Ohmori T. Effect of intestinal microflora and dietary fat on 3,2'-dimethyl-4-aminobiphenyl-induced colon carcinogenesis in F344 rats. *Cancer Res* 1981; **41**: 1363-1367 [PMID: 7194137]

29 **Sugimura T**, Terada M. Experimental chemical carcinogenesis in the stomach and colon. *Jpn J Clin Oncol* 1998; **28**: 163-167 [PMID: 9614437 DOI: 10.1093/jjco/28.3.163]

30 **Bouvard V**, Loomis D, Guyton KZ, Grosse Y, Ghissassi FE, Benbrahim-Tallaa L, Guha N, Mattock H, Straif K; International Agency for Research on Cancer Monograph Working Group. Carcinogenicity of consumption of red and processed meat. *Lancet Oncol* 2015; **16**: 1599-1600 [PMID: 26514947 DOI: 10.1016/S1470-2045(15)00444-1]

31 **Jägerstad M**, Skog K. Genotoxicity of heat-processed foods. *Mutat Res* 2005; **574**: 156-172 [PMID: 15914214 DOI: 10.1016/j.mrfmmm.2005.01.030]

32 **Turesky RJ**. Formation and biochemistry of carcinogenic heterocyclic aromatic amines in cooked meats. *Toxicol Lett* 2007; **168**: 219-227 [PMID: 17174486 DOI: 10.1016/j.toxlet.2006.10.018]

33 **Cheung C**, Ma X, Krausz KW, Kimura S, Feigenbaum L, Dalton TP, Nebert DW, Idle JR, Gonzalez FJ. Differential metabolism of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) in mice humanized for CYP1A1 and CYP1A2. *Chem Res Toxicol* 2005; **18**: 1471-1478 [PMID: 16167840 DOI: 10.1021/tx050136g]

34 **Kaderlik KR**, Mulder GJ, Shaddock JG, Casciano DA, Teitel CH, Kadlubar FF. Effect of glutathione depletion and inhibition of glucuronidation and sulfation on 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) metabolism, PhIP-DNA adduct formation and unscheduled DNA synthesis in primary rat hepatocytes. *Carcinogenesis* 1994; **15**: 1711-1716 [PMID: 8055653 DOI: 10.1093/carcin/15.8.1711]

35 **Alexander J**, Wallin H, Rossland OJ, Solberg KE, Holme JA, Becher G, Andersson R, Grivas S. Formation of a glutathione conjugate and a semistable transportable glucuronide conjugate of N2-oxidized species of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) in rat liver. *Carcinogenesis* 1991; **12**: 2239-2245 [PMID: 1747923 DOI: 10.1093/carcin/12.12.2239]

36 **Malfatti MA**, Kulp KS, Knize MG, Davis C, Massengill JP, Williams S, Nowell S, MacLeod S, Dingley KH, Turteltaub KW, Lang NP, Felton JS. The identification of [2-(14)C]2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine metabolites in humans. *Carcinogenesis* 1999; **20**: 705-713 [PMID: 10223203 DOI: 10.1093/carcin/20.4.705]

37 **Zhang J**, Lacroix C, Wortmann E, Ruscheweyh HJ, Sunagawa S, Sturla SJ, Schwab C. Gut microbial beta-glucuronidase and glycerol/diol dehydratase activity contribute to dietary heterocyclic amine biotransformation. *BMC Microbiol* 2019; **19**: 99 [PMID: 31096909 DOI: 10.1186/s12866-019-1483-x]

38 **Frandsen H**. Biomonitoring of urinary metabolites of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) following human consumption of cooked chicken. *Food Chem Toxicol* 2008; **46**: 3200-3205 [PMID: 18692111 DOI: 10.1016/j.fct.2008.07.008]

39 **Nakagama H**, Ochiai M, Ubagai T, Tajima R, Fujiwara K, Sugimura T, Nagao M. A rat colon cancer model induced by 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine, PhIP. *Mutat Res* 2002; **506-507**: 137-144 [PMID: 12351153 DOI: 10.1016/s0027-5107(02)00160-4]

40 **Chen JX**, Wang H, Liu A, Zhang L, Reuhl K, Yang CS. From the Cover: PhIP/DSS-Induced Colon Carcinogenesis in CYP1A-Humanized Mice and the Possible Role of Lgr5+ Stem Cells. *Toxicol Sci* 2017; **155**: 224-233 [PMID: 27664423 DOI: 10.1093/toxsci/kfw190]

41 **Sugimura T**, Wakabayashi K, Nakagama H, Nagao M. Heterocyclic amines: Mutagens/carcinogens produced during cooking of meat and fish. *Cancer Sci* 2004; **95**: 290-299 [PMID: 15072585 DOI: 10.1111/j.1349-7006.2004.tb03205.x]

42 **Huderson AC**, Myers JN, Niaz MS, Washington MK, Ramesh A. Chemoprevention of benzo(a)pyrene-induced colon polyps in ApcMin mice by resveratrol. *J Nutr Biochem* 2013; **24**: 713-724 [PMID: 22889612 DOI: 10.1016/j.jnutbio.2012.04.005]

43 **Diggs DL**, Harris KL, Rekhadevi PV, Ramesh A. Tumor microsomal metabolism of the food toxicant, benzo(a)pyrene, in ApcMin mouse model of colon cancer. *Tumour Biol* 2012; **33**: 1255-1260 [PMID: 22430258 DOI: 10.1007/s13277-012-0375-6]

44 **Okayasu I**, Hatakeyama S, Yamada M, Ohkusa T, Inagaki Y, Nakaya R. A novel method in the induction of reliable experimental acute and chronic ulcerative colitis in mice. *Gastroenterology* 1990; **98**: 694-702 [PMID: 1688816 DOI: 10.1016/0016-5085(90)90290-h]

45 **Kawada M**, Arihiro A, Mizoguchi E. Insights from advances in research of chemically induced experimental models of human inflammatory bowel disease. *World J Gastroenterol* 2007; **13**: 5581-5593 [PMID: 17948932 DOI: 10.3748/wjg.v13.i42.5581]

46 **Eichele DD**, Kharbanda KK. Dextran sodium sulfate colitis murine model: An indispensable tool for advancing our understanding of inflammatory bowel diseases pathogenesis. *World J Gastroenterol* 2017; **23**: 6016-6029 [PMID: 28970718 DOI: 10.3748/wjg.v23.i33.6016]

47 **Munyaka PM**, Rabbi MF, Khafipour E, Ghia JE. Acute dextran sulfate sodium (DSS)-induced colitis promotes gut microbial dysbiosis in mice. *J Basic Microbiol* 2016; **56**: 986-998 [PMID: 27112251 DOI: 10.1002/jobm.201500726]

48 **He X**, Wei Z, Wang J, Kou J, Liu W, Fu Y, Yang Z. Alpinetin attenuates inflammatory responses by suppressing TLR4 and NLRP3 signaling pathways in DSS-induced acute colitis. *Sci Rep* 2016; **6**: 28370 [PMID: 27321991 DOI: 10.1038/srep28370]

49 **Dieleman LA**, Palmen MJ, Akol H, Bloemena E, Peña AS, Meuwissen SG, Van Rees EP. Chronic experimental colitis induced by dextran sulphate sodium (DSS) is characterized by Th1 and Th2 cytokines. *Clin Exp Immunol* 1998; **114**: 385-391 [PMID: 9844047 DOI: 10.1046/j.1365-2249.1998.00728.x]

50 **Hoffmann M**, Schwertassek U, Seydel A, Weber K, Falk W, Hauschildt S, Lehmann J. A refined and translationally relevant model of chronic DSS colitis in BALB/c mice. *Lab Anim* 2018; **52**: 240-252 [PMID: 29192559 DOI: 10.1177/0023677217742681]

51 **Sussman DA**, Santaolalla R, Strobel S, Dheer R, Abreu MT. Cancer in inflammatory bowel disease: lessons from animal models. *Curr Opin Gastroenterol* 2012; **28**: 327-333 [PMID: 22614440 DOI: 10.1097/MOG.0b013e328354cc36]

52 **Tanaka T**, Kohno H, Suzuki R, Hata K, Sugie S, Niho N, Sakano K, Takahashi M, Wakabayashi K. Dextran sodium sulfate strongly promotes colorectal carcinogenesis in Apc(Min/+) mice: inflammatory stimuli by dextran sodium sulfate results in development of multiple colonic neoplasms. *Int J Cancer* 2006; **118**: 25-34 [PMID: 16049979 DOI: 10.1002/ijc.21282]

53 **Cooper HS**, Everley L, Chang WC, Pfeiffer G, Lee B, Murthy S, Clapper ML. The role of mutant Apc in the development of dysplasia and cancer in the mouse model of dextran sulfate sodium-induced colitis. *Gastroenterology* 2001; **121**: 1407-1416 [PMID: 11729120 DOI: 10.1053/gast.2001.29609]

54 **De Robertis M**, Massi E, Poeta ML, Carotti S, Morini S, Cecchetelli L, Signori E, Fazio VM. The AOM/DSS murine model for the study of colon carcinogenesis: From pathways to diagnosis and therapy studies. *J Carcinog* 2011; **10**: 9 [PMID: 21483655 DOI: 10.4103/1477-3163.78279]

55 **Velázquez KT**, Enos RT, Carson MS, Cranford TL, Bader JE, Chatzistamou I, Singh UP, Nagarkatti PS, Nagarkatti M, Davis JM, Carson JA, Murphy EA. Weight loss following diet-induced obesity does not alter colon tumorigenesis in the AOM mouse model. *Am J Physiol Gastrointest Liver Physiol* 2016; **311**: G699-G712 [PMID: 27609769 DOI: 10.1152/ajpgi.00207.2016]

56 **Cuellar-Nuñez ML**, Luzardo-Ocampo I, Campos-Vega R, Gallegos-Corona MA, González de Mejía E, Loarca-Piña G. Physicochemical and nutraceutical properties of moringa (Moringa oleifera) leaves and their effects in an in vivo AOM/DSS-induced colorectal carcinogenesis model. *Food Res Int* 2018; **105**: 159-168 [PMID: 29433203 DOI: 10.1016/j.foodres.2017.11.004]

57 **Canene-Adams K**, Sfanos KS, Liang CT, Yegnasubramanian S, Nelson WG, Brayton C, De Marzo AM. Dietary chemoprevention of PhIP induced carcinogenesis in male Fischer 344 rats with tomato and broccoli. *PLoS One* 2013; **8**: e79842 [PMID: 24312188 DOI: 10.1371/journal.pone.0079842]

58 **Chen JX**, Liu A, Lee MJ, Wang H, Yu S, Chi E, Reuhl K, Suh N, Yang CS. δ- and γ-tocopherols inhibit phIP/DSS-induced colon carcinogenesis by protection against early cellular and DNA damages. *Mol Carcinog* 2017; **56**: 172-183 [PMID: 27175800 DOI: 10.1002/mc.22481]

59 **Bi W**, Liu H, Shen J, Zhang LH, Li P, Peng B, Cao L, Zhang P, He C, Xiao P. Chemopreventive effects of Ku-jin tea against AOM-induced precancerous colorectal lesions in rats and metabolomic analysis. *Sci Rep* 2017; **7**: 15893 [PMID: 29162930 DOI: 10.1038/s41598-017-16237-0]

60 **Sun MC**, Zhang FC, Yin X, Cheng BJ, Zhao CH, Wang YL, Zhang ZZ, Hao HW, Zhang TH, Ye HQ. Lactobacillus reuteri F-9-35 Prevents DSS-Induced Colitis by Inhibiting Proinflammatory Gene Expression and Restoring the Gut Microbiota in Mice. *J Food Sci* 2018; **83**: 2645-2652 [PMID: 30216448 DOI: 10.1111/1750-3841.14326]

61 **Snider AJ**, Bialkowska AB, Ghaleb AM, Yang VW, Obeid LM, Hannun YA. Murine Model for Colitis-Associated Cancer of the Colon. *Methods Mol Biol* 2016; **1438**: 245-254 [PMID: 27150094 DOI: 10.1007/978-1-4939-3661-8\_14]

62 **Yang J**, Shikata N, Mizuoka H, Tsubura A. Colon carcinogenesis in shrews by intrarectal infusion of N-methyl-N-nitrosourea. *Cancer Lett* 1996; **110**: 105-112 [PMID: 9018088 DOI: 10.1016/s0304-3835(96)04468-0]

63 **Derry MM**, Raina K, Agarwal R, Agarwal C. Characterization of azoxymethane-induced colon tumor metastasis to lung in a mouse model relevant to human sporadic colorectal cancer and evaluation of grape seed extract efficacy. *Exp Toxicol Pathol* 2014; **66**: 235-242 [PMID: 24670932 DOI: 10.1016/j.etp.2014.02.003]

64 **Papanikolaou A**, Wang QS, Papanikolaou D, Whiteley HE, Rosenberg DW. Sequential and morphological analyses of aberrant crypt foci formation in mice of differing susceptibility to azoxymethane-induced colon carcinogenesis. *Carcinogenesis* 2000; **21**: 1567-1572 [PMID: 10910960]

65 **Nambiar PR**, Girnun G, Lillo NA, Guda K, Whiteley HE, Rosenberg DW. Preliminary analysis of azoxymethane induced colon tumors in inbred mice commonly used as transgenic/knockout progenitors. *Int J Oncol* 2003; **22**: 145-150 [PMID: 12469197 DOI: 10.3892/ijo.22.1.145]

66 **Clapp NK**, Henke MA, London JF, Shock TL. Enhancement of 1,2-dimethylhydrazine-induced large bowel tumorigenesis in Balb/c mice by corn, soybean, and wheat brans. *Nutr Cancer* 1984; **6**: 77-85 [PMID: 6100660 DOI: 10.1080/0163558850951381]

67 **Neufert C**, Becker C, Neurath MF. An inducible mouse model of colon carcinogenesis for the analysis of sporadic and inflammation-driven tumor progression. *Nat Protoc* 2007; **2**: 1998-2004 [PMID: 17703211 DOI: 10.1038/nprot.2007.279]

68 **Piñol V**, Andreu M, Castells A, Payá A, Bessa X, Jover R; Gastrointestinal Oncology Group of the Spanish Gastroenterological Association. Synchronous colorectal neoplasms in patients with colorectal cancer: predisposing individual and familial factors. *Dis Colon Rectum* 2004; **47**: 1192-1200 [PMID: 15164252 DOI: 10.1007/s10350-004-0562-7]

69 **Marqués-Lespier JM**, Soto-Salgado M, González-Pons M, Méndez V, Freyre K, Beltrán C, Pericchi LR, Cruz-Correa M. Prevalence of Synchronous Oligopolyposis in Incident Colorectal Cancer: A Population-Based Study. *P R Health Sci J* 2018; **37**: 39-45 [PMID: 29547683]

70 **Thaker AI**, Shaker A, Rao MS, Ciorba MA. Modeling colitis-associated cancer with azoxymethane (AOM) and dextran sulfate sodium (DSS). *J Vis Exp* 2012; **(67)** [PMID: 22990604 DOI: 10.3791/4100]

71 **Matsumoto K**, Iwase T, Hirono I, Nishida Y, Iwahori Y, Hori T, Asamoto M, Takasuka N, Kim DJ, Ushijima T, Nagao M, Tsuda H. Demonstration of ras and p53 gene mutations in carcinomas in the forestomach and intestine and soft tissue sarcomas induced by N-methyl-N-nitrosourea in the rat. *Jpn J Cancer Res* 1997; **88**: 129-136 [PMID: 9119740 DOI: 10.1111/j.1349-7006.1997.tb00357.x]

72 **Nordlinger B**, Panis Y, Puts JP, Herve JP, Delelo R, Ballet F. Experimental model of colon cancer: recurrences after surgery alone or associated with intraperitoneal 5-fluorouracil chemotherapy. *Dis Colon Rectum* 1991; **34**: 658-663 [PMID: 1855422 DOI: 10.1007/bf02050346]

73 **Narisawa T**, Weisburger JH. Colon cancer induction in mice by intrarectal instillation of N-methylnitosorurea (38498). *Proc Soc Exp Biol Med* 1975; **148**: 166-169 [PMID: 1129255 DOI: 10.3181/00379727-148-38498]

74 **Hasegawa R**, Sano M, Tamano S, Imaida K, Shirai T, Nagao M, Sugimura T, Ito N. Dose-dependence of 2-amino-1-methyl-6-phenylimidazo[4,5-b]-pyridine (PhIP) carcinogenicity in rats. *Carcinogenesis* 1993; **14**: 2553-2557 [PMID: 8269626 DOI: 10.1093/carcin/14.12.2553]

75 **Leslie A**, Carey FA, Pratt NR, Steele RJ. The colorectal adenoma-carcinoma sequence. *Br J Surg* 2002; **89**: 845-860 [PMID: 12081733 DOI: 10.1046/j.1365-2168.2002.02120.x]

76 **Jacoby RF**, Llor X, Teng BB, Davidson NO, Brasitus TA. Mutations in the K-ras oncogene induced by 1,2-dimethylhydrazine in preneoplastic and neoplastic rat colonic mucosa. *J Clin Invest* 1991; **87**: 624-630 [PMID: 1991846 DOI: 10.1172/JCI115039]

77 **De Filippo C**, Caderni G, Bazzicalupo M, Briani C, Giannini A, Fazi M, Dolara P. Mutations of the Apc gene in experimental colorectal carcinogenesis induced by azoxymethane in F344 rats. *Br J Cancer* 1998; **77**: 2148-2151 [PMID: 9649126 DOI: 10.1038/bjc.1998.359]

78 **Aaltonen LA**, Peltomäki P, Leach FS, Sistonen P, Pylkkänen L, Mecklin JP, Järvinen H, Powell SM, Jen J, Hamilton SR. Clues to the pathogenesis of familial colorectal cancer. *Science* 1993; **260**: 812-816 [PMID: 8484121 DOI: 10.1126/science.8484121]

79 **Pan Q**, Lou X, Zhang J, Zhu Y, Li F, Shan Q, Chen X, Xie Y, Su S, Wei H, Lin L, Wu L, Liu S. Genomic variants in mouse model induced by azoxymethane and dextran sodium sulfate improperly mimic human colorectal cancer. *Sci Rep* 2017; **7**: 25 [PMID: 28154415 DOI: 10.1038/s41598-017-00057-3]

80 **Cheung C**, Loy S, Li GX, Liu AB, Yang CS. Rapid induction of colon carcinogenesis in CYP1A-humanized mice by 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine and dextran sodium sulfate. *Carcinogenesis* 2011; **32**: 233-239 [PMID: 21081470 DOI: 10.1093/carcin/bgq235]

81 **Brinster RL**, Chen HY, Messing A, van Dyke T, Levine AJ, Palmiter RD. Transgenic mice harboring SV40 T-antigen genes develop characteristic brain tumors. *Cell* 1984; **37**: 367-379 [PMID: 6327063 DOI: 10.1016/0092-8674(84)90367-2]

82 **Adams JM**, Harris AW, Pinkert CA, Corcoran LM, Alexander WS, Cory S, Palmiter RD, Brinster RL. The c-myc oncogene driven by immunoglobulin enhancers induces lymphoid malignancy in transgenic mice. *Nature* 1985; **318**: 533-538 [PMID: 3906410 DOI: 10.1038/318533a0]

83 **Ornitz DM**, Palmiter RD, Messing A, Hammer RE, Pinkert CA, Brinster RL. Elastase I promoter directs expression of human growth hormone and SV40 T antigen genes to pancreatic acinar cells in transgenic mice. *Cold Spring Harb Symp Quant Biol* 1985; **50**: 399-409 [PMID: 3006998 DOI: 10.1101/sqb.1985.050.01.050]

84 **Quaife CJ**, Pinkert CA, Ornitz DM, Palmiter RD, Brinster RL. Pancreatic neoplasia induced by ras expression in acinar cells of transgenic mice. *Cell* 1987; **48**: 1023-1034 [PMID: 3470144 DOI: 10.1016/0092-8674(87)90710-0]

85 **Stewart TA**, Pattengale PK, Leder P. Spontaneous mammary adenocarcinomas in transgenic mice that carry and express MTV/myc fusion genes. *Cell* 1984; **38**: 627-637 [PMID: 6488314 DOI: 10.1016/0092-8674(84)90257-5]

86 **Rüther U**, Garber C, Komitowski D, Müller R, Wagner EF. Deregulated c-fos expression interferes with normal bone development in transgenic mice. *Nature* 1987; **325**: 412-416 [PMID: 3027573 DOI: 10.1038/325412a0]

87 **Hanahan D**. Heritable formation of pancreatic beta-cell tumours in transgenic mice expressing recombinant insulin/simian virus 40 oncogenes. *Nature* 1985; **315**: 115-122 [PMID: 2986015 DOI: 10.1038/315115a0]

88 **Schäfer C**. Nobel Price for Medicine 2007. Knockout mice are revolutionizing genetics. *Ophthalmologe* 2007; **104**: 1080-1082 [PMID: 18074163 DOI: 10.1007/s00347-007-1663-1]

89 **Jacks T**, Fazeli A, Schmitt EM, Bronson RT, Goodell MA, Weinberg RA. Effects of an Rb mutation in the mouse. *Nature* 1992; **359**: 295-300 [PMID: 1406933 DOI: 10.1038/359295a0]

90 **Donehower LA**, Harvey M, Slagle BL, McArthur MJ, Montgomery CA Jr, Butel JS, Bradley A. Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. *Nature* 1992; **356**: 215-221 [PMID: 1552940 DOI: 10.1038/356215a0]

91 **Lakso M**, Sauer B, Mosinger B Jr, Lee EJ, Manning RW, Yu SH, Mulder KL, Westphal H. Targeted oncogene activation by site-specific recombination in transgenic mice. *Proc Natl Acad Sci U S A* 1992; **89**: 6232-6236 [PMID: 1631115 DOI: 10.1073/pnas.89.14.6232]

92 **Meuwissen R**, Linn SC, van der Valk M, Mooi WJ, Berns A. Mouse model for lung tumorigenesis through Cre/lox controlled sporadic activation of the K-Ras oncogene. *Oncogene* 2001; **20**: 6551-6558 [PMID: 11641780 DOI: 10.1038/sj.onc.1204837]

93 **Hingorani SR**, Petricoin EF, Maitra A, Rajapakse V, King C, Jacobetz MA, Ross S, Conrads TP, Veenstra TD, Hitt BA, Kawaguchi Y, Johann D, Liotta LA, Crawford HC, Putt ME, Jacks T, Wright CV, Hruban RH, Lowy AM, Tuveson DA. Preinvasive and invasive ductal pancreatic cancer and its early detection in the mouse. *Cancer Cell* 2003; **4**: 437-450 [PMID: 14706336 DOI: 10.1016/s1535-6108(03)00309-x]

94 **Moser AR**, Pitot HC, Dove WF. A dominant mutation that predisposes to multiple intestinal neoplasia in the mouse. *Science* 1990; **247**: 322-324 [PMID: 2296722 DOI: 10.1126/science.2296722]

95 **Shoemaker AR**, Gould KA, Luongo C, Moser AR, Dove WF. Studies of neoplasia in the Min mouse. *Biochim Biophys Acta* 1997; **1332**: F25-F48 [PMID: 9141462 DOI: 10.1016/s0304-419x(96)00041-8]

96 **Yamada Y**, Mori H. Multistep carcinogenesis of the colon in Apc(Min/+) mouse. *Cancer Sci* 2007; **98**: 6-10 [PMID: 17052257 DOI: 10.1111/j.1349-7006.2006.00348.x]

97 **Moser AR**, Shoemaker AR, Connelly CS, Clipson L, Gould KA, Luongo C, Dove WF, Siggers PH, Gardner RL. Homozygosity for the Min allele of Apc results in disruption of mouse development prior to gastrulation. *Dev Dyn* 1995; **203**: 422-433 [PMID: 7496034 DOI: 10.1002/aja.1002030405]

98 **Møllersen L**, Paulsen JE, Alexander J. Loss of heterozygosity and nonsense mutation in Apc in azoxymethane-induced colonic tumours in min mice. *Anticancer Res* 2004; **24**: 2595-2599 [PMID: 15517863]

99 **Ju J**, Nolan B, Cheh M, Bose M, Lin Y, Wagner GC, Yang CS. Voluntary exercise inhibits intestinal tumorigenesis in Apc(Min/+) mice and azoxymethane/dextran sulfate sodium-treated mice. *BMC Cancer* 2008; **8**: 316 [PMID: 18976499 DOI: 10.1186/1471-2407-8-316]

100 **Tanaka T**, Suzuki R, Kohno H, Sugie S, Takahashi M, Wakabayashi K. Colonic adenocarcinomas rapidly induced by the combined treatment with 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine and dextran sodium sulfate in male ICR mice possess beta-catenin gene mutations and increases immunoreactivity for beta-catenin, cyclooxygenase-2 and inducible nitric oxide synthase. *Carcinogenesis* 2005; **26**: 229-238 [PMID: 15459021 DOI: 10.1093/carcin/bgh292]

101 **Jin D**, Liu T, Dong W, Zhang Y, Wang S, Xie R, Wang B, Cao H. Dietary feeding of freeze-dried whole cranberry inhibits intestinal tumor development in *Apc*min/+ mice. *Oncotarget* 2017; **8**: 97787-97800 [PMID: 29228651 DOI: 10.18632/oncotarget.22081]

102 **Ni Y**, Wong VH, Tai WC, Li J, Wong WY, Lee MM, Fong FL, El-Nezami H, Panagiotou G. A metagenomic study of the preventive effect of Lactobacillus rhamnosus GG on intestinal polyp formation in ApcMin/+ mice. *J Appl Microbiol* 2017; **122**: 770-784 [PMID: 28004480 DOI: 10.1111/jam.13386]

103 **Zhang L**, Shay JW. Multiple Roles of APC and its Therapeutic Implications in Colorectal Cancer. *J Natl Cancer Inst* 2017; **109**: [PMID: 28423402 DOI: 10.1093/jnci/djw332]

104 **Halberg RB**, Waggoner J, Rasmussen K, White A, Clipson L, Prunuske AJ, Bacher JW, Sullivan R, Washington MK, Pitot HC, Petrini JH, Albertson DG, Dove WF. Long-lived Min mice develop advanced intestinal cancers through a genetically conservative pathway. *Cancer Res* 2009; **69**: 5768-5775 [PMID: 19584276 DOI: 10.1158/0008-5472.CAN-09-0446]

105 **Sødring M**, Gunnes G, Paulsen JE. Spontaneous initiation, promotion and progression of colorectal cancer in the novel A/J Min/+ mouse. *Int J Cancer* 2016; **138**: 1936-1946 [PMID: 26566853 DOI: 10.1002/ijc.29928]

106 **Fodde R**, Edelmann W, Yang K, van Leeuwen C, Carlson C, Renault B, Breukel C, Alt E, Lipkin M, Khan PM. A targeted chain-termination mutation in the mouse Apc gene results in multiple intestinal tumors. *Proc Natl Acad Sci U S A* 1994; **91**: 8969-8973 [PMID: 8090754 DOI: 10.1073/pnas.91.19.8969]

107 **Smits R**, Kartheuser A, Jagmohan-Changur S, Leblanc V, Breukel C, de Vries A, van Kranen H, van Krieken JH, Williamson S, Edelmann W, Kucherlapati R, KhanPM, Fodde R. Loss of Apc and the entire chromosome 18 but absence of mutations at the Ras and Tp53 genes in intestinal tumors from Apc1638N, a mouse model for Apc-driven carcinogenesis. *Carcinogenesis* 1997; **18**: 321-327 [PMID: 9054624 DOI: 10.1093/carcin/18.2.321]

108 **Oshima M**, Oshima H, Kitagawa K, Kobayashi M, Itakura C, Taketo M. Loss of Apc heterozygosity and abnormal tissue building in nascent intestinal polyps in mice carrying a truncated Apc gene. *Proc Natl Acad Sci U S A* 1995; **92**: 4482-4486 [PMID: 7753829 DOI: 10.1073/pnas.92.10.4482]

109 **Crist RC**, Roth JJ, Baran AA, McEntee BJ, Siracusa LD, Buchberg AM. The armadillo repeat domain of Apc suppresses intestinal tumorigenesis. *Mamm Genome* 2010; **21**: 450-457 [PMID: 20886217 DOI: 10.1007/s00335-010-9288-0]

110 **Colnot S**, Niwa-Kawakita M, Hamard G, Godard C, Le Plenier S, Houbron C, Romagnolo B, Berrebi D, Giovannini M, Perret C. Colorectal cancers in a new mouse model of familial adenomatous polyposis: influence of genetic and environmental modifiers. *Lab Invest* 2004; **84**: 1619-1630 [PMID: 15502862 DOI: 10.1038/labinvest.3700180]

111 **Fazeli A**, Steen RG, Dickinson SL, Bautista D, Dietrich WF, Bronson RT, Bresalier RS, Lander ES, Costa J, Weinberg RA. Effects of p53 mutations on apoptosis in mouse intestinal and human colonic adenomas. *Proc Natl Acad Sci U S A* 1997; **94**: 10199-10204 [PMID: 9294187 DOI: 10.1073/pnas.94.19.10199]

112 **Clarke AR**, Cummings MC, Harrison DJ. Interaction between murine germline mutations in p53 and APC predisposes to pancreatic neoplasia but not to increased intestinal malignancy. *Oncogene* 1995; **11**: 1913-1920 [PMID: 7478622]

113 **Halberg RB**, Katzung DS, Hoff PD, Moser AR, Cole CE, Lubet RA, Donehower LA, Jacoby RF, Dove WF. Tumorigenesis in the multiple intestinal neoplasia mouse: redundancy of negative regulators and specificity of modifiers. *Proc Natl Acad Sci U S A* 2000; **97**: 3461-3466 [PMID: 10716720 DOI: 10.1073/pnas.050585597]

114 **Maslon MM**, Hupp TR. Drug discovery and mutant p53. *Trends Cell Biol* 2010; **20**: 542-555 [PMID: 20656489 DOI: 10.1016/j.tcb.2010.06.005]

115 **Billant O**, Léon A, Le Guellec S, Friocourt G, Blondel M, Voisset C. The dominant-negative interplay between p53, p63 and p73: A family affair. *Oncotarget* 2016; **7**: 69549-69564 [PMID: 27589690 DOI: 10.18632/oncotarget.11774]

116 **Tuveson DA**, Shaw AT, Willis NA, Silver DP, Jackson EL, Chang S, Mercer KL, Grochow R, Hock H, Crowley D, Hingorani SR, Zaks T, King C, Jacobetz MA, Wang L, Bronson RT, Orkin SH, DePinho RA, Jacks T. Endogenous oncogenic K-ras(G12D) stimulates proliferation and widespread neoplastic and developmental defects. *Cancer Cell* 2004; **5**: 375-387 [PMID: 15093544 DOI: 10.1016/s1535-6108(04)00085-6]

117 **Guerra C**, Mijimolle N, Dhawahir A, Dubus P, Barradas M, Serrano M, Campuzano V, Barbacid M. Tumor induction by an endogenous K-ras oncogene is highly dependent on cellular context. *Cancer Cell* 2003; **4**: 111-120 [PMID: 12957286 DOI: 10.1016/s1535-6108(03)00191-0]

118 **el Marjou F**, Janssen KP, Chang BH, Li M, Hindie V, Chan L, Louvard D, Chambon P, Metzger D, Robine S. Tissue-specific and inducible Cre-mediated recombination in the gut epithelium. *Genesis* 2004; **39**: 186-193 [PMID: 15282745 DOI: 10.1002/gene.20042]

119 **Saam JR**, Gordon JI. Inducible gene knockouts in the small intestinal and colonic epithelium. *J Biol Chem* 1999; **274**: 38071-38082 [PMID: 10608876 DOI: 10.1074/jbc.274.53.38071]

120 **Robanus-Maandag EC**, Koelink PJ, Breukel C, Salvatori DC, Jagmohan-Changur SC, Bosch CA, Verspaget HW, Devilee P, Fodde R, Smits R. A new conditional Apc-mutant mouse model for colorectal cancer. *Carcinogenesis* 2010; **31**: 946-952 [PMID: 20176656 DOI: 10.1093/carcin/bgq046]

121 **Ireland H**, Kemp R, Houghton C, Howard L, Clarke AR, Sansom OJ, Winton DJ. Inducible Cre-mediated control of gene expression in the murine gastrointestinal tract: effect of loss of beta-catenin. *Gastroenterology* 2004; **126**: 1236-1246 [PMID: 15131783 DOI: 10.1053/j.gastro.2004.03.020]

122 **Sansom OJ**, Meniel V, Wilkins JA, Cole AM, Oien KA, Marsh V, Jamieson TJ, Guerra C, Ashton GH, Barbacid M, Clarke AR. Loss of Apc allows phenotypic manifestation of the transforming properties of an endogenous K-ras oncogene in vivo. *Proc Natl Acad Sci U S A* 2006; **103**: 14122-14127 [PMID: 16959882 DOI: 10.1073/pnas.0604130103]

123 **Sansom OJ**, Griffiths DF, Reed KR, Winton DJ, Clarke AR. Apc deficiency predisposes to renal carcinoma in the mouse. *Oncogene* 2005; **24**: 8205-8210 [PMID: 16116480 DOI: 10.1038/sj.onc.1208956]

124 **Hinoi T**, Akyol A, Theisen BK, Ferguson DO, Greenson JK, Williams BO, Cho KR, Fearon ER. Mouse model of colonic adenoma-carcinoma progression based on somatic Apc inactivation. *Cancer Res* 2007; **67**: 9721-9730 [PMID: 17942902 DOI: 10.1158/0008-5472.CAN-07-2735]

125 **Akyol A**, Hinoi T, Feng Y, Bommer GT, Glaser TM, Fearon ER. Generating somatic mosaicism with a Cre recombinase-microsatellite sequence transgene. *Nat Methods* 2008; **5**: 231-233 [PMID: 18264107 DOI: 10.1038/nmeth.1182]

126 **Xue Y**, Johnson R, Desmet M, Snyder PW, Fleet JC. Generation of a transgenic mouse for colorectal cancer research with intestinal cre expression limited to the large intestine. *Mol Cancer Res* 2010; **8**: 1095-1104 [PMID: 20663863 DOI: 10.1158/1541-7786.MCR-10-0195]

127 **Shibata H**, Toyama K, Shioya H, Ito M, Hirota M, Hasegawa S, Matsumoto H, Takano H, Akiyama T, Toyoshima K, Kanamaru R, Kanegae Y, Saito I, Nakamura Y, Shiba K, Noda T. Rapid colorectal adenoma formation initiated by conditional targeting of the Apc gene. *Science* 1997; **278**: 120-123 [PMID: 9311916 DOI: 10.1126/science.278.5335.120]

128 **Roper J**, Tammela T, Cetinbas NM, Akkad A, Roghanian A, Rickelt S, Almeqdadi M, Wu K, Oberli MA, Sánchez-Rivera FJ, Park YK, Liang X, Eng G, Taylor MS, Azimi R, Kedrin D, Neupane R, Beyaz S, Sicinska ET, Suarez Y, Yoo J, Chen L, Zukerberg L, Katajisto P, Deshpande V, Bass AJ, Tsichlis PN, Lees J, Langer R, Hynes RO, Chen J, Bhutkar A, Jacks T, Yilmaz ÖH. In vivo genome editing and organoid transplantation models of colorectal cancer and metastasis. *Nat Biotechnol* 2017; **35**: 569-576 [PMID: 28459449 DOI: 10.1038/nbt.3836]

129 **Hadac JN**, Leystra AA, Paul Olson TJ, Maher ME, Payne SN, Yueh AE, Schwartz AR, Albrecht DM, Clipson L, Pasch CA, Matkowskyj KA, Halberg RB, Deming DA. Colon Tumors with the Simultaneous Induction of Driver Mutations in APC, KRAS, and PIK3CA Still Progress through the Adenoma-to-carcinoma Sequence. *Cancer Prev Res (Phila)* 2015; **8**: 952-961 [PMID: 26276752 DOI: 10.1158/1940-6207.CAPR-15-0003]

130 **Hung KE**, Maricevich MA, Richard LG, Chen WY, Richardson MP, Kunin A, Bronson RT, Mahmood U, Kucherlapati R. Development of a mouse model for sporadic and metastatic colon tumors and its use in assessing drug treatment. *Proc Natl Acad Sci U S A* 2010; **107**: 1565-1570 [PMID: 20080688 DOI: 10.1073/pnas.0908682107]

131 **Sansom OJ**, Reed KR, Hayes AJ, Ireland H, Brinkmann H, Newton IP, Batlle E, Simon-Assmann P, Clevers H, Nathke IS, Clarke AR, Winton DJ. Loss of Apc in vivo immediately perturbs Wnt signaling, differentiation, and migration. *Genes Dev* 2004; **18**: 1385-1390 [PMID: 15198980 DOI: 10.1101/gad.287404]

132 **Cheung AF**, Carter AM, Kostova KK, Woodruff JF, Crowley D, Bronson RT, Haigis KM, Jacks T. Complete deletion of Apc results in severe polyposis in mice. *Oncogene* 2010; **29**: 1857-1864 [PMID: 20010873 DOI: 10.1038/onc.2009.457]

133 **Feng Y**, Sentani K, Wiese A, Sands E, Green M, Bommer GT, Cho KR, Fearon ER. Sox9 induction, ectopic Paneth cells, and mitotic spindle axis defects in mouse colon adenomatous epithelium arising from conditional biallelic Apc inactivation. *Am J Pathol* 2013; **183**: 493-503 [PMID: 23769888 DOI: 10.1016/j.ajpath.2013.04.013]

134 **Calcagno SR**, Li S, Colon M, Kreinest PA, Thompson EA, Fields AP, Murray NR. Oncogenic K-ras promotes early carcinogenesis in the mouse proximal colon. *Int J Cancer* 2008; **122**: 2462-2470 [PMID: 18271008 DOI: 10.1002/ijc.23383]

135 **Luo F**, Brooks DG, Ye H, Hamoudi R, Poulogiannis G, Patek CE, Winton DJ, Arends MJ. Mutated K-ras(Asp12) promotes tumourigenesis in Apc(Min) mice more in the large than the small intestines, with synergistic effects between K-ras and Wnt pathways. *Int J Exp Pathol* 2009; **90**: 558-574 [PMID: 19765110 DOI: 10.1111/j.1365-2613.2009.00667.x]

136 **Haigis KM**, Kendall KR, Wang Y, Cheung A, Haigis MC, Glickman JN, Niwa-Kawakita M, Sweet-Cordero A, Sebolt-Leopold J, Shannon KM, Settleman J, Giovannini M, Jacks T. Differential effects of oncogenic K-Ras and N-Ras on proliferation, differentiation and tumor progression in the colon. *Nat Genet* 2008; **40**: 600-608 [PMID: 18372904 DOI: 10.1038/ng.115]

137 **Russo A**, Bazan V, Iacopetta B, Kerr D, Soussi T, Gebbia N; TP53-CRC Collaborative Study Group. The TP53 colorectal cancer international collaborative study on the prognostic and predictive significance of p53 mutation: influence of tumor site, type of mutation, and adjuvant treatment. *J Clin Oncol* 2005; **23**: 7518-7528 [PMID: 16172461 DOI: 10.1200/JCO.2005.00.471]

138 **Iacopetta B**, Russo A, Bazan V, Dardanoni G, Gebbia N, Soussi T, Kerr D, Elsaleh H, Soong R, Kandioler D, Janschek E, Kappel S, Lung M, Leung CS, Ko JM, Yuen S, Ho J, Leung SY, Crapez E, Duffour J, Ychou M, Leahy DT, O'Donoghue DP, Agnese V, Cascio S, Di Fede G, Chieco-Bianchi L, Bertorelle R, Belluco C, Giaretti W, Castagnola P, Ricevuto E, Ficorella C, Bosari S, Arizzi CD, Miyaki M, Onda M, Kampman E, Diergaarde B, Royds J, Lothe RA, Diep CB, Meling GI, Ostrowski J, Trzeciak L, Guzinska-Ustymowicz K, Zalewski B, Capellá GM, Moreno V, Peinado MA, Lönnroth C, Lundholm K, Sun XF, Jansson A, Bouzourene H, Hsieh LL, Tang R, Smith DR, Allen-Mersh TG, Khan ZA, Shorthouse AJ, Silverman ML, Kato S, Ishioka C; TP53-CRC Collaborative Group. Functional categories of TP53 mutation in colorectal cancer: results of an International Collaborative Study. *Ann Oncol* 2006; **17**: 842-847 [PMID: 16524972 DOI: 10.1093/annonc/mdl035]

139 **Nakayama M**, Sakai E, Echizen K, Yamada Y, Oshima H, Han TS, Ohki R, Fujii S, Ochiai A, Robine S, Voon DC, Tanaka T, Taketo MM, Oshima M. Intestinal cancer progression by mutant p53 through the acquisition of invasiveness associated with complex glandular formation. *Oncogene* 2017; **36**: 5885-5896 [PMID: 28628120 DOI: 10.1038/onc.2017.194]

140 **Sakai E**, Nakayama M, Oshima H, Kouyama Y, Niida A, Fujii S, Ochiai A, Nakayama KI, Mimori K, Suzuki Y, Hong CP, Ock CY, Kim SJ, Oshima M. Combined Mutation of *Apc, Kras*, and *Tgfbr2* Effectively Drives Metastasis of Intestinal Cancer. *Cancer Res* 2018; **78**: 1334-1346 [PMID: 29282223 DOI: 10.1158/0008-5472.CAN-17-3303]

141 **Chanrion M**, Kuperstein I, Barrière C, El Marjou F, Cohen D, Vignjevic D, Stimmer L, Paul-Gilloteaux P, Bièche I, Tavares Sdos R, Boccia GF, Cacheux W, Meseure D, Fre S, Martignetti L, Legoix-Né P, Girard E, Fetler L, Barillot E, Louvard D, Zinovyev A, Robine S. Concomitant Notch activation and p53 deletion trigger epithelial-to-mesenchymal transition and metastasis in mouse gut. *Nat Commun* 2014; **5**: 5005 [PMID: 25295490 DOI: 10.1038/ncomms6005]

142 **Fre S**, Pallavi SK, Huyghe M, Laé M, Janssen KP, Robine S, Artavanis-Tsakonas S, Louvard D. Notch and Wnt signals cooperatively control cell proliferation and tumorigenesis in the intestine. *Proc Natl Acad Sci U S A* 2009; **106**: 6309-6314 [PMID: 19251639 DOI: 10.1073/pnas.0900427106]

143 **Dow LE**, O'Rourke KP, Simon J, Tschaharganeh DF, van Es JH, Clevers H, Lowe SW. Apc Restoration Promotes Cellular Differentiation and Reestablishes Crypt Homeostasis in Colorectal Cancer. *Cell* 2015; **161**: 1539-1552 [PMID: 26091037 DOI: 10.1016/j.cell.2015.05.033]

144 **Mao JH**, Perez-Losada J, Wu D, Delrosario R, Tsunematsu R, Nakayama KI, Brown K, Bryson S, Balmain A. Fbxw7/Cdc4 is a p53-dependent, haploinsufficient tumour suppressor gene. *Nature* 2004; **432**: 775-779 [PMID: 15592418 DOI: 10.1038/nature03155]

145 **Welcker M**, Clurman BE. FBW7 ubiquitin ligase: a tumour suppressor at the crossroads of cell division, growth and differentiation. *Nat Rev Cancer* 2008; **8**: 83-93 [PMID: 18094723 DOI: 10.1038/nrc2290]

146 **Babaei-Jadidi R**, Li N, Saadeddin A, Spencer-Dene B, Jandke A, Muhammad B, Ibrahim EE, Muraleedharan R, Abuzinadah M, Davis H, Lewis A, Watson S, Behrens A, Tomlinson I, Nateri AS. FBXW7 influences murine intestinal homeostasis and cancer, targeting Notch, Jun, and DEK for degradation. *J Exp Med* 2011; **208**: 295-312 [PMID: 21282377 DOI: 10.1084/jem.20100830]

147 **Sancho R**, Jandke A, Davis H, Diefenbacher ME, Tomlinson I, Behrens A. F-box and WD repeat domain-containing 7 regulates intestinal cell lineage commitment and is a haploinsufficient tumor suppressor. *Gastroenterology* 2010; **139**: 929-941 [PMID: 20638938 DOI: 10.1053/j.gastro.2010.05.078]

148 **Grim JE**, Knoblaugh SE, Guthrie KA, Hagar A, Swanger J, Hespelt J, Delrow JJ, Small T, Grady WM, Nakayama KI, Clurman BE. Fbw7 and p53 cooperatively suppress advanced and chromosomally unstable intestinal cancer. *Mol Cell Biol* 2012; **32**: 2160-2167 [PMID: 22473991 DOI: 10.1128/MCB.00305-12]

149 **Faes S**, Dormond O. PI3K and AKT: Unfaithful Partners in Cancer. *Int J Mol Sci* 2015; **16**: 21138-21152 [PMID: 26404259 DOI: 10.3390/ijms160921138]

150 **Leystra AA**, Deming DA, Zahm CD, Farhoud M, Olson TJ, Hadac JN, Nettekoven LA, Albrecht DM, Clipson L, Sullivan R, Washington MK, Torrealba JR, Weichert JP, Halberg RB. Mice expressing activated PI3K rapidly develop advanced colon cancer. *Cancer Res* 2012; **72**: 2931-2936 [PMID: 22525701 DOI: 10.1158/0008-5472.CAN-11-4097]

151 **Marsh V**, Winton DJ, Williams GT, Dubois N, Trumpp A, Sansom OJ, Clarke AR. Epithelial Pten is dispensable for intestinal homeostasis but suppresses adenoma development and progression after Apc mutation. *Nat Genet* 2008; **40**: 1436-1444 [PMID: 19011632 DOI: 10.1038/ng.256]

152 **Davies EJ**, Marsh Durban V, Meniel V, Williams GT, Clarke AR. PTEN loss and KRAS activation leads to the formation of serrated adenomas and metastatic carcinoma in the mouse intestine. *J Pathol* 2014; **233**: 27-38 [PMID: 24293351 DOI: 10.1002/path.4312]

153 **Yu M**, Trobridge P, Wang Y, Kanngurn S, Morris SM, Knoblaugh S, Grady WM. Inactivation of TGF-β signaling and loss of PTEN cooperate to induce colon cancer in vivo. *Oncogene* 2014; **33**: 1538-1547 [PMID: 23604118 DOI: 10.1038/onc.2013.102]

154 **Syed V**. TGF-β Signaling in Cancer. *J Cell Biochem* 2016; **117**: 1279-1287 [PMID: 26774024 DOI: 10.1002/jcb.25496]

155 **Nagaraj NS**, Datta PK. Targeting the transforming growth factor-beta signaling pathway in human cancer. *Expert Opin Investig Drugs* 2010; **19**: 77-91 [PMID: 20001556 DOI: 10.1517/13543780903382609]

156 **Alberici P**, Jagmohan-Changur S, De Pater E, Van Der Valk M, Smits R, Hohenstein P, Fodde R. Smad4 haploinsufficiency in mouse models for intestinal cancer. *Oncogene* 2006; **25**: 1841-1851 [PMID: 16288217 DOI: 10.1038/sj.onc.1209226]

157 **Takaku K**, Oshima M, Miyoshi H, Matsui M, Seldin MF, Taketo MM. Intestinal tumorigenesis in compound mutant mice of both Dpc4 (Smad4) and Apc genes. *Cell* 1998; **92**: 645-656 [PMID: 9506519 DOI: 10.1016/s0092-8674(00)81132-0]

158 **Tetteh PW**, Kretzschmar K, Begthel H, van den Born M, Korving J, Morsink F, Farin H, van Es JH, Offerhaus GJ, Clevers H. Generation of an inducible colon-specific Cre enzyme mouse line for colon cancer research. *Proc Natl Acad Sci U S A* 2016; **113**: 11859-11864 [PMID: 27708166 DOI: 10.1073/pnas.1614057113]

159 **Takaku K**, Wrana JL, Robertson EJ, Taketo MM. No effects of Smad2 (madh2) null mutation on malignant progression of intestinal polyps in Apc(delta716) knockout mice. *Cancer Res* 2002; **62**: 4558-4561 [PMID: 12183405]

160 **Zhu Y**, Richardson JA, Parada LF, Graff JM. Smad3 mutant mice develop metastatic colorectal cancer. *Cell* 1998; **94**: 703-714 [PMID: 9753318 DOI: 10.1016/s0092-8674(00)81730-4]

161 **Sodir NM**, Chen X, Park R, Nickel AE, Conti PS, Moats R, Bading JR, Shibata D, Laird PW. Smad3 deficiency promotes tumorigenesis in the distal colon of ApcMin/+ mice. *Cancer Res* 2006; **66**: 8430-8438 [PMID: 16951153 DOI: 10.1158/0008-5472.CAN-06-1437]

162 **Fleming NI**, Jorissen RN, Mouradov D, Christie M, Sakthianandeswaren A, Palmieri M, Day F, Li S, Tsui C, Lipton L, Desai J, Jones IT, McLaughlin S, Ward RL, Hawkins NJ, Ruszkiewicz AR, Moore J, Zhu HJ, Mariadason JM, Burgess AW, Busam D, Zhao Q, Strausberg RL, Gibbs P, Sieber OM. SMAD2, SMAD3 and SMAD4 mutations in colorectal cancer. *Cancer Res* 2013; **73**: 725-735 [PMID: 23139211 DOI: 10.1158/0008-5472.CAN-12-2706]

163 **Engle SJ**, Hoying JB, Boivin GP, Ormsby I, Gartside PS, Doetschman T. Transforming growth factor beta1 suppresses nonmetastatic colon cancer at an early stage of tumorigenesis. *Cancer Res* 1999; **59**: 3379-3386 [PMID: 10416598]

164 **Biswas S**, Guix M, Rinehart C, Dugger TC, Chytil A, Moses HL, Freeman ML, Arteaga CL. Inhibition of TGF-beta with neutralizing antibodies prevents radiation-induced acceleration of metastatic cancer progression. *J Clin Invest* 2007; **117**: 1305-1313 [PMID: 17415413 DOI: 10.1172/JCI30740]

165 **Oshima H**, Nakayama M, Han TS, Naoi K, Ju X, Maeda Y, Robine S, Tsuchiya K, Sato T, Sato H, Taketo MM, Oshima M. Suppressing TGFβ signaling in regenerating epithelia in an inflammatory microenvironment is sufficient to cause invasive intestinal cancer. *Cancer Res* 2015; **75**: 766-776 [PMID: 25687406 DOI: 10.1158/0008-5472.CAN-14-2036]

166 **Trobridge P**, Knoblaugh S, Washington MK, Munoz NM, Tsuchiya KD, Rojas A, Song X, Ulrich CM, Sasazuki T, Shirasawa S, Grady WM. TGF-beta receptor inactivation and mutant Kras induce intestinal neoplasms in mice via a beta-catenin-independent pathway. *Gastroenterology* 2009; **136**: 1680-8.e7 [PMID: 19208363 DOI: 10.1053/j.gastro.2009.01.066]

167 **Muñoz NM**, Upton M, Rojas A, Washington MK, Lin L, Chytil A, Sozmen EG, Madison BB, Pozzi A, Moon RT, Moses HL, Grady WM. Transforming growth factor beta receptor type II inactivation induces the malignant transformation of intestinal neoplasms initiated by Apc mutation. *Cancer Res* 2006; **66**: 9837-9844 [PMID: 17047044 DOI: 10.1158/0008-5472.CAN-06-0890]

168 **Rajagopalan H**, Bardelli A, Lengauer C, Kinzler KW, Vogelstein B, Velculescu VE. Tumorigenesis: RAF/RAS oncogenes and mismatch-repair status. *Nature* 2002; **418**: 934 [PMID: 12198537 DOI: 10.1038/418934a]

169 **Hinoue T**, Weisenberger DJ, Lange CP, Shen H, Byun HM, Van Den Berg D, Malik S, Pan F, Noushmehr H, van Dijk CM, Tollenaar RA, Laird PW. Genome-scale analysis of aberrant DNA methylation in colorectal cancer. *Genome Res* 2012; **22**: 271-282 [PMID: 21659424 DOI: 10.1101/gr.117523.110]

170 **Bond CE**, Whitehall VLJ. How the *BRAF* V600E Mutation Defines a Distinct Subgroup of Colorectal Cancer: Molecular and Clinical Implications. *Gastroenterol Res Pract* 2018; **2018**: 9250757 [PMID: 30598662 DOI: 10.1155/2018/9250757]

171 **Carragher LA**, Snell KR, Giblett SM, Aldridge VS, Patel B, Cook SJ, Winton DJ, Marais R, Pritchard CA. V600EBraf induces gastrointestinal crypt senescence and promotes tumour progression through enhanced CpG methylation of p16INK4a. *EMBO Mol Med* 2010; **2**: 458-471 [PMID: 20941790 DOI: 10.1002/emmm.201000099]

172 **Coffee EM**, Faber AC, Roper J, Sinnamon MJ, Goel G, Keung L, Wang WV, Vecchione L, de Vriendt V, Weinstein BJ, Bronson RT, Tejpar S, Xavier RJ, Engelman JA, Martin ES, Hung KE. Concomitant BRAF and PI3K/mTOR blockade is required for effective treatment of BRAF(V600E) colorectal cancer. *Clin Cancer Res* 2013; **19**: 2688-2698 [PMID: 23549875 DOI: 10.1158/1078-0432.CCR-12-2556]

173 **Anderson RL**, Balasas T, Callaghan J, Coombes RC, Evans J, Hall JA, Kinrade S, Jones D, Jones PS, Jones R, Marshall JF, Panico MB, Shaw JA, Steeg PS, Sullivan M, Tong W, Westwell AD, Ritchie JWA; Cancer Research UK and Cancer Therapeutics CRC Australia Metastasis Working Group. A framework for the development of effective anti-metastatic agents. *Nat Rev Clin Oncol* 2019; **16**: 185-204 [PMID: 30514977 DOI: 10.1038/s41571-018-0134-8]

174 **Rad R**, Cadiñanos J, Rad L, Varela I, Strong A, Kriegl L, Constantino-Casas F, Eser S, Hieber M, Seidler B, Price S, Fraga MF, Calvanese V, Hoffman G, Ponstingl H, Schneider G, Yusa K, Grove C, Schmid RM, Wang W, Vassiliou G, Kirchner T, McDermott U, Liu P, Saur D, Bradley A. A genetic progression model of Braf(V600E)-induced intestinal tumorigenesis reveals targets for therapeutic intervention. *Cancer Cell* 2013; **24**: 15-29 [PMID: 23845441 DOI: 10.1016/j.ccr.2013.05.014]

175 **Anwar M**, Kochhar R, Singh R, Bhatia A, Vaiphei K, Mahmood A, Mahmood S. Frequent activation of the β-catenin gene in sporadic colorectal carcinomas: A mutational & expression analysis. *Mol Carcinog* 2016; **55**: 1627-1638 [PMID: 26373808 DOI: 10.1002/mc.22414]

176 **Abdelmaksoud-Damak R**, Miladi-Abdennadher I, Triki M, Khabir A, Charfi S, Ayadi L, Frikha M, Sellami-Boudawara T, Mokdad-Gargouri R. Expression and mutation pattern of β-catenin and adenomatous polyposis coli in colorectal cancer patients. *Arch Med Res* 2015; **46**: 54-62 [PMID: 25660336 DOI: 10.1016/j.arcmed.2015.01.001]

177 **Schwitalla S**, Fingerle AA, Cammareri P, Nebelsiek T, Göktuna SI, Ziegler PK, Canli O, Heijmans J, Huels DJ, Moreaux G, Rupec RA, Gerhard M, Schmid R, Barker N, Clevers H, Lang R, Neumann J, Kirchner T, Taketo MM, van den Brink GR, Sansom OJ, Arkan MC, Greten FR. Intestinal tumorigenesis initiated by dedifferentiation and acquisition of stem-cell-like properties. *Cell* 2013; **152**: 25-38 [PMID: 23273993 DOI: 10.1016/j.cell.2012.12.012]

178 **Harada N**, Tamai Y, Ishikawa T, Sauer B, Takaku K, Oshima M, Taketo MM. Intestinal polyposis in mice with a dominant stable mutation of the beta-catenin gene. *EMBO J* 1999; **18**: 5931-5942 [PMID: 10545105 DOI: 10.1093/emboj/18.21.5931]

179 **Perekatt AO**, Shah PP, Cheung S, Jariwala N, Wu A, Gandhi V, Kumar N, Feng Q, Patel N, Chen L, Joshi S, Zhou A, Taketo MM, Xing J, White E, Gao N, Gatza ML, Verzi MP. SMAD4 Suppresses WNT-Driven Dedifferentiation and Oncogenesis in the Differentiated Gut Epithelium. *Cancer Res* 2018; **78**: 4878-4890 [PMID: 29986996 DOI: 10.1158/0008-5472.CAN-18-0043]

180 **Ionov Y**, Peinado MA, Malkhosyan S, Shibata D, Perucho M. Ubiquitous somatic mutations in simple repeated sequences reveal a new mechanism for colonic carcinogenesis. *Nature* 1993; **363**: 558-561 [PMID: 8505985 DOI: 10.1038/363558a0]

181 **Thibodeau SN**, Bren G, Schaid D. Microsatellite instability in cancer of the proximal colon. *Science* 1993; **260**: 816-819 [PMID: 8484122 DOI: 10.1126/science.8484122]

182 **Peltomäki P**, Lothe RA, Aaltonen LA, Pylkkänen L, Nyström-Lahti M, Seruca R, David L, Holm R, Ryberg D, Haugen A. Microsatellite instability is associated with tumors that characterize the hereditary non-polyposis colorectal carcinoma syndrome. *Cancer Res* 1993; **53**: 5853-5855 [PMID: 8261393]

183 **Yamamoto H**, Imai K. Microsatellite instability: an update. *Arch Toxicol* 2015; **89**: 899-921 [PMID: 25701956 DOI: 10.1007/s00204-015-1474-0]

184 **Edelmann W**, Yang K, Kuraguchi M, Heyer J, Lia M, Kneitz B, Fan K, Brown AM, Lipkin M, Kucherlapati R. Tumorigenesis in Mlh1 and Mlh1/Apc1638N mutant mice. *Cancer Res* 1999; **59**: 1301-1307 [PMID: 10096563]

185 **Reitmair AH**, Schmits R, Ewel A, Bapat B, Redston M, Mitri A, Waterhouse P, Mittrücker HW, Wakeham A, Liu B. MSH2 deficient mice are viable and susceptible to lymphoid tumours. *Nat Genet* 1995; **11**: 64-70 [PMID: 7550317 DOI: 10.1038/ng0995-64]

186 **de Wind N**, Dekker M, Berns A, Radman M, te Riele H. Inactivation of the mouse Msh2 gene results in mismatch repair deficiency, methylation tolerance, hyperrecombination, and predisposition to cancer. *Cell* 1995; **82**: 321-330 [PMID: 7628020 DOI: 10.1016/0092-8674(95)90319-4]

187 **Edelmann W**, Yang K, Umar A, Heyer J, Lau K, Fan K, Liedtke W, Cohen PE, Kane MF, Lipford JR, Yu N, Crouse GF, Pollard JW, Kunkel T, Lipkin M, Kolodner R, Kucherlapati R. Mutation in the mismatch repair gene Msh6 causes cancer susceptibility. *Cell* 1997; **91**: 467-477 [PMID: 9390556 DOI: 10.1016/s0092-8674(00)80433-x]

188 **Prolla TA**, Baker SM, Harris AC, Tsao JL, Yao X, Bronner CE, Zheng B, Gordon M, Reneker J, Arnheim N, Shibata D, Bradley A, Liskay RM. Tumour susceptibility and spontaneous mutation in mice deficient in Mlh1, Pms1 and Pms2 DNA mismatch repair. *Nat Genet* 1998; **18**: 276-279 [PMID: 9500552 DOI: 10.1038/ng0398-276]

189 **Baker SM**, Plug AW, Prolla TA, Bronner CE, Harris AC, Yao X, Christie DM, Monell C, Arnheim N, Bradley A, Ashley T, Liskay RM. Involvement of mouse Mlh1 in DNA mismatch repair and meiotic crossing over. *Nat Genet* 1996; **13**: 336-342 [PMID: 8673133 DOI: 10.1038/ng0796-336]

190 **Yang G**, Scherer SJ, Shell SS, Yang K, Kim M, Lipkin M, Kucherlapati R, Kolodner RD, Edelmann W. Dominant effects of an Msh6 missense mutation on DNA repair and cancer susceptibility. *Cancer Cell* 2004; **6**: 139-150 [PMID: 15324697 DOI: 10.1016/j.ccr.2004.06.024]

191 **Reitmair AH**, Cai JC, Bjerknes M, Redston M, Cheng H, Pind MT, Hay K, Mitri A, Bapat BV, Mak TW, Gallinger S. MSH2 deficiency contributes to accelerated APC-mediated intestinal tumorigenesis. *Cancer Res* 1996; **56**: 2922-2926 [PMID: 8674041]

192 **Smits R**, Hofland N, Edelmann W, Geugien M, Jagmohan-Changur S, Albuquerque C, Breukel C, Kucherlapati R, Kielman MF, Fodde R. Somatic Apc mutations are selected upon their capacity to inactivate the beta-catenin downregulating activity. *Genes Chromosomes Cancer* 2000; **29**: 229-239 [PMID: 10992298]

193 **Kucherlapati M**, Yang K, Kuraguchi M, Zhao J, Lia M, Heyer J, Kane MF, Fan K, Russell R, Brown AM, Kneitz B, Edelmann W, Kolodner RD, Lipkin M, Kucherlapati R. Haploinsufficiency of Flap endonuclease (Fen1) leads to rapid tumor progression. *Proc Natl Acad Sci U S A* 2002; **99**: 9924-9929 [PMID: 12119409 DOI: 10.1073/pnas.152321699]

194 **Kucherlapati M**, Nguyen A, Kuraguchi M, Yang K, Fan K, Bronson R, Wei K, Lipkin M, Edelmann W, Kucherlapati R. Tumor progression in Apc(1638N) mice with Exo1 and Fen1 deficiencies. *Oncogene* 2007; **26**: 6297-6306 [PMID: 17452984 DOI: 10.1038/sj.onc.1210453]

195 **Panarelli NC**, Vaughn CP, Samowitz WS, Yantiss RK. Sporadic microsatellite instability-high colon cancers rarely display immunohistochemical evidence of Wnt signaling activation. *Am J Surg Pathol* 2015; **39**: 313-317 [PMID: 25602793 DOI: 10.1097/PAS.0000000000000380]

196 **Marisa L**, de Reyniès A, Duval A, Selves J, Gaub MP, Vescovo L, Etienne-Grimaldi MC, Schiappa R, Guenot D, Ayadi M, Kirzin S, Chazal M, Fléjou JF, Benchimol D, Berger A, Lagarde A, Pencreach E, Piard F, Elias D, Parc Y, Olschwang S, Milano G, Laurent-Puig P, Boige V. Gene expression classification of colon cancer into molecular subtypes: characterization, validation, and prognostic value. *PLoS Med* 2013; **10**: e1001453 [PMID: 23700391 DOI: 10.1371/journal.pmed.1001453]

197 **Kucherlapati MH**, Lee K, Nguyen AA, Clark AB, Hou H Jr, Rosulek A, Li H, Yang K, Fan K, Lipkin M, Bronson RT, Jelicks L, Kunkel TA, Kucherlapati R, Edelmann W. An Msh2 conditional knockout mouse for studying intestinal cancer and testing anticancer agents. *Gastroenterology* 2010; **138**: 993-1002.e1 [PMID: 19931261 DOI: 10.1053/j.gastro.2009.11.009]

198 **Bennecke M**, Kriegl L, Bajbouj M, Retzlaff K, Robine S, Jung A, Arkan MC, Kirchner T, Greten FR. Ink4a/Arf and oncogene-induced senescence prevent tumor progression during alternative colorectal tumorigenesis. *Cancer Cell* 2010; **18**: 135-146 [PMID: 20708155 DOI: 10.1016/j.ccr.2010.06.013]

199 **Huang D**, Sun W, Zhou Y, Li P, Chen F, Chen H, Xia D, Xu E, Lai M, Wu Y, Zhang H. Mutations of key driver genes in colorectal cancer progression and metastasis. *Cancer Metastasis Rev* 2018; **37**: 173-187 [PMID: 29322354 DOI: 10.1007/s10555-017-9726-5]

200 **Starr TK**, Allaei R, Silverstein KA, Staggs RA, Sarver AL, Bergemann TL, Gupta M, O'Sullivan MG, Matise I, Dupuy AJ, Collier LS, Powers S, Oberg AL, Asmann YW, Thibodeau SN, Tessarollo L, Copeland NG, Jenkins NA, Cormier RT, Largaespada DA. A transposon-based genetic screen in mice identifies genes altered in colorectal cancer. *Science* 2009; **323**: 1747-1750 [PMID: 19251594 DOI: 10.1126/science.1163040]

201 **Takeda H**, Wei Z, Koso H, Rust AG, Yew CC, Mann MB, Ward JM, Adams DJ, Copeland NG, Jenkins NA. Transposon mutagenesis identifies genes and evolutionary forces driving gastrointestinal tract tumor progression. *Nat Genet* 2015; **47**: 142-150 [PMID: 25559195 DOI: 10.1038/ng.3175]

202 **Morris SM**, Davison J, Carter KT, O'Leary RM, Trobridge P, Knoblaugh SE, Myeroff LL, Markowitz SD, Brett BT, Scheetz TE, Dupuy AJ, Starr TK, Grady WM. Transposon mutagenesis identifies candidate genes that cooperate with loss of transforming growth factor-beta signaling in mouse intestinal neoplasms. *Int J Cancer* 2017; **140**: 853-863 [PMID: 27790711 DOI: 10.1002/ijc.30491]

203 **Shabad LM**. Mstislav Novinsky, pioneer of tumour transplantation. *Cancer Lett* 1976; **2**: 1-3 [PMID: 797444 DOI: 10.1016/s0304-3835(76)80002-x]

204 **Hanau A**. Erfolgreiche Übertragung von Carcinom. *Fortschr d Med* 1889; **7**: 321

205 **Triolo VA**. Nineteenth century foundations of cancer research. Origins of experimental research. *Cancer Res* 1964; **24**: 4-27 [PMID: 14106160]

206 **DeVita VT Jr**, Chu E. A history of cancer chemotherapy. *Cancer Res* 2008; **68**: 8643-8653 [PMID: 18974103 DOI: 10.1158/0008-5472.CAN-07-6611]

207 **Thorsby E**. A short history of HLA. *Tissue Antigens* 2009; **74**: 101-116 [PMID: 19523022 DOI: 10.1111/j.1399-0039.2009.01291.x]

208 **Wyke JA**. Oncogenic viruses. *J Pathol* 1981; **135**: 39-85 [PMID: 6271940 DOI: 10.1002/path.1711350105]

209 **TOOLAN HW**. Successful subcutaneous growth and transplantation of human tumors in X-irradiated laboratory animals. *Proc Soc Exp Biol Med* 1951; **77**: 572-578 [PMID: 14864665 DOI: 10.3181/00379727-77-18854]

210 **TOOLAN HW**. Transplantable human neoplasms maintained in cortisone-treated laboratory animals: H.S. No. 1; H.Ep. No. 1; H.Ep. No. 2; H.Ep. No. 3; and H.Emb.Rh. No. 1. *Cancer Res* 1954; **14**: 660-666 [PMID: 13209540]

211 **Yong KSM**, Her Z, Chen Q. Humanized Mice as Unique Tools for Human-Specific Studies. *Arch Immunol Ther Exp (Warsz)* 2018; **66**: 245-266 [PMID: 29411049 DOI: 10.1007/s00005-018-0506-x]

212 **Flanagan SP**. 'Nude', a new hairless gene with pleiotropic effects in the mouse. *Genet Res* 1966; **8**: 295-309 [PMID: 5980117 DOI: 10.1017/s0016672300010168]

213 **Rygaard J**, Povlsen CO. Heterotransplantation of a human malignant tumour to "Nude" mice. *Acta Pathol Microbiol Scand* 1969; **77**: 758-760 [PMID: 5383844 DOI: 10.1111/j.1699-0463.1969.tb04520.x]

214 **Bosma GC**, Custer RP, Bosma MJ. A severe combined immunodeficiency mutation in the mouse. *Nature* 1983; **301**: 527-530 [PMID: 6823332 DOI: 10.1038/301527a0]

215 **Makino S**, Kunimoto K, Muraoka Y, Mizushima Y, Katagiri K, Tochino Y. Breeding of a non-obese, diabetic strain of mice. *Jikken Dobutsu* 1980; **29**: 1-13 [PMID: 6995140 DOI: 10.1538/expanim1978.29.1\_1]

216 **Greiner DL**, Shultz LD, Yates J, Appel MC, Perdrizet G, Hesselton RM, Schweitzer I, Beamer WG, Shultz KL, Pelsue SC. Improved engraftment of human spleen cells in NOD/LtSz-scid/scid mice as compared with C.B-17-scid/scid mice. *Am J Pathol* 1995; **146**: 888-902 [PMID: 7717456]

217 **Christianson SW**, Greiner DL, Hesselton RA, Leif JH, Wagar EJ, Schweitzer IB, Rajan TV, Gott B, Roopenian DC, Shultz LD. Enhanced human CD4+ T cell engraftment in beta2-microglobulin-deficient NOD-scid mice. *J Immunol* 1997; **158**: 3578-3586 [PMID: 9103418]

218 **Ueda T**, Tsuji K, Yoshino H, Ebihara Y, Yagasaki H, Hisakawa H, Mitsui T, Manabe A, Tanaka R, Kobayashi K, Ito M, Yasukawa K, Nakahata T. Expansion of human NOD/SCID-repopulating cells by stem cell factor, Flk2/Flt3 ligand, thrombopoietin, IL-6, and soluble IL-6 receptor. *J Clin Invest* 2000; **105**: 1013-1021 [PMID: 10749580 DOI: 10.1172/JCI8583]

219 **Santagostino SF**, Arbona RJR, Nashat MA, White JR, Monette S. Pathology of Aging in NOD scid gamma Female Mice. *Vet Pathol* 2017; **54**: 855-869 [PMID: 28355107 DOI: 10.1177/0300985817698210]

220 **Nonoyama S**, Smith FO, Bernstein ID, Ochs HD. Strain-dependent leakiness of mice with severe combined immune deficiency. *J Immunol* 1993; **150**: 3817-3824 [PMID: 8473734]

221 **Shultz LD**, Ishikawa F, Greiner DL. Humanized mice in translational biomedical research. *Nat Rev Immunol* 2007; **7**: 118-130 [PMID: 17259968 DOI: 10.1038/nri2017]

222 **Ito M**, Hiramatsu H, Kobayashi K, Suzue K, Kawahata M, Hioki K, Ueyama Y, Koyanagi Y, Sugamura K, Tsuji K, Heike T, Nakahata T. NOD/SCID/gamma(c)(null) mouse: an excellent recipient mouse model for engraftment of human cells. *Blood* 2002; **100**: 3175-3182 [PMID: 12384415 DOI: 10.1182/blood-2001-12-0207]

223 **Shultz LD**, Lyons BL, Burzenski LM, Gott B, Chen X, Chaleff S, Kotb M, Gillies SD, King M, Mangada J, Greiner DL, Handgretinger R. Human lymphoid and myeloid cell development in NOD/LtSz-scid IL2R gamma null mice engrafted with mobilized human hemopoietic stem cells. *J Immunol* 2005; **174**: 6477-6489 [PMID: 15879151 DOI: 10.4049/jimmunol.174.10.6477]

224 **Suzuki K**, Nakajima H, Saito Y, Saito T, Leonard WJ, Iwamoto I. Janus kinase 3 (Jak3) is essential for common cytokine receptor gamma chain (gamma(c))-dependent signaling: comparative analysis of gamma(c), Jak3, and gamma(c) and Jak3 double-deficient mice. *Int Immunol* 2000; **12**: 123-132 [PMID: 10653847 DOI: 10.1093/intimm/12.2.123]

225 **Shultz LD**, Banuelos S, Lyons B, Samuels R, Burzenski L, Gott B, Lang P, Leif J, Appel M, Rossini A, Greiner DL. NOD/LtSz-Rag1nullPfpnull mice: a new model system with increased levels of human peripheral leukocyte and hematopoietic stem-cell engraftment. *Transplantation* 2003; **76**: 1036-1042 [PMID: 14557749 DOI: 10.1097/01.TP.0000083041.44829.2C]

226 **Pearson T**, Greiner DL, Shultz LD. Creation of "humanized" mice to study human immunity. *Curr Protoc Immunol* 2008; **Chapter 15**: Unit 15.21 [PMID: 18491294 DOI: 10.1002/0471142735.im1521s81]

227 **Foreman O**, Kavirayani AM, Griffey SM, Reader R, Shultz LD. Opportunistic bacterial infections in breeding colonies of the NSG mouse strain. *Vet Pathol* 2011; **48**: 495-499 [PMID: 20817888 DOI: 10.1177/0300985810378282]

228 **Gock M**, Kühn F, Mullins CS, Krohn M, Prall F, Klar E, Linnebacher M. Tumor Take Rate Optimization for Colorectal Carcinoma Patient-Derived Xenograft Models. *Biomed Res Int* 2016; **2016**: 1715053 [PMID: 27999790 DOI: 10.1155/2016/1715053]

229 **Linnebacher M**, Maletzki C, Ostwald C, Klier U, Krohn M, Klar E, Prall F. Cryopreservation of human colorectal carcinomas prior to xenografting. *BMC Cancer* 2010; **10**: 362 [PMID: 20615215 DOI: 10.1186/1471-2407-10-362]

230 **Simon MM**, Greenaway S, White JK, Fuchs H, Gailus-Durner V, Wells S, Sorg T, Wong K, Bedu E, Cartwright EJ, Dacquin R, Djebali S, Estabel J, Graw J, Ingham NJ, Jackson IJ, Lengeling A, Mandillo S, Marvel J, Meziane H, Preitner F, Puk O, Roux M, Adams DJ, Atkins S, Ayadi A, Becker L, Blake A, Brooker D, Cater H, Champy MF, Combe R, Danecek P, di Fenza A, Gates H, Gerdin AK, Golini E, Hancock JM, Hans W, Hölter SM, Hough T, Jurdic P, Keane TM, Morgan H, Müller W, Neff F, Nicholson G, Pasche B, Roberson LA, Rozman J, Sanderson M, Santos L, Selloum M, Shannon C, Southwell A, Tocchini-Valentini GP, Vancollie VE, Westerberg H, Wurst W, Zi M, Yalcin B, Ramirez-Solis R, Steel KP, Mallon AM, de Angelis MH, Herault Y, Brown SD. A comparative phenotypic and genomic analysis of C57BL/6J and C57BL/6N mouse strains. *Genome Biol* 2013; **14**: R82 [PMID: 23902802 DOI: 10.1186/gb-2013-14-7-r82]

231 **Houghton PJ**, Morton CL, Tucker C, Payne D, Favours E, Cole C, Gorlick R, Kolb EA, Zhang W, Lock R, Carol H, Tajbakhsh M, Reynolds CP, Maris JM, Courtright J, Keir ST, Friedman HS, Stopford C, Zeidner J, Wu J, Liu T, Billups CA, Khan J, Ansher S, Zhang J, Smith MA. The pediatric preclinical testing program: description of models and early testing results. *Pediatr Blood Cancer* 2007; **49**: 928-940 [PMID: 17066459 DOI: 10.1002/pbc.21078]

232 **Johnson JI**, Decker S, Zaharevitz D, Rubinstein LV, Venditti JM, Schepartz S, Kalyandrug S, Christian M, Arbuck S, Hollingshead M, Sausville EA. Relationships between drug activity in NCI preclinical in vitro and in vivo models and early clinical trials. *Br J Cancer* 2001; **84**: 1424-1431 [PMID: 11355958 DOI: 10.1054/bjoc.2001.1796]

233 **Talmadge JE**, Singh RK, Fidler IJ, Raz A. Murine models to evaluate novel and conventional therapeutic strategies for cancer. *Am J Pathol* 2007; **170**: 793-804 [PMID: 17322365 DOI: 10.2353/ajpath.2007.060929]

234 **Boven E**, Winograd B, Berger DP, Dumont MP, Braakhuis BJ, Fodstad O, Langdon S, Fiebig HH. Phase II preclinical drug screening in human tumor xenografts: a first European multicenter collaborative study. *Cancer Res* 1992; **52**: 5940-5947 [PMID: 1394220]

235 **Voskoglou-Nomikos T**, Pater JL, Seymour L. Clinical predictive value of the in vitro cell line, human xenograft, and mouse allograft preclinical cancer models. *Clin Cancer Res* 2003; **9**: 4227-4239 [PMID: 14519650]

236 **Golovko D**, Kedrin D, Yilmaz ÖH, Roper J. Colorectal cancer models for novel drug discovery. *Expert Opin Drug Discov* 2015; **10**: 1217-1229 [PMID: 26295972 DOI: 10.1517/17460441.2015.1079618]

237 **Katsiampoura A**, Raghav K, Jiang ZQ, Menter DG, Varkaris A, Morelli MP, Manuel S, Wu J, Sorokin AV, Rizi BS, Bristow C, Tian F, Airhart S, Cheng M, Broom BM, Morris J, Overman MJ, Powis G, Kopetz S. Modeling of Patient-Derived Xenografts in Colorectal Cancer. *Mol Cancer Ther* 2017; **16**: 1435-1442 [PMID: 28468778 DOI: 10.1158/1535-7163.MCT-16-0721]

238 **Brattain MG**, Fine WD, Khaled FM, Thompson J, Brattain DE. Heterogeneity of malignant cells from a human colonic carcinoma. *Cancer Res* 1981; **41**: 1751-1756 [PMID: 7214343]

239 **Horbach SPJM**, Halffman W. The ghosts of HeLa: How cell line misidentification contaminates the scientific literature. *PLoS One* 2017; **12**: e0186281 [PMID: 29023500 DOI: 10.1371/journal.pone.0186281]

240 **Mouradov D**, Sloggett C, Jorissen RN, Love CG, Li S, Burgess AW, Arango D, Strausberg RL, Buchanan D, Wormald S, O'Connor L, Wilding JL, Bicknell D, Tomlinson IP, Bodmer WF, Mariadason JM, Sieber OM. Colorectal cancer cell lines are representative models of the main molecular subtypes of primary cancer. *Cancer Res* 2014; **74**: 3238-3247 [PMID: 24755471 DOI: 10.1158/0008-5472.CAN-14-0013]

241 **Wilding JL**, Bodmer WF. Cancer cell lines for drug discovery and development. *Cancer Res* 2014; **74**: 2377-2384 [PMID: 24717177 DOI: 10.1158/0008-5472.CAN-13-2971]

242 **Tanaka Y**, Wu AY, Ikekawa N, Iseki K, Kawai M, Kobayashi Y. Inhibition of HT-29 human colon cancer growth under the renal capsule of severe combined immunodeficient mice by an analogue of 1,25-dihydroxyvitamin D3, DD-003. *Cancer Res* 1994; **54**: 5148-5153 [PMID: 7923132]

243 **Lawrentschuk N**, Rigopoulos A, Lee FT, Davis ID, Scott AM, Bolton DM. Xenografting tumour beneath the renal capsule using modern surgical equipment. *Eur Surg Res* 2006; **38**: 340-346 [PMID: 16791005 DOI: 10.1159/000094093]

244 **Edelstein MB**. The subrenal capsule assay: a critical commentary. *Eur J Cancer Clin Oncol* 1986; **22**: 757-760 [PMID: 3533556 DOI: 10.1016/0277-5379(86)90359-7]

245 **Wang Y**, Wang JX, Xue H, Lin D, Dong X, Gout PW, Gao X, Pang J. Subrenal capsule grafting technology in human cancer modeling and translational cancer research. *Differentiation* 2016; **91**: 15-19 [PMID: 26547391 DOI: 10.1016/j.diff.2015.10.012]

246 **Li C**, Wang J, Kong J, Tang J, Wu Y, Xu E, Zhang H, Lai M. GDF15 promotes EMT and metastasis in colorectal cancer. *Oncotarget* 2016; **7**: 860-872 [PMID: 26497212 DOI: 10.18632/oncotarget.6205]

247 **Murdocca M**, Capuano R, Pucci S, Cicconi R, Polidoro C, Catini A, Martinelli E, Paolesse R, Orlandi A, Mango R, Novelli G, Di Natale C, Sangiuolo F. Targeting LOX-1 Inhibits Colorectal Cancer Metastasis in an Animal Model. *Front Oncol* 2019; **9**: 927 [PMID: 31608230 DOI: 10.3389/fonc.2019.00927]

248 **Hanahan D**, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011; **144**: 646-674 [PMID: 21376230 DOI: 10.1016/j.cell.2011.02.013]

249 **Brand MI**, Casillas S, Dietz DW, Milsom JW, Vladisavljevic A. Development of a reliable colorectal cancer liver metastasis model. *J Surg Res* 1996; **63**: 425-432 [PMID: 8661237 DOI: 10.1006/jsre.1996.0287]

250 **Fleten KG**, Bakke KM, Mælandsmo GM, Abildgaard A, Redalen KR, Flatmark K. Use of non-invasive imaging to monitor response to aflibercept treatment in murine models of colorectal cancer liver metastases. *Clin Exp Metastasis* 2017; **34**: 51-62 [PMID: 27812769 DOI: 10.1007/s10585-016-9829-3]

251 **Li Z**, Wang J, Zhou T, Ye X. Establishment of a colorectal cancer nude mouse visualization model of HIF-1α overexpression. *Oncol Lett* 2016; **11**: 2725-2732 [PMID: 27073543 DOI: 10.3892/ol.2016.4287]

252 **Mullins CS**, Micheel B, Matschos S, Leuchter M, Bürtin F, Krohn M, Hühns M, Klar E, Prall F, Linnebacher M. Integrated Biobanking and Tumor Model Establishment of Human Colorectal Carcinoma Provides Excellent Tools for Preclinical Research. *Cancers (Basel)* 2019; **11**: [PMID: 31601052 DOI: 10.3390/cancers11101520]

253 **Prall F**, Maletzki C, Hühns M, Krohn M, Linnebacher M. Colorectal carcinoma tumour budding and podia formation in the xenograft microenvironment. *PLoS One* 2017; **12**: e0186271 [PMID: 29040282 DOI: 10.1371/journal.pone.0186271]

254 **Guenot D**, Guérin E, Aguillon-Romain S, Pencreach E, Schneider A, Neuville A, Chenard MP, Duluc I, Du Manoir S, Brigand C, Oudet P, Kedinger M, Gaub MP. Primary tumour genetic alterations and intra-tumoral heterogeneity are maintained in xenografts of human colon cancers showing chromosome instability. *J Pathol* 2006; **208**: 643-652 [PMID: 16450341 DOI: 10.1002/path.1936]

255 **Burgenske DM**, Monsma DJ, Dylewski D, Scott SB, Sayfie AD, Kim DG, Luchtefeld M, Martin KR, Stephenson P, Hostetter G, Dujovny N, MacKeigan JP. Establishment of genetically diverse patient-derived xenografts of colorectal cancer. *Am J Cancer Res* 2014; **4**: 824-837 [PMID: 25520871]

256 **Mattie M**, Christensen A, Chang MS, Yeh W, Said S, Shostak Y, Capo L, Verlinsky A, An Z, Joseph I, Zhang Y, Kumar-Ganesan S, Morrison K, Stover D, Challita-Eid P. Molecular characterization of patient-derived human pancreatic tumor xenograft models for preclinical and translational development of cancer therapeutics. *Neoplasia* 2013; **15**: 1138-1150 [PMID: 24204193 DOI: 10.1593/neo.13922]

257 **Cho YB**, Hong HK, Choi YL, Oh E, Joo KM, Jin J, Nam DH, Ko YH, Lee WY. Colorectal cancer patient-derived xenografted tumors maintain characteristic features of the original tumors. *J Surg Res* 2014; **187**: 502-509 [PMID: 24332554 DOI: 10.1016/j.jss.2013.11.010]

258 **Ben-David U**, Ha G, Tseng YY, Greenwald NF, Oh C, Shih J, McFarland JM, Wong B, Boehm JS, Beroukhim R, Golub TR. Patient-derived xenografts undergo mouse-specific tumor evolution. *Nat Genet* 2017; **49**: 1567-1575 [PMID: 28991255 DOI: 10.1038/ng.3967]

259 **Sebolt-Leopold JS**. Development of Preclinical Models to Understand and Treat Colorectal Cancer. *Clin Colon Rectal Surg* 2018; **31**: 199-204 [PMID: 29720906 DOI: 10.1055/s-0037-1602240]

260 **Moro M**, Bertolini G, Tortoreto M, Pastorino U, Sozzi G, Roz L. Patient-derived xenografts of non small cell lung cancer: resurgence of an old model for investigation of modern concepts of tailored therapy and cancer stem cells. *J Biomed Biotechnol* 2012; **2012**: 568567 [PMID: 22547927 DOI: 10.1155/2012/568567]

261 **Hylander BL**, Punt N, Tang H, Hillman J, Vaughan M, Bshara W, Pitoniak R, Repasky EA. Origin of the vasculature supporting growth of primary patient tumor xenografts. *J Transl Med* 2013; **11**: 110 [PMID: 23639003 DOI: 10.1186/1479-5876-11-110]

262 **Blomme A**, Van Simaeys G, Doumont G, Costanza B, Bellier J, Otaka Y, Sherer F, Lovinfosse P, Boutry S, Palacios AP, De Pauw E, Hirano T, Yokobori T, Hustinx R, Bellahcène A, Delvenne P, Detry O, Goldman S, Nishiyama M, Castronovo V, Turtoi A. Murine stroma adopts a human-like metabolic phenotype in the PDX model of colorectal cancer and liver metastases. *Oncogene* 2018; **37**: 1237-1250 [PMID: 29242606 DOI: 10.1038/s41388-017-0018-x]

263 **Lazzari L**, Corti G, Picco G, Isella C, Montone M, Arcella P, Durinikova E, Zanella ER, Novara L, Barbosa F, Cassingena A, Cancelliere C, Medico E, Sartore-Bianchi A, Siena S, Garnett MJ, Bertotti A, Trusolino L, Di Nicolantonio F, Linnebacher M, Bardelli A, Arena S. Patient-Derived Xenografts and Matched Cell Lines Identify Pharmacogenomic Vulnerabilities in Colorectal Cancer. *Clin Cancer Res* 2019; **25**: 6243-6259 [PMID: 31375513 DOI: 10.1158/1078-0432.CCR-18-3440]

264 **Pauli C**, Hopkins BD, Prandi D, Shaw R, Fedrizzi T, Sboner A, Sailer V, Augello M, Puca L, Rosati R, McNary TJ, Churakova Y, Cheung C, Triscott J, Pisapia D, Rao R, Mosquera JM, Robinson B, Faltas BM, Emerling BE, Gadi VK, Bernard B, Elemento O, Beltran H, Demichelis F, Kemp CJ, Grandori C, Cantley LC, Rubin MA. Personalized *In Vitro* and *In Vivo* Cancer Models to Guide Precision Medicine. *Cancer Discov* 2017; **7**: 462-477 [PMID: 28331002 DOI: 10.1158/2159-8290.CD-16-1154]

265 **Hidalgo M**, Bruckheimer E, Rajeshkumar NV, Garrido-Laguna I, De Oliveira E, Rubio-Viqueira B, Strawn S, Wick MJ, Martell J, Sidransky D. A pilot clinical study of treatment guided by personalized tumorgrafts in patients with advanced cancer. *Mol Cancer Ther* 2011; **10**: 1311-1316 [PMID: 21673092 DOI: 10.1158/1535-7163.MCT-11-0233]

266 **Bertotti A**, Migliardi G, Galimi F, Sassi F, Torti D, Isella C, Corà D, Di Nicolantonio F, Buscarino M, Petti C, Ribero D, Russolillo N, Muratore A, Massucco P, Pisacane A, Molinaro L, Valtorta E, Sartore-Bianchi A, Risio M, Capussotti L, Gambacorta M, Siena S, Medico E, Sapino A, Marsoni S, Comoglio PM, Bardelli A, Trusolino L. A molecularly annotated platform of patient-derived xenografts ("xenopatients") identifies HER2 as an effective therapeutic target in cetuximab-resistant colorectal cancer. *Cancer Discov* 2011; **1**: 508-523 [PMID: 22586653 DOI: 10.1158/2159-8290.CD-11-0109]

267 **Migliardi G**, Sassi F, Torti D, Galimi F, Zanella ER, Buscarino M, Ribero D, Muratore A, Massucco P, Pisacane A, Risio M, Capussotti L, Marsoni S, Di Nicolantonio F, Bardelli A, Comoglio PM, Trusolino L, Bertotti A. Inhibition of MEK and PI3K/mTOR suppresses tumor growth but does not cause tumor regression in patient-derived xenografts of RAS-mutant colorectal carcinomas. *Clin Cancer Res* 2012; **18**: 2515-2525 [PMID: 22392911 DOI: 10.1158/1078-0432.CCR-11-2683]

268 **Gao H**, Korn JM, Ferretti S, Monahan JE, Wang Y, Singh M, Zhang C, Schnell C, Yang G, Zhang Y, Balbin OA, Barbe S, Cai H, Casey F, Chatterjee S, Chiang DY, Chuai S, Cogan SM, Collins SD, Dammassa E, Ebel N, Embry M, Green J, Kauffmann A, Kowal C, Leary RJ, Lehar J, Liang Y, Loo A, Lorenzana E, Robert McDonald E 3rd, McLaughlin ME, Merkin J, Meyer R, Naylor TL, Patawaran M, Reddy A, Röelli C, Ruddy DA, Salangsang F, Santacroce F, Singh AP, Tang Y, Tinetto W, Tobler S, Velazquez R, Venkatesan K, Von Arx F, Wang HQ, Wang Z, Wiesmann M, Wyss D, Xu F, Bitter H, Atadja P, Lees E, Hofmann F, Li E, Keen N, Cozens R, Jensen MR, Pryer NK, Williams JA, Sellers WR. High-throughput screening using patient-derived tumor xenografts to predict clinical trial drug response. *Nat Med* 2015; **21**: 1318-1325 [PMID: 26479923 DOI: 10.1038/nm.3954]

269 **Witkiewicz AK**, Balaji U, Eslinger C, McMillan E, Conway W, Posner B, Mills GB, O'Reilly EM, Knudsen ES. Integrated Patient-Derived Models Delineate Individualized Therapeutic Vulnerabilities of Pancreatic Cancer. *Cell Rep* 2016; **16**: 2017-2031 [PMID: 27498862 DOI: 10.1016/j.celrep.2016.07.023]

270 **Cescon D**. Personalized Patient Derived Xenograft (pPDX) Modeling to Test Drug Response in Matching Host (REFLECT). [accessed 2019 Oct 25]. In: ClinicalTrials.gov [Internet]. Bethesda (MD): U.S. National Library of Medicine. Available from: https://clinicaltrials.gov/ct2/show/record/NCT02732860 ClinicalTrials.gov Identifier: NCT02732860

271 **Heinemann V**. Optimization of Individualized Therapy for CRCs With Secondary RESISTance Towards Anti-EGFR Targeted Therapy Using an Avatar Model. [accessed 2019 Oct 25]. In: ClinicalTrials.gov [Internet]. Bethesda (MD): U.S. National Library of Medicine. Available from: https://clinicaltrials.gov/ct2/show/NCT03263663 ClinicalTrials.gov Identifier: NCT03263663

272 **Perea S,** Sarno F. Personalised Therapy for Metastatic ADPC Determined by Genetic Testing and Avatar Model Generation (AVATAR). [accessed 2019 Oct 25]. In: ClinicalTrials.gov [Internet]. Bethesda (MD): U.S. National Library of Medicine. Available from: https://clinicaltrials.gov/ct2/show/NCT02795650 ClinicalTrials.gov Identifier: NCT02795650

273 **Oh BY**, Lee WY, Jung S, Hong HK, Nam DH, Park YA, Huh JW, Yun SH, Kim HC, Chun HK, Cho YB. Correlation between tumor engraftment in patient-derived xenograft models and clinical outcomes in colorectal cancer patients. *Oncotarget* 2015; **6**: 16059-16068 [PMID: 25965827 DOI: 10.18632/oncotarget.3863]

274 **Puig I**, Chicote I, Tenbaum SP, Arqués O, Herance JR, Gispert JD, Jimenez J, Landolfi S, Caci K, Allende H, Mendizabal L, Moreno D, Charco R, Espín E, Prat A, Elez ME, Argilés G, Vivancos A, Tabernero J, Rojas S, Palmer HG. A personalized preclinical model to evaluate the metastatic potential of patient-derived colon cancer initiating cells. *Clin Cancer Res* 2013; **19**: 6787-6801 [PMID: 24170545 DOI: 10.1158/1078-0432.CCR-12-1740]

275 **Julien S**, Merino-Trigo A, Lacroix L, Pocard M, Goéré D, Mariani P, Landron S, Bigot L, Nemati F, Dartigues P, Weiswald LB, Lantuas D, Morgand L, Pham E, Gonin P, Dangles-Marie V, Job B, Dessen P, Bruno A, Pierré A, De Thé H, Soliman H, Nunes M, Lardier G, Calvet L, Demers B, Prévost G, Vrignaud P, Roman-Roman S, Duchamp O, Berthet C. Characterization of a large panel of patient-derived tumor xenografts representing the clinical heterogeneity of human colorectal cancer. *Clin Cancer Res* 2012; **18**: 5314-5328 [PMID: 22825584 DOI: 10.1158/1078-0432.CCR-12-0372]

276 **Collins AT**, Lang SH. A systematic review of the validity of patient derived xenograft (PDX) models: the implications for translational research and personalised medicine. *PeerJ* 2018; **6**: e5981 [PMID: 30498642 DOI: 10.7717/peerj.5981]

277 **Dangles-Marie V**, Pocard M, Richon S, Weiswald LB, Assayag F, Saulnier P, Judde JG, Janneau JL, Auger N, Validire P, Dutrillaux B, Praz F, Bellet D, Poupon MF. Establishment of human colon cancer cell lines from fresh tumors versus xenografts: comparison of success rate and cell line features. *Cancer Res* 2007; **67**: 398-407 [PMID: 17210723 DOI: 10.1158/0008-5472.CAN-06-0594]

278 **Prasetyanti PR**, van Hooff SR, van Herwaarden T, de Vries N, Kalloe K, Rodermond H, van Leersum R, de Jong JH, Franitza M, Nürnberg P, Todaro M, Stassi G, Medema JP. Capturing colorectal cancer inter-tumor heterogeneity in patient-derived xenograft (PDX) models. *Int J Cancer* 2019; **144**: 366-371 [PMID: 30151914 DOI: 10.1002/ijc.31767]

279 **Wetterauer C**, Vlajnic T, Schüler J, Gsponer JR, Thalmann GN, Cecchini M, Schneider J, Zellweger T, Pueschel H, Bachmann A, Ruiz C, Dirnhofer S, Bubendorf L, Rentsch CA. Early development of human lymphomas in a prostate cancer xenograft program using triple knock-out immunocompromised mice. *Prostate* 2015; **75**: 585-592 [PMID: 25585936 DOI: 10.1002/pros.22939]

280 **Zhang L**, Liu Y, Wang X, Tang Z, Li S, Hu Y, Zong X, Wu X, Bu Z, Wu A, Li Z, Li Z, Huang X, Jia L, Kang Q, Liu Y, Sutton D, Wang L, Luo L, Ji J. The extent of inflammatory infiltration in primary cancer tissues is associated with lymphomagenesis in immunodeficient mice. *Sci Rep* 2015; **5**: 9447 [PMID: 25819560 DOI: 10.1038/srep09447]

281 **Bondarenko G**, Ugolkov A, Rohan S, Kulesza P, Dubrovskyi O, Gursel D, Mathews J, O'Halloran TV, Wei JJ, Mazar AP. Patient-Derived Tumor Xenografts Are Susceptible to Formation of Human Lymphocytic Tumors. *Neoplasia* 2015; **17**: 735-741 [PMID: 26476081 DOI: 10.1016/j.neo.2015.09.004]

282 **Chen K**, Ahmed S, Adeyi O, Dick JE, Ghanekar A. Human solid tumor xenografts in immunodeficient mice are vulnerable to lymphomagenesis associated with Epstein-Barr virus. *PLoS One* 2012; **7**: e39294 [PMID: 22723990 DOI: 10.1371/journal.pone.0039294]

283 **Taurozzi AJ**, Beekharry R, Wantoch M, Labarthe MC, Walker HF, Seed RI, Simms M, Rodrigues G, Bradford J, van der Horst G, van der Pluijm G, Collins AT. Spontaneous development of Epstein-Barr Virus associated human lymphomas in a prostate cancer xenograft program. *PLoS One* 2017; **12**: e0188228 [PMID: 29145505 DOI: 10.1371/journal.pone.0188228]

284 **John T**, Yanagawa N, Kohler D, Craddock KJ, Bandarchi-Chamkhaleh B, Pintilie M, Sykes J, To C, Li M, Panchal D, Chen W, Shepherd FA, Tsao MS. Characterization of lymphomas developing in immunodeficient mice implanted with primary human non-small cell lung cancer. *J Thorac Oncol* 2012; **7**: 1101-1108 [PMID: 22617243 DOI: 10.1097/JTO.0b013e3182519d4d]

285 **McCormick KH**, Giovanella BC, Klein G, Nilsson K, Stehlin JS. Diploid human lymphoblastoid and Burkitt lymphoma cell lines: susceptibility to murine NK cells and heterotransplantation to nude mice. *Int J Cancer* 1981; **28**: 455-458 [PMID: 6273332 DOI: 10.1002/ijc.2910280410]

286 **Butler KA**, Hou X, Becker MA, Zanfagnin V, Enderica-Gonzalez S, Visscher D, Kalli KR, Tienchaianada P, Haluska P, Weroha SJ. Prevention of Human Lymphoproliferative Tumor Formation in Ovarian Cancer Patient-Derived Xenografts. *Neoplasia* 2017; **19**: 628-636 [PMID: 28658648 DOI: 10.1016/j.neo.2017.04.007]

287 **Snipes RL**. Anatomy of the cecum of the laboratory mouse and rat. *Anat Embryol (Berl)* 1981; **162**: 455-474 [PMID: 7347499 DOI: 10.1007/bf00301871]

288 **Bresalier RS**, Raper SE, Hujanen ES, Kim YS. A new animal model for human colon cancer metastasis. *Int J Cancer* 1987; **39**: 625-630 [PMID: 3032811 DOI: 10.1002/ijc.2910390514]

289 **Fu XY**, Besterman JM, Monosov A, Hoffman RM. Models of human metastatic colon cancer in nude mice orthotopically constructed by using histologically intact patient specimens. *Proc Natl Acad Sci U S A* 1991; **88**: 9345-9349 [PMID: 1924398]

290 **Rajput A**, Agarwal E, Leiphrakpam P, Brattain MG, Chowdhury S. Establishment and Validation of an Orthotopic Metastatic Mouse Model of Colorectal Cancer. *ISRN Hepatol* 2013; **2013**: 206875 [PMID: 27340651 DOI: 10.1155/2013/206875]

291 **Chow AK**, Cheng NS, Lam CS, Ng L, Wong SK, Wan TM, Man JH, Cheung AH, Yau TC, Poon JT, Law WL, Pang RW. Preclinical analysis of the anti-tumor and anti-metastatic effects of Raf265 on colon cancer cells and CD26(+) cancer stem cells in colorectal carcinoma. *Mol Cancer* 2015; **14**: 80 [PMID: 25884645 DOI: 10.1186/s12943-015-0352-y]

292 **Abou-Elkacem L**, Arns S, Brix G, Gremse F, Zopf D, Kiessling F, Lederle W. Regorafenib inhibits growth, angiogenesis, and metastasis in a highly aggressive, orthotopic colon cancer model. *Mol Cancer Ther* 2013; **12**: 1322-1331 [PMID: 23619301 DOI: 10.1158/1535-7163.MCT-12-1162]

293 **Zhao L**, Liu L, Wang S, Zhang YF, Yu L, Ding YQ. Differential proteomic analysis of human colorectal carcinoma cell lines metastasis-associated proteins. *J Cancer Res Clin Oncol* 2007; **133**: 771-782 [PMID: 17503081 DOI: 10.1007/s00432-007-0222-0]

294 **Chunhua L**, Donglan L, Xiuqiong F, Lihua Z, Qin F, Yawei L, Liang Z, Ge W, Linlin J, Ping Z, Kun L, Xuegang S. Apigenin up-regulates transgelin and inhibits invasion and migration of colorectal cancer through decreased phosphorylation of AKT. *J Nutr Biochem* 2013; **24**: 1766-1775 [PMID: 23773626 DOI: 10.1016/j.jnutbio.2013.03.006]

295 **Wang J**, Rajput A, Kan JL, Rose R, Liu XQ, Kuropatwinski K, Hauser J, Beko A, Dominquez I, Sharratt EA, Brattain L, Levea C, Sun FL, Keane DM, Gibson NW, Brattain MG. Knockdown of Ron kinase inhibits mutant phosphatidylinositol 3-kinase and reduces metastasis in human colon carcinoma. *J Biol Chem* 2009; **284**: 10912-10922 [PMID: 19224914 DOI: 10.1074/jbc.M809551200]

296 **Lin W**, Zhuang Q, Zheng L, Cao Z, Shen A, Li Q, Fu C, Feng J, Peng J. Pien Tze Huang inhibits liver metastasis by targeting TGF-β signaling in an orthotopic model of colorectal cancer. *Oncol Rep* 2015; **33**: 1922-1928 [PMID: 25653118 DOI: 10.3892/or.2015.3784]

297 **Kochall S**, Thepkaysone ML, García SA, Betzler AM, Weitz J, Reissfelder C, Schölch S. Isolation of Circulating Tumor Cells in an Orthotopic Mouse Model of Colorectal Cancer. *J Vis Exp* 2017; **(125)** [PMID: 28745637 DOI: 10.3791/55357]

298 **Yang JL,** Seetoo DQ, Wang Y, Ranson M, Berney CR, Ham JM, Russell PJ, Crowe PJ. Urokinase-type plasminogen activator and its receptor in colorectal cancer: Independent prognostic factors of metastasis and cancer-specific survival and potential therapeutic targets. *Int J Cancer* 2000; **89**: 431–439 [DOI: 10.1002/1097-0215(20000920)89:53.0.CO;2-V]

299 **Céspedes MV**, Espina C, García-Cabezas MA, Trias M, Boluda A, Gómez del Pulgar MT, Sancho FJ, Nistal M, Lacal JC, Mangues R. Orthotopic microinjection of human colon cancer cells in nude mice induces tumor foci in all clinically relevant metastatic sites. *Am J Pathol* 2007; **170**: 1077-1085 [PMID: 17322390 DOI: 10.2353/ajpath.2007.060773]

300 **Klose J**, Eissele J, Volz C, Schmitt S, Ritter A, Ying S, Schmidt T, Heger U, Schneider M, Ulrich A. Salinomycin inhibits metastatic colorectal cancer growth and interferes with Wnt/β-catenin signaling in CD133+ human colorectal cancer cells. *BMC Cancer* 2016; **16**: 896 [PMID: 27855654 DOI: 10.1186/s12885-016-2879-8]

301 **Alamo P**, Gallardo A, Pavón MA, Casanova I, Trias M, Mangues MA, Vázquez E, Villaverde A, Mangues R, Céspedes MV. Subcutaneous preconditioning increases invasion and metastatic dissemination in mouse colorectal cancer models. *Dis Model Mech* 2014; **7**: 387-396 [PMID: 24487410 DOI: 10.1242/dmm.013995]

302 **Schulz P,** Dierkes C, Wiedenmann B, Grötzinger C. Near-Infrared Confocal Laser Endomicroscopy Detects Colorectal Cancer via an Integrin αvβ3 Optical Probe. *Mol Imaging Bio* 2015; **17**: 450–460 [DOI: 10.1007/s11307-015-0825-9]

303 **Künzli BM**, Bernlochner MI, Rath S, Käser S, Csizmadia E, Enjyoji K, Cowan P, d'Apice A, Dwyer K, Rosenberg R, Perren A, Friess H, Maurer CA, Robson SC. Impact of CD39 and purinergic signalling on the growth and metastasis of colorectal cancer. *Purinergic Signal* 2011; **7**: 231-241 [PMID: 21484085 DOI: 10.1007/s11302-011-9228-9]

304 **Lee WY**, Hong HK, Ham SK, Kim CI, Cho YB. Comparison of colorectal cancer in differentially established liver metastasis models. *Anticancer Res* 2014; **34**: 3321-3328 [PMID: 24982336]

305 **Sasaki H**, Miura K, Horii A, Kaneko N, Fujibuchi W, Kiseleva L, Gu Z, Murata Y, Karasawa H, Mizoi T, Kobayashi T, Kinouchi M, Ohnuma S, Yazaki N, Unno M, Sasaki I. Orthotopic implantation mouse model and cDNA microarray analysis indicates several genes potentially involved in lymph node metastasis of colorectal cancer. *Cancer Sci* 2008; **99**: 711-719 [PMID: 18307535 DOI: 10.1111/j.1349-7006.2008.00725.x]

306 **Tang W**, Zhu Y, Gao J, Fu J, Liu C, Liu Y, Song C, Zhu S, Leng Y, Wang G, Chen W, Du P, Huang S, Zhou X, Kang J, Cui L. MicroRNA-29a promotes colorectal cancer metastasis by regulating matrix metalloproteinase 2 and E-cadherin via KLF4. *Br J Cancer* 2014; **110**: 450-458 [PMID: 24281002 DOI: 10.1038/bjc.2013.724]

307 **Alamo P**, Gallardo A, Di Nicolantonio F, Pavón MA, Casanova I, Trias M, Mangues MA, Lopez-Pousa A, Villaverde A, Vázquez E, Bardelli A, Céspedes MV, Mangues R. Higher metastatic efficiency of KRas G12V than KRas G13D in a colorectal cancer model. *FASEB J* 2015; **29**: 464-476 [PMID: 25359494 DOI: 10.1096/fj.14-262303]

308 **Chen HN**, Yuan K, Xie N, Wang K, Huang Z, Chen Y, Dou Q, Wu M, Nice EC, Zhou ZG, Huang C. PDLIM1 Stabilizes the E-Cadherin/β-Catenin Complex to Prevent Epithelial-Mesenchymal Transition and Metastatic Potential of Colorectal Cancer Cells. *Cancer Res* 2016; **76**: 1122-1134 [PMID: 26701804 DOI: 10.1158/0008-5472.CAN-15-1962]

309 **Meunier K**, Ferron M, Calmel C, Fléjou JF, Pocard M, Praz F. Impact of MLH1 expression on tumor evolution after curative surgical tumor resection in a murine orthotopic xenograft model for human MSI colon cancer. *Genes Chromosomes Cancer* 2017; **56**: 681-690 [PMID: 28512763 DOI: 10.1002/gcc.22472]

310 **Xu H**, Zhang Y, Peña MM, Pirisi L, Creek KE. Six1 promotes colorectal cancer growth and metastasis by stimulating angiogenesis and recruiting tumor-associated macrophages. *Carcinogenesis* 2017; **38**: 281-292 [PMID: 28199476 DOI: 10.1093/carcin/bgw121]

311 **Paulson B**, Kim IH, Namgoong JM, Kim YG, Lee S, Moon Y, Shin DM, Choo MS, Kim JK. Longitudinal micro-endoscopic monitoring of high-success intramucosal xenografts for mouse models of colorectal cancer. *Int J Med Sci* 2019; **16**: 1453-1460 [PMID: 31673236 DOI: 10.7150/ijms.35666]

312 **Enquist IB**, Good Z, Jubb AM, Fuh G, Wang X, Junttila MR, Jackson EL, Leong KG. Lymph node-independent liver metastasis in a model of metastatic colorectal cancer. *Nat Commun* 2014; **5**: 3530 [PMID: 24667486 DOI: 10.1038/ncomms4530]

313 **Rapic S**, Vangestel C, Verhaeghe J, Van den Wyngaert T, Hinz R, Verhoye M, Pauwels P, Staelens S, Stroobants S. Characterization of an Orthotopic Colorectal Cancer Mouse Model and Its Feasibility for Accurate Quantification in Positron Emission Tomography. *Mol Imaging Biol* 2017; **19**: 762-771 [PMID: 28194632 DOI: 10.1007/s11307-017-1051-4]

314 **Mira A**, Morello V, Céspedes MV, Perera T, Comoglio PM, Mangues R, Michieli P. Stroma-derived HGF drives metabolic adaptation of colorectal cancer to angiogenesis inhibitors. *Oncotarget* 2017; **8**: 38193-38213 [PMID: 28445144 DOI: 10.18632/oncotarget.16942]

315 **Afik R**, Zigmond E, Vugman M, Klepfish M, Shimshoni E, Pasmanik-Chor M, Shenoy A, Bassat E, Halpern Z, Geiger T, Sagi I, Varol C. Tumor macrophages are pivotal constructors of tumor collagenous matrix. *J Exp Med* 2016; **213**: 2315-2331 [PMID: 27697834 DOI: 10.1084/jem.20151193]

316 **Hu CT**, Guo LL, Feng N, Zhang L, Zhou N, Ma LL, Shen L, Tong GH, Yan QW, Zhu SJ, Bian XW, Lai MD, Deng YJ, Ding YQ. MIF, secreted by human hepatic sinusoidal endothelial cells, promotes chemotaxis and outgrowth of colorectal cancer in liver prometastasis. *Oncotarget* 2015; **6**: 22410-22423 [PMID: 26087187 DOI: 10.18632/oncotarget.4198]

317 **Basilico C**, Hultberg A, Blanchetot C, de Jonge N, Festjens E, Hanssens V, Osepa SI, De Boeck G, Mira A, Cazzanti M, Morello V, Dreier T, Saunders M, de Haard H, Michieli P. Four individually druggable MET hotspots mediate HGF-driven tumor progression. *J Clin Invest* 2014; **124**: 3172-3186 [PMID: 24865428 DOI: 10.1172/JCI72316]

318 **Margolin DA**, Myers T, Zhang X, Bertoni DM, Reuter BA, Obokhare I, Borgovan T, Grimes C, Green H, Driscoll T, Lee CG, Davis NK, Li L. The critical roles of tumor-initiating cells and the lymph node stromal microenvironment in human colorectal cancer extranodal metastasis using a unique humanized orthotopic mouse model. *FASEB J* 2015; **29**: 3571-3581 [PMID: 25962655 DOI: 10.1096/fj.14-268938]

319 **Devaud C**, Rousseau B, Netzer S, Pitard V, Paroissin C, Khairallah C, Costet P, Moreau JF, Couillaud F, Dechanet-Merville J, Capone M. Anti-metastatic potential of human Vδ1(+) γδ T cells in an orthotopic mouse xenograft model of colon carcinoma. *Cancer Immunol Immunother* 2013; **62**: 1199-1210 [PMID: 23619975 DOI: 10.1007/s00262-013-1402-1]

320 **Schölch S**, García SA, Iwata N, Niemietz T, Betzler AM, Nanduri LK, Bork U, Kahlert C, Thepkaysone ML, Swiersy A, Büchler MW, Reissfelder C, Weitz J, Rahbari NN. Circulating tumor cells exhibit stem cell characteristics in an orthotopic mouse model of colorectal cancer. *Oncotarget* 2016; **7**: 27232-27242 [PMID: 27029058 DOI: 10.18632/oncotarget.8373]

321 **Kashtan H**, Rabau M, Mullen JB, Wong AH, Roder JC, Shpitz B, Stern HS, Gallinger S. Intra-rectal injection of tumour cells: a novel animal model of rectal cancer. *Surg Oncol* 1992; **1**: 251-256 [PMID: 1341258 DOI: 10.1016/0960-7404(92)90072-s]

322 **Donigan M**, Norcross LS, Aversa J, Colon J, Smith J, Madero-Visbal R, Li S, McCollum N, Ferrara A, Gallagher JT, Baker CH. Novel murine model for colon cancer: non-operative trans-anal rectal injection. *J Surg Res* 2009; **154**: 299-303 [PMID: 19101690 DOI: 10.1016/j.jss.2008.05.028]

323 **Cohen G**, Lecht S, Arien-Zakay H, Ettinger K, Amsalem O, Oron-Herman M, Yavin E, Prus D, Benita S, Nissan A, Lazarovici P. Bio-imaging of colorectal cancer models using near infrared labeled epidermal growth factor. *PLoS One* 2012; **7**: e48803 [PMID: 23144978 DOI: 10.1371/journal.pone.0048803]

324 **Kim YI**, Jeong S, Jung KO, Song MG, Lee CH, Chung SJ, Park JY, Cha MG, Lee SG, Jun BH, Lee YS, Hwang DW, Youn H, Kang KW, Lee YS, Jeong DH, Lee DS. Simultaneous Detection of EGFR and VEGF in Colorectal Cancer using Fluorescence-Raman Endoscopy. *Sci Rep* 2017; **7**: 1035 [PMID: 28432289 DOI: 10.1038/s41598-017-01020-y]

325 **Zigmond E**, Halpern Z, Elinav E, Brazowski E, Jung S, Varol C. Utilization of murine colonoscopy for orthotopic implantation of colorectal cancer. *PLoS One* 2011; **6**: e28858 [PMID: 22174916 DOI: 10.1371/journal.pone.0028858]

326 **Zaytseva YY**, Elliott VA, Rychahou P, Mustain WC, Kim JT, Valentino J, Gao T, O'Connor KL, Neltner JM, Lee EY, Weiss HL, Evers BM. Cancer cell-associated fatty acid synthase activates endothelial cells and promotes angiogenesis in colorectal cancer. *Carcinogenesis* 2014; **35**: 1341-1351 [PMID: 24510238 DOI: 10.1093/carcin/bgu042]

327 **Bettenworth D**, Mücke MM, Schwegmann K, Faust A, Poremba C, Schäfers M, Domagk D, Lenz P. Endoscopy-guided orthotopic implantation of colorectal cancer cells results in metastatic colorectal cancer in mice. *Clin Exp Metastasis* 2016; **33**: 551-562 [PMID: 27146063 DOI: 10.1007/s10585-016-9797-7]

328 **Kishimoto H**, Momiyama M, Aki R, Kimura H, Suetsugu A, Bouvet M, Fujiwara T, Hoffman RM. Development of a clinically-precise mouse model of rectal cancer. *PLoS One* 2013; **8**: e79453 [PMID: 24265772 DOI: 10.1371/journal.pone.0079453]

329 **Hite N**, Klinger A, Hellmers L, Maresh GA, Miller PE, Zhang X, Li L, Margolin DA. An Optimal Orthotopic Mouse Model for Human Colorectal Cancer Primary Tumor Growth and Spontaneous Metastasis. *Dis Colon Rectum* 2018; **61**: 698-705 [PMID: 29722728 DOI: 10.1097/DCR.0000000000001096]

330 **Prasad S**, Yadav VR, Sung B, Reuter S, Kannappan R, Deorukhkar A, Diagaradjane P, Wei C, Baladandayuthapani V, Krishnan S, Guha S, Aggarwal BB. Ursolic acid inhibits growth and metastasis of human colorectal cancer in an orthotopic nude mouse model by targeting multiple cell signaling pathways: chemosensitization with capecitabine. *Clin Cancer Res* 2012; **18**: 4942-4953 [PMID: 22832932 DOI: 10.1158/1078-0432.CCR-11-2805]

331 **Wang J**, Chen C, Wang S, Zhang Y, Yin P, Gao Z, Xu J, Feng D, Zuo Q, Zhao R, Chen T. Bufalin Inhibits HCT116 Colon Cancer Cells and Its Orthotopic Xenograft Tumor in Mice Model through Genes Related to Apoptotic and PTEN/AKT Pathways. *Gastroenterol Res Pract* 2015; **2015**: 457193 [PMID: 26770191 DOI: 10.1155/2015/457193]

332 **Bhome R**, Goh RW, Bullock MD, Pillar N, Thirdborough SM, Mellone M, Mirnezami R, Galea D, Veselkov K, Gu Q, Underwood TJ, Primrose JN, De Wever O, Shomron N, Sayan AE, Mirnezami AH. Exosomal microRNAs derived from colorectal cancer-associated fibroblasts: role in driving cancer progression. *Aging (Albany NY)* 2017; **9**: 2666-2694 [PMID: 29283887 DOI: 10.18632/aging.101355]

333 **Tan X**, Zhang Z, Yao H, Shen L. Tim-4 promotes the growth of colorectal cancer by activating angiogenesis and recruiting tumor-associated macrophages via the PI3K/AKT/mTOR signaling pathway. *Cancer Lett* 2018; **436**: 119-128 [PMID: 30118845 DOI: 10.1016/j.canlet.2018.08.012]

334 **Mizukoshi K**, Okazawa Y, Haeno H, Koyama Y, Sulidan K, Komiyama H, Saeki H, Ohtsuji N, Ito Y, Kojima Y, Goto M, Habu S, Hino O, Sakamoto K, Orimo A. Metastatic seeding of human colon cancer cell clusters expressing the hybrid epithelial/mesenchymal state. *Int J Cancer* 2019 [PMID: 31506938 DOI: 10.1002/ijc.32672]

335 **Russell WMS,** Burch RL. The principles of humane experimental technique. London: Methuen&Co. Ltd., 1959

336 **Wege AK**, Ernst W, Eckl J, Frankenberger B, Vollmann-Zwerenz A, Männel DN, Ortmann O, Kroemer A, Brockhoff G. Humanized tumor mice--a new model to study and manipulate the immune response in advanced cancer therapy. *Int J Cancer* 2011; **129**: 2194-2206 [PMID: 21544806 DOI: 10.1002/ijc.26159]

337 **Choi Y**, Lee S, Kim K, Kim SH, Chung YJ, Lee C. Studying cancer immunotherapy using patient-derived xenografts (PDXs) in humanized mice. *Exp Mol Med* 2018; **50**: 99 [PMID: 30089794 DOI: 10.1038/s12276-018-0115-0]

338 **Bird GA**, Polsky A, Estes P, Hanlon T, Hamilton H, Morton JJ, Gutman J, Jimeno A, Turner BC, Refaeli Y. Expansion of human and murine hematopoietic stem and progenitor cells ex vivo without genetic modification using MYC and Bcl-2 fusion proteins. *PLoS One* 2014; **9**: e105525 [PMID: 25170611 DOI: 10.1371/journal.pone.0105525]

339 **Morton JJ**, Bird G, Keysar SB, Astling DP, Lyons TR, Anderson RT, Glogowska MJ, Estes P, Eagles JR, Le PN, Gan G, McGettigan B, Fernandez P, Padilla-Just N, Varella-Garcia M, Song JI, Bowles DW, Schedin P, Tan AC, Roop DR, Wang XJ, Refaeli Y, Jimeno A. XactMice: humanizing mouse bone marrow enables microenvironment reconstitution in a patient-derived xenograft model of head and neck cancer. *Oncogene* 2016; **35**: 290-300 [PMID: 25893296 DOI: 10.1038/onc.2015.94]

340 **McIntosh BE**, Brown ME, Duffin BM, Maufort JP, Vereide DT, Slukvin II, Thomson JA. Nonirradiated NOD,B6.SCID Il2rγ-/- Kit(W41/W41) (NBSGW) mice support multilineage engraftment of human hematopoietic cells. *Stem Cell Reports* 2015; **4**: 171-180 [PMID: 25601207 DOI: 10.1016/j.stemcr.2014.12.005]

341 **Jespersen H**, Lindberg MF, Donia M, Söderberg EMV, Andersen R, Keller U, Ny L, Svane IM, Nilsson LM, Nilsson JA. Clinical responses to adoptive T-cell transfer can be modeled in an autologous immune-humanized mouse model. *Nat Commun* 2017; **8**: 707 [PMID: 28955032 DOI: 10.1038/s41467-017-00786-z]

342 **Jangalwe S**, Shultz LD, Mathew A, Brehm MA. Improved B cell development in humanized NOD*-scid IL2Rγnull* mice transgenically expressing human stem cell factor, granulocyte-macrophage colony-stimulating factor and interleukin-3. *Immun Inflamm Dis* 2016; **4**: 427-440 [PMID: 27980777 DOI: 10.1002/iid3.124]

343 **Herndler-Brandstetter D**, Shan L, Yao Y, Stecher C, Plajer V, Lietzenmayer M, Strowig T, de Zoete MR, Palm NW, Chen J, Blish CA, Frleta D, Gurer C, Macdonald LE, Murphy AJ, Yancopoulos GD, Montgomery RR, Flavell RA. Humanized mouse model supports development, function, and tissue residency of human natural killer cells. *Proc Natl Acad Sci U S A* 2017; **114**: E9626-E9634 [PMID: 29078283 DOI: 10.1073/pnas.1705301114]

344 **Capasso A**, Lang J, Pitts TM, Jordan KR, Lieu CH, Davis SL, Diamond JR, Kopetz S, Barbee J, Peterson J, Freed BM, Yacob BW, Bagby SM, Messersmith WA, Slansky JE, Pelanda R, Eckhardt SG. Characterization of immune responses to anti-PD-1 mono and combination immunotherapy in hematopoietic humanized mice implanted with tumor xenografts. *J Immunother Cancer* 2019; **7**: 37 [PMID: 30736857 DOI: 10.1186/s40425-019-0518-z]

345 **Overman MJ**, Lonardi S, Wong KYM, Lenz HJ, Gelsomino F, Aglietta M, Morse MA, Van Cutsem E, McDermott R, Hill A, Sawyer MB, Hendlisz A, Neyns B, Svrcek M, Moss RA, Ledeine JM, Cao ZA, Kamble S, Kopetz S, André T. Durable Clinical Benefit With Nivolumab Plus Ipilimumab in DNA Mismatch Repair-Deficient/Microsatellite Instability-High Metastatic Colorectal Cancer. *J Clin Oncol* 2018; **36**: 773-779 [PMID: 29355075 DOI: 10.1200/JCO.2017.76.9901]

346 **Barretina J**, Caponigro G, Stransky N, Venkatesan K, Margolin AA, Kim S, Wilson CJ, Lehár J, Kryukov GV, Sonkin D, Reddy A, Liu M, Murray L, Berger MF, Monahan JE, Morais P, Meltzer J, Korejwa A, Jané-Valbuena J, Mapa FA, Thibault J, Bric-Furlong E, Raman P, Shipway A, Engels IH, Cheng J, Yu GK, Yu J, Aspesi P Jr, de Silva M, Jagtap K, Jones MD, Wang L, Hatton C, Palescandolo E, Gupta S, Mahan S, Sougnez C, Onofrio RC, Liefeld T, MacConaill L, Winckler W, Reich M, Li N, Mesirov JP, Gabriel SB, Getz G, Ardlie K, Chan V, Myer VE, Weber BL, Porter J, Warmuth M, Finan P, Harris JL, Meyerson M, Golub TR, Morrissey MP, Sellers WR, Schlegel R, Garraway LA. The Cancer Cell Line Encyclopedia enables predictive modelling of anticancer drug sensitivity. *Nature* 2012; **483**: 603-607 [PMID: 22460905 DOI: 10.1038/nature11003]

347 **Krbal L**, Soukup J, Stanislav J, Hanusova V. Derivation and basic characterization of colorectal carcinoma primary cell lines. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub* 2017; **161**: 360-368 [PMID: 29042709 DOI: 10.5507/bp.2017.040]

348 **Bolck HA**, Pauli C, Göbel E, Mühlbauer K, Dettwiler S, Moch H, Schraml P. Cancer Sample Biobanking at the Next Level: Combining Tissue With Living Cell Repositories to Promote Precision Medicine. *Front Cell Dev Biol* 2019; **7**: 246 [PMID: 31696117 DOI: 10.3389/fcell.2019.00246]

349 **Kang Y**, Zhang R, Suzuki R, Li SQ, Roife D, Truty MJ, Chatterjee D, Thomas RM, Cardwell J, Wang Y, Wang H, Katz MH, Fleming JB. Two-dimensional culture of human pancreatic adenocarcinoma cells results in an irreversible transition from epithelial to mesenchymal phenotype. *Lab Invest* 2015; **95**: 207-222 [PMID: 25485535 DOI: 10.1038/labinvest.2014.143]

350 **Hickman JA**, Graeser R, de Hoogt R, Vidic S, Brito C, Gutekunst M, van der Kuip H; IMI PREDECT Consortium. Three-dimensional models of cancer for pharmacology and cancer cell biology: capturing tumor complexity in vitro/ex vivo. *Biotechnol J* 2014; **9**: 1115-1128 [PMID: 25174503 DOI: 10.1002/biot.201300492]

351 **Luca AC**, Mersch S, Deenen R, Schmidt S, Messner I, Schäfer KL, Baldus SE, Huckenbeck W, Piekorz RP, Knoefel WT, Krieg A, Stoecklein NH. Impact of the 3D microenvironment on phenotype, gene expression, and EGFR inhibition of colorectal cancer cell lines. *PLoS One* 2013; **8**: e59689 [PMID: 23555746 DOI: 10.1371/journal.pone.0059689]

352 **van Tienderen GS**, Groot Koerkamp B, IJzermans JNM, van der Laan LJW, Verstegen MMA. Recreating Tumour Complexity in a Dish: Organoid Models to Study Liver Cancer Cells and their Extracellular Environment. *Cancers (Basel)* 2019; **11**: [PMID: 31683901 DOI: 10.3390/cancers11111706]

353 **van de Wetering M**, Francies HE, Francis JM, Bounova G, Iorio F, Pronk A, van Houdt W, van Gorp J, Taylor-Weiner A, Kester L, McLaren-Douglas A, Blokker J, Jaksani S, Bartfeld S, Volckman R, van Sluis P, Li VS, Seepo S, Sekhar Pedamallu C, Cibulskis K, Carter SL, McKenna A, Lawrence MS, Lichtenstein L, Stewart C, Koster J, Versteeg R, van Oudenaarden A, Saez-Rodriguez J, Vries RG, Getz G, Wessels L, Stratton MR, McDermott U, Meyerson M, Garnett MJ, Clevers H. Prospective derivation of a living organoid biobank of colorectal cancer patients. *Cell* 2015; **161**: 933-945 [PMID: 25957691 DOI: 10.1016/j.cell.2015.03.053]

354 **Vlachogiannis G**, Hedayat S, Vatsiou A, Jamin Y, Fernández-Mateos J, Khan K, Lampis A, Eason K, Huntingford I, Burke R, Rata M, Koh DM, Tunariu N, Collins D, Hulkki-Wilson S, Ragulan C, Spiteri I, Moorcraft SY, Chau I, Rao S, Watkins D, Fotiadis N, Bali M, Darvish-Damavandi M, Lote H, Eltahir Z, Smyth EC, Begum R, Clarke PA, Hahne JC, Dowsett M, de Bono J, Workman P, Sadanandam A, Fassan M, Sansom OJ, Eccles S, Starling N, Braconi C, Sottoriva A, Robinson SP, Cunningham D, Valeri N. Patient-derived organoids model treatment response of metastatic gastrointestinal cancers. *Science* 2018; **359**: 920-926 [PMID: 29472484 DOI: 10.1126/science.aao2774]

355 **Tiriac H**, Belleau P, Engle DD, Plenker D, Deschênes A, Somerville TDD, Froeling FEM, Burkhart RA, Denroche RE, Jang GH, Miyabayashi K, Young CM, Patel H, Ma M, LaComb JF, Palmaira RLD, Javed AA, Huynh JC, Johnson M, Arora K, Robine N, Shah M, Sanghvi R, Goetz AB, Lowder CY, Martello L, Driehuis E, LeComte N, Askan G, Iacobuzio-Donahue CA, Clevers H, Wood LD, Hruban RH, Thompson E, Aguirre AJ, Wolpin BM, Sasson A, Kim J, Wu M, Bucobo JC, Allen P, Sejpal DV, Nealon W, Sullivan JD, Winter JM, Gimotty PA, Grem JL, DiMaio DJ, Buscaglia JM, Grandgenett PM, Brody JR, Hollingsworth MA, O'Kane GM, Notta F, Kim E, Crawford JM, Devoe C, Ocean A, Wolfgang CL, Yu KH, Li E, Vakoc CR, Hubert B, Fischer SE, Wilson JM, Moffitt R, Knox J, Krasnitz A, Gallinger S, Tuveson DA. Organoid Profiling Identifies Common Responders to Chemotherapy in Pancreatic Cancer. *Cancer Discov* 2018; **8**: 1112-1129 [PMID: 29853643 DOI: 10.1158/2159-8290.CD-18-0349]

356 **Yan HHN**, Siu HC, Law S, Ho SL, Yue SSK, Tsui WY, Chan D, Chan AS, Ma S, Lam KO, Bartfeld S, Man AHY, Lee BCH, Chan ASY, Wong JWH, Cheng PSW, Chan AKW, Zhang J, Shi J, Fan X, Kwong DLW, Mak TW, Yuen ST, Clevers H, Leung SY. A Comprehensive Human Gastric Cancer Organoid Biobank Captures Tumor Subtype Heterogeneity and Enables Therapeutic Screening. *Cell Stem Cell* 2018; **23**: 882-897.e11 [PMID: 30344100 DOI: 10.1016/j.stem.2018.09.016]

357 **Lee SH**, Hong JH, Park HK, Park JS, Kim BK, Lee JY, Jeong JY, Yoon GS, Inoue M, Choi GS, Lee IK. Colorectal cancer-derived tumor spheroids retain the characteristics of original tumors. *Cancer Lett* 2015; **367**: 34-42 [PMID: 26185002 DOI: 10.1016/j.canlet.2015.06.024]

358 **Sato T**, Vries RG, Snippert HJ, van de Wetering M, Barker N, Stange DE, van Es JH, Abo A, Kujala P, Peters PJ, Clevers H. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. *Nature* 2009; **459**: 262-265 [PMID: 19329995 DOI: 10.1038/nature07935]

359 **Drost J**, van Jaarsveld RH, Ponsioen B, Zimberlin C, van Boxtel R, Buijs A, Sachs N, Overmeer RM, Offerhaus GJ, Begthel H, Korving J, van de Wetering M, Schwank G, Logtenberg M, Cuppen E, Snippert HJ, Medema JP, Kops GJ, Clevers H. Sequential cancer mutations in cultured human intestinal stem cells. *Nature* 2015; **521**: 43-47 [PMID: 25924068 DOI: 10.1038/nature14415]

360 **Fumagalli A**, Drost J, Suijkerbuijk SJ, van Boxtel R, de Ligt J, Offerhaus GJ, Begthel H, Beerling E, Tan EH, Sansom OJ, Cuppen E, Clevers H, van Rheenen J. Genetic dissection of colorectal cancer progression by orthotopic transplantation of engineered cancer organoids. *Proc Natl Acad Sci U S A* 2017; **114**: E2357-E2364 [PMID: 28270604 DOI: 10.1073/pnas.1701219114]

361 **Gao D**, Vela I, Sboner A, Iaquinta PJ, Karthaus WR, Gopalan A, Dowling C, Wanjala JN, Undvall EA, Arora VK, Wongvipat J, Kossai M, Ramazanoglu S, Barboza LP, Di W, Cao Z, Zhang QF, Sirota I, Ran L, MacDonald TY, Beltran H, Mosquera JM, Touijer KA, Scardino PT, Laudone VP, Curtis KR, Rathkopf DE, Morris MJ, Danila DC, Slovin SF, Solomon SB, Eastham JA, Chi P, Carver B, Rubin MA, Scher HI, Clevers H, Sawyers CL, Chen Y. Organoid cultures derived from patients with advanced prostate cancer. *Cell* 2014; **159**: 176-187 [PMID: 25201530 DOI: 10.1016/j.cell.2014.08.016]

362 **Agnoletto C**, Corrà F, Minotti L, Baldassari F, Crudele F, Cook WJJ, Di Leva G, d'Adamo AP, Gasparini P, Volinia S. Heterogeneity in Circulating Tumor Cells: The Relevance of the Stem-Cell Subset. *Cancers (Basel)* 2019; **11**: [PMID: 30959764 DOI: 10.3390/cancers11040483]

363 **Aleman J**, Skardal A. A multi-site metastasis-on-a-chip microphysiological system for assessing metastatic preference of cancer cells. *Biotechnol Bioeng* 2019; **116**: 936-944 [PMID: 30450540 DOI: 10.1002/bit.26871]

364 **Miller PG**, Shuler ML. Design and demonstration of a pumpless 14 compartment microphysiological system. *Biotechnol Bioeng* 2016; **113**: 2213-2227 [PMID: 27070809 DOI: 10.1002/bit.25989]

365 **Guinney J**, Dienstmann R, Wang X, de Reyniès A, Schlicker A, Soneson C, Marisa L, Roepman P, Nyamundanda G, Angelino P, Bot BM, Morris JS, Simon IM, Gerster S, Fessler E, De Sousa E Melo F, Missiaglia E, Ramay H, Barras D, Homicsko K, Maru D, Manyam GC, Broom B, Boige V, Perez-Villamil B, Laderas T, Salazar R, Gray JW, Hanahan D, Tabernero J, Bernards R, Friend SH, Laurent-Puig P, Medema JP, Sadanandam A, Wessels L, Delorenzi M, Kopetz S, Vermeulen L, Tejpar S. The consensus molecular subtypes of colorectal cancer. *Nat Med* 2015; **21**: 1350-1356 [PMID: 26457759 DOI: 10.1038/nm.3967]

366 **Mooi JK**, Wirapati P, Asher R, Lee CK, Savas P, Price TJ, Townsend A, Hardingham J, Buchanan D, Williams D, Tejpar S, Mariadason JM, Tebbutt NC. The prognostic impact of consensus molecular subtypes (CMS) and its predictive effects for bevacizumab benefit in metastatic colorectal cancer: molecular analysis of the AGITG MAX clinical trial. *Ann Oncol* 2018; **29**: 2240-2246 [PMID: 30247524 DOI: 10.1093/annonc/mdy410]

367 **Jean-Quartier C**, Jeanquartier F, Jurisica I, Holzinger A. In silico cancer research towards 3R. *BMC Cancer* 2018; **18**: 408 [PMID: 29649981 DOI: 10.1186/s12885-018-4302-0]

368 **Christopher R**, Dhiman A, Fox J, Gendelman R, Haberitcher T, Kagle D, Spizz G, Khalil IG, Hill C. Data-driven computer simulation of human cancer cell. *Ann N Y Acad Sci* 2004; **1020**: 132-153 [PMID: 15208190 DOI: 10.1196/annals.1310.014]

369 **Uhlen M**, Zhang C, Lee S, Sjöstedt E, Fagerberg L, Bidkhori G, Benfeitas R, Arif M, Liu Z, Edfors F, Sanli K, von Feilitzen K, Oksvold P, Lundberg E, Hober S, Nilsson P, Mattsson J, Schwenk JM, Brunnström H, Glimelius B, Sjöblom T, Edqvist PH, Djureinovic D, Micke P, Lindskog C, Mardinoglu A, Ponten F. A pathology atlas of the human cancer transcriptome. *Science* 2017; **357**: [PMID: 28818916 DOI: 10.1126/science.aan2507]

370 **Farshidfar F**, Weljie AM, Kopciuk KA, Hilsden R, McGregor SE, Buie WD, MacLean A, Vogel HJ, Bathe OF. A validated metabolomic signature for colorectal cancer: exploration of the clinical value of metabolomics. *Br J Cancer* 2016; **115**: 848-857 [PMID: 27560555 DOI: 10.1038/bjc.2016.243]

371 **Porta-Pardo E**, Garcia-Alonso L, Hrabe T, Dopazo J, Godzik A. A Pan-Cancer Catalogue of Cancer Driver Protein Interaction Interfaces. *PLoS Comput Biol* 2015; **11**: e1004518 [PMID: 26485003 DOI: 10.1371/journal.pcbi.1004518]

372 **Subramanian A**, Narayan R, Corsello SM, Peck DD, Natoli TE, Lu X, Gould J, Davis JF, Tubelli AA, Asiedu JK, Lahr DL, Hirschman JE, Liu Z, Donahue M, Julian B, Khan M, Wadden D, Smith IC, Lam D, Liberzon A, Toder C, Bagul M, Orzechowski M, Enache OM, Piccioni F, Johnson SA, Lyons NJ, Berger AH, Shamji AF, Brooks AN, Vrcic A, Flynn C, Rosains J, Takeda DY, Hu R, Davison D, Lamb J, Ardlie K, Hogstrom L, Greenside P, Gray NS, Clemons PA, Silver S, Wu X, Zhao WN, Read-Button W, Wu X, Haggarty SJ, Ronco LV, Boehm JS, Schreiber SL, Doench JG, Bittker JA, Root DE, Wong B, Golub TR. A Next Generation Connectivity Map: L1000 Platform and the First 1,000,000 Profiles. *Cell* 2017; **171**: 1437-1452.e17 [PMID: 29195078 DOI: 10.1016/j.cell.2017.10.049]

373 **Kim YA**, Cho DY, Przytycka TM. Understanding Genotype-Phenotype Effects in Cancer via Network Approaches. *PLoS Comput Biol* 2016; **12**: e1004747 [PMID: 26963104 DOI: 10.1371/journal.pcbi.1004747]

374 **Aoki K**, Tamai Y, Horiike S, Oshima M, Taketo MM. Colonic polyposis caused by mTOR-mediated chromosomal instability in Apc+/Delta716 Cdx2+/- compound mutant mice. *Nat Genet* 2003; **35**: 323-330 [PMID: 14625550 DOI: 10.1038/ng1265]

375 **Rao CV**, Yang YM, Swamy MV, Liu T, Fang Y, Mahmood R, Jhanwar-Uniyal M, Dai W. Colonic tumorigenesis in BubR1+/-ApcMin/+ compound mutant mice is linked to premature separation of sister chromatids and enhanced genomic instability. *Proc Natl Acad Sci U S A* 2005; **102**: 4365-4370 [PMID: 15767571 DOI: 10.1073/pnas.0407822102]

376 **Hahn MM**, Vreede L, Bemelmans SA, van der Looij E, van Kessel AG, Schackert HK, Ligtenberg MJ, Hoogerbrugge N, Kuiper RP, de Voer RM. Prevalence of germline mutations in the spindle assembly checkpoint gene BUB1B in individuals with early-onset colorectal cancer. *Genes Chromosomes Cancer* 2016; **55**: 855-863 [PMID: 27239782 DOI: 10.1002/gcc.22385]

377 **Batlle E**, Bacani J, Begthel H, Jonkheer S, Gregorieff A, van de Born M, Malats N, Sancho E, Boon E, Pawson T, Gallinger S, Pals S, Clevers H. EphB receptor activity suppresses colorectal cancer progression. *Nature* 2005; **435**: 1126-1130 [PMID: 15973414 DOI: 10.1038/nature03626]

378 **Paul Olson TJ**, Hadac JN, Sievers CK, Leystra AA, Deming DA, Zahm CD, Albrecht DM, Nomura A, Nettekoven LA, Plesh LK, Clipson L, Sullivan R, Newton MA, Schelman WR, Halberg RB. Dynamic tumor growth patterns in a novel murine model of colorectal cancer. *Cancer Prev Res (Phila)* 2014; **7**: 105-113 [PMID: 24196829 DOI: 10.1158/1940-6207.CAPR-13-0163]

379 **Suzui M**, Okuno M, Tanaka T, Nakagama H, Moriwaki H. Enhanced colon carcinogenesis induced by azoxymethane in min mice occurs via a mechanism independent of beta-catenin mutation. *Cancer Lett* 2002; **183**: 31-41 [PMID: 12049812 DOI: 10.1016/S0304-3835(02)00114-3]

380 **Møllersen L**, Vikse R, Andreassen A, Steffensen IL, Mikalsen A, Paulsen JE, Alexander J. Adenomatous polyposis coli truncation mutations in 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP)-induced intestinal tumours of multiple intestinal neoplasia mice. *Mutat Res* 2004; **557**: 29-40 [PMID: 14706516 DOI: 10.1016/j.mrgentox.2003.09.008]

381 **Smits R**, van der Houven van Oordt W, Luz A, Zurcher C, Jagmohan-Changur S, Breukel C, Khan PM, Fodde R. Apc1638N: a mouse model for familial adenomatous polyposis-associated desmoid tumors and cutaneous cysts. *Gastroenterology* 1998; **114**: 275-283 [PMID: 9453487 DOI: 10.1016/s0016-5085(98)70478-0]

382 **Xu J**, Cortellino S, Tricarico R, Chang WC, Scher G, Devarajan K, Slifker M, Moore R, Bassi MR, Caretti E, Clapper M, Cooper H, Bellacosa A. *Thymine DNA Glycosylase (TDG)* is involved in the pathogenesis of intestinal tumors with reduced *APC* expression. *Oncotarget* 2017; **8**: 89988-89997 [PMID: 29163805 DOI: 10.18632/oncotarget.21219]

383 **Kawaguchi Y**, Hinoi T, Saito Y, Adachi T, Miguchi M, Niitsu H, Sasada T, Shimomura M, Egi H, Oka S, Tanaka S, Chayama K, Sentani K, Oue N, Yasui W, Ohdan H. Mouse model of proximal colon-specific tumorigenesis driven by microsatellite instability-induced Cre-mediated inactivation of Apc and activation of Kras. *J Gastroenterol* 2016; **51**: 447-457 [PMID: 26361962 DOI: 10.1007/s00535-015-1121-9]

384 **Roper J**, Richardson MP, Wang WV, Richard LG, Chen W, Coffee EM, Sinnamon MJ, Lee L, Chen PC, Bronson RT, Martin ES, Hung KE. The dual PI3K/mTOR inhibitor NVP-BEZ235 induces tumor regression in a genetically engineered mouse model of PIK3CA wild-type colorectal cancer. *PLoS One* 2011; **6**: e25132 [PMID: 21966435 DOI: 10.1371/journal.pone.0025132]

385 **Byun AJ**, Hung KE, Fleet JC, Bronson RT, Mason JB, Garcia PE, Crott JW. Colon-specific tumorigenesis in mice driven by Cre-mediated inactivation of Apc and activation of mutant Kras. *Cancer Lett* 2014; **347**: 191-195 [PMID: 24632531 DOI: 10.1016/j.canlet.2014.03.004]

386 **Kang DW**, Lee SW, Hwang WC, Lee BH, Choi YS, Suh YA, Choi KY, Min DS. Phospholipase D1 Acts through Akt/TopBP1 and RB1 to Regulate the E2F1-Dependent Apoptotic Program in Cancer Cells. *Cancer Res* 2017; **77**: 142-152 [PMID: 27793841 DOI: 10.1158/0008-5472.CAN-15-3032]

387 **Kitamura T**, Biyajima K, Aoki M, Oshima M, Taketo MM. Matrix metalloproteinase 7 is required for tumor formation, but dispensable for invasion and fibrosis in SMAD4-deficient intestinal adenocarcinomas. *Lab Invest* 2009; **89**: 98-105 [PMID: 19002110 DOI: 10.1038/labinvest.2008.107]

388 **Hamamoto T**, Beppu H, Okada H, Kawabata M, Kitamura T, Miyazono K, Kato M. Compound disruption of smad2 accelerates malignant progression of intestinal tumors in apc knockout mice. *Cancer Res* 2002; **62**: 5955-5961 [PMID: 12384562]

389 **Luo F**, Brooks DG, Ye H, Hamoudi R, Poulogiannis G, Patek CE, Winton DJ, Arends MJ. Conditional expression of mutated K-ras accelerates intestinal tumorigenesis in Msh2-deficient mice. *Oncogene* 2007; **26**: 4415-4427 [PMID: 17297472 DOI: 10.1038/sj.onc.1210231]

390 **Edelmann W**, Umar A, Yang K, Heyer J, Kucherlapati M, Lia M, Kneitz B, Avdievich E, Fan K, Wong E, Crouse G, Kunkel T, Lipkin M, Kolodner RD, Kucherlapati R. The DNA mismatch repair genes Msh3 and Msh6 cooperate in intestinal tumor suppression. *Cancer Res* 2000; **60**: 803-807 [PMID: 10706084]

391 **Sakamoto K**, Tominaga Y, Yamauchi K, Nakatsu Y, Sakumi K, Yoshiyama K, Egashira A, Kura S, Yao T, Tsuneyoshi M, Maki H, Nakabeppu Y, Tsuzuki T. MUTYH-null mice are susceptible to spontaneous and oxidative stress induced intestinal tumorigenesis. *Cancer Res* 2007; **67**: 6599-6604 [PMID: 17638869 DOI: 10.1158/0008-5472.CAN-06-4802]

**Footnotes**

**Conflict-of-interest statement:** Dr. Linnebacher reports grants from Ministerium für Wirtschaft, Arbeit und Gesundheit Mecklenburg-Vorpommern during the conduct of the study.

**Open-Access:** This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/

**Manuscript source:** Invited manuscript

**Peer-review started:** December 18, 2019

**First decision:** February 18, 2020

**Article in press:**

**Specialty type:** Gastroenterology and Hepatology

**Country of origin:** Germany

**Peer-review report classification**

Grade A (Excellent): 0

Grade B (Very good): B, B, B

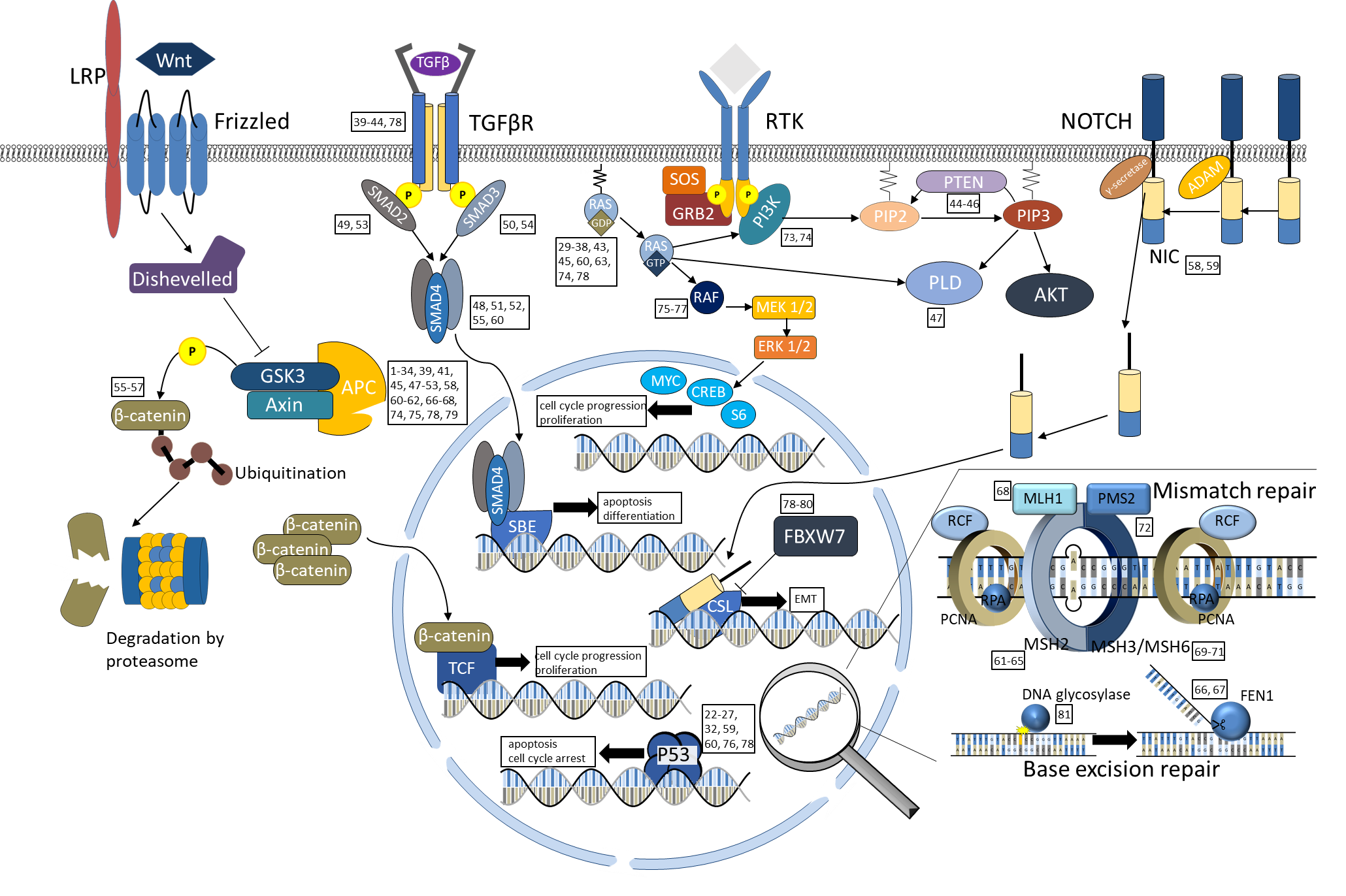
Grade C (Good): 0

Grade D (Fair): 0

Grade E (Poor): 0

**P- Reviewer:** Li Y, Lin JM, Huang ZH **S- Editor:** Dou Y **L- Editor:** **E- Editor:**

**Figure Legends**

****

**Figure 1 Overview of the frequently altered pathways in colorectal cancer.** The numbers in square brackets label the corresponding model descriptions as given in Table 1.



**Figure 2 After surgery, a small sample of the tumor, which is not needed for pathological diagnosis, is obtained and cut into pieces of 27 mm3.** These can be either implanted immediately in recipient mice or vitally cryopreserved in liquid nitrogen. The resulting patient-derived xenograft (b) closely reflects the histology of the donor tumor (a) (Previously published in[229]). Patient-derived xenograft can be further processed for subsequent implantation or cryopreservation.

## **Table 1 Overview of genetically engineered mouse models**

|  |  |  |  |
| --- | --- | --- | --- |
| **Link to Figure 1** | **Ref.** | **Methods** | **Results** |
| 1 | [127] | *loxP* flanked *Apc exon 14 (Apc580S)* | Adenoma formation in the distal rectum in most of the *Apc*580S homozygotes. 50% of animals show invasive adenocarcinoma after 1 yr without lymphatic or distant metastases |
| Colorectal tumor induction by rectal infection with *Cre-*delivering Adenovirus *(AxSRαCre)* |
| 2 | [109] | *ApcΔ242*/+ | Adenomas: higher in numbers but smaller in size and no differences in histology compared to *Apc*Min/+ mice |
| 3 | [110] | *ApcΔ14/+* | Shift of tumor distribution, more severe phenotype, invasion of muscularis propria, 50% dead after 12 mo |
| 4 | [120] | *Fabpl-Cre; Apc15lox*/+ | Increased survival due to lower number of tumors, but larger tumors predominantly in the colon, 91% at least low-grade adenoma, 50% carcinoma; invasiveness and metastases not reported |
| 5 | [108] | *Apc+/Δ716*(C57BL/6J background) | Intestinal polyposis with emphasis on the small intestine |
| 6 | [126] | *Cac; Apc580S/+* | Transgene expression limited to the large intestine. Adenoma formation without malignancy |
| 7 | [125] | *CDX2P9.5-G22Cre* | Frameshifted Cre-recombinase with a long guanine nucleotide tract under control of the homeobox promotor *CDX2P9.5* leads to limited activation of Cre by spontaneous somatic mutations in the large intestine: *Apc*flox/flox homozygotes dye rapidly from florid polyposis of proximal colon and cecum |
| *Apc flox/flox* |
| 8 | [374] | *Apc*+/*Δ716 Cdx2+/−* | Increased adenoma formation in the colon, reduced number of polyps in the small intestine |
| 9 | [132] | *Apc+/fle1−15; Villin-Cre* mice (conditional) and *ApcΔe1−15* constitutive null allele | More severe polyposis compared to *Apc*Min/+ mice |
| 10 | [375] | *BubR1*+/– *ApcMin*/+ | Increased tumor formation in the large intestine and higher malignancy through increased chromosomal instability (invasiveness and metastases not reported). Note, that *BUBR1* mutations are uncommon in CRC[376] |
| 11 | [377] | *ΔcyEphb2;ApcMin/+* | Reduced tumor formation in the small intestine, but large adenocarcinomas of the colorectum |
| *Ephb3+/-;ApcMin/+* |
| *Ephb3-/-;ApcMin/+* |
| 12 | [133] | *CDX2P-CreERT2Apcflox/flox* | Tamoxifen inducible *Apc*-knockout in the distal intestine |
| 13 | [378] | long living *ApcMin/+* mice | Some adenomas progress to adenocarcinomas |
| 14 | [104] | C57BL/6J *ApcMin/+* × SWR/J or C57BR/cdcJ | Hybrid *ApcMin/+* micesurvive longer due to decreased adenoma frequency. After one-year high amount of invasive adenocarcinomas. 3% metastasis to lymph nodes |
| 15 | [105] | Change of the *ApcMin/+* genetic background from C57Bl6/J to A/J mice | Increased tumor formation in the intestine. 50% adenocarcinomas in the small intestine and 20% in the colon |
| 16 | [131] | *AhCre+; Apcflox/flox* | β-naphthoflavone-inducible Cyp1A promoter *Cre*-transgene (*AhCre*). Rapid death upon induction due to disruption of intestinal architecture |
| 17 | [98] | *Apc*Min/+ + AOM | 6-fold increase of colonic tumor formation compared to *Apc*Min/+ mice |
| 18 | [379] | *Apc*Min/+ + AOM | Increased incidence of colonic adenocarcinomas |
| 19 | [52] | *ApcMin/+*+ DSS | High incidence of well differentiated colonic carcinomas |
| 20 | [380] | *Apc*Min/+ + PhIP | 2- to 3-fold increase of tumor formation compared to *Apc*Min/+ mice |
| 21 | [99] | *Apc*Min/+ + AOM + DSS | Mainly small intestinal tumor formation |
| 22 | [111] | *Apc*Min*/+ P53-/-* | No increased adenoma formation or malignancy compared to *ApcMin/+ 53-/+ -* and *ApcMin/+ P53+/+*-mice |
| 23 | [112] | *ApcMin/+ P53-/-* | No increased malignancy or adenoma formation compared to *ApcMin/+* mice |
| 24 | [381] | *ApcMin/+ P53-/-* | Slight, but not significant, increase in malignancy |
| 25 | [113] | *ApcMin/+ Mom1R/R P53−/−* | P53 deficiency increases intestinal adenoma multiplicity and malignancy |
| *ApcMin/+ Mom1R/S P53−/−* |
| 26 | [139] | *ApcΔ716* *Trp53+/LSL•R270H* *Villin-CreER* | Homozygotes die rapidly from lymphoma while heterozygous *P53R*270H leads to invasive adenocarcinomas with features of EMT |
| *ApcΔ716* *Trp53LSL•R270H/LSL•R270H* *Villin-CreER* |
| 27 | [128] | Deletion of *Apc and P53* by viral delivery of corresponding sgRNA into *Rosa26LSL-Cas9-eGFP/+; VillinCreER* | *In vivo* editing of *Apc* alone or in combination with *P53* *via* *Cre* mediated Cas9-expression and provision of sgRNA by viral infection of the colonic epithelium leads to tumor formation without metastatic properties |
| 28 | [382] | *Fabpl:*Cre+/o *Tdg*flox/- *Apc*Min/+ | TDG knockout increases adenoma formation, no carcinomas |
| 29 | [122] | *AhCre*+/*T*; *Kras*+/*LSLV12* | Cytochrome p450 mediated Cre expression in the liver and intestine induced by β-naphthoflavone (*AhCre*). *Kras*V12 mutation does not alter the intestinal epithelium, but combined with APC-loss, accelerates tumorigenesis in the intestine. 17% of the tumors are invasive adenocarcinomas. |
| *AhCre*+/*T*; *Kras*+/*LSLV12*, *Apc+/fl* |
| 30 | [383] | *CDX2P9.5*-*G22Cre; Apc* *flox/flox; LSL*-*Kras* *G12D* | Severe debilities in mice with reduced weight and lifespan and anal bulging. *Kras* mutation does not increase malignancy |
| 31 | [136] | *Fapbl-Cre; Apc2lox14/+*;*KrasLSL-G12D/+* | *Kras*G12D, but not *Nras*G12D drives colon cancer progression. *Nras* indistinct from *Apc*Min/+ mice |
| *Fapbl-Cre; Apc2lox14/+; NrasLSL-G12D/+* |
| 32 | [143] | *shApc/KrasG12D/P53fl/fl/Lgr5* | Mice with inducible and reversible *Apc* deletion *via* short hairpin RNA show duodenal and colonic tumor formation. Additional, conditional mutations drive cancer progression, but upon *Apc* restoration by withdrawal of doxycycline rapid tumor regression can be induced |
| 33 | [130,384] | *ApcCKO/LSL-Kras* | *Cre*-mediated knockout of *Apc* and *Kras*G12D activation by surgical application of *AdenoCre* to the colonic epithelium leads to adenocarcinomas with 20% liver metastases after 20 weeks |
| 34 | [385] | *Apc*+/580S, *Kras*+/LSL, CAC+ | Only adenomas |
| 35 | [116] | *CMV-cre* × *LSL- KrasG12D* | Germline embryonic expression of an endogenous *Kras*G12D allele is uniformly lethal. Diffuse colonic hyper- and dysplasia |
| *LSL-KrasG12D; Fabpl-Cre* |
| 36 | [117] | *Kras*+/V12 × *CMV-Cre*+/T | High embryonic lethality; adult animals succumb to pulmonary neoplasia, no phenotypic changes in the intestine |
| 37 | [134] | *LSL-KrasG12D*/*Villin-cre* +AOM | Increased number of microadenomas in the proximal colon |
| 38 | [198] | *Villin-Cre/K-rasG12Dint/Ink4a/Arf−/−* | Within 12 wk progression to invasive adenocarcinomas (79%) with 60% lung metastases |
| 39 | [165] | *ApcΔ716 Tgfbr2flox/flox; villin-CreER* | Disruption of TGFβ-signaling leads to locally invasive adenocarcinomas |
| 40 | [164] | AOM-treatment of *Fabpl*4xat-132 *Cre; Tgfbr2flx/flx* mice | Higher incidence of colonic adenomas and adenocarcinomas |
| 41 | [167] | *Villin*-Cre; Apc1638N/wt; *Tgfbr2flx/flx* | Compared to *Apc1638N/wt* similar tumor incidence but increased progression to locally invasive adenocarcinoma |
| 42 | [163] | *Tgfb1*−/− *Rag2*−/− | Rapid formation of adenomas and adenocarcinomas |
| 43 | [166] | *LSL-KrasG12D/wt; Villin-Cre; Tgfbr2E2flx/E2flx* | Wnt-independent induction of invasive carcinomas in the intestine with 15% distant metastases |
| 44 | [153] | *Villin-Cre; Ptenflx/flx*; *Tgfbr2flx/flx* | Mice with inactivation of TGFβR2 combined with loss of PTEN show high number of mucinous adenocarcinomas throughout the intestine. 8% show distant metastases (not Wnt, but deregulation of CDK inhibitor expression). *Pten* loss without mutation has no effect |
| 45 | [152] | *Villin-CreER*T*; Apcfl/+; Ptenfl/fl; KrasLSL/+* | *Villin-CreERT; Apcfl/+; Ptenfl/fl; KrasLSL/+* mice show rapid morbidity due to invasive small intestinal tumors |
| 46 | [151] | *AhCre*; *Pten*f/f | PTEN is dispensable in the intestinal epithelium, but increases tumorigenesis in the context of APC deficiency |
| 47 | [386] | *ApcMin/+Pld1−/− vs ApcMin/+Pld1+/+* +AOM/DSS | *Pld1*-knockout/suppression leads to decreased tumor burden |
| 48 | [157] | *Dpc4+/-: Apc+/Δ716* | *Dpc4* and *ApcΔ716* cis-compound heterozygote mice show adenoma to carcinoma progression in the small intestine and colon with submucosal infiltration |
| 49 | [159] | *Smad2+/-; Apc+/Δ716* | Combination of *Apc* mutation and loss of *Smad2* leads to no changes in tumor size or properties compared to *Apc*+/Δ716 mice |
| 50 | [161] | *ApcMin/+; Smad3−/−* | Reduced lifespan of 2 months due to rapid tumor development in the distal colon with mixed histology but no metastases |
| 51 | [156] | *Apc*+/1638N/*Smad4*+/E6sad (cis and trans) | *Smad4* mutation leads to intestinal tumors without malignant properties. Both mutations lead to high tumor burden in the upper GI (cis>trans); some show invasion of the submucosa |
| 52 | [387] | *cis-Apc+/-/Smad4+/- Mmp7−/−* | *Mmp7* knockout reduces tumor size but does not affect invasiveness |
| 53 | [388] | *Smad2+/-; Apc580D/+* (cis) | Larger tumors, higher incidence of malignant phenotype (compared to *Apc580D/+*mice) |
| 54 | [160] | *Smad3*-/- (129/Sv genetic background) | Adenocarcinomas of the intestine with penetration of the whole intestinal wall and lymphatic spread. Lower tumor burden in C57/BL6 × 129/Sv *Smad3-/-*hybrids. Note, that *Smad3* mutations occur only in 2% of CRC (Fleming *et al*[162], 2013) |
| 55 | [179] | *Smad4f/f;Catnblox(ex3)/+;Lgr5-CreERT2-IRES-GFP* | Mosaic *Cre-*expression leads to adenoma formation |
| 56 | [178] | *Catnb+/lox(ex3): Krt1-19+/cre*  *Catnb+/lox(ex3): Tg·Fabplcre* | Constitutional Cre-mediated excision of ß-catenin phosphorylation site leads to a plethora of small intestine adenomas |
| 57 | [177] | *Villin-cre*ERT2/*Catnb*loxEx3/WT | Expression of GSK3β-resistant β-catenin leads to substitution of enterocytes by highly proliferative crypt stem cells (rapid death) |
| 58 | [142] | *Nicd/Apc*+/1638N | NOTCH-signaling does not influence adenoma formation |
| 59 | [141] | *Nicd/P53−/−* | *Villin-CreERT2* tamoxifen-dependent *P53* deletion in constitutively active NOTCH-signaling background leads to intestinal tumor formation and metastasis |
| 60 | [158] | *Car1*CreER/+; *Apc*fl/fl; *Kras*LSL–G12D/+; *P53*KO*; Smad4*fl/fl | Rapid tumor formation in cecum and proximal colon, but high mortality in triple and quadruple mutants |
| 61 | [192] | *Msh2Δ7N/Δ7N* /*Apc*+/1638N.*Msh2Δ7N/Δ7N* /*Apc*Min/+ | Rapid tumor formation in the small intestine, early death (2-3 months), more tumors in *Msh2Δ7N/Δ7N* /*Apc*Min/+ |
| 62 | [191] | *ApcMin/+/Msh2+/+; ApcMin/+/Msh2+/-; ApcMin/+/Msh2-/-* | Accelerated tumor formation in the small intestine in MSH2-deficient mice. Mice homozygous for Msh2-/- dye rapidly from lymphomas |
| 63 | [389] | *KrasV12/Cre/Msh2*−/− | β-naphthoflavone inducible Kras mutation (*AhCre*+/*T*, *Kras*+/*LSLV12*) combined with homozygous *Msh2*-knockout leads to increased number of intestinal adenomas. No carcinomas, relevant number of thymic lymphomas |
| 64 | [197] | *VCMsh2LoxP/LoxP* | Villin-controlled *Cre*-expression leads to intestinal MMR-deficiency, similar to Lynch syndrome. 50% of tumors in the small intestine are malignant. A high proportion *of carcinomas in VCMsh2*LoxP/null mice are resistant to cisplatin and FOLFOX |
| *VCMsh2LoxP/G674D* |
| *VCMsh2LoxP/null* |
| 65 | [185,186] | *Msh2-/-* | Death due to lymphoma |
| 66 | [194] | *Apc1638N/+ Exo1 +/− Fen1+/−* | Increased tumor multiplicity and incidence, higher progression to malignancy, high incidence of hematopoietic cancers |
| 67 | [193] | *Fen1null/Apc1638N* | Increased malignancy of intestinal tumors compared to *Apc1638N* mice through MSI |
| 68 | [184] | *Mlh1-/-*/*Apc1638N* | Increased tumor incidence and multiplicity, 30% adenocarcinomas, reduced lifespan of 3.3 mo. High amount of extraintestinal tumors |
| 69 | [187] | *Msh6-/+* | Reduced life span in hetero- and homozygotes due to lymphomas and gastrointestinal tumors. Tumors show no signs of MSI |
| *Msh6-/-* |
| 70 | [390] | *Msh3−/−; Msh6−/−* | Decreased life span, death due to intestinal adenocarcinomas or lymphomas |
| 71 | [190] | *Msh6TD/TD; Msh6TD/+* | B or T cell non-Hodgkin lymphomas, adenomas of the small intestine, basal cell carcinomas |
| 72 | [388] | *Pms2-/-; Pms1-/-* | *Pms2-*deficient mice develop lymphomas and sarcomas, but no intestinal tumors; Pms1 deficiency does not cause tumor development |
| 73 | [150] | *Fc*+*; Pik3ca*\*+ (FVB/N-Tg(Fabp1-Cre)1Jig;*Gt(ROSA)26Sortm7(Pik3ca\*,EGFP)Rsky* | Constitutively active PI3K causes mucinous adenocarcinomas of the proximal colon with infiltration of the whole intestinal wall |
| 74 | [129] | *Apcfl/fl KrasG12D/+ Pik3cap110\*+ Cre-*Adenovirus *via* coloscopic injection | Additional driver mutations do not increase tumor proliferation, but cause progression to adenocarcinoma and metastatic disease |
| 75 | [172] | *ApcCKO/CKO; BrafCA/+*, *AdenoCre* delivery *via* colonoscopy | *Cre-*mediated *Apc*-knockout combined with latent *BrafV600E* cause neoplasia of the distal colon (50% adenocarcinomas) |
| 76 | [174] | *Villin-Cre;BrafLSL-V637E*/+ | Some invasive adenocarcinomas (13%), dominant negative P53 mutation leads to 60% cancers with 2% metastases. Also, *p16ink4a* mutation causes carcinomas in a *Braf* mutational background |
| *Villin-Cre;BrafLSL-V637E*/+*;P53LSL-R172H/+* |
| *Villin-Cre;BrafLSL-V637E*/+*;p16Ink4∗* |
| 77 | [171] | *Braf+/LSL-V600E*; *AhCreERT+/o x* | *CypA1*-promotor-driven, tamoxifen-inducible *Cre*-recombinase facilitates *BrafV600E* expression in the small intestine with consecutive crypt hyperplasia. Repression of *p16Ink4A* leads to tumor formation in various tissues and decreased survival (6 wk) |
| *Cdkn2a* (Ink4a/ArfΔEx2,3) = VE;Cdkn2aΔEx2,3/ΔEx2,3 |
| 78 | [140] | *ApcΔ716* (A), *Kras*+/*LSL-G12D* (K), *Tgfbr2flox/flox* (T), *Trp53*+/*LSL-R270H* (P), *Fbxw7flox/flox* (F), and V*illin-CreER* | *Kras* mutation increases multiplicity of tumors, whilst *P53* gain-of-function mutation and *Tgfβr*-knockout leads to invasiveness, no spontaneous metastases |
| 79 | [147] | *ApcMin/+; Fbw7ΔG* | Reduced survival for *Fbw7* deficient mice, also in heterozygous setting |
| 80 | [148] | *Fbw7flox/flox; P53flox/flox; Villin-Cre* | Aggressive carcinomas with metastatic spread to lymph nodes and liver |
| 81 | [391] | *Mutyh-/-* | Spontaneous adenoma and adenocarcinoma development in the intestine; predominantly in the upper small intestine. Tumorigenesis increased by oxidative stress (KBrO3) |