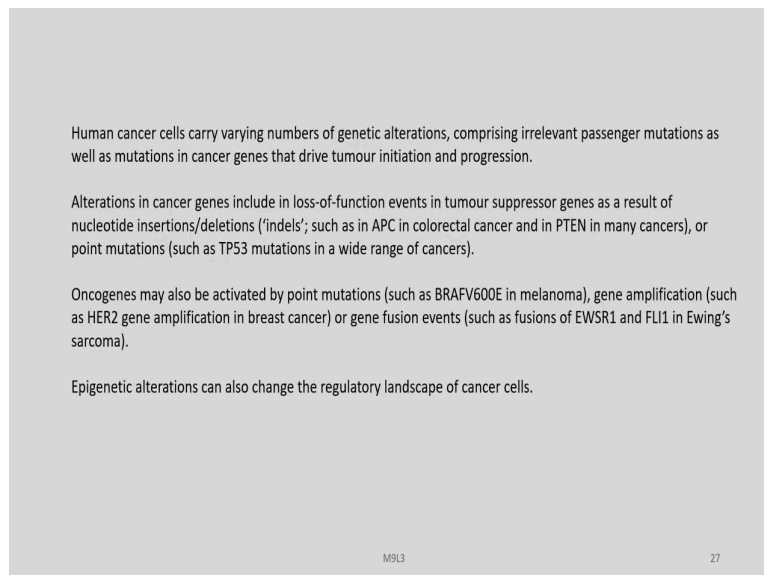


**Genome Editing and Engineering**  
**Prof. Utpal Bora**  
**Department of Bioscience and Bioengineering**  
**Indian Institute of Technology, Guwahati**

**Module - 09**  
**Genome Engineered Disease Modelling**  
**Lecture - 01**  
**Cancer Disease Models - Part B**

Welcome to my course on Genome Editing and Engineering. We are discussing module 9 Genome Engineered Disease Modelling and we have been discussing about Cancer Disease Models and we continue our discussion in Part B of this lecture.

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Human cancer cells carry varying numbers of genetic alterations, comprising irrelevant passenger mutations as well as mutations in cancer genes that drive tumour initiation and progression.

Alterations in cancer genes include in loss-of-function events in tumour suppressor genes as a result of nucleotide insertions/deletions ('indels'; such as in APC in colorectal cancer and in PTEN in many cancers), or point mutations (such as TP53 mutations in a wide range of cancers).

Oncogenes may also be activated by point mutations (such as BRAFV600E in melanoma), gene amplification (such as HER2 gene amplification in breast cancer) or gene fusion events (such as fusions of EWSR1 and FL11 in Ewing's sarcoma).

Epigenetic alterations can also change the regulatory landscape of cancer cells.

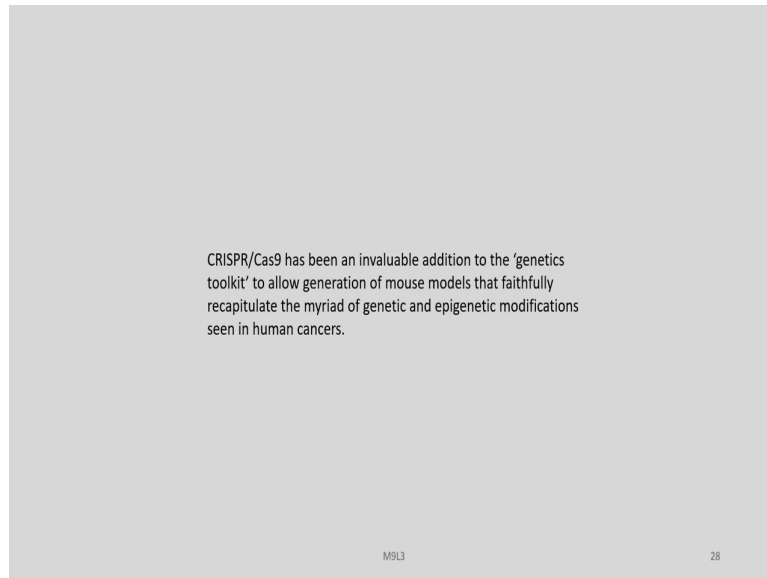
M9L3 27

We have learnt in the last unit that human cancer cells carry varying numbers of genetic alterations comprising irrelevant passenger mutations as well as mutations in cancer genes that drive tumour initiation and progression.

Alteration in cancer genes include loss of function events in tumour suppression genes as a result of nucleotide insertion deletions. Indels such as in APC in colorectal cancer and in PTEN in many cancers or point mutation such as TP53 mutations in a wide range of cancers. Oncogenes may also be activated by point mutations such as BRAFV600E in melanoma. Gene and amplification such as HER2 gene amplification is breast cancer or gene diffusion event such as fusions of EWSR1 and FL11 in Ewing's sarcoma.

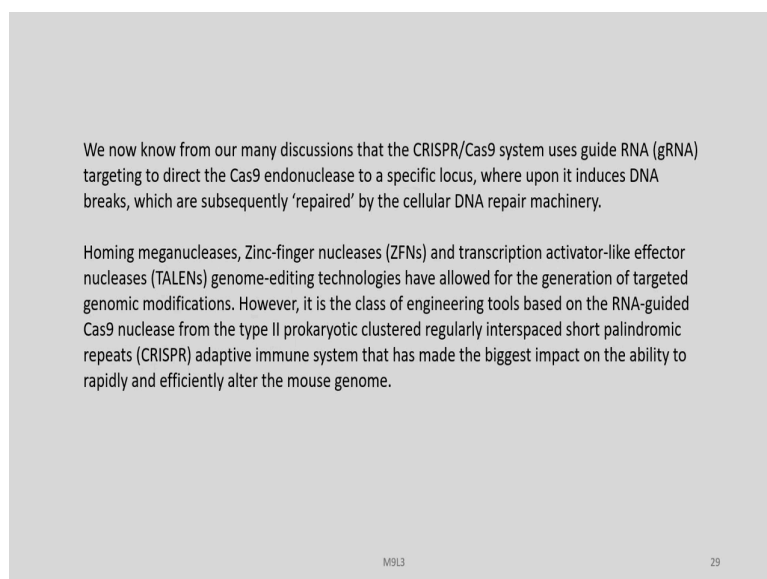
Epigenetic alterations can also change the regulatory landscape of cancer cells. And in the modelling of a cancer we have to imitate all these kind of genetic changes and built up these regulatory landscape of the genes and genetic elements inside in the cancer cells.

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In this context CRISPR Cas9 has been an invaluable addition to the genetics toolkit to allow generation of mouse models that faithfully recapitulate the myriad of genetic and epigenetic modifications seen in human cancers.

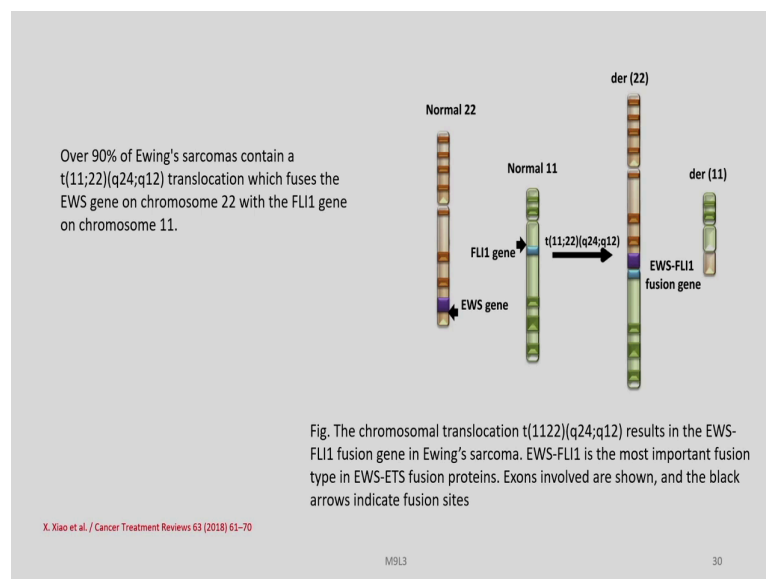
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From our innumerable discussions in the past, we now know that CRISPR Cas9 system uses guide RNA targeting to direct the Cas9 endonuclease to a specific locus where upon it induces DNA breaks which are subsequently repaired by the cellular DNA repair mechanism machinery.

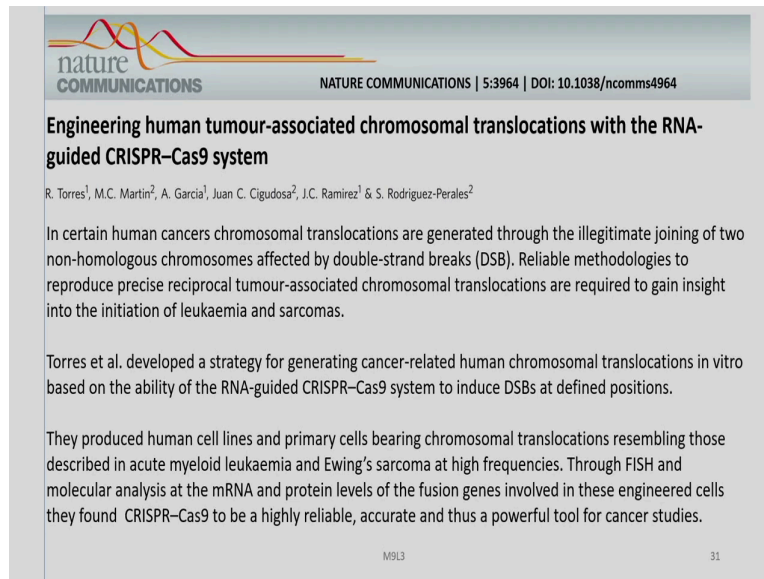
Homing meganucleases ZFNs and TALENs genome editing technologies have allowed for a generation of targeted genomic modifications. However, it is the class of engineering tools based on the RNA-guided Cas9 nuclease from the type II prokaryotic clustered regularly interspaced short palindromic repeats CRISPR adaptive immune system that has made the biggest impact on the ability to rapidly and efficiently alter the mouse genome.

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We have discussed about the Ewing's sarcoma in the earlier part of this lecture and there we have discussed about the use of ZFN in generating a translocation which is found in the diseased case.

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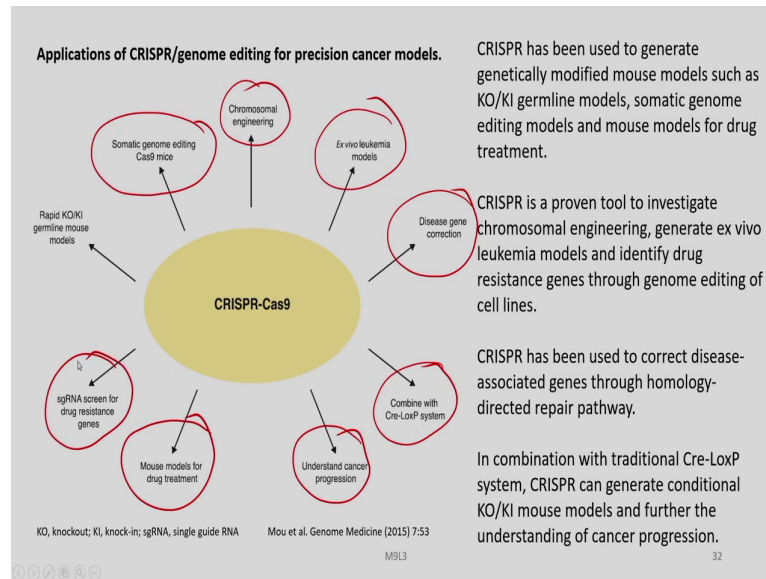
The slide displays the title and abstract of a research paper. At the top left is the 'nature COMMUNICATIONS' logo. To its right, the journal information reads 'NATURE COMMUNICATIONS | 5:3964 | DOI: 10.1038/ncomms4964'. The title of the paper is 'Engineering human tumour-associated chromosomal translocations with the RNA-guided CRISPR-Cas9 system'. Below the title, the authors are listed as 'R. Torres<sup>1</sup>, M.C. Martín<sup>2</sup>, A. García<sup>1</sup>, Juan C. Cigudosa<sup>2</sup>, J.C. Ramirez<sup>1</sup> & S. Rodríguez-Perales<sup>2</sup>'. The abstract text follows, starting with 'In certain human cancers chromosomal translocations are generated through the illegitimate joining of two non-homologous chromosomes affected by double-strand breaks (DSB). Reliable methodologies to reproduce precise reciprocal tumour-associated chromosomal translocations are required to gain insight into the initiation of leukaemia and sarcomas.' A second paragraph states 'Torres et al. developed a strategy for generating cancer-related human chromosomal translocations in vitro based on the ability of the RNA-guided CRISPR-Cas9 system to induce DSBs at defined positions.' The final paragraph describes the results: 'They produced human cell lines and primary cells bearing chromosomal translocations resembling those described in acute myeloid leukaemia and Ewing's sarcoma at high frequencies. Through FISH and molecular analysis at the mRNA and protein levels of the fusion genes involved in these engineered cells they found CRISPR-Cas9 to be a highly reliable, accurate and thus a powerful tool for cancer studies.' At the bottom of the slide, the number 'M913' is on the left and '31' is on the right.

CRISPR Cas9 has also been efficiently used for doing the similar kind of modelling by Torres and his colleagues in certain human cancers chromosomal translocations are generated through the illegitimate joining of two non homologous chromosomes affected by double strand breaks. Reliable methodologies to reproduce precise reciprocal tumour associated chromosomal translocations are required to gain insight into the initiation of leukaemia and sarcomas.

The methodology developed by Torres et al, for generating cancer related human chromosomal translocations in vitro based on the ability of the RNA guided CRISPR Cas9 system induce DSB at the defined positions.

Through these they produced human cell lines and primary cells bearing chromosomal translocations resembling those described in acute myeloid leukaemia and Ewing's sarcoma at very high frequencies through fish and molecular analysis at the mRNA and protein levels of the fusion genes involved in these engineered cells. They found CRISPR Cas9 to be highly reliable accurate and a powerful tool for cancer modelling studies.

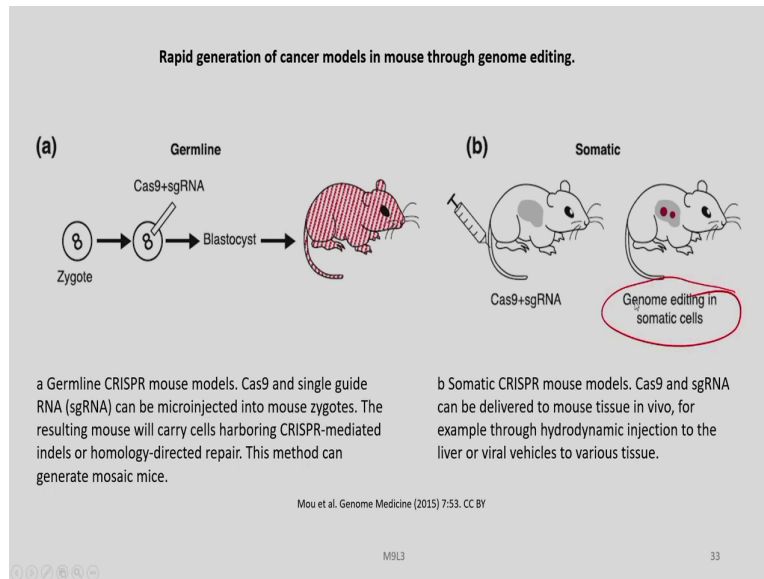
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So, CRISPR Cas9 can have huge impact in cancer research. The various applications of CRISPR genome editing for precision cancer models can be seen in this figure. So, it is used for the rapid knockout knock in germline mouse model generation. Somatic genome editing a Cas9 mice then for chromosomal engineering ex vivo leukemia models development, disease gene correction combine with Cre-LoxP system and for understanding cancer progression and developing mouse models for drug testing and treatment, then sgRNA screen for drug resistant genes.

CRISPR has been used to generate all these kind of studies and it is a proven tool to investigate chromosomal engineering, generate ex vivo leukemia models and identify drug resistance genes through genome editing in cell lines. It can be used to correct disease associated genes through homology directed repair pathway and in combination with the traditional Cre-LoxP system, CRISPR can generate conditional knockout knock in mouse models and further the understanding of cancer progression.

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Rapid generation of cancer models in mouse through genome editing is possible using CRISPR Cas9. So, here in this figure a, you can see the production of germline CRISPR mouse models. Cas9 and single guide RNA can be micro injected into mouse zygotes. The resulting mouse will carry cells harboring CRISPR mediated indels or homology directed repair and this method generates mosaic mice. In the case of figure b you can see the somatic CRISPR mouse models production Cas9 and sgRNA can be delivered to mouse tissue in vivo.

For example through hydro dynamic injection to the liver or viral vehicles to various tissues and we obtain genome editing in the somatic cells as a outcome of this procedure.

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The engineering of CRISPR-mediated genomic deletions ranging from 1.3 kb to greater than 1 megabase (Mb) in mammalian cells has been reported and an inverse relationship exists between deletion frequency and deletion size.

Canver et al. J Biol Chem. 2014;289:21312–24.

**Characterization of Genomic Deletion Efficiency Mediated by Clustered Regularly Interspaced Palindromic Repeats (CRISPR)/Cas9 Nuclease System in Mammalian Cells\*\***

Received for publication, March 12, 2014, and in revised form, May 27, 2014. Published, JBC Papers in Press, June 6, 2014, DOI 10.1074/jbc.M114.564625

Matthew C. Canver<sup>1,2</sup>, Daniel E. Bauer<sup>1,3,4</sup>, Abhishek Dass<sup>1</sup>, Yvette Y. Yien<sup>1</sup>, Jacky Chung<sup>1,2</sup>, Takeshi Masuda<sup>1</sup>, Takahiro Maeda<sup>1</sup>, Barry H. Paw<sup>1,5</sup>, and Stuart H. Orkin<sup>1,4,6,7</sup>

From the <sup>1</sup>Harvard Medical School, the <sup>2</sup>Division of Hematology/Oncology, Boston Children's Hospital, the <sup>3</sup>Department of Pediatric Oncology, Dana-Farber Cancer Institute, the <sup>4</sup>Division of Hematology, Brigham and Women's Hospital, and the <sup>5</sup>Howard Hughes Medical Institute, Boston, Massachusetts 02115

**Background:** CRISPR/Cas9-directed cleavages may result in genomic deletion.  
**Results:** CRISPR/Cas9-produced genomic deletion frequency is inversely related to deletion size, with large deletions and inversions practicable and biallelic deletions exceeding probabilistic expectation.  
**Conclusion:** Biallelic, large genomic deletions are efficiently engineered in mammalian cells by CRISPR/Cas9.  
**Significance:** CRISPR/Cas9-mediated genomic deletion represents a robust method for loss-of-function studies in mammalian cells.

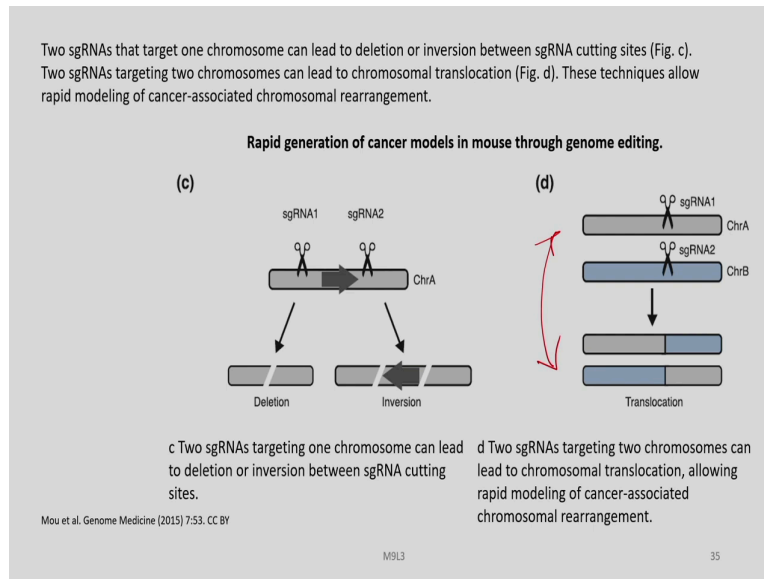
Canver and associates showed that that CRISPR/Cas9 is a robust system to produce a spectrum of genomic deletions to allow investigation of genes and genetic elements.

M913 34

The engineering of CRISPR mediated genomic deletions ranging from 1.3 kb to greater than 1 mega bases in mammalian cells has been reported and an inverse relationship exists between deletion frequency and deletion size.

This work by Canver et al and in journal of Biological Chemistry on the characterization of genomic deletion efficiency mediated by CRISPR Cas9 nuclease system in mammalian cells is considered as a very important study in this regard and they showed that CRISPR Cas9 is a robust system to produce a spectrum of genomic deletions to allow investigations of genes and a genetic elements.

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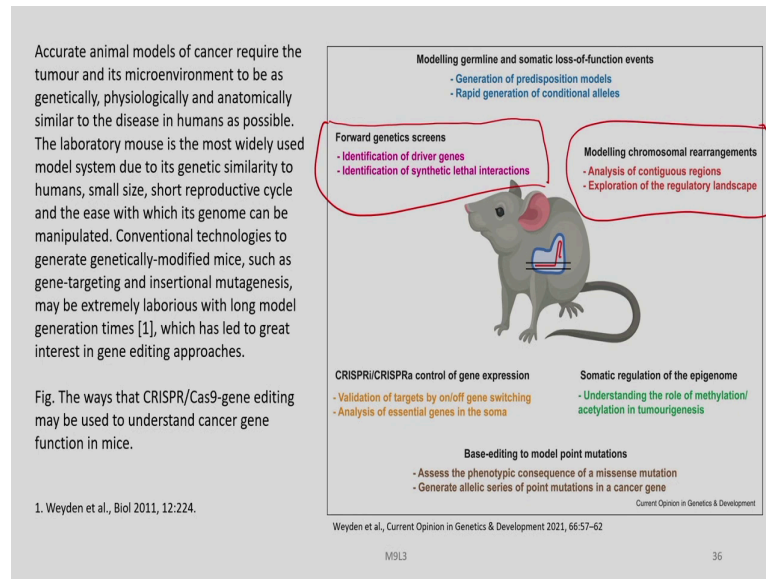


Two sgRNAs that target one chromosome can lead to deletion or inversion between sgRNA cutting sites as seen in this figure c. Two sgRNAs targeting two different chromosomes can lead to a chromosomal translocation as shown in the figure d. These techniques allow rapid modelling of cancer associated chromosomal rearrangement and we have seen in the case of Ewing's sarcoma, how these translocations are utilized.

So, in figure c you can see two sgRNAs are targeting one chromosome due to which there is either a gap or deletion or this is getting inverted and while in figure d there is a occurrence of translocation.



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Accurate animal models of cancer require the tumour and its micro environment to be as genetically physiologically and anatomically similar to the disease in humans as possible.

And while studying the cyclic process in cancer modelling there is a step of a model refinement or model improvement. So, we may not be able to build up the perfect model in the first circle, but after learning about the various problems and false fallacies we can improve upon the model and make them as much similar to the disease in humans and generate a real model.

The laboratory mouse is the most widely used model system due to its genetic similarity to humans. Small size short reproductive cycle which we have already discussed. And above all the ease with which its genome can be manipulated convention and technologies to generate genetically modified mice such gene targeting and insertional and mutagenesis may be extremely laborious with long model generation times which has led to great interest in gene editing approaches. In this figure we can see the various ways CRISPR Cas9 gene editing is used to understand cancer gene function in mice.

The modelling germline and somatic loss of function events for the generation of predisposition model, rapid generation of conditional alleles, we may have forward genetic screen identification of driver genes and identification of synthetic lethal interactions. In a modelling of chromosomal rearrangements the analysis of contiguous regions and exploration of the regulatory landscape is involved. In the case of CRISPRi and CRISPRa control of gene

expression is possible. Validation of targets by on off gene switching analysis of essential genes in the body or soma.

Somatic regulation of the epigenome for understanding the role of methylation acetylation in tumour genesis. We may also use the base editing to model point mutations here we have access to the phenotypic consequences of a missense mutation and generate allelic series of point mutations in a cancer gene.

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**Modelling germline and somatic loss-of function events**  
Use of CRISPR/Cas9 technology to generate germline 'knockout' (KO) mouse models of cancer.

Hydrodynamic tail vein injection (HTVI; to deliver CRISPR/Cas9 component to the liver) of a plasmid expressing both Cas9 and sgRNAs against Pten and Trp53, resulted in the development of hepatocellular carcinomas that mimicked those seen in traditionally-generated mice with liver-specific loss of Pten and Trp53 [3].

A landmark study that was the first to show the power of in vivo genome editing for identifying and validating driver genes.

Intraductal injections of WapCre;Cdh1flox/flox;Cas9 female mice with a lenti virus carrying a sgRNA for Pten or Myh9 resulted in a proportion of the mice developing invasive lobular breast carcinomas (ILCs) that closely resembled human ILCs [4,5]. Kas et al study is a large-scale example of how insertion mutagenesis can be used to identify and validate cancer genes and pathways in an unbiased way.

3. Xue et al.: Nature 2014, 514:380-384  
4. Annunziato S, Kas SM, Nethé M, Yu<sup>2</sup> cel H, Del Bravo J, Pritchard C, Bin Ali R, van Gerwen B, Siteur B, Drenth AP et al.: Genes Dev 2016, 30:1470-1480.  
5. Kas et al.: Nat Genet 2017, 49:1219-1230

Weyden et al., Current Opinion in Genetics & Development 2021, 66:57-62

M9L3 37

Let us now discuss these various aspects of CRISPR Cas9 gene editing in the generation of cancer models one by one. Let us start with the modelling of germline and somatic loss of function events. CRISPR Cas9 technology can be used to generate germline knockout mouse models of cancer.

Hydrodynamic tail vein injection of a plasmid expressing both Cas9 and sgRNAs against Pten and Trp53 has resulted in the development of hepatocellular carcinoma that mimicked those seen in traditionally generated mice with liver specific loss of Pten and Trp53. This was a landmark study which for the first time showed the power of in vivo genome editing for identifying and validating driver genes.

Intraductal injections of WapCre;Cdh1flox/flox;Cas9 female mice with a lenti virus carrying a sgRNA for Pten or Myh9 resulted in a proportion of the mice developing invasive lobular breast carcinomas that closely resemble human ILCs

Kas et al studied a large scale example of how insertion mutagenesis can be used to identify and validate cancer genes and pathways in an unbiased way.

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**Modelling germline and somatic loss-of function events**  
Use of CRISPR/Cas9 technology to generate germline 'knockout' (KO) mouse models of cancer.

The prosencephalon refers to the future forebrain, which includes the telencephalon and the diencephalon; these structures give rise to the cerebral hemispheres and the thalamus/hypothalamus.

To model brain cancer, wildtype mice underwent in Utero electroporation of the developing prosencephalon with three plasmids carrying Cas9 together with sgRNAs target-ing Nf1, Trp53 or Pten which led to the development of highly aggressive glioblastomas, similar to those seen in human glioblastoma patients [6].

6. Zuckermann et al., Nat Commun 2015, 6:7391.

Weyden et al., Current Opinion in Genetics & Development 2021, 66:57-62

M9L3 38

The prosencephalon refers to the future forebrain which includes the telencephalon and the diencephalon; these structures give rise to the cerebral hemisphere and the thalamus or hypothalamus.

For modelling brain cancer wild type mouse underwent in Utero electroporation of the developing prosencephalon with three plasmids carrying Cas9 together with sgRNAs targeting Nf1, Trp53 or Pten which led to the development of highly aggressive glioblastomas similar to those seen in human glioblastoma patients.

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Extending this approach using multiplexing, Alb-Cre;KrasLSL-G12D/+ mice were HTVI-administered 10 individual CRISPR-SB vectors (each vector carrying Cas9 and one of 10 different sgRNAs, Flanked by Sleeping Beauty(SB) transposon repeats) and an SB transposase vector to promote genomic integration of the CRISPR-SB vectors, resulting in the development of hepa-tocellular carcinoma and intrahepatic cholangio carcinoma[7]. This system allows for assessment of the oncogenic effects of multiple genes and genetic interactions in a single experiment.

7. Weber et al. Proc Natl Acad Sci 2015, 112:13982-13987.

Weyden et al., Current Opinion in Genetics & Development 2021, 66:57-62

M9L3 39

Extending this approach using multiplexing Alb-Cre;KrasLSL-G12D oblique positive mice where HTV administered as 10 individual CRISPR-SB vectors. Each vector carrying Cas9 and one of the 10 different sgRNAs is flanked by sleeping beauty transposon repeats and an SB transposase vector to promote genomic integration of the CRISPR-SB vectors resulting in the development of hepatocellular carcinoma and intra hepatic cholangio carcinoma. This system allows for assessment of the oncogenic effects of multiple genes and genetic interactions in a single experiment.

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Conditional in vivo genome editing allows both temporal and spatial control of the induction of genetic alterations. The mouse 'genetics toolkit' has several ways to generate conditional alleles, of which a commonly used approach is the Cre recombinase (Cre) enzyme which can inactivate or activate genes by excising loxP-flanked ( flox) exons or LoxP-Stop-LoxP (LSL) transcriptional terminators, respectively[1]. The CRISPR/Cas9 system can be used in conditional mouse models of cancer to study cooperation between cancer driver genes.

For example, KrasLSL-G12D/+;Trp53flox/flox (KP) mice intratracheally administered a lentivirus encoding a sgRNA targeting Nkx2.1 or Pten, Cas9 and Cre, developed lung adenocarcinomas faster than KP mice treated with a lentivirus encoding Cre alone [10].

10. Sánchez-Rivera et al. Nature 2015, 516:428-431.

Weyden et al., Current Opinion in Genetics & Development 2021, 66:57-62

M9L3 40

Conditional in vivo genome editing allows both temporal and spatial control of the induction of genetic alterations. The mouse genetics toolkit has several ways to generate conditional alleles of which a commonly used approach is the Cre recombinase Cre enzyme which can inactivate or activate genes by excising LoxP flanked exons or LoxP-Stop-LoxP transcriptional terminators respectively. The CRISPR Cas9 system can be used in conditional mouse models of cancer to study cooperation between cancer driver genes.

Some examples are *Kras*<sup>LSL-G12D/+</sup>; *Trp53* flox/flox (KP) mice intra tracheally administered a lentivirus encoding a sgRNA targeting *Nkx2.1* or *Pten* Cas9 and Cre developed lung adenocarcinomas faster than KP mice treated with a lentivirus encoding Cre alone.

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Other studies have used transgenic mice carrying a LSL-Cas9 cassette 'knocked in' to a safe/inert targeting site, such as *Hipp11* (H11) or *Rosa26* (R26). For example, *Kras*<sup>LSL-G12D/+</sup>; *R26*<sup>LSL-Tom</sup>; *H11*<sup>LSL-Cas9(KT;Cas9)</sup> mice given retrograde pancreatic ductal injections of a lentivirus carrying Cre and a sgRNA against *Lkb1* developed extensive tumour growth in the pancreas as early as 2 months after tumour initiation, with histological features that were indistinguishable from those found in *KT;Lkb1*<sup>flox/-</sup> mice injected with a Cre lentivirus [11]

11. Chiou et al. *Genes Dev* 2015, 29:1576-1585.

Weyden et al., *Current Opinion in Genetics & Development* 2021, 66:57-62

In other studies they have used transgenic mice carrying a LSL Cas9 cassette knocked in to a safe inert targeting site such as *Hipp11* or *Rosa26* and examples are *kras*<sup>LSL-G12D/+</sup>; *R26*<sup>LSL-Tom</sup>; *H11*<sup>LSL-Cas9(KT;Cas9)</sup> mice given retrograde pancreatic ductal injections of a lentivirus carrying Cre and a sgRNA against *Lkb1* developed extensive tumour growth in the pancreas as early as 2 months after tumour initiation, with histological features that were indistinguishable from those found in *KT Lkb1* flox or flox mice injected with a Cre lentivirus.

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#### Modelling chromosomal rearrangements

The CRISPR/Cas9 system can be used to accurately recapitulate the complex genomic rearrangements seen in human cancers, such as chromosomal deletions, translocations and gene fusion events.

One group utilised the replication-competent avian leukosis virus splice-acceptor (RCAS)-TVA-based approach, which uses RCAS vectors to target individual cells engineered to express the TVA cell surface receptor. To create a chromosomal deletion, an RCAS virus carrying sgRNAs targeting intron 13 of *Bcan* and intron 10 of *Ntrk1* was used to infect neural stem cells (NSCs) isolated from *Gtv-a*;GFAP-Cre;LSL-Cas9;Trp53lox/lox pups, had brain-specific p53-loss and expression of TVA and Cas9 [20].

Intracranial injection of the infected NSCs into NOD/SCID mice resulted in tumours that showed histological features of high-grade glioma, including a high percentage of Ki67-positive cells, microvascular proliferation and pseudopalisading necrosis [20].

20. Oldrini et al., Nat Commun 2018, 9:1466.

Weyden et al., Current Opinion in Genetics & Development 2021, 66:57-62

M9L3

42

Another application of CRISPR Cas9 is in the modelling chromosomal rearrangements about which we have already discussed in the case of Ewing sarcoma. One group utilized the replication competent avian leukosis virus splice acceptor a based approach which uses RCAS vector to target individual cells engineer to express the TVA cell surface receptor.

To create a chromosomal deletion and RCAS virus carrying sgRNA targeting intron 13 of *Bcan* and intron 10 of *Ntrk1* was used to infect neural stem cells isolated from *Gtv-a*;GFAP-Cre;LSL-Cas9;Trp53 lox/lox pups, had brain specific p53 loss and expression of TVA and Cas9.

Intracranial injection of the infected NSCs into NOD/SCID mice resulting in tumours that showed histological features of high grade glioma including a high percentage of Ki67 positive cells microvascular proliferation and pseudopalisading necrosis.

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Another group used the above-mentioned KC-RIK mice to mimic a large homozygous chromosomal deletion seen on chromosome 9 in human PDAC. Two sgRNAs (sgDel-A and sgDel-B) against target sites separated by 1.2Mb on the syntenic region of mouse chromosome 4 were cloned into the 3GIC9 plasmid, which was then inserted into the Col1a1 locus of KC-RIK ES cells and transmitted through the germline [18]. The pancreas of dox-treated KC-RIK-sgDel-A/B mice showed the presence of multiple foci of PDAC, of poor to moderate differentiation.

18. Mishra et al. *Carcinogenesis* 2020, 41:334-344.

Weyden et al., *Current Opinion in Genetics & Development* 2021, 66:57-62

M9L3 43

Another group used the above mentioned KC-RIK mice to mimic a large homozygous chromosomal deletion seen on chromosome 9 in human PDAC. Two sgRNAs against target site separated by 1.2 Mb on the syntenic region of mouse chromosome 4 cloned into the 3GIC9 plasmid, which was then inserted into the Col1a1 locus of KC RIK ES cells and transmitted throughout the germline. The pancreas of dox treated KC-RIK-sgDel-A/B mice showed the presence of multiple foci of PDAC of poor to moderate differentiation.

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The CRISPR/Cas9 system has also been used to generate chromosomal inversions in vivo, with one study generating a mouse model of Eml4-Alk-driven lung cancer; using a plasmid that simultaneously expressed Cas9 and two distinct sgRNAs (Eml4 and Alk) from tandem U6 promoters.

Recombinant adenoviruses expressing Cas9 and both sgRNAs (Ad-EA) were administered to mice by intratracheal instillation and at 12-14 weeks post-infection, the lungs of Ad-EA-infected mice showed the presence of multiple adenocarcinomas [21]. Importantly, the Ad-EA-induced lung tumours were sensitive to crizotinib, which is an ALK/MET inhibitor used in the clinic to treat patients with ALK-positive non-small cell lung cancer [21].

21. Maddalo et al., *Nature* 2014, 516:423-427.

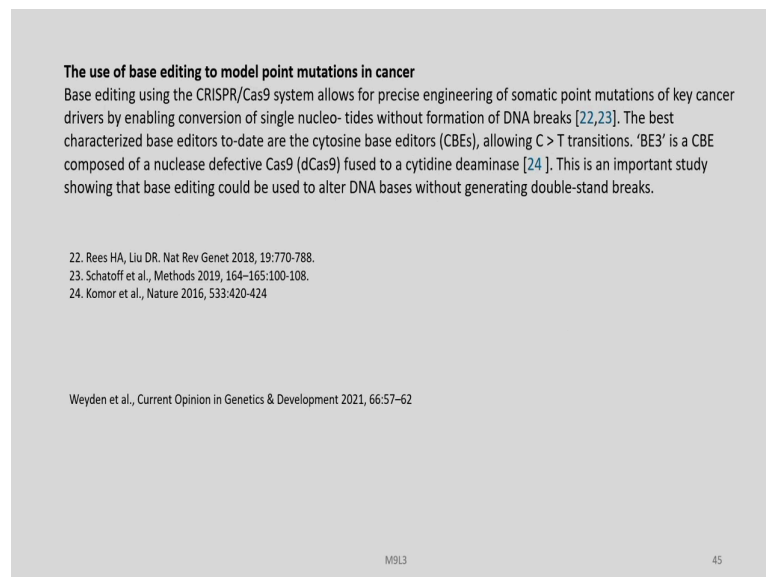
Weyden et al., *Current Opinion in Genetics & Development* 2021, 66:57-62

M9L3 44

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**The use of base editing to model point mutations in cancer**

Base editing using the CRISPR/Cas9 system allows for precise engineering of somatic point mutations of key cancer drivers by enabling conversion of single nucleotides without formation of DNA breaks [22,23]. The best characterized base editors to-date are the cytosine base editors (CBEs), allowing C > T transitions. 'BE3' is a CBE composed of a nuclease defective Cas9 (dCas9) fused to a cytidine deaminase [24]. This is an important study showing that base editing could be used to alter DNA bases without generating double-strand breaks.

22. Rees HA, Liu DR. Nat Rev Genet 2018, 19:770-788.  
23. Schatoff et al., Methods 2019, 164-165:100-108.  
24. Komor et al., Nature 2016, 533:420-424

Weyden et al., Current Opinion in Genetics & Development 2021, 66:57-62

M9L3 45

The use of base editing to model point mutation in cancer has also been actively pursued using CRISPR Cas9 system which allows for precise engineering of somatic point mutations of key cancer drivers by enabling conversion of single nucleotides without formation of DNA breaks.

The best characterized base editors to date are the cytosine base editors, allowing C to T transitions. BE3 is a CBE composed of a nuclease defective Cas9 fused to a cytidine deaminase. This is an important study showing that base editing could be used to alter DNA bases without generating double strand breaks.



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**The use of base editing to model point mutations in cancer**

A conditional BE3 allele was introduced into the Col1a1 locus of ES cells from WapCre;Brca1flox/flox;Trp53flox/flox (WB1P) mice and transmitted through the germline to produce WB1P-BE3 mice [25]. Intraductal injection of these mice with a lentivirus carrying either a non-targeting (NT) sgRNA or an sgRNA targeting the third exon of Akt1 (in order to generate an oncogenic Akt1E17K missense mutation by base editing), as well as a Myc-overexpression cassette (Lenti-sgAkt1E17K-Myc), resulted in the development of mammary tumours; however, the Lenti-sgAkt1E17K-Myc mice developed tumours with much shorter latency than the Lenti-sgNT-Myc mice [25].

25. Annunziato et al. EMBO J 2020, 39:e102169.

Weyden et al., Current Opinion in Genetics & Development 2021, 66:57-62

M913 46

A conditional BE3 allele was introduced into the Col1a1 locus of ES cells from WapCre; Brca1flox/flox;Trp53flox/flox WB1P mice and transmitted through the germline to produce WB1P-BE3 mice. Intraductal injection of these mice with a lentiviral virus carrying either a non-targeting sgRNA or an sgRNA targeting the third exon of Akt1 in order to generate an oncogenic AktE17K missense mutation by base editing as well as by Myc-overexpression cassette resulted in the development of mammary tumours.

However, the Lenti-sgAkt1E17K-Myc mice developed tumours with much shorter latency than the Lenti-sgNT-Myc mice. So, these are some of the achievements of CRISPR Cas9 in the field of base editing to model point mutations in cancer.

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This same study also generated an allelic series of missense mutations of *Pik3ca* in vivo, and showed that Lenti-sgPik3caE542K-Myc, Lenti-sgPik3caE545K-Myc and Lenti-sgPik3caE453K-Myc-dosed WB1P-BE3 females developed mammary tumours with a significantly shorter latency than Lenti-sgNT-Myc-dosed females, underscoring the fact that whilst the E453 K mutation is not found as commonly as the other 2 mutations in human tumours, it has similar cooperative effects in this setting [25]. The feasibility of multiplexed base editing was also demonstrated when WB1P-BE3 mice carrying a *Trp53F/+* allele were injected with a tandem Lenti-sgPik3caE545K/s<sub>g</sub>Trp53Q97\*-Myc vector that harbours two arrayed sgRNA cassettes, to simultaneously introduce the *Pik3caE545K* missense mutation and inactivate the residual wildtype copy of *Trp53*; the mice developed mammary tumours significantly faster than Lenti-sgTrp53Q97\*-Myc-dosed mice [25].

25. Annunziato et al. EMBO J 2020, 39:e102169.

Weyden et al., Current Opinion in Genetics & Development 2021, 66:57-62

M913 47

The same study also generated an allelic series of missense mutations in *Pik3ca* in vivo and showed that Lenti-sgPik3caE542K-Myc, then Lenti-sgPik3caE545K-Myc and Lenti-sgPik3caE453K-Myc dosed WB1P-BE3 females developed mammary tumours with a significantly shorter latency than Lenti-sgNT-Myc dosed females underscoring the fact that whilst the E453 K mutation is not found as commonly as the other two mutations in human tumours, it has similar cooperative effects in this setting. The feasibility of multiplexed base editing was also demonstrated when WB1P-BE3 mice carrying *Trp53F/+* allele were injected with a tandem Lenti-sgPik3caE545K/s<sub>g</sub>Trp53Q97\*-Myc vector that harbours two arrayed sgRNA, to simultaneously introduce the *Pik3caE545K* missense mutations and inactivate the residual wild type copy of *Trp53*.

The mice develop mammary tumours significantly faster than Lenti-sgTrp53Q97\*-Myc dosed mice. The majority of these studies discussed here has been taken from this review by Weyden et al in Current Opinion in Genetics and Development and the original references are also quoted in exact numbers in the text as references. So, for further details kindly consult the original articles from which these case studies have been collected and compiled by Weyden et al.

Overall to conclude the various gene editing technologies like ZFN Talen and CRISPR Cas9 has been successfully used in creating models for cancer in cells as well as in animals. And this makes the study of cancer the discovery of cancer related genes as well as the

development of anti cancer drugs quite easy than in earlier times. So, genome editing technologies for cancer disease modelling has a very big impact in the development of various cancer based therapeutics. With this we come to the conclusion of module 9.

Thank you for your patient hearing.