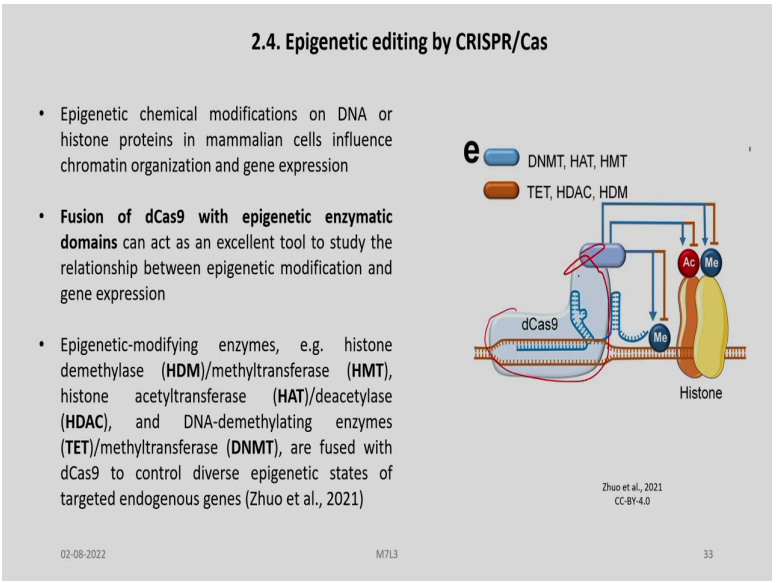
Genome Editing and Engineering
Prof. Utpal Bora
Department of Bioscience and Bioengineering
Indian Institute of Technology, Guwahati

Module - 07
Clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 technology
Lecture - 03

Applications of CRISPR/Cas9 Part - B

Welcome to my course on Genome Editing and Engineering. We are discussing module 7 on CRISPR Cas9 technology and in lecture number 3 we were discussing the Applications of CRISPR Cas9 to various fields. We are continuing our discussion in this Part B of lecture 2. One of the areas where CRISPR Cas9 is used, particularly the dCas9, is the epigenetic editing by CRISPR Cas. We must be little bit careful over here because in general CRISPR Cas9 wild type is used for genome editing while, the dead Cas9 is used for epigenetic editing.

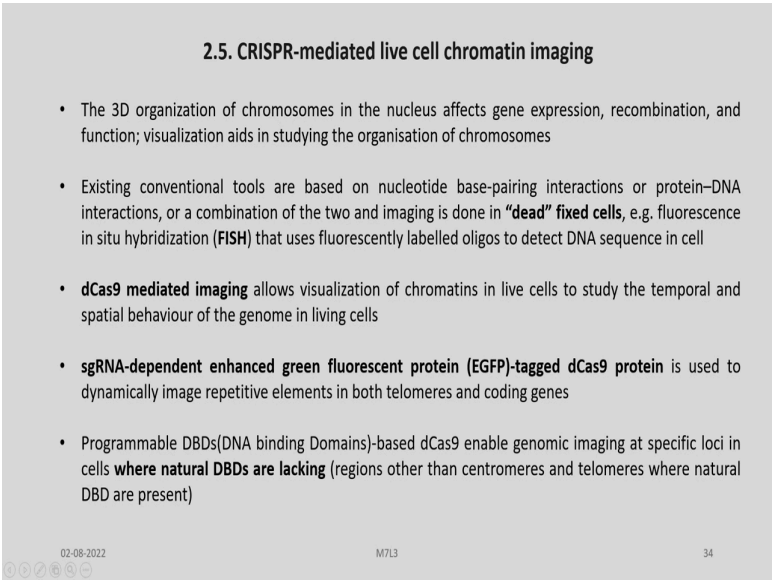
(Refer Slide Time: 01:30)



Epigenetic chemical modification on DNA or histone proteins in mammalian cells influence chromatin organization and gene expression. Fusion of dCas9 with epigenetic enzymatic domains can act as an excellent tool to study the relationship between epigenetic modifications and gene expressions.

Epigenetic-modifying enzymes example, histone demethylase (HDM) or methyltransferases (HMT), histone acetyltransferases (HAT), deacetylase (HDAC) and DNA-demethylating enzymes (TET), methyltransferases (DNMT), are fused with dCas9 to control diverse epigenetic states of targeted endogenous genes. So, you can see here the various epigenetic modifying enzyme modules, and these are fused with the dCas9.

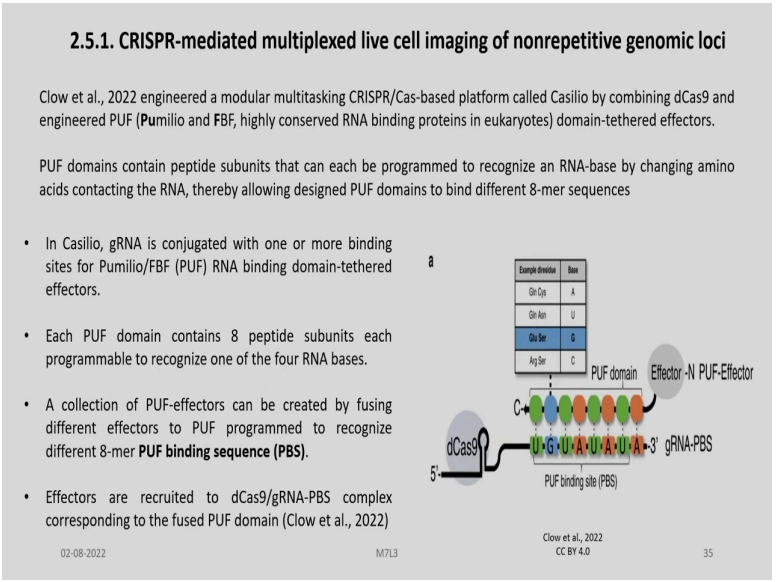
(Refer Slide Time: 02:37)



The 3D organization of chromosomes in the nucleus affect gene expression, recombination, and function; visualization aids in studying the organization of the chromosomes. Existing conventional tools are based on nucleotide base-pairing interactions or protein-DNA interactions, or a combination of the two and imaging is done in "dead" fixed cells, example fluorescence in situ hybridization (FISH) that uses fluorescently labelled oligos to detect DNA sequences in cells.

We can use CRISPR for live cell chromatin imaging by this modification of the CRISPR Cas system. So, in this case the dCas9 mediated imaging allows visualization of chromatins in live cells to study the temporal and spatial behavior of the genome in living cells. Single guide RNA dependent enhanced green fluorescent protein or (EGFP)-tagged dCas9 protein is used to dynamically image repetitive elements in both telomeres and coding genes. Programmable DBDs (DNA binding domains)-based dCas9 enable genomic imaging at specific loci in cells where natural DBDs are lacking.

(Refer Slide Time: 04:05)

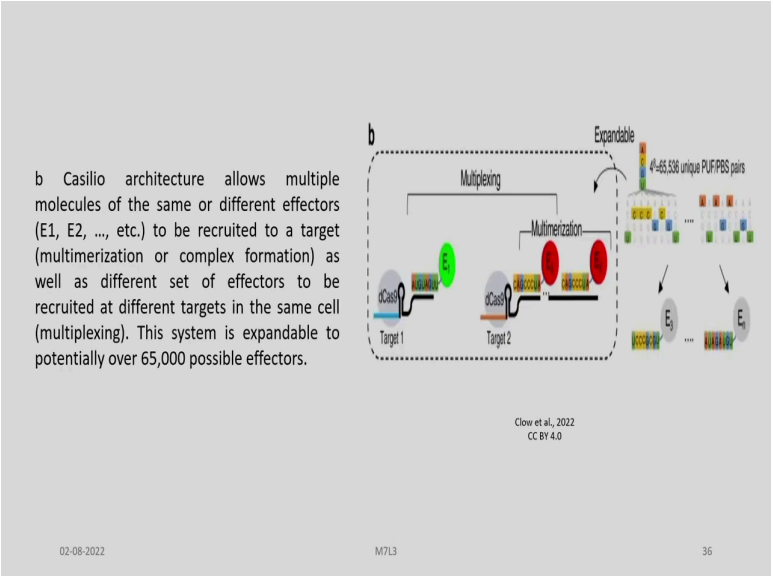


We can also use CRISPR for multiplexed live cell imaging of non repetitive genomic loci. Clow et al recently engineered a modular multitasking CRISPR Cas based platform called Casilio by combining dCas9 and engineered PUF (**Pu**milio and **F**BF, highly conserved RNA binding proteins in eukaryotes) domain-tethered effectors.

PUF domains containe peptide subunits that can be programmed to recognize an RNA base by changing amino acids contacting the RNA, thereby allowing designed PUF domains to bind different octamer sequences. In Casilio, guide RNA is conjugated with one or more binding sites for Pumilio FBF RNA binding domain tethered effectors.

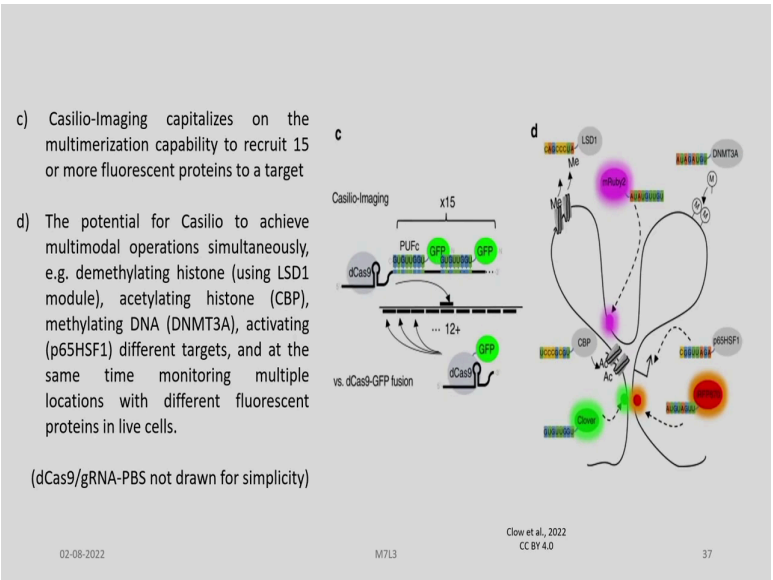
Each PUF domain contains 8 peptide subunits, each programmable to recognize one of the four RNA basis. A collection of PUF-effectors can be created by fusing different effectors to PUF programmed to recognize different octamer PUF binding sequences (PBS). Effectors are recruited to dCas9 gRNA-PBS complex corresponding to the fused PUF domain.

(Refer Slide Time: 05:35)



The Casilio architecture allows multiple molecules of the same or different effectors to be recruited to a target as well as different set of effectors, but to be recruited at different targets in the same cell. This system is expandable to potentially over 65,000 possible effectors.

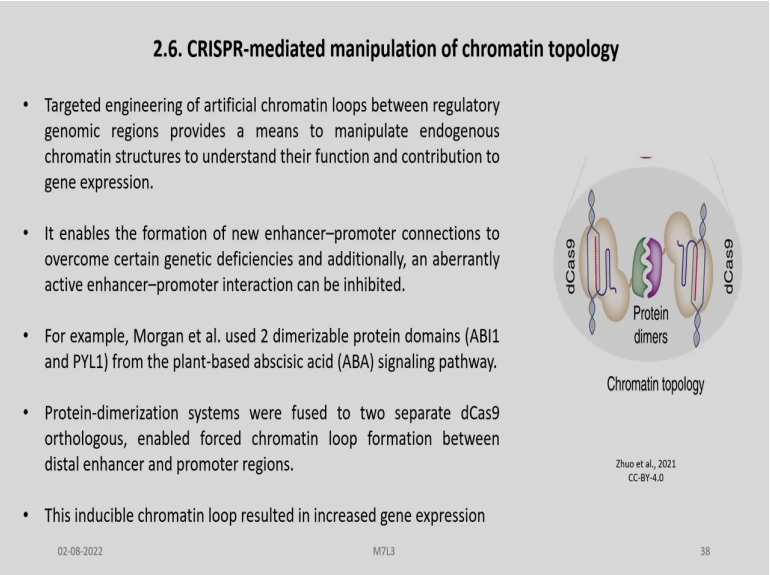
(Refer Slide Time: 06:02)



The Casilio-imaging capitalizes on the multimerization capability to recruit 15 or more fluorescent proteins to a target. The potential for Casilio to achieve multi modal operations simultaneously, example demethylating histone using LSD1 module, acetylating histone and

methylating DNA activating different targets, and at the same time monitoring multiple locations with different fluorescent proteins in the live cells.

(Refer Slide Time: 06:40)

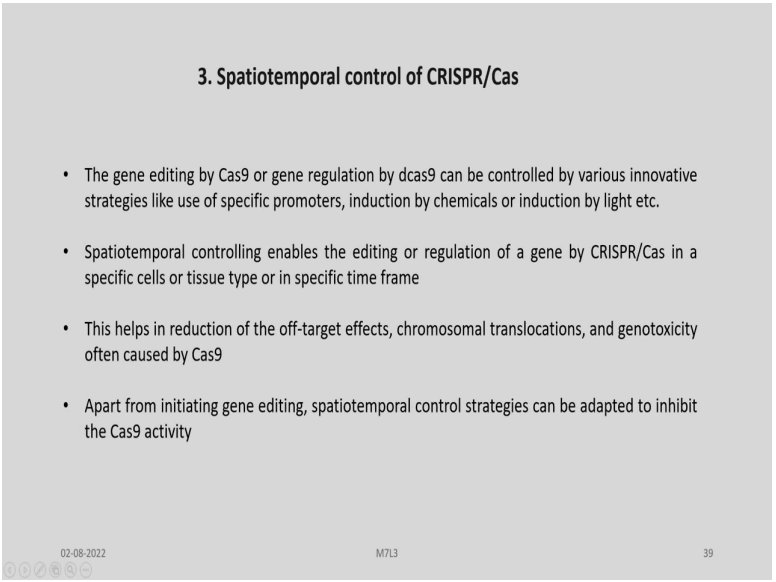


Another application is the CRISPR mediated manipulation of chromatin topology. So, you can see here a dCas9 bound to a protein and another dCas9 bound to another protein and these two proteins can dimerize. Let us see how this is being used for the manipulation of chromatin topology. Targeted engineering of artificial chromatin loops between regulatory genomic regions provides a means to manipulate endogenous chromatin structures to understand their function and contributions to gene expression.

This enables the formation of new enhancer promoter connections to overcome certain genetic deficiencies and additionally, an aberrantly active enhancer promoter interaction can be inhibited. Morgan et al used 2 dimerizable protein domains, ABI1 and PYL1, from the plant based abscisic acid signaling pathway.

Protein-dimerization systems were fused to two separate dCas9 orthologous, enabled forced chromatin loop formation between distal enhancer and promoter region. This inducible chromatin loop resulted in increased gene expression, as we can see that the promoter region and the enhancer regions are brought into close proximity due to this dimerization reaction.

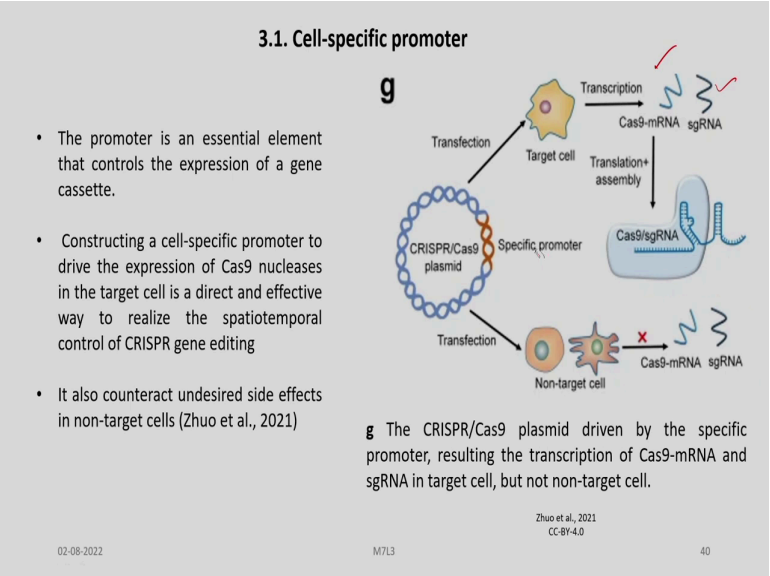
(Refer Slide Time: 08:22)



Another application is the spatiotemporal control of CRISPR Cas9. The gene editing by Cas9 or gene regulation by dCas9 can be controlled by various innovative strategies like the use of specific promoters, induction by chemicals or induction by light etc. Spatiotemporal controlling enables the editing or regulation of a gene by CRISPR Cas in specific cells or tissue type or in a specific time frame.

This helps in reduction of the off-target effects, chromosomal translocations and genotoxicity which often is caused by the Cas9. Apart from initiating gene editing, spatiotemporal control strategies can be adapted to inhibit the Cas9 activity.

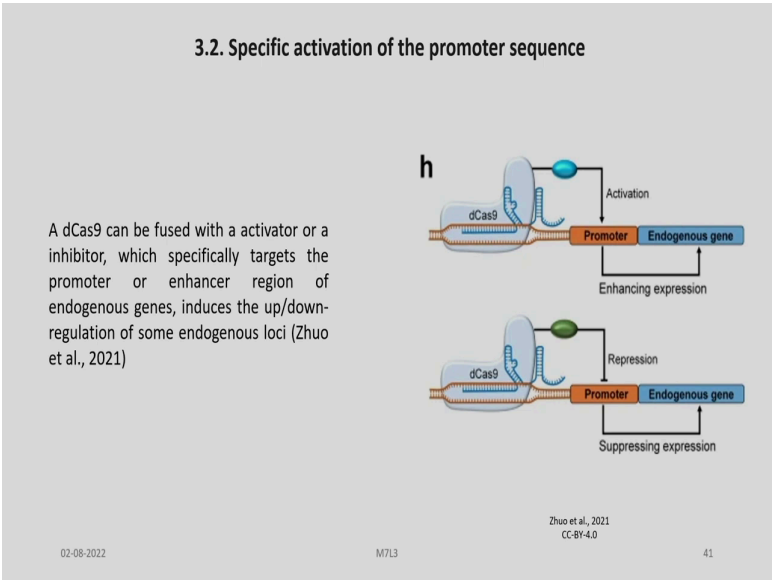
(Refer Slide Time: 09:15)



Let us see the cell specific promoter application. We know the promoter is an essential element that controls the expression of a gene cassette. Constructing a cell-specific promoter to drive the expression of Cas9 nucleases in the target cell is a direct and effective way to realize the spatiotemporal control of CRISPR gene editing.

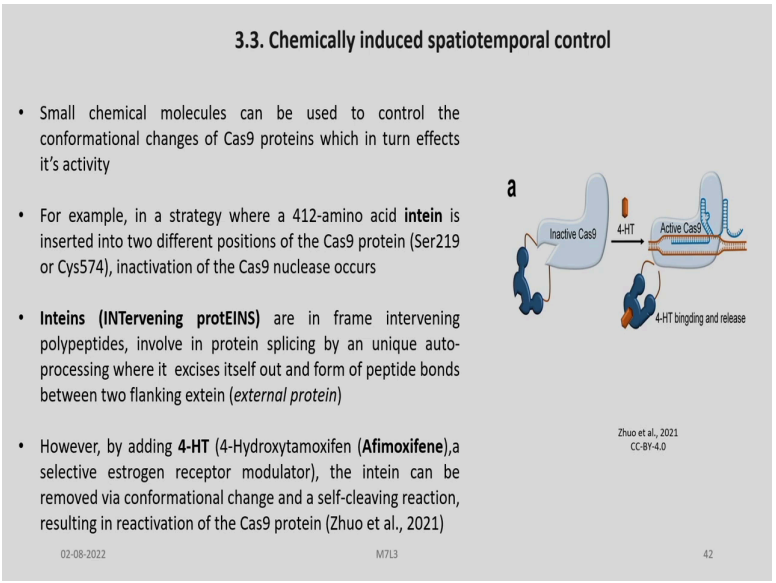
It also counteracts undesired side effects in non-target cells because, we have made the expression specific to a particular cell. Here in (g) you can see the CRISPR Cas9 plasmid driven by the specific promoter resulting the transcription of Cas9 mRNA and sgRNA in the target cell, but this is not happening in the non-target cell.

(Refer Slide Time: 10:19)



A dCas9 can be fused with an activator or an inhibitor, which specifically targets the promoter or enhancer region of endogenous genes, which induces the up and down regulation of some endogenous loci. So, we have an enhancer or activator here which binds to the promoter and there is activation and here we have a repressure which represses, or which suppresses the expression. So, this is a method by which we can simply switch off and switch on a gene easily.

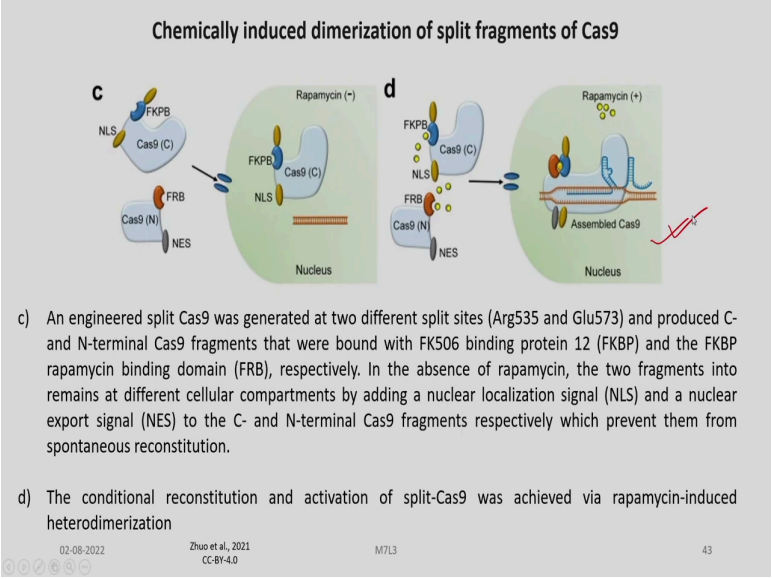
(Refer Slide Time: 11:04)



Small chemical molecules can be used can be used to control the conformational changes of Cas9 proteins which in turn effects its activity. For example, in a strategy where a 412- amino acid intein is inserted into two different positions of the Cas9 protein (Ser219 and Cys574), inactivation of the Cas9 nucleus occurs.

Inteins or intervening proteins are in frame intervening polypeptides, involved in protein splicing by a unique auto-processing where it excises itself out and forms a peptide bonds between two flanking extein. However, by adding 4-HT, a selective estrogen receptor modulator, the intein can be removed via conformational change and the self cleaving reaction, results in reactivation of the Cas9 protein.

(Refer Slide Time: 12:07)



We can also go for chemically induced dimerization of split fragments of Cas9. So, in figure C you can see an engineered split Cas9 which was generated at two different split sites and produced C and N terminal Cas9 fragments that were bound with FK504 binding proteins 12 and the FKBP rapamycin binding domain or FRB.

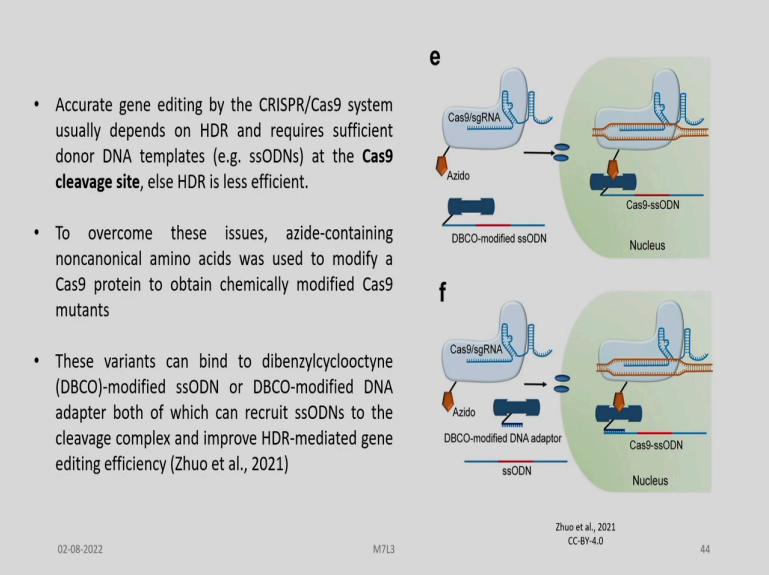
In the absence of rapamycin, the two fragments remain at different cellular compartments by adding a nuclear localization signal and a nuclear export signal, NES and NLS as you can see here. So, chemically induced dimerization of split fragments of Cas9, in figure C you can see there is a Cas9 C terminus fragment and a Cas9 N terminus fragment and they are having various components like nuclear export signal, a nuclear localization signal then, FB506

binding protein 12 for FKBP in Cas9 C terminal and FKB rapamycin binding domain in the N terminal.

So, there is a condition where you have rapamycin absent and another condition where you have rapamycin present. So, these split Cas9 was generated at two different split sites and in the absence of rapamycin the two fragments remain at different cellular compartments and by adding a nuclear localization signal and a nuclear export signal to the C and N terminal fragments respectively, which prevent them from spontaneous reconstitution.

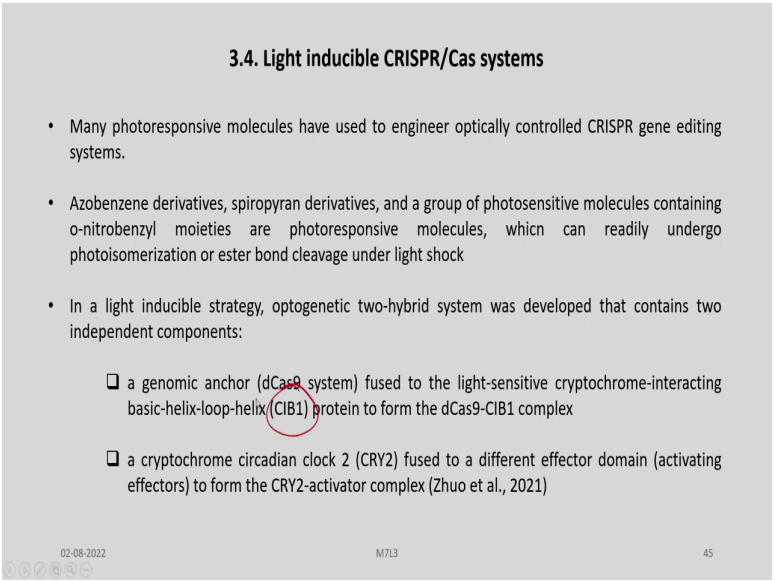
In figure D, you can see the conditional reconstitution and activation of split Cas9 which was achieved via rapamycin induced heterodimerization. Here both these N and C terminal got dimerized and this become a assemble Cas9.

(Refer Slide Time: 14:47)



Accurate gene editing by the CRISPR Cas9 system usually depends on HDR and requires sufficient donor templates at the Cas9 and cleavage site, otherwise the reaction is inefficient or less efficient. To overcome these issues, azide-containing non canonical amino acids was used to modify a Cas9 protein to obtain chemically modified Cas9 mutants. These variants can bind to dibenzylcyclooctyne or DBCO modified ssODN or DBCO modified DNA adapter, both of which can recruit ssODNs to the cleavage complex and improve HDR mediated gene editing efficiency.

(Refer Slide Time: 15:35)

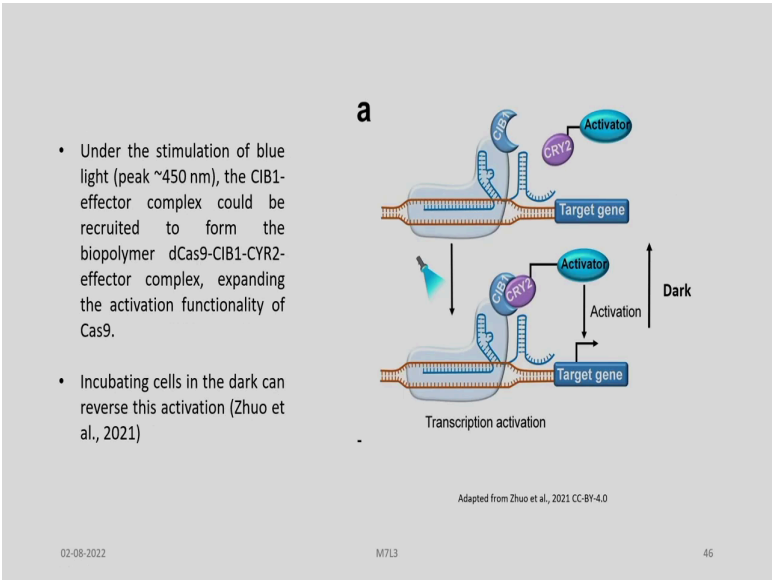


One very interesting application of a CRISPR Cas system is development of light inducible CRISPR Cas systems.

Many photosresponsive molecules have been used to engineer optically controlled CRISPR gene editing systems. Azobenzene derivatives, spiropyran derivatives, and a group of photosensitive molecules containing o-nitrobenzyl moieties are photosensitive, which can readily undergo photoisomerization or ester bone cleavage under light shock.

In a light inducible strategy, optogenetic two-hybrid system was developed that contains two independent components. A genomic anchor (dCas9 system) fused to the light sensitive cryptochrome-interacting basic helix loop helix (CIB1) protein to form the dCas9-CIB1 complex. A cryptochrome circadian clock 2 (CRY2) fused to a different effector domain to form the CRY2 activator complex was carried out by Zhuo et al in 2021.

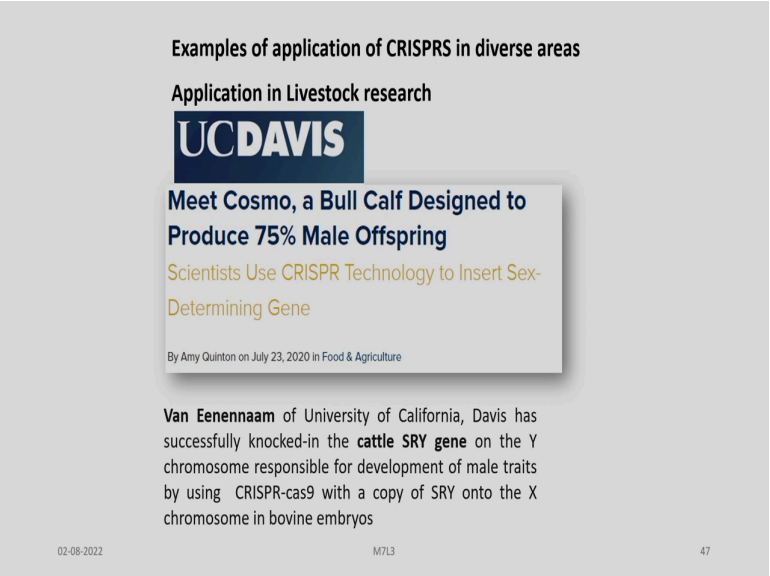
(Refer Slide Time: 16:50)



So, you can see here this CIB1 and you can see here the CRY2 and you have a target gene over here. Let us discuss what is happening in this particular reaction. And you can see here also some source of light and then, this reaction is actually reversible in the absence of light in dark the opposite reaction will take place.

So, under the stimulation of blue light at around 400 nanometers, the CIB1 effector complex could be recruited to form the biopolymer dCas9-CIB1-CRY2 effector complex, expanding the activation functionality of the Cas9. And incubating the cells in the dark can reverse this activation as already discussed earlier.

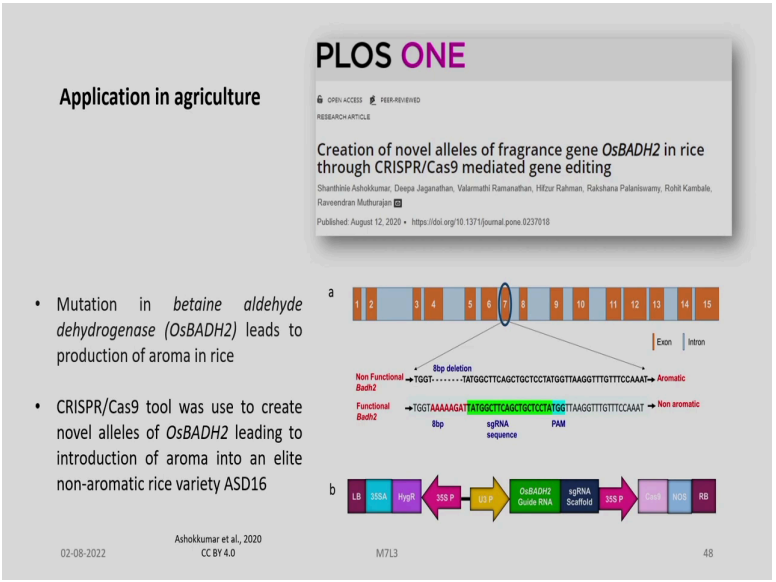
(Refer Slide Time: 17:54)



There are many other applications of CRISPRS and CRISPR Cas9 or CRISPR Cas systems or modified Cas-CRISPR systems. We're not going to discuss each and every one of them in detail. Some of them are alarmingly very dangerous although they may have some kind of economic benefit. For example, there is a phenomenon called genetic sexing or molecular sexing where a particular population can be engineered to have a dominating type of a sex or gender.

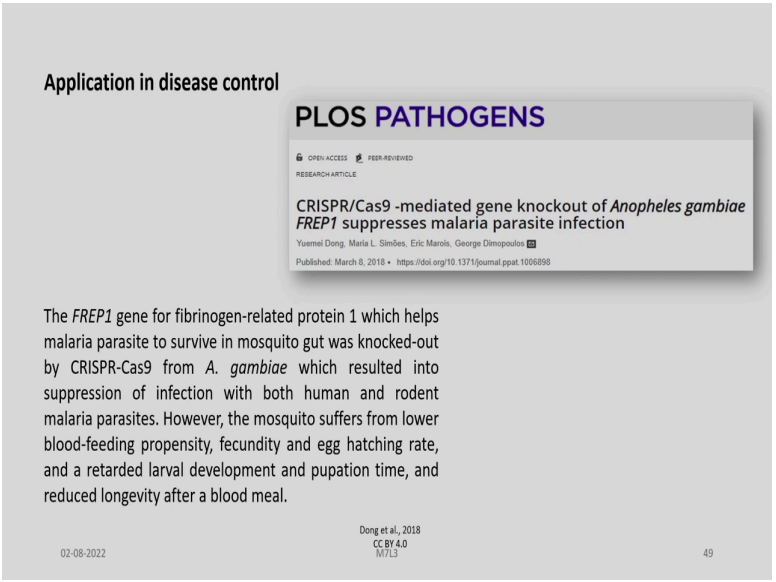
For example, here a Cosmo, a bull calf, has been designed to produce 75 percent male offspring's, similar experiments has also been carried out in silkworms where the engineering is done in such a way that the male silkworm production is more. So, these are all matters of ethics which we will be dealing it with in the towards the end of this course.

(Refer Slide Time: 19:05)



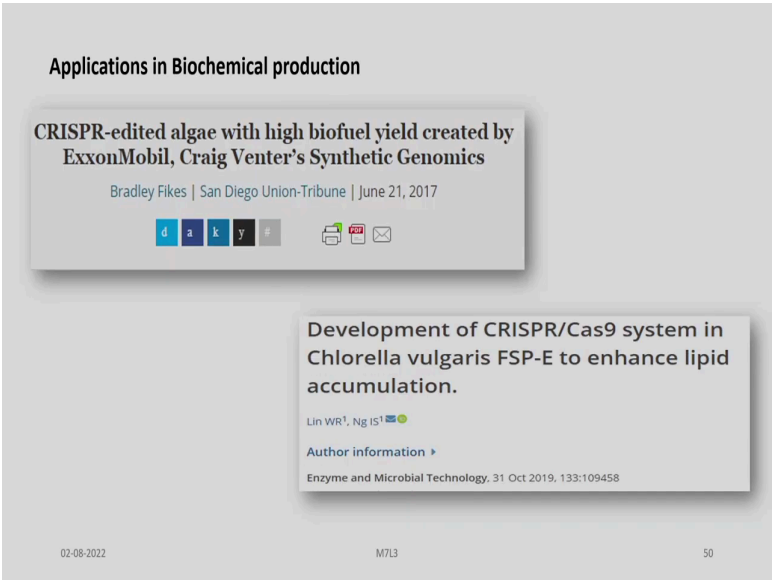
Then there are various other applications in agriculture where you have the creation of novel alleles of fragrance genes in rice through CRISPR Cas9 mediated gene editing.

(Refer Slide Time: 19:23)



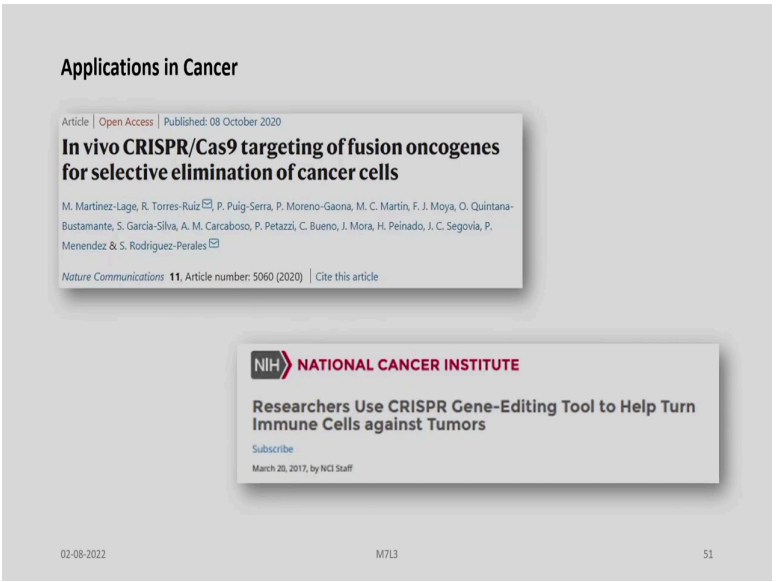
Then various other applications in disease control, particularly say knocked out of *Anopheles gambiae* which suppresses the malaria parasite infection.

(Refer Slide Time: 19:33)



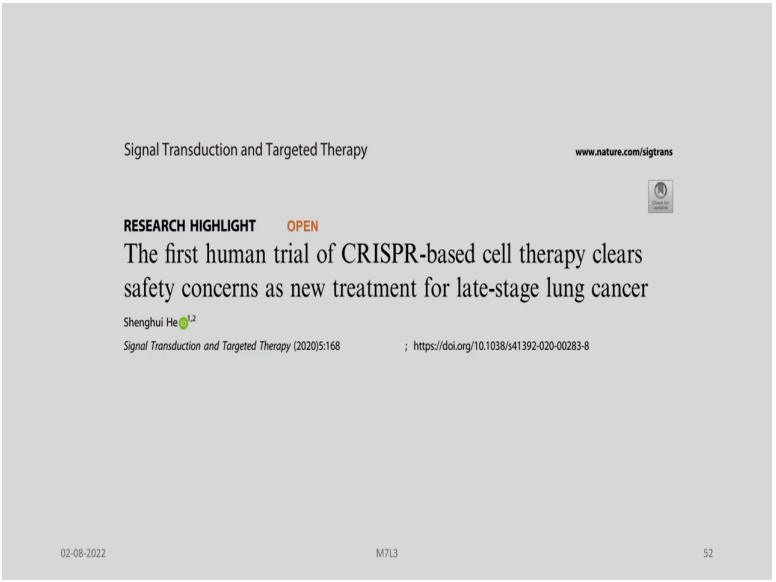
And then, CRISPR-edited algae with high biofuel yield has been created by ExxoneMobil and development of CRISPR Cas9 system in *Chlorella vulgaris* to enhance lipid accumulation. So, many of these are being looked at as solutions to energy crisis in future.

(Refer Slide Time: 19:55)



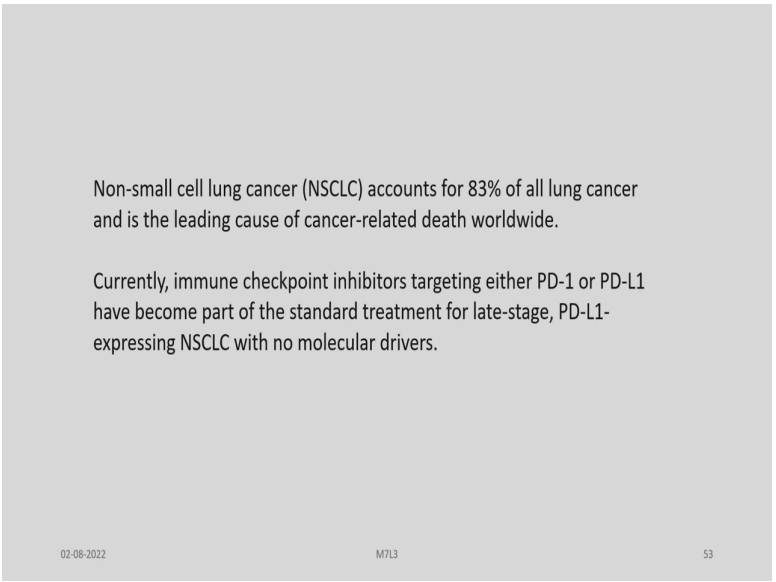
As well as food crisis and there are a lot of applications in cancer research.

(Refer Slide Time: 20:07)



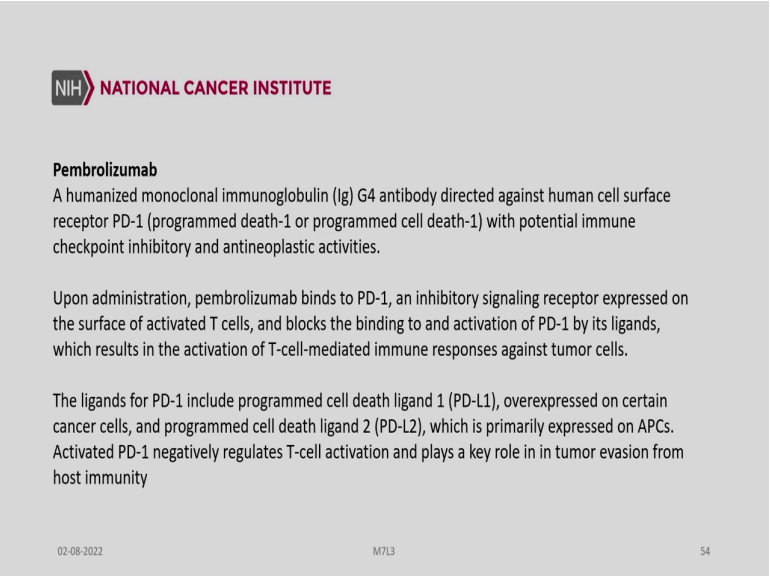
And recently the first human trial of CRISPR-based cell therapy was cleared and particularly for the late-stage lung cancer and this is being reported here in this signal transaction and targeted therapy by He.

(Refer Slide Time: 20:27)



We know that non-small cell lung cancer accounts for 83 percent of all lung cancer and is the leading cause of cancer related deaths worldwide. Currently, immune checkpoint inhibitors targeting either PD-1 or PD-L1 have become part of the standard treatment for late stage, PD-L1 expressing NSCLC with no molecular drivers.

(Refer Slide Time: 20:56)

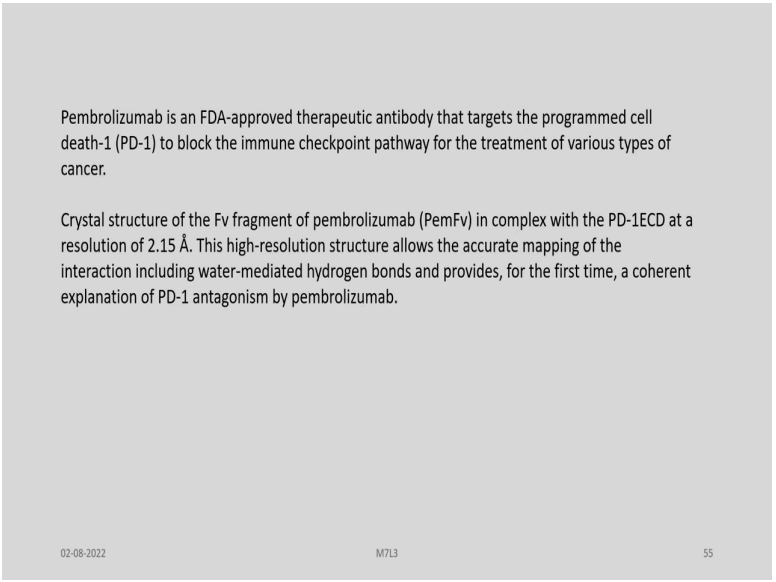


And this is a drug Pembrolizumab which has been defined by national cancer institute. As a humanized monoclonal immunoglobulin G4 antibody which is directed against human cell surface receptor PD-1 with potential immune checkpoint inhibition and anti-antineoplastic activities.

These particular drug binds to binds to PD-1, an inhibitory signaling receptor expressed on the surface of activated T cells and blocks the binding to and activation of PD-1 by its ligands, which results in the activation of T-cell-mediated immune response against tumor cells.

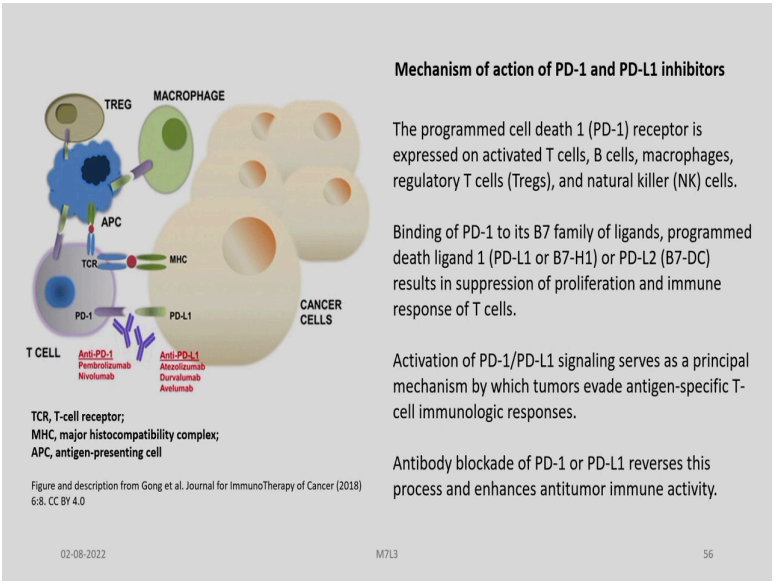
The ligands for PD-1 include programmed cell that ligand (PD-L1), over expressed on certain cancer cells and program cell that ligand 2 (PD-L2), which is primarily expressed on APCs. Activated PD-1 negatively regulates T cell activation and plays a key role in tumor evasion from host immunity.

(Refer Slide Time: 22:09)



This molecular antibody is FDA approved therapeutic antibody and it is used in various cancers. And the crystal structure of the Fv fragment of this particular mono coronal antibody, pembrolizumab (PemFv) in complex with the PD-1ECD has been resolved and this shows are some of the interactions at the molecular level.

(Refer Slide Time: 22:49)

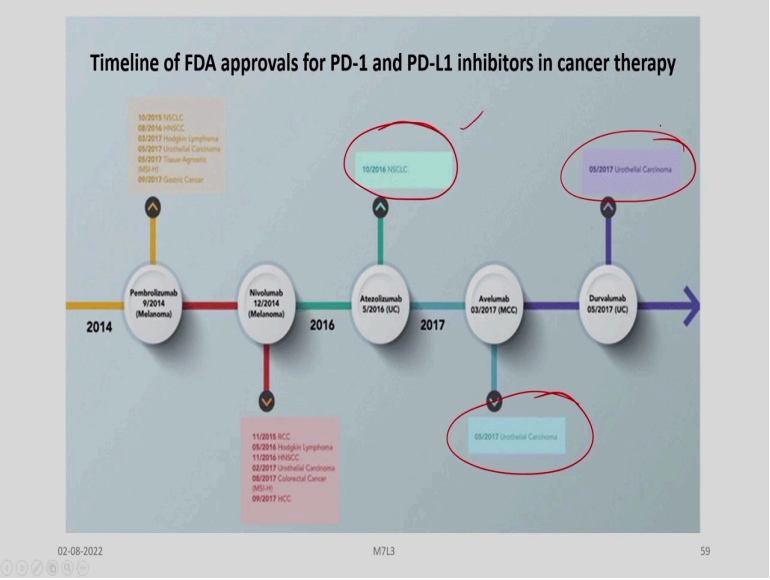


So, we have already discussed about the mechanism of action of PD-1 and PD-L1 activators. So, you can see here these PD-1s and these are the PD antibodies and then, TCR is the T cell receptor, MHC is the major histocompatibility complex and APC is the antigen presenting

cell and you have the macrophage here and we have the cancer cells. So, the program cell that one or PD-1 receptor is expressed on the activated T cell, then also in the B cells, macrophages, regulatory T cell or tracks and natural killer cells.

Binding of PD-1 to its B7 family of ligands, programmed death ligand 1 (PD-L1 or B7-H1) or PD-L2 (B7-DC) results in suppression of proliferation and immune responses of the T cells. The activation of PD-1 oblique PD-L1 signaling serves as a principal mechanism by which tumors evade antigen specific T cell immunological responses. Antibody blockade of PD-1 or PD-L1 reverses this process and enhances anti tumor immune activity.

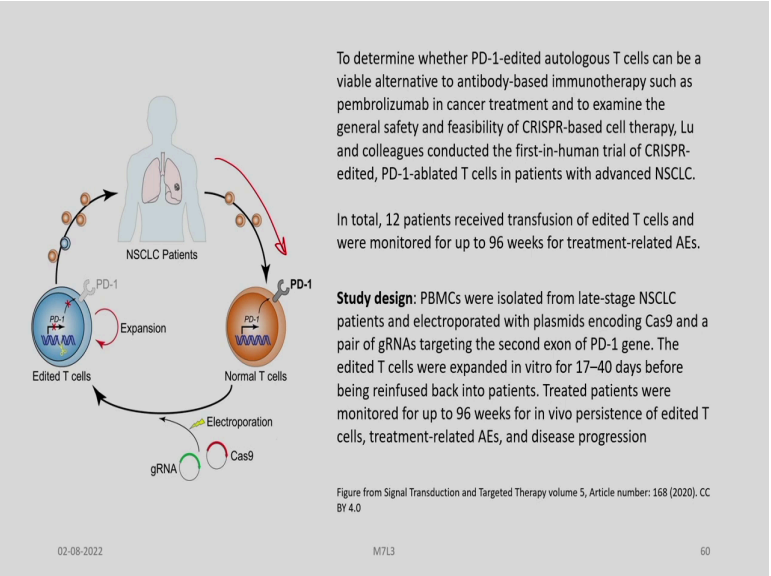
(Refer Slide Time: 24:24)



So, here is the timeline of FDA approvals of PD-1 and PD-L1 inhibitors in cancer therapy starting from 2014 through 2016 to 2017. Many different kinds of monoclonal antibodies have been developed over the years and these are used for different kinds of cancers as you can see in this list.

And some of them are used against multiple cancers while some of them are very specific. So, there are so many kinds of monoclonal antibodies being developed against cancer therapy. However, monoclonal antibodies have been found out to be very expensive and one of the alternative approach that is being thought of is the gene therapy of cancer.

(Refer Slide Time: 25:30)

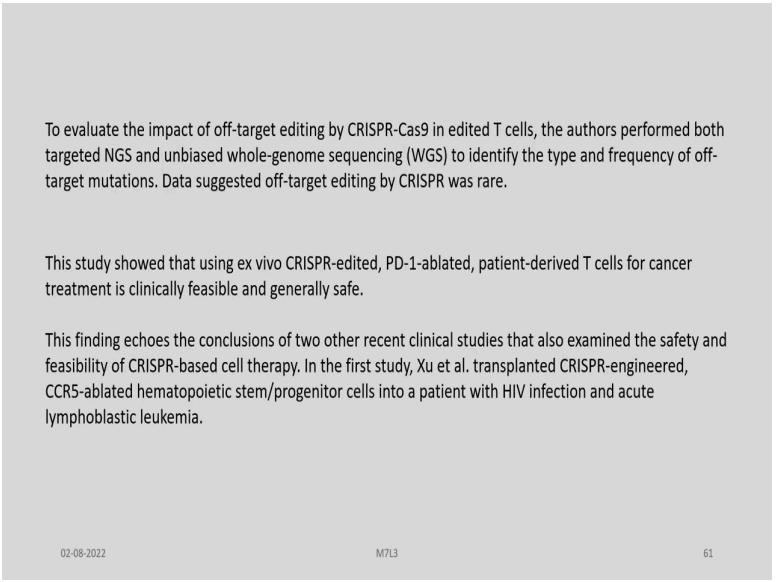


A group led by Lou wanted to determine whether the PD-1 edited autologous T cells can be viable alternative to antibody-based immunotherapy as we have discussed in the earlier slide. They took 12 patients and administered transfusion of edited T cells who were monitored for up to 96 weeks for treatment-related AEs and this is the study design.

Now, PBMCs were isolated from patients with late-stage NACLC and these were electroporated with plasmids containing Cas9 and a pair of gRNAs targeting the second exon of PD-1 gene. The edited T cells were expanded in vitro for 17-40 days before being reinfused back into the patients.

And were monitored up to 96 weeks for in vivo persistence of additive T cells and this is progression.

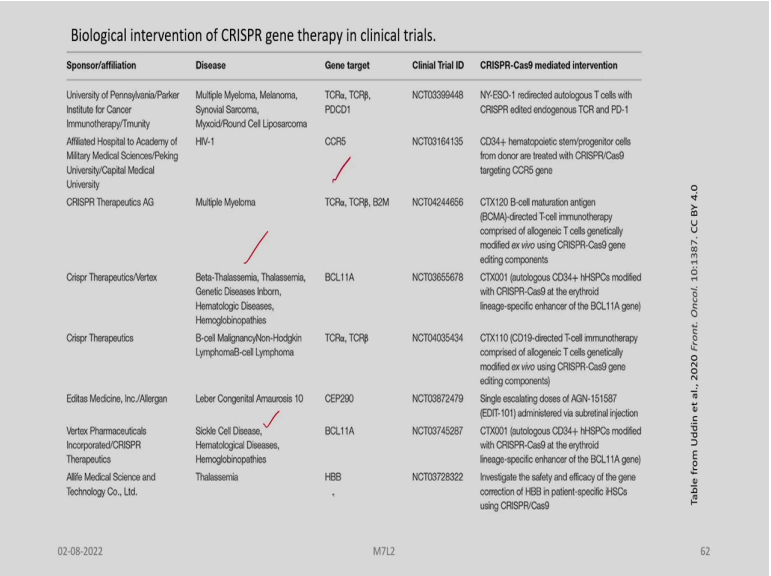
(Refer Slide Time: 26:48)



To evaluate the impact of off-target editing by CRISPR Cas9 in edited T cells, Lou and colleagues performed both targeted NGS and unbiased whole genome sequencing to identify the type and frequency of off target mutations. Data suggested off-target editing by CRISPR was rare or very low.

This study by Lou and colleagues showed that using ex vivo CRISPR-edited, PD-1 ablated, patient derived T cells for cancer treatment is clinically feasible and generally safe. This finding echoes the conclusions of two other recent clinical studies that also examine the safety and feasibility of CRISPR Cas based cell therapy. In the first study, Xu et al transplanted CRISPR engineered CCR5 ablative hematopoietic stem progenitor cells into a patient with HIV infection and acute lymphoblastic leukemia.

(Refer Slide Time: 27:55)



Biological intervention of CRISPR gene therapy in clinical trials.

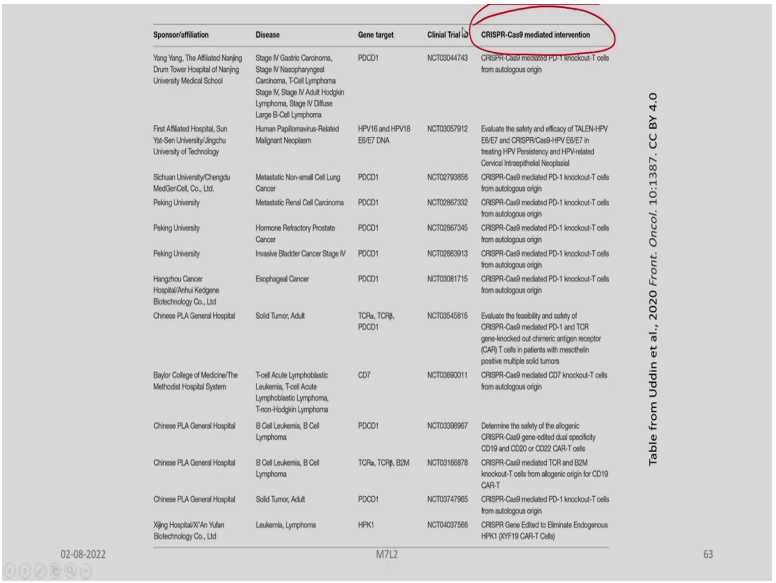
Sponsor/affiliation	Disease	Gene target	Clinial Trial ID	CRISPR-Cas9 mediated intervention
University of Pennsylvania/Parker Institute for Cancer Immunotherapy/Tmunity	Multiple Myeloma, Melanoma, Synovial Sarcoma, Myxoid/Round Cell Liposarcoma	TCRα, TCRβ, PDCD1	NCT03399448	NY-ESO-1 redirected autologous T cells with CRISPR edited endogenous TCR and PD-1
Affiliated Hospital to Academy of Military Medical Sciences/Peking University/Capital Medical University	HIV-1	CCR5	NCT03164135	CD34+ hematopoietic stem/progenitor cells from donor are treated with CRISPR/Cas9 targeting CCR5 gene
CRISPR Therapeutics AG	Multiple Myeloma	TCRα, TCRβ, B2M	NCT04244656	CTX120 B-cell maturation antigen (BCMA)-directed T-cell immunotherapy comprised of allogeneic T cells genetically modified *ex vivo* using CRISPR-Cas9 gene editing components
Crispr Therapeutics/Vertex	Beta-Thalassemia, Thalassemia, Genetic Diseases Inborn, Hematologic Diseases, Hemoglobinopathies	BCL11A	NCT03655678	CTX001 (autologous CD34+ hHSPCs modified with CRISPR-Cas9 at the erythroid lineage-specific enhancer of the BCL11A gene)
Crispr Therapeutics	B-cell MalignancyNon-Hodgkin LymphomaB-cell Lymphoma	TCRα, TCRβ	NCT04035434	CTX110 (CD19-directed T-cell immunotherapy comprised of allogeneic T cells genetically modified *ex vivo* using CRISPR-Cas9 gene editing components)
Editas Medicine, Inc./Allergan	Leber Congenital Amaurosis 10	CEP290	NCT03872479	Single escalating doses of AGN-151587 (EDIT-101) administered via subretinal injection
Vertex Pharmaceuticals Incorporated/CRISPR Therapeutics	Sickle Cell Disease, Hematological Diseases, Hemoglobinopathies	BCL11A	NCT03745287	CTX001 (autologous CD34+ hHSPCs modified with CRISPR-Cas9 at the erythroid lineage-specific enhancer of the BCL11A gene)
Allife Medical Science and Technology Co., Ltd.	Thalassemia	HBB	NCT03728322	Investigate the safety and efficacy of the gene correction of HBB in patient-specific HSCs using CRISPR/Cas9

Table from Uddin et al., 2020 *Front. Oncol.* 10:1387. CC BY 4.0

There are now many CRISPR based gene therapy in various stages of clinical trials and the list is quite huge and you can refer to Front (Refer Slide Time: 28:13) Oncology by and this article by Uddin et al. So, you can see the different type of diseases for example, multiple myeloma, melanoma, beta thalassemia and sickle cell disease have been attempted to be intervened with CRISPR Cas9 technology.

And here are the various clinical trial ids and each disease has some of the popular gene targets like in the case of multiple myeloma, TCR alpha and then, in thalassemia you have HBB and so on.

(Refer Slide Time: 29:00)

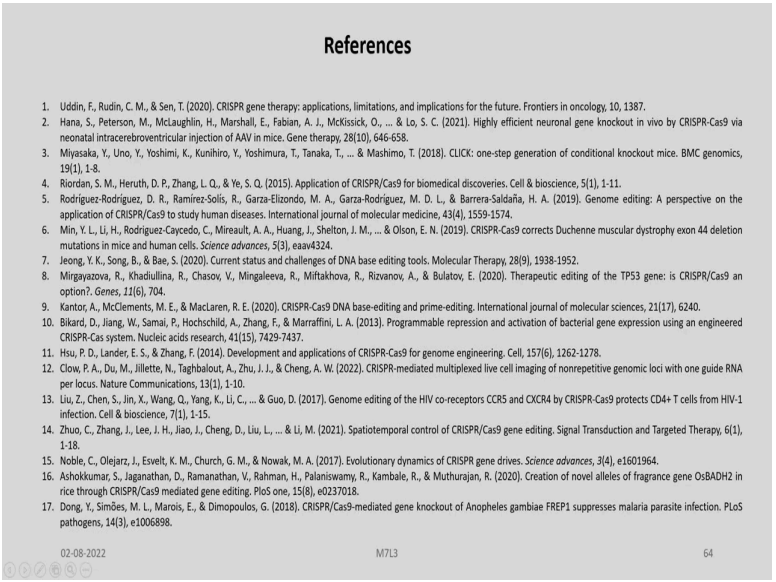


Sponsor/affiliation	Disease	Gene target	Clinial Trial ID	CRISPR-Cas9 mediated intervention
Yong Yang, The Affiliated Nanjing Drum Tower Hospital of Nanjing University Medical School	Stage IV Gastric Carcinoma, Stage IV Nasopharyngeal Carcinoma, T-Cell Lymphoma Stage IV, Stage IV Adult Hodgkin Lymphoma, Stage IV Diffuse Large B-Cell Lymphoma	PDCD1	NCT03044743	CRISPR-Cas9 mediated PD-1 knockout-T cells from autologous origin
First Affiliated Hospital, Sun Yat-Sen University/Jingchu University of Technology	Human Papillomavirus-Related Malignant Neoplasm	HPV16 and HPV18 E6/E7 DNA	NCT03057912	Evaluate the safety and efficacy of TALEN-HPV E6/E7 and CRISPR/Cas9-HPV E6/E7 in treating HPV Persistency and HPV-related Cervical Intraepithelial Neoplasial
Sichuan University/Chengdu MedGenCell, Co., Ltd.	Metastatic Non-small Cell Lung Cancer	PDCD1	NCT02793856	CRISPR-Cas9 mediated PD-1 knockout-T cells from autologous origin
Peking University	Metastatic Renal Cell Carcinoma	PDCD1	NCT02867332	CRISPR-Cas9 mediated PD-1 knockout-T cells from autologous origin
Peking University	Hormone Refractory Prostate Cancer	PDCD1	NCT02867345	CRISPR-Cas9 mediated PD-1 knockout-T cells from autologous origin
Peking University	Invasive Bladder Cancer Stage IV	PDCD1	NCT02863913	CRISPR-Cas9 mediated PD-1 knockout-T cells from autologous origin
Hangzhou Cancer Hospital/Anhui Kedgene Biotechnology Co., Ltd	Esophageal Cancer	PDCD1	NCT03081715	CRISPR-Cas9 mediated PD-1 knockout-T cells from autologous origin
Chinese PLA General Hospital	Solid Tumor, Adult	TCRα, TCRβ, PDCD1	NCT03545815	Evaluate the feasibility and safety of CRISPR-Cas9 mediated PD-1 and TCR gene-knocked out chimeric antigen receptor (CAR) T cells in patients with mesothelin positive multiple solid tumors
Baylor College of Medicine/The Methodist Hospital System	T-cell Acute Lymphoblastic Leukemia, T-cell Acute Lymphoblastic Lymphoma, T-non-Hodgkin Lymphoma	CD7	NCT03690011	CRISPR-Cas9 mediated CD7 knockout-T cells from autologous origin
Chinese PLA General Hospital	B Cell Leukemia, B Cell Lymphoma	PDCD1	NCT03398967	Determine the safety of the allogenic CRISPR-Cas9 gene-edited dual specificity CD19 and CD20 or CD22 CAR-T cells
Chinese PLA General Hospital	B Cell Leukemia, B Cell Lymphoma	TCRα, TCRβ, B2M	NCT03166878	CRISPR-Cas9 mediated TCR and B2M knockout-T cells from allogenic origin for CD19 CAR-T
Chinese PLA General Hospital	Solid Tumor, Adult	PDCD1	NCT03747965	CRISPR-Cas9 mediated PD-1 knockout-T cells from autologous origin
Xijing Hospital/XI'An Yufan Biotechnology Co., Ltd	Leukemia, Lymphoma	HPK1	NCT04037566	CRISPR Gene Edited to Eliminate Endogenous HPK1 (XYF19 CAR-T Cells)

Table from Uddin et al., 2020 *Front. Oncol.* 10:1387. CC BY 4.0

This is quite a big list as you can see which shows the power of the CRISPR Cas9 mediated therapeutic technologies.

(Refer Slide Time: 29:13)



References

1. Uddin, F., Rudin, C. M., & Sen, T. (2020). CRISPR gene therapy: applications, limitations, and implications for the future. Frontiers in oncology, 10, 1387.
2. Hana, S., Peterson, M., McLaughlin, H., Marshall, E., Fabian, A. J., McKissick, O., ... & Lo, S. C. (2021). Highly efficient neuronal gene knockout in vivo by CRISPR-Cas9 via neonatal intracerebroventricular injection of AAV in mice. Gene therapy, 28(10), 646-658.
3. Miyasaka, Y., Uno, Y., Yoshimi, K., Kunihiro, Y., Yoshimura, T., Tanaka, T., ... & Mashimo, T. (2018). CLICK: one-step generation of conditional knockout mice. BMC genomics, 19(1), 1-8.
4. Riordan, S. M., Heruth, D. P., Zhang, L. Q., & Ye, S. Q. (2015). Application of CRISPR/Cas9 for biomedical discoveries. Cell & bioscience, 5(1), 1-11.
5. Rodríguez-Rodríguez, D. R., Ramírez-Solís, R., Garza-Elizondo, M. A., Garza-Rodríguez, M. D. L., & Barrera-Saldaña, H. A. (2019). Genome editing: A perspective on the application of CRISPR/Cas9 to study human diseases. International journal of molecular medicine, 43(4), 1559-1574.
6. Min, Y. L., Li, H., Rodriguez-Caycedo, C., Mireault, A. A., Huang, J., Shelton, J. M., ... & Olson, E. N. (2019). CRISPR-Cas9 corrects Duchenne muscular dystrophy exon 44 deletion mutations in mice and human cells. *Science advances*, 5(3), eaav4324.
7. Jeong, Y. K., Song, B., & Bae, S. (2020). Current status and challenges of DNA base editing tools. Molecular Therapy, 28(9), 1938-1952.
8. Mirgayazova, R., Khadiullina, R., Chasov, V., Mingaleeva, R., Miftakhova, R., Rizvanov, A., & Bulatov, E. (2020). Therapeutic editing of the TP53 gene: is CRISPR/Cas9 an option?. *Genes*, *11*(6), 704.
9. Kantor, A., McClements, M. E., & MacLaren, R. E. (2020). CRISPR-Cas9 DNA base-editing and prime-editing. International journal of molecular sciences, 21(17), 6240.
10. Bikard, D., Jiang, W., Samai, P., Hochschild, A., Zhang, F., & Marraffini, L. A. (2013). Programmable repression and activation of bacterial gene expression using an engineered CRISPR-Cas system. Nucleic acids research, 41(15), 7429-7437.
11. Hsu, P. D., Lander, E. S., & Zhang, F. (2014). Development and applications of CRISPR-Cas9 for genome engineering. Cell, 157(6), 1262-1278.
12. Clow, P. A., Du, M., Jillette, N., Taghbalout, A., Zhu, J. J., & Cheng, A. W. (2022). CRISPR-mediated multiplexed live cell imaging of nonrepetitive genomic loci with one guide RNA per locus. Nature Communications, 13(1), 1-10.
13. Liu, Z., Chen, S., Jin, X., Wang, Q., Yang, K., Li, C., ... & Guo, D. (2017). Genome editing of the HIV co-receptors CCR5 and CXCR4 by CRISPR-Cas9 protects CD4+ T cells from HIV-1 infection. Cell & bioscience, 7(1), 1-15.
14. Zhuo, C., Zhang, J., Lee, J. H., Jiao, J., Cheng, D., Liu, L., ... & Li, M. (2021). Spatiotemporal control of CRISPR/Cas9 gene editing. Signal Transduction and Targeted Therapy, 6(1), 1-18.
15. Noble, C., Olejarz, J., Esvelt, K. M., Church, G. M., & Nowak, M. A. (2017). Evolutionary dynamics of CRISPR gene drives. *Science advances*, *3*(4), e1601964.
16. Ashokkumar, S., Jaganathan, D., Ramanathan, V., Rahman, H., Palaniswamy, R., Kambale, R., & Muthurajan, R. (2020). Creation of novel alleles of fragrance gene OsBADH2 in rice through CRISPR/Cas9 mediated gene editing. PloS one, 15(8), e0237018.
17. Dong, Y., Simões, M. L., Marois, E., & Dimopoulos, G. (2018). CRISPR/Cas9-mediated gene knockout of Anopheles gambiae FREP1 suppresses malaria parasite infection. PLoS pathogens, 14(3), e1006898.

So, here are some references which you can go back to for further details of the various topics that we have discussed in these lectures.

Thank you.