THERAPEUTIC STRATEGIES OF CERVICAL CANCER IN NEW ERA

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Joint Graduate Seminar

11th Dec 2019

Agenda

- Background
- Therapeutic strategies
- Perspectives and Challenges

Facts you need to know about Cervical Cancer

4th
most common cancer in women

500,000
cases occur each year around world

250,000
deaths of cervical cancer each year

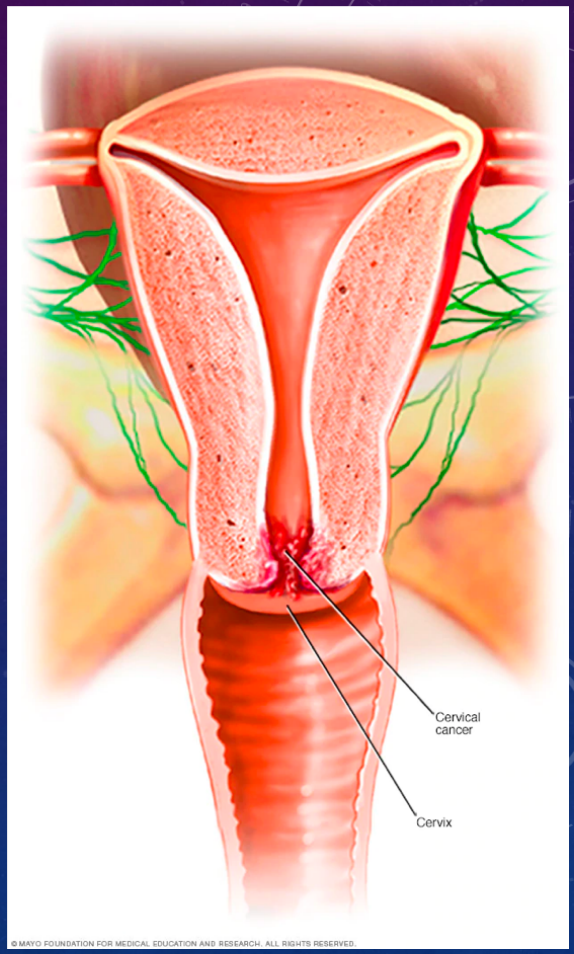
<50
Most cases of cervical cancer occur at ages under 50

75%
cervical cancer can be prevented by screening

(Harden and Munger 2017)

Overview of Cervical Cancer

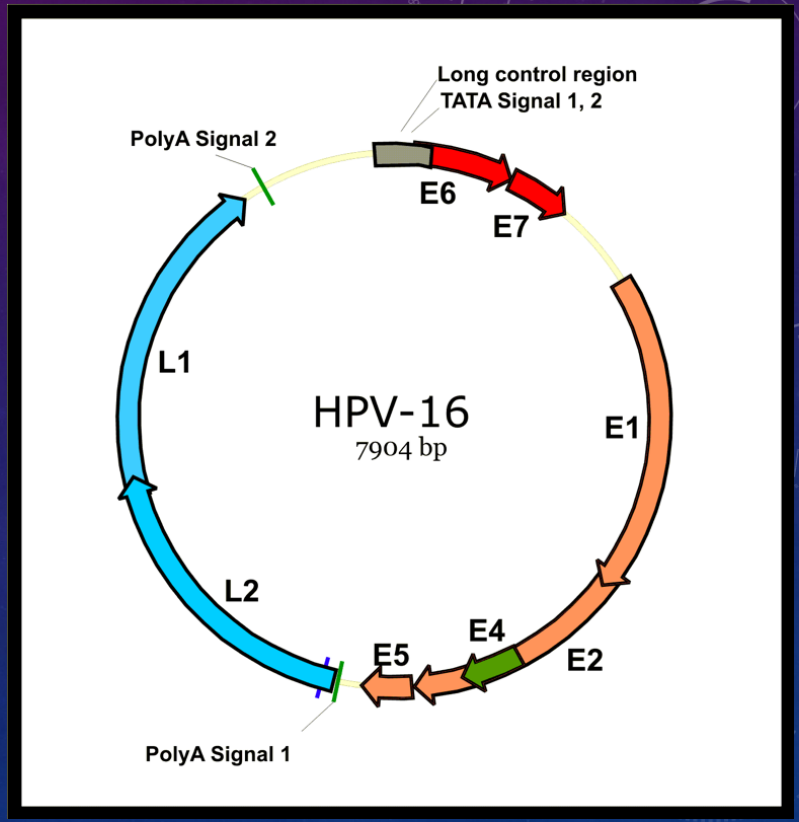
- Two types of cervical cancer begin in two types of cervical cell.
  - ✓ Squamous cell carcinoma (70%)—squamous cells.
  - ✓ Adenocarcinoma (30%)—glandular cells.
- Cervical cancer is slow-growing, its progression through precancerous changes provides opportunities for:
  1. Prevention;
  2. Early detection and;
  3. Treatment
- Cervical cancer can be altogether eliminated (75%).



World Health Organization

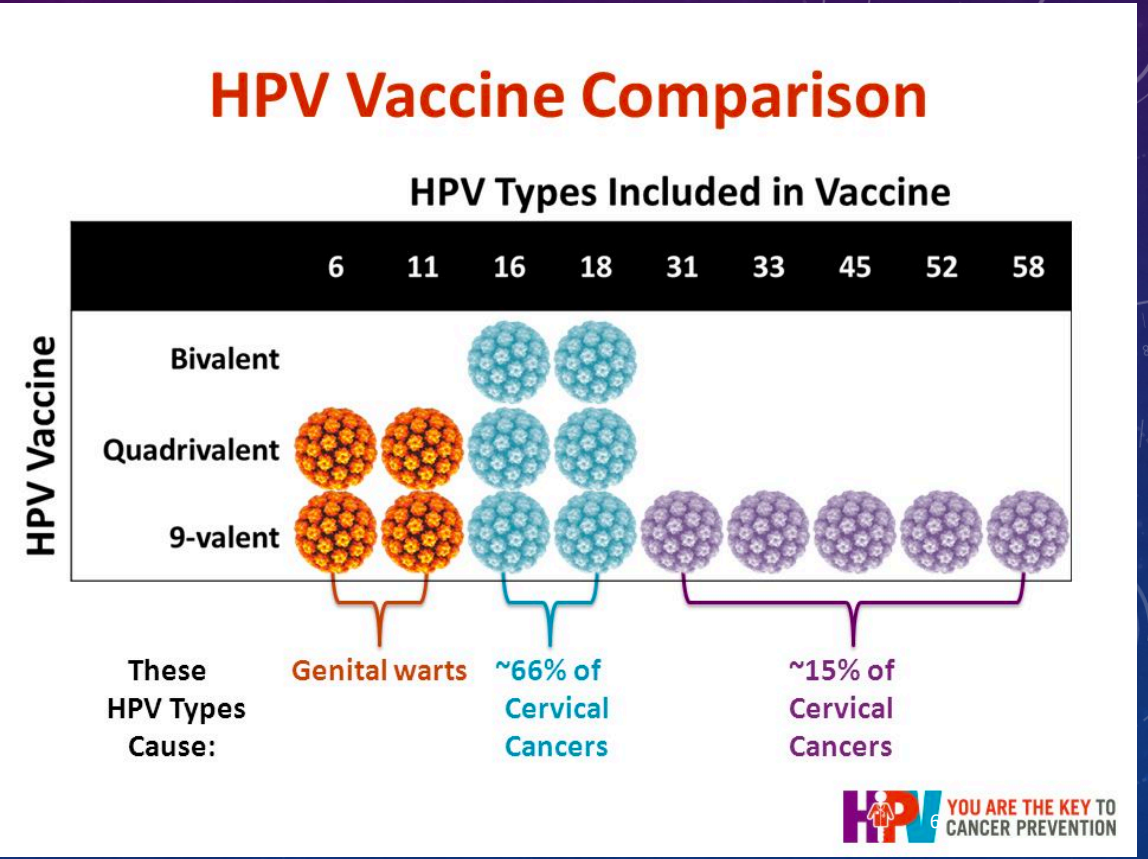
Structure of Human Papillomavirus (HPV)

- Mostly caused by persistent HPV infections
- Cervical cancer is caused by high-risk types of HPV
  - ✓ HPV 16 and 18: most common high-risk HPV types;
  - ✓ Responsible for approximately 70% of cervical cancer cases.
- HPV infection is currently the most common sexually transmitted infection (STI)
  - ✓ 80% of women can be infected at some point in their lifetime;
  - ✓ Most of infection clear naturally

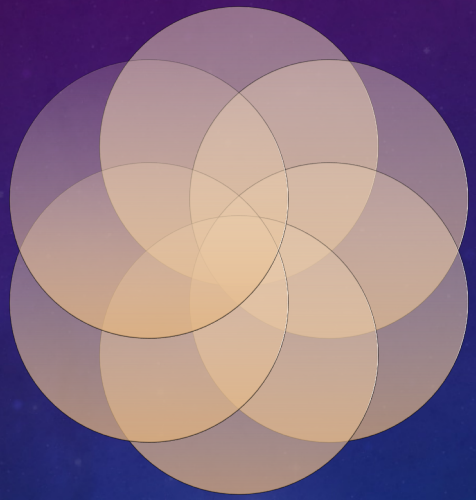


Prevention of Cervical Cancer

- Vaccination is one of the most effective method to prevent HPV infection;
- The 9-valent vaccine could prevent up to 90% of cervical cancer.



Treatment of Cervical Cancer



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What is gene therapy?

1. Replacing a mutated gene that causes disease with a healthy copy of the gene.

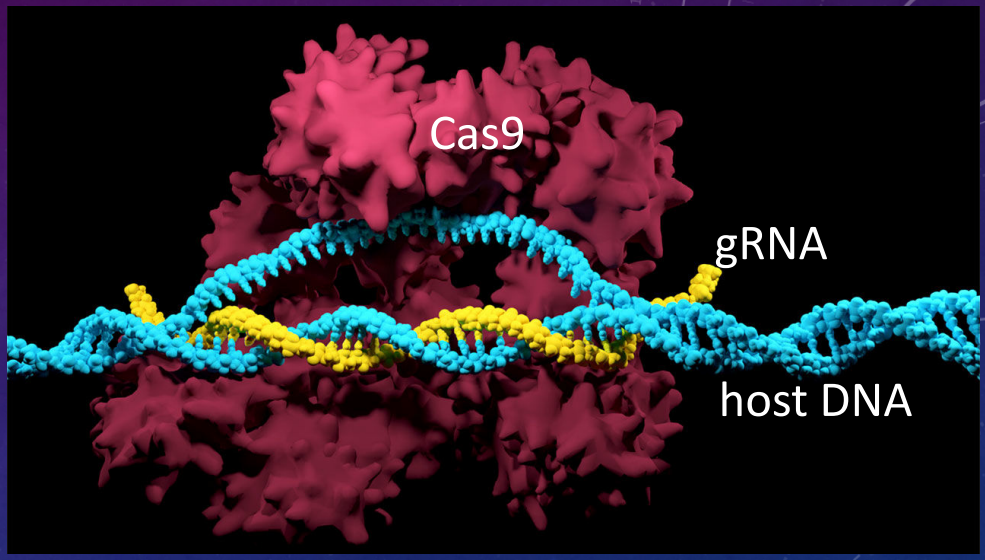
2. Inactivating or “knocking out” a mutated gene that is functioning improperly.

3. Introducing a new gene into the body to help fight a disease.

CRISPR-Cas9 could be more targeted and safer therapeutic method.

What is CRISPR-Cas9 and how it works?

- The CRISPR/Cas system is prokaryotic immune system that confers resistance to foreign genetic elements and phages.
- The sequences contain snippets of DNA from attacking viruses.



1. The guide RNA (gRNA) containing the target sequence will find the right place for ‘scissors’ to bind;
2. Cas9 binds to the target and generates site-specific double-strand breaks;
3. Change the genes via homology-directed repair (HDR), and remove the genes via non-homologous end joining (NHEJ).

Gene Therapy for Cervical Cancer

"This is the first cure for any cancer using this technology."

- The Griffith University scientists used CRISPR/Cas9 to successfully target and treat cervical cancer tumors *in vivo.*

2014-2019

Journals.ASM.

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Translational Oncology
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Molecular Therapy
Original Article

AMERICAN SOCIETY of GENE & CELL THERAPY

Systemic Delivery of CRISPR/Cas9 Targeting HPV Oncogenes Is Effective at Eliminating Established Tumors

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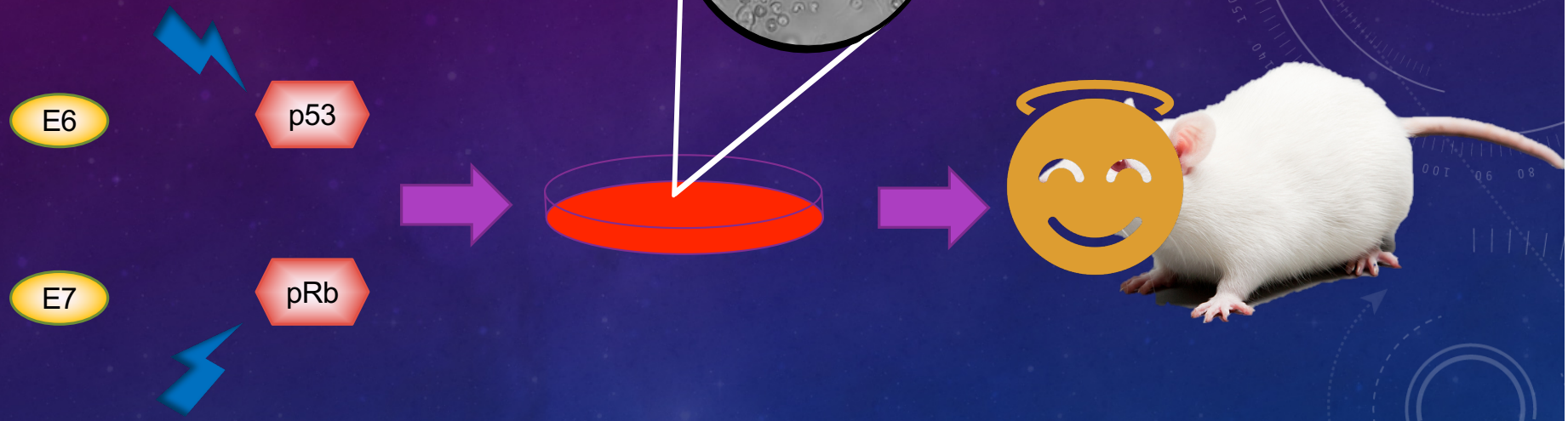
Workflow

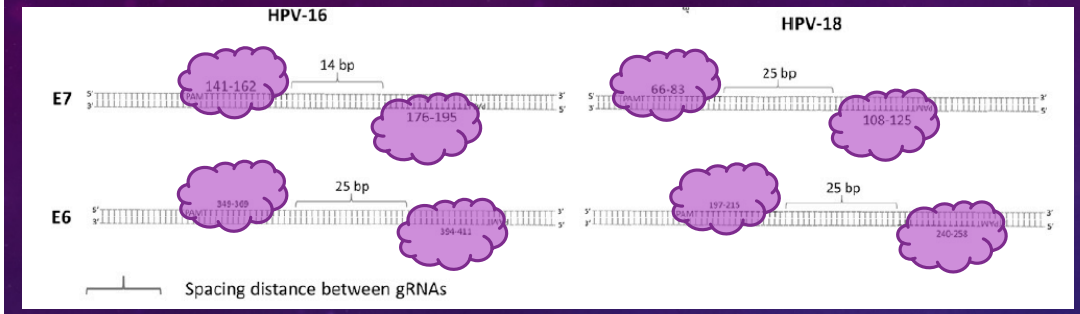


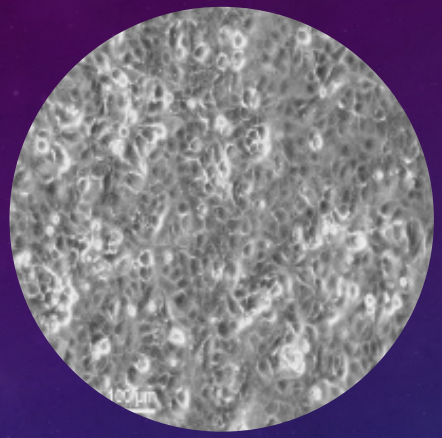
- E6/E7 binds p53/pRb to degradation.
- E6/E7-specific CRISPR-Cas9 to knock out oncogenes to restore p53/pRb.

- Restoration of p53/pRb induces cell apoptosis and death.

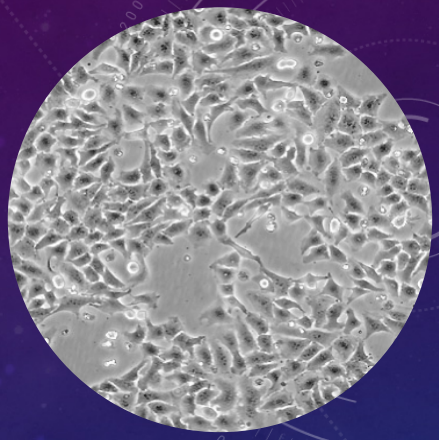
- Application of E6/E6-specific CRISPR-Cas9 *in vivo* prolongs the cancerous mice survival.

Tumor Cell line used in study



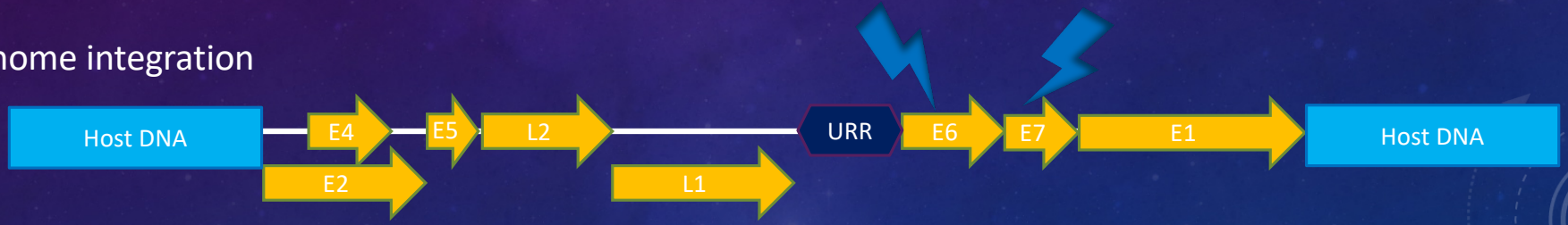


CasKi



HeLa

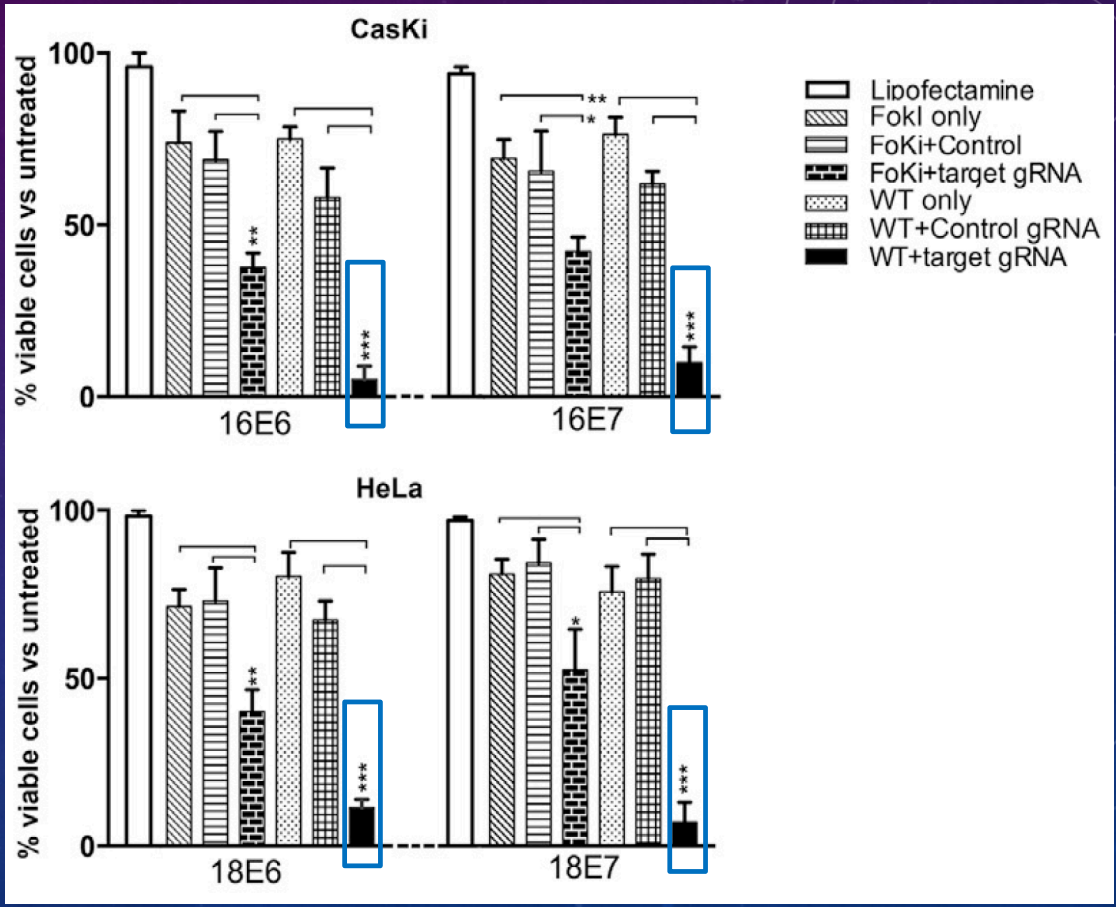
Genome integration



- **CasKi:** HPV16 positive; **HeLa**: HPV18 positive
- Using specific CRISPR-Cas9 to knock out E6 and E7

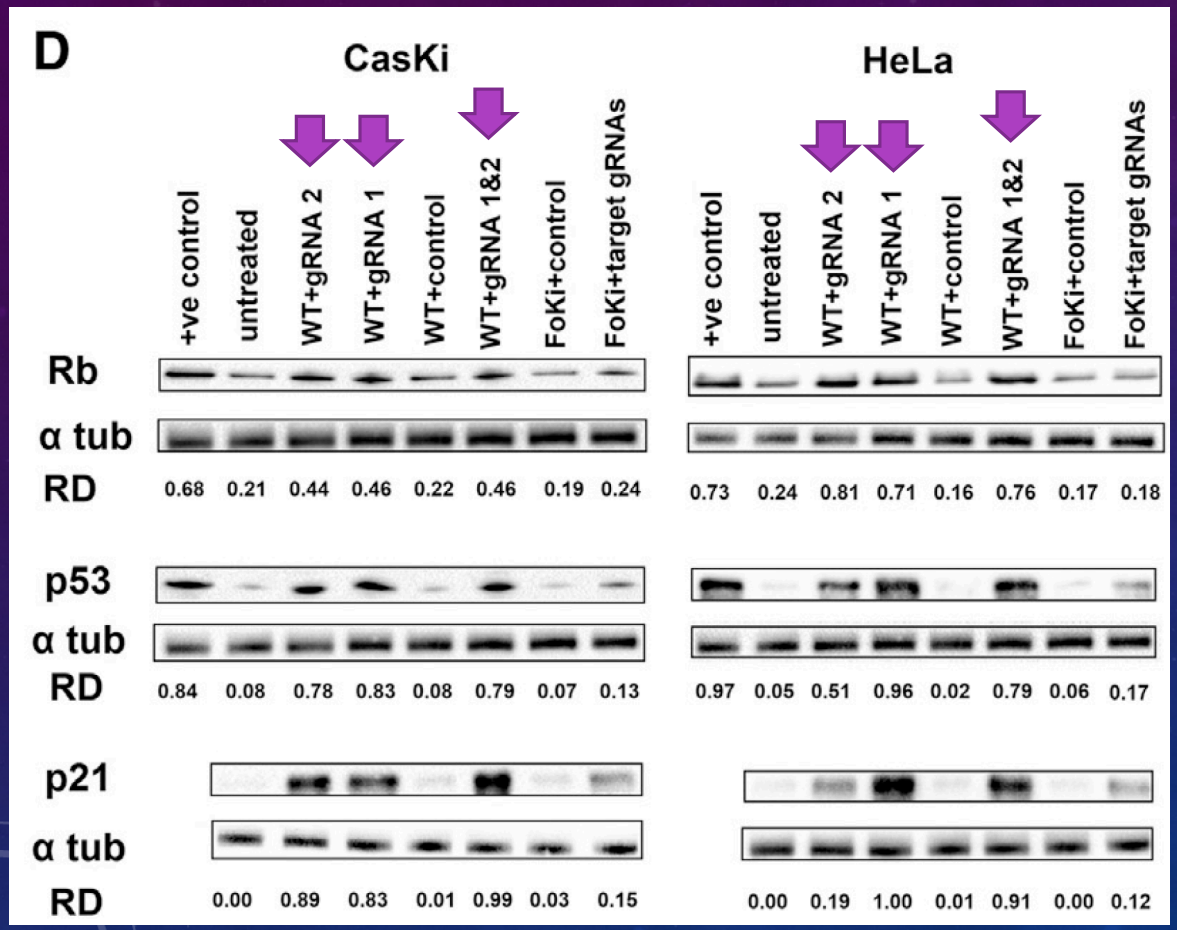
Compatibility of E6/E7 CRISPR-Cas9 *in vitro*

- The transfected E6/E7-specific guide RNA (gRNA) and Cas9 plasmids worked well *in vitro*.
- The Knocked-out of E6/E7 induced cell death.



(Jubair, Fallaha, and McMillan 2019)

p53/p21 restoration by E6-specific CRISPR-Cas9

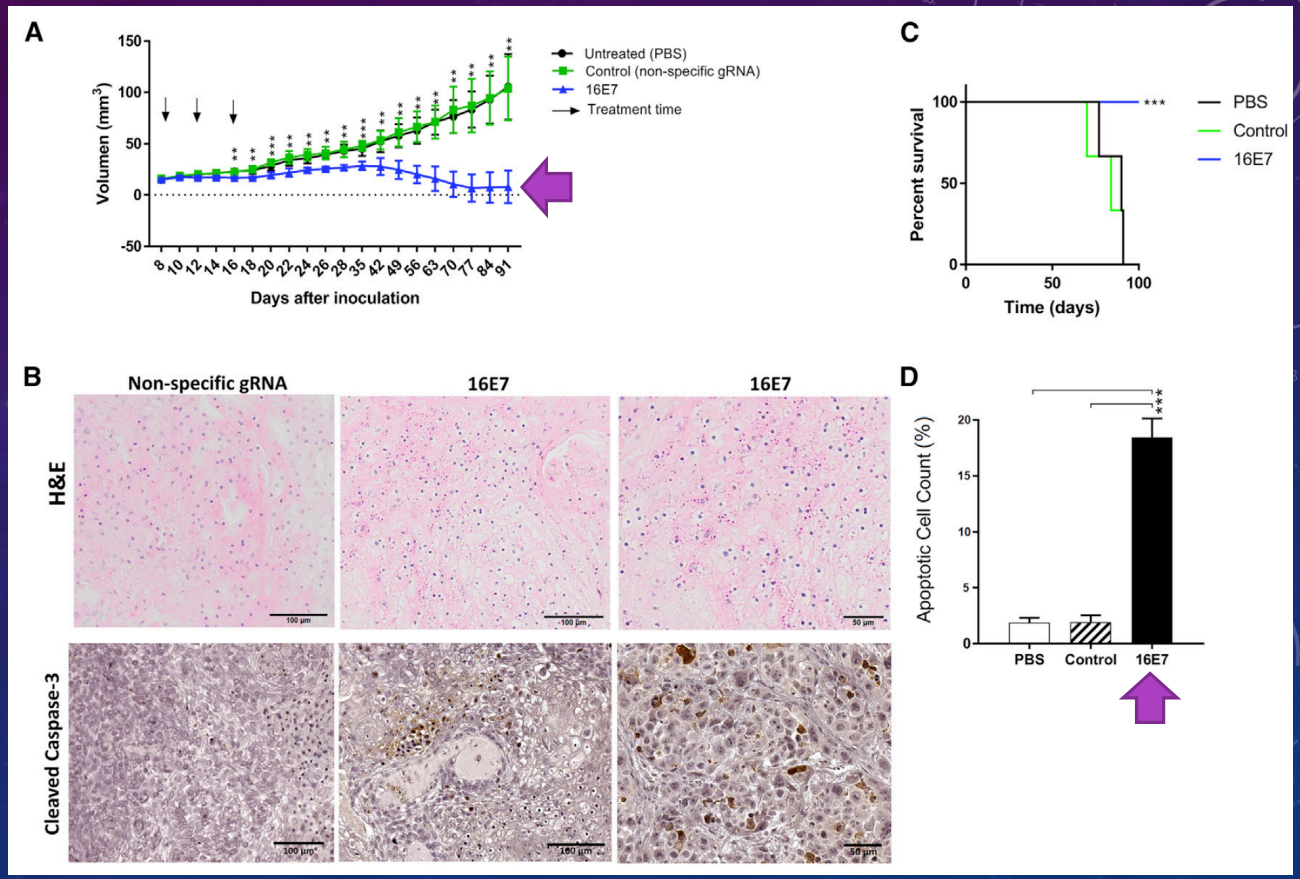


- E6/E7-specific CRISIPR-Cas9 system restores both p53 and pRb expression.

(Jubair, Fallaha, and McMillan 2019)

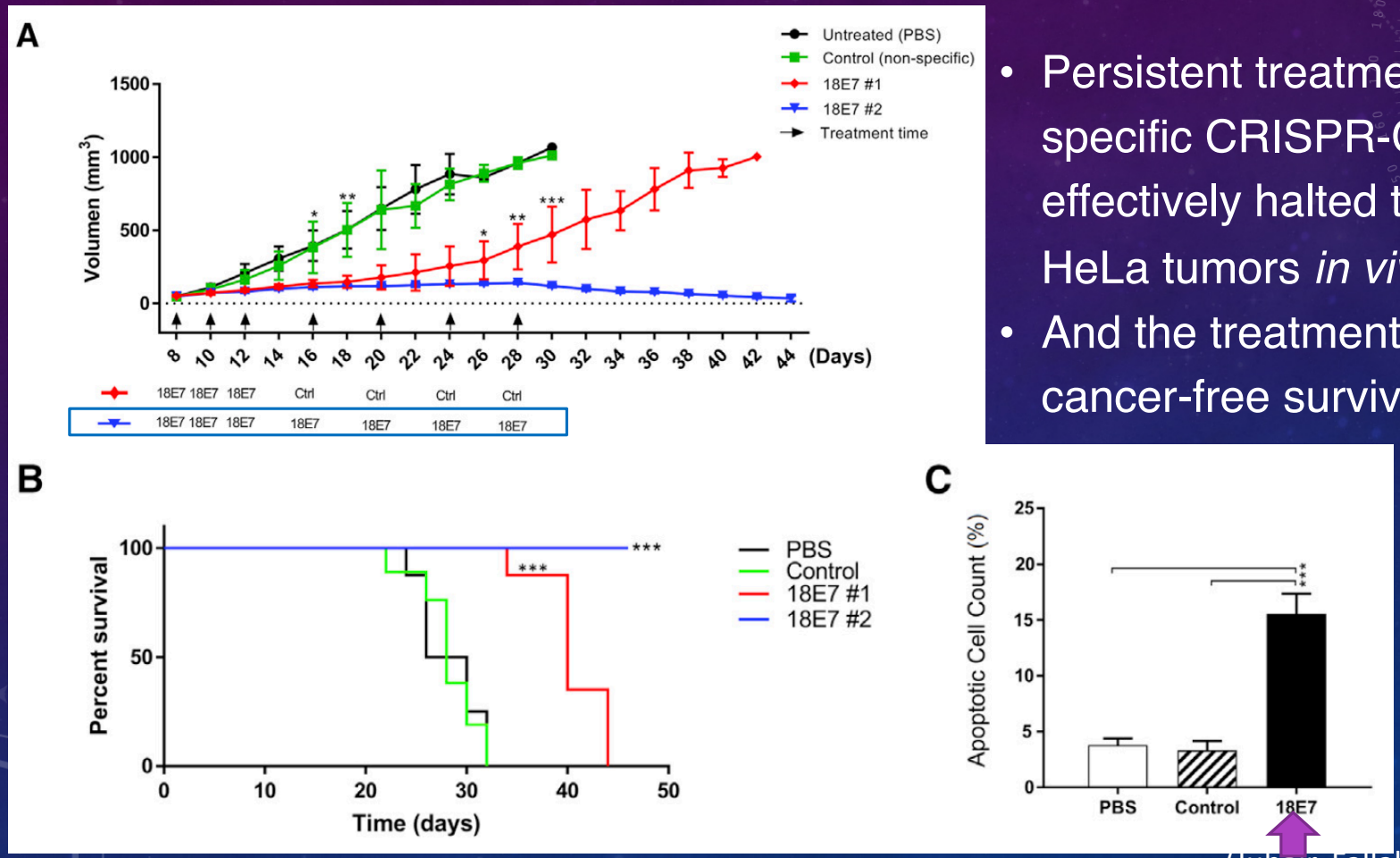
CRISPR-Cas9 targeting oncogenes eliminates established tumors *in vivo*

- Intravenous infection with lipoplexes of specific gRNA and Cas9 plasmid.
- 16E7-specific CRISPR-Cas9 treatment halted the growth of Caski Tumors *in vivo.*
- The treatment induced more apoptosis.



(Jubair, Fallaha, and McMillan 2019)

Persistent treatment effectively prolonged survival



- Persistent treatment of 18E7-specific CRISPR-Cas9 (7 doses) effectively halted the growth of HeLa tumors *in vivo.*
- And the treatment prolonged the cancer-free survival.

(Jubair, Fallaha, and McMillan 2019)

Summary

- E6/E7-specific CRISPR-Cas9 knocked out HPV E6 and E7 genes that integrated in tumor cell lines (Caski and HeLa).
- Knockout of oncogenes restores p53 and pRb expression, which induces apoptosis and cellular death.
- Intravenous injection of lipoplexed CRISPR-Cas9 could be well translated *in vivo* and effectively eliminate established tumors, which prolonged survival of host.

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Challenges in future

- Specificity of CRISPR-Cas9.
  - ✓ Off-target effects.
- Efficiency of *in vivo* delivery methods.
  - ✓ Viral vectors, e.g., AAVs.
  - ✓ Translatability of delivery methods.
- Immunogenicity of CRISPR-Cas9 and delivery vehicles
  - ✓ Immunogenicity of viral vectors.
  - ✓ Host immune responses may attenuate therapeutic effects and cause inflammatory reactions.
  - ✓ Humanizing Cas9 protein.
- Fitness of edited cells.

THANKS FOR ATTENTION!

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