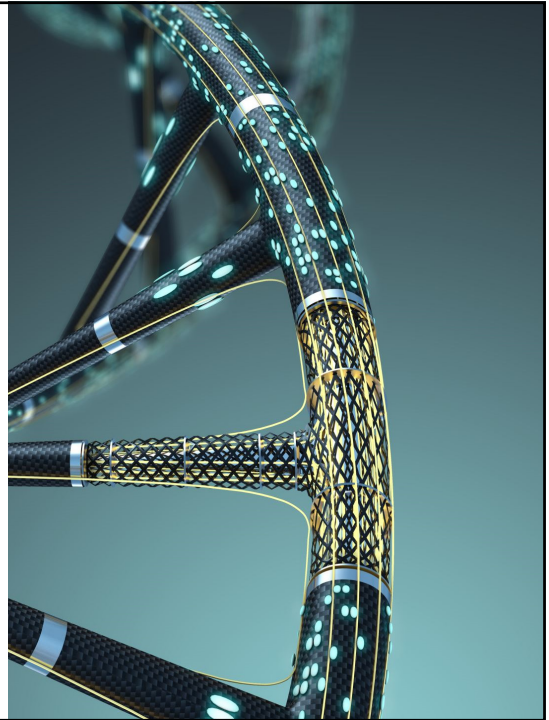


Engineering TALENs to permanently disable HBV replication

Tiffany Smith

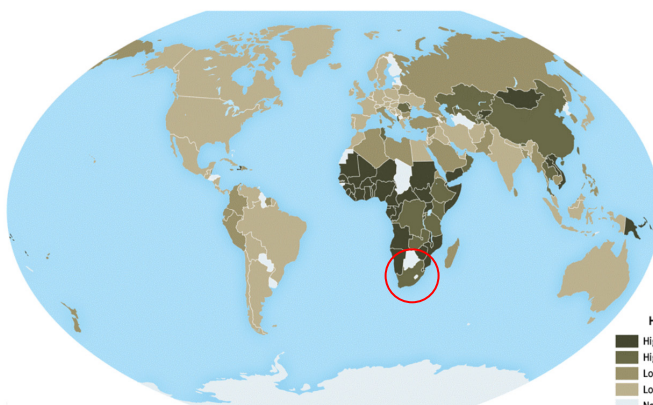
4th SUSTAINABLE BIO-INNOVATION
SYMPOSIUM

28 September 2023



1

HBV epidemiology



Transmission of HBV infection:
Blood or other body fluids of an infected person

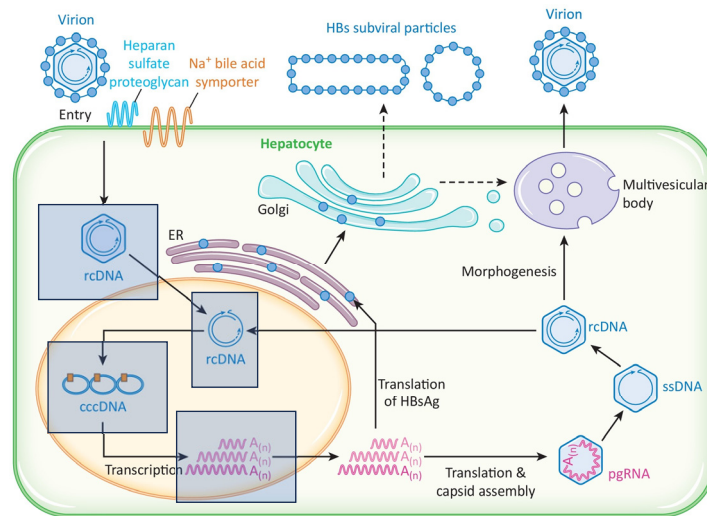
- Mother to child
- Sexual contact
- Sharing of equipment between intravenous drug users

Chronic sufferers are at risk of developing liver scarring and/or liver cancer ~600 000 deaths annually

Source: Centers for Disease Control and Prevention. CDC Yellow Book 2020: Health Information for International Travel. New York: Oxford University Press; 2019.

2

HBV genome



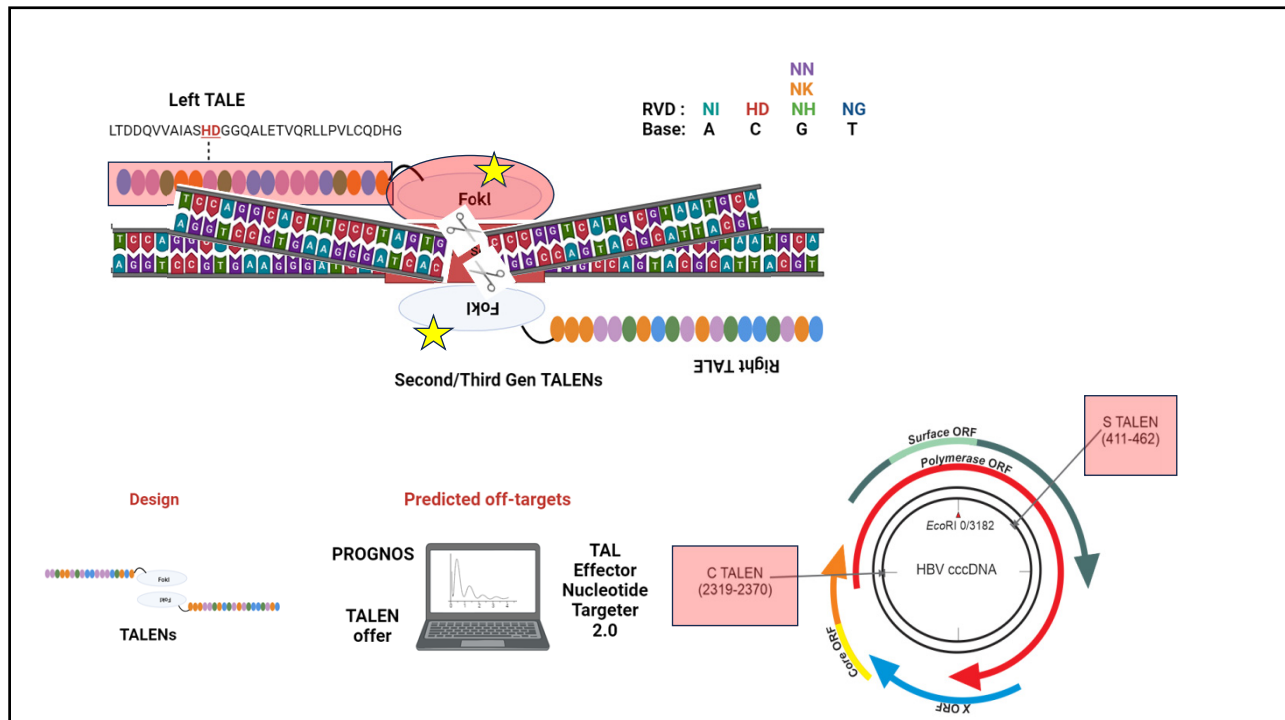
Several FDA approved drugs rarely leads to cure primarily because of the stable cccDNA reservoir

Source: Shih, C., Yang, C.C., Chojilsuren, G., Chang, C.H. and Liou, A.T., 2018. Hepatitis B virus. Trends in microbiology, 26(4), pp.386-387.

3

TALEN Design

4



5

Previous publications

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MDPI

original article

Inactivation of Hepatitis B Virus Replication in Cultured Cells and *In Vivo* with Engineered Transcription Activator-Like Effector Nucleases

Kristie Bloom¹, Abdullah Ely¹, Claudio Mussolino², Toni Cathomen² and Patrick Arbuthnot¹

¹Artificial Gene Therapy Research Unit, School of Pathology and African Network for Drugs and Diagnostics Innovation (ANDDI) Centre of Excellence, Health Sciences faculty, University of the Witwatersrand, Johannesburg, South Africa; ²Institute for Cell and Gene Therapy, Center for Chronic Immunodeficiency, University Medical Center Freiburg, Freiburg, Germany

viruses

MDPI

Article

Improved Specificity and Safety of Anti-Hepatitis B Virus TALENs Using Obligate Heterodimeric FokI Nuclease Domains

Tiffany Smith¹, Prashika Singh¹, Kay Ole Chmielewski², Kristie Bloom¹, Toni Cathomen², Patrick Arbuthnot¹ and Abdullah Ely^{1,*}

¹Wits/SAMRC Artificial Gene Therapy Research Unit, School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, Parktown 2010, South Africa; tiffsmith0777@gmail.com (T.S.); prashika.singh@wits.ac.za (P.S.); kristie.bloom@wits.ac.za (K.B.); patrick.arbuthnot@wits.ac.za (P.A.)
²Institute for Translational Medicine and Gene Therapy, Medical Center University of Freiburg & Medical Faculty, University of Freiburg, 79106 Freiburg, Germany; kay.chmielewski@uniklinik-freiburg.de (K.O.C.); toni.cathomen@uniklinik-freiburg.de (T.C.)
*Correspondence: abduhah-ely@wits.ac.za; Tel.: +27-031-1-717-2561

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original article

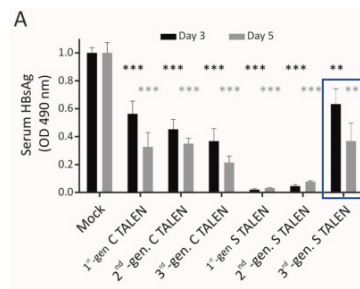
An Efficient Antiviral Strategy for Targeting Hepatitis B Virus Genome Using Transcription Activator-Like Effector Nucleases

Jieliang Chen^{1,2}, Wen Zhang^{1,2}, Junyu Lin¹, Fan Wang^{1,2}, Min Wu², Cuncun Chen^{1,3}, Ye Zheng², Xiuhua Peng², Jianhua Li² and Zhenghong Yuan^{1,2,3}

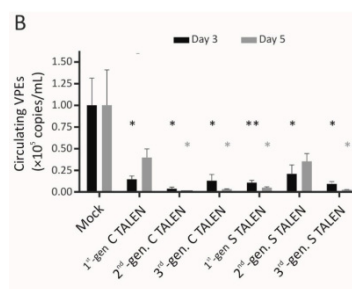
¹Key Laboratory of Medical Molecular Virology, School of Basic Medical Sciences, Shanghai Medical College of Fudan University, Shanghai, China; ²Shanghai Public Health Clinical Center, Fudan University, Shanghai, China; ³Institute of Medical Microbiology and Biomedical Sciences, Fudan University, Shanghai, China

6

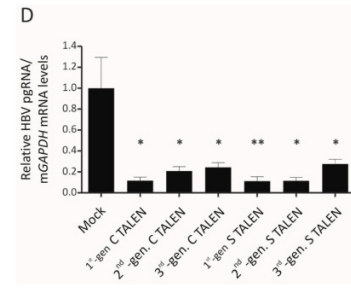
Anti HBV effects-Viral markers



Hepatitis B surface antigen (HBsAg)= marker of active replication



Viral Particle equivalents



pgRNA: Template for reverse transcription= rcDNA
rcDNA: Precursor for cccDNA
pgRNA: Viral persistence and active gene expression

7

Gene therapy



Degradation by intra/extracellular enzymes

Difficulties in passing through the plasma membrane

1.

DNA→mRNA

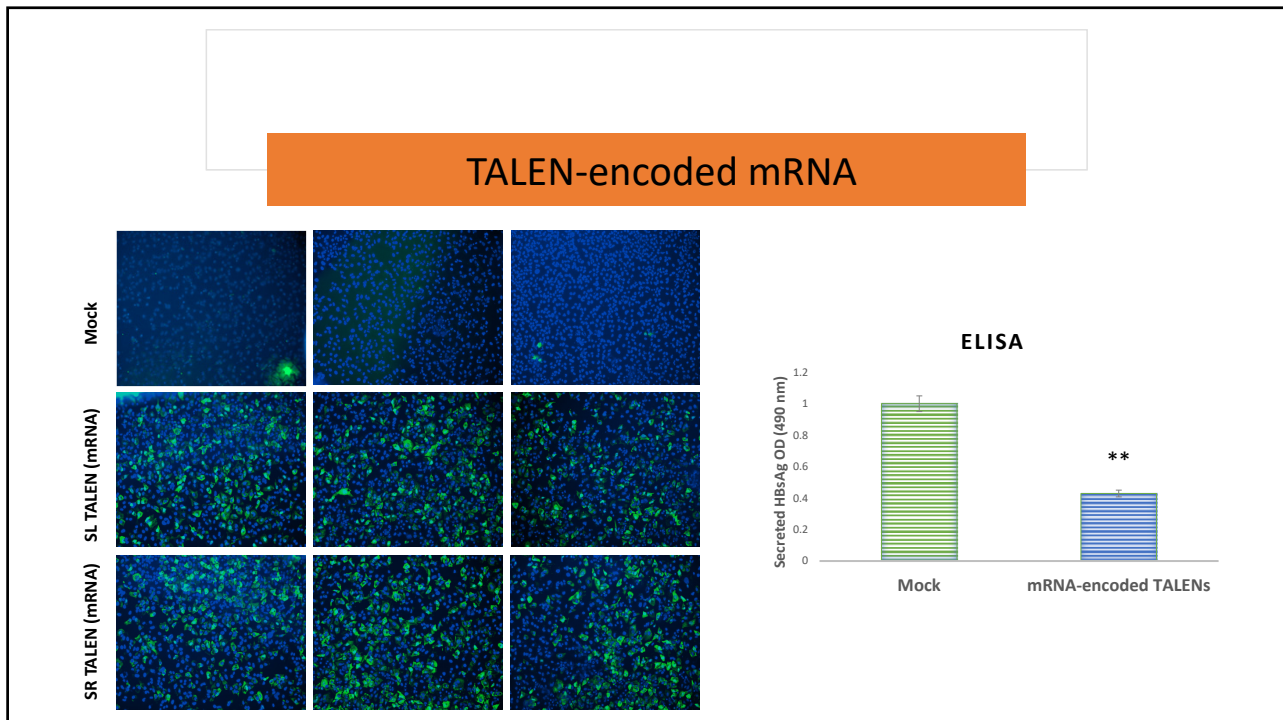
- No risk of genome integration upon entering the cell
- Easily scalable as transcription can take place *in vitro*

2.

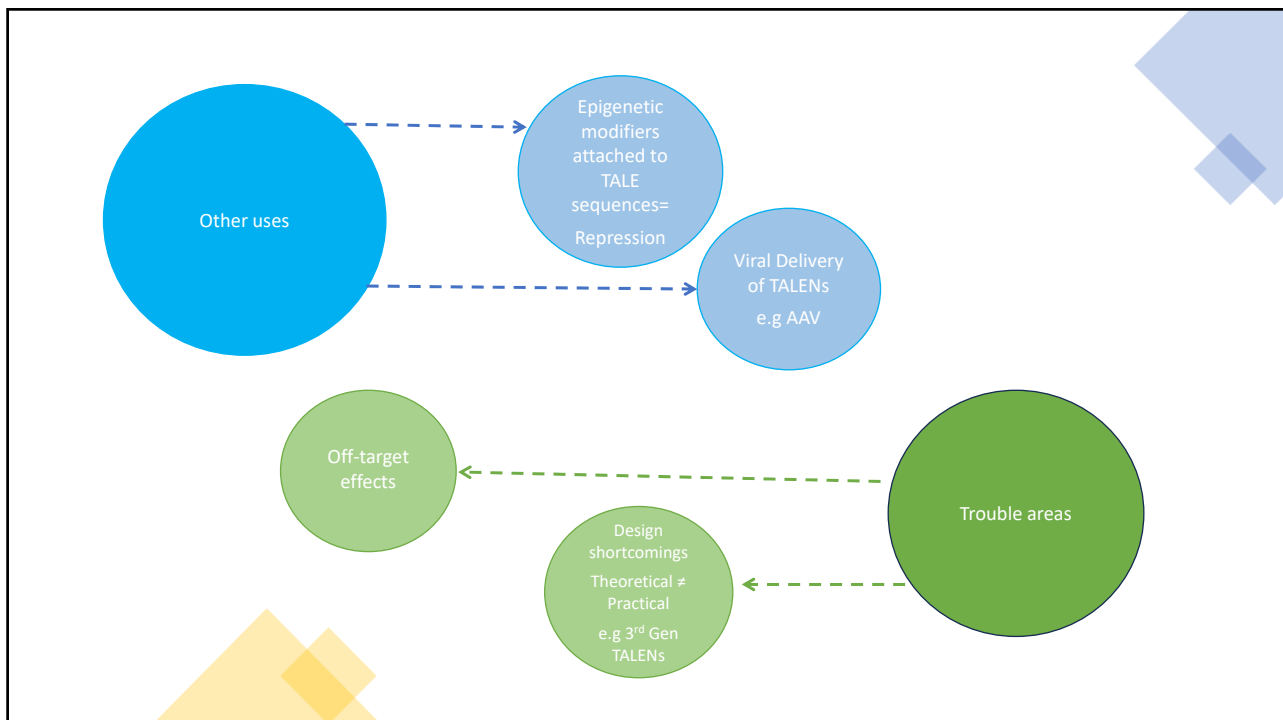
Delivery vector: Liposomes

- Non-viral vector systems are biodegradable and endogenous enzymes break down lipid components
- Synthetic-large scale synthesis

8



9



10

Acknowledgements

- Prof. Patrick Arbuthnot, Prof. Abdullah Ely and Dr Betty Maepa
- Prashika Singh
- Antiviral Gene Therapy Research Unit

