UNIVERSITI PUTRA MALAYSIA

CHARACTERISATION AND RESCUE OF A RECOMBINANT NEWCASTLE DISEASE VIRUS STRAIN AF2240-I

KAVITHA MURULITHARAN

IB 2016 18

CHARACTERISATION AND RESCUE OF A RECOMBINANT NEWCASTLE DISEASE VIRUS STRAIN AF2240-I

By

KAVITHA MURULITHARAN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

February 2016

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the Degree of Doctor of Philosophy

CHARACTERISATION AND RESCUE OF A RECOMBINANT NEWCASTLE DISEASE VIRUS STRAIN AF2240-I

By

KAVITHA MURULITHARAN

February 2016

Chairman : Professor Datin Paduka Khatijah Mohamad Yusoff, PhD
Institute : Bioscience

Newcastle disease virus (NDV) or avian paramyxovirus 1 remains a constant major threat to commercial poultry production. The virus has shown remarkable effect as an oncolytic agent as well as a promising vaccine candidate against animal diseases. Although various studies have produced positive response rates for these two applications, there are still some important issues to be addressed which requires reverse genetics application. The rescue of a Malaysian NDV would help in the development of a recombinant vaccine; one which is phylogenetically closer to those viruses from current outbreaks with a potential to be used as a novel oncolytic agent. One such candidate strain with these potentials is the strain AF2240-I, a derivative of the viscerotropic velogenic local NDV strain AF2240. The main objectives of this study are to sequence the full genome of NDV strain AF2240-I and perform *in-silico* characterisation and to generate a reverse genetics system to rescue stably virulent NDV strain AF2240-I. Firstly, full genome amplification was performed using PCR and rapid amplification of cDNA ends (RACE) method followed by sequencing to verify the sequence. Full length sequence analysis showed that the strain AF2240-I belongs to genotype VIII at a length of 15,192 bp which follows the rule of six. The amplification of the haemagglutinin-neuraminidase (HN) gene of the strain given (initially presumed AF2240) has indicated the presence of Arg 403 residue in the HN gene which was reported to be absent in the HN gene of strain AF2240. A frameshift was also observed between the amplified matrix (M) gene of AF2240-I and the published M gene sequence of AF2240. It was concluded from that the frameshifts in both HN and M gene were likely due to quasi-species interference. Meanwhile, rescue of virus was done using the helper plasmid method. Three helper plasmids consisting of nucleoprotein (NP), phosphoprotein (P) and large protein (L) genes were prepared in pCI-Neo expression vector. The full length NDV anti-genome was synthesized and cloned into a transcription vector, pOLTV_phiX. The synthesised genome contained a silenced *BsmB*I RE site, at position 6741, by replacing G with A (CGTCTC to CATCTC) to serve as a genetic marker. These plasmids (helper plasmids & full

genome plasmid) were co-transfected into Baby Hamster Kidney (BHK) cells stably expressing T7 RNA polymerase by lipofectamine transfection reagent using 5 different ratios and harvested at different time. The recombinant AF2240-I (rAF) recorded an intracerebral pathogenicity index (ICPI) and mean death time (MDT) values between 1.83 - 1.85 and between 47 h to 49 h respectively for passage 1 to passage 5. In summary, 16 h post transfection is sufficient to produce infectious viral particles in cell supernatant and that all 5 ratios showed no preference in the production of AF2240-I infectious virus particles. Also, both pathogenicity indices showed that rAF possesses a stable virulence capability even after 5 passages. Thus the reverse genetics system of a local NDV strain AF2240-I was successfully achieved.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

PENCIRIAN DAN PENYELAMATAN VIRUS REKOMBINAN PENYAKIT NEWCASTLE STRAIN AF2240-I

Oleh

KAVITHA MURULITHARAN

Februari 2016

Pengerusi : Profesor Datin Paduka Khatijah Mohamad Yusoff, PhD
Institut : Biosains

Virus penyakit Newcastle (NDV) atau avian paramyxovirus 1 masih merupakan menjadi ancaman yang utama bagi pengeluaran ayam komersial. Virus ini telah menunjukkan kesan yang luar biasa sebagai agen onkolitik dan juga sebagai calon vaksin haiwan. Walaupun pelbagai kajian telah menghasilkan keputusan pada kadar positif bagi aplikasi-aplikasi yang dinyatakan, masih terdapat beberapa isu-isu penting yang perlu ditangani. Oleh itu, penyelamatan virus NDV Malaysia berperanan dalam membantu pembangunan vaksin rekombinan; yang secara filogenetik lebih dekat kepada wabak-wabak semasa dengan potensi untuk digunakan sebagai agen oncolytik yang baharu. Salah satu calon dengan ciri-ciri ini adalah strain AF2240-I terbitan daripada strain NDV tempatan AF2240 yang viscerotropik velogenic. Oleh itu objektif utama kajian ini adalah untuk mengenal pasti jujukan genom penuh strain NDV AF2240-I dan melaksanakan pencirian in-silico bagi menjana sistem genetik pembalikkan untuk menyelamatkan strain NDV AF2240-I yang stabil. Pertama, genom aplifikasi penuh telah dilakukan dengan menggunakan PCR dan kaedah aplifikasi pesat hujung cDNA (RACE) diikuti oleh pengesahkan turutan itu dengan cara penjujukan. Analisis penuh jujukan menunjukkan bahawa strain AF2240-I kepunyaan genotip VIII dan panjanganya adalah 15,192 pasangan bes yang mengikut undang-undang enam. Aplifikasi daripada haemagglutinin-neuraminidase (HN) gen daripada strain yang diberikan (pada mulanya dianggap AF2240) telah menunjukkan kehadiran asid amino Arg 403 dalam gen HN, yang sebelum ini dilaporkan tiada dalam gen HN di strain AF2240. Mutasi sebingkai juga telah diperhatikan di antara matriks (M) gen AF2240-I dan jujukan M gen yang diterbitkan bagi AF2240. Secara kesimpulan, mutasi dalam gen HN and M dianggap disebabkan oleh gangguan kuasi-spesis. Sementara itu, pemulihan virus telah dilakukan dengan menggunakan kaedah plasmid bantuan. Tiga plasmid bantuan yang terdiri daripada gen nucleoprotein (NP), phosphoprotein (P) dan protein yang besar (L) telah disediakan di dalam vector pCI-Neo. Anti-genom NDV telah disintesis dan diklon ke dalam vektor transkripsi, pOLTV_phiX. Genom yang disintesis mengandungi tapak BsmBI enzim yang disenyapkan,

pada kedudukan 6741, dengan menggantikan G dengan A (CGTCTC ke CATCTC) untuk bertujuan sebagai penanda genetik. Plasmid-plasmid ini (plasmid bantuan & plasmid anti-genom penuh) telah bersama-sama ditransfek menggunakan reagen lipofectamine ke dalam sel-sel Buah Pinggang Hamster Bayi (BHK) yang secara stabil boleh mengekspreskan T7 RNA polymerase menggunakan 5 ratio and masa pengumpulan supernatant yang berlainan. AF2240-I rekombinan (rAF) mencatatkan indeks patogenitasi intraserebrum (ICPI) dan masa kematian minimum (MDT) antara 1.83-1.85 dan antara 47 j hingga 49 j masing-masing untuk laluan 1 hingga 5 kali di dalam telur. Kesimpulan dibuat bahawa 16 j selepas transfeksi, supernatan yang dikumpul cukup menghasilkan zarah virus berjangkit dan kesemua 5 ratio tiada keutamaan menghasilkan virus. Juga kedua-dua indeks patogenitasi intraserebrum menunjukkan bahawa rAF mempunyai keupayaan yang stabil untuk menyebabkan jangkitan walaupun selepas 5 laluan di dalam telur SPF. Oleh sedemikian, satu system pembalikan gen bagi NDV tempatan AF2240-I berjaya dihasilkan.

ACKNOWLEDGEMENTS

I would like to express my heartfelt gratitude to my supervisor, Prof. Datin Paduka Dr. Khatijah Mohamad Yusoff for giving me the trust to embark on this research during which I had very limited molecular biology knowledge. I appreciate her expertise, immense knowledge and skills that has assisted me throughout this research and in writing this thesis. On many occasions, her optimism has been my inspiration and has truly made difference in my life. I could not have imagined having another advisor for my PhD study.

I would like to thank the other members of my committee, Prof. Dr. Abdul Rahman Omar and Dr. Ben Peeters for the assistance they provided at all levels of the research project. Prof. Dr. Abdul Rahman Omar was never shy of time to help me in my research doubts throughout this four years. His understanding, and patience, added considerably to my graduate experience. Dr. Ben Peeters on the other hand, a researcher with vast experience had humbly accepted my request to serve as my external supervisor upon my first request. Without their support I would have not successfully completed this research.

My sincere thanks goes to Dr. Aidin Molouki for playing an important role in my support team. He provided me with direction, technical support and became more of a mentor and friend. It was through his, persistence, understanding and kindness that I was able to grasp the technical and molecular skills over a short span. I doubt that I will ever be able to convey my appreciation fully, but I owe him my eternal gratitude.

A very special thanks goes out to Dr. Tan Sheau Wei, Dr. Yeap Swee Keong together with other members of Vaccine and Immunotherapeutic Lab, Puan Nancy Liew, Puan Norhaszalina, and Puan Nurhafiza for always considering my request for access to the laboratory and research facilities. Without their precious support it would not be possible to conduct this research. Appreciation also goes out to all office staff of Institute Bioscience, UPM for all the instances in which their assistance helped me along the way.

I would also like to thank my fellow lab mates for the exchanges of knowledge, skills and the memories that we have had in the past four years. This particularly goes out to Dr. How Chee Wun, Ms.Yap Huan Yoong, Ms. Choong Oi Kuan, Dr. Foo Jhi Biau, Dr. Ng Wei Keat and Dr. Tor Yin Sim whom have proven that friends in need are friends in deed during my tough times.

I would also like to thank my father and my mother for the support they provided me through my entire life and had never doubted in my decision to pursue this PhD. My brothers and sisters-in-law for supporting me emotionally throughout writing this thesis and my life in general. In particular, I must acknowledge my husband and best friend, Roy Mithran, without whose love, encouragement and editing assistance, I would not have finished this thesis. Last but not least, I thank God for being my strength and guide in the writing of this thesis whom without I would not have had the wisdom or the physical ability to do so.

In conclusion, I recognize that this research would not have been possible without the financial assistance by Graduate Research Fellowship (GRF) and MyBrain15 Scholarship.

I certify that a Thesis Examination Committee has met on 19 February 2016 to conduct the final examination of Kavitha a/p Murulitharan on her thesis entitled "Characterisation and Rescue of a Recombinant Newcastle Disease Virus Strain AF2240-I" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

Muhajir bin Hamid, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Abhimanyu a/l Veerakumarasivam, PhD
Senior Lecturer
Faculty of Medicine and Health Science
Universiti Putra Malaysia
(Internal Examiner)

Syahrilnizam bin Abdullah, PhD
Associate Professor
Faculty of Medicine and Health Science
Universiti Putra Malaysia
(Internal Examiner)

Chew Fook Tim, PhD
Associate Professor
National University of Singapore
Singapore
(External Examiner)

ZULKARNAIN ZAINAL, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 21 April 2016

This thesis was submitted to the Senate of the Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

Datin Paduka Khatijah Mohamad Yusoff, PhD
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia,
(Chairman)

Abdul Rahman Omar, PhD
Professor
Institute Bioscience,
Universiti Putra Malaysia,
(Member)

Ben Peeters, PhD
Central Veterinary Institute,
Wageningen University and Research Centre,
Lelystad, The Netherlands
(Member)

ROBIAH BINTI YUNUS, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

Declaration by graduate student

I hereby confirm that:

- this thesis is my original work;
- quotations, illustrations and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any institutions;
- intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software

Signature: _____ Date: _____

Name and Matric No.: Kavitha Murulitharan (GS30844)

Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) were adhered to.

Signature: _____
Name of Chairman of Supervisory Committee: Professor Dr. Datin Paduka Khatijah Mohamad Yusoff

Signature: _____
Name of Member of Supervisory Committee: Professor Dr. Abdul Rahman Omar

Signature: _____
Name of Member of Supervisory Committee: Dr. Ben Peeters

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

NDV	Newcastle disease virus
aa	Amino acid
AIV	Avian influenza virus
APS	Ammonium persulfate
BHK	Baby hamster kidney
bp	Base pairs
BSA	Bovine serum albumin
cDNA	Complementary deoxyribonucleic acid
CNS	Central nervous system
DEPC	Diethylpyrocarbonate
dH_20	Distilled water
DIVA	Differentiating vaccinated animals from their infected
DMEM	Dulbecco's modified eagle medium
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide
DTT	1,4-dithiothritol
EDTA	Ethylenediaminetetraacetic acid
EGFP	Enhanced green fluorescent protein
F	Fusion
FBS	Foetal bovine serum
GFP	Green fluorescent protein
GMEM	Glasgow modified eagle medium
h	Hour

HA	Haemagglutination
HAU	Haemagglutination units
HDV	Hepatitis delta virus
HN	Haemagglutinin-neuraminidase
IBDV	Infectious bursal disease virus
ICPI	Intra-cerebral pathogenicity index
IFN	Interferon
Ig	Immunoglobulin
IGS	Intergenic sequences
IL	Interleukin
kDa	Kilo Dalton
L	Large
LB	Luria broth
M	Matrix
MDT	Mean death time
mRNA	Messenger ribonucleic acid
NA	Neuraminidase
NBT	Nitro blue tetrazolium
NCS	Newborn calf serum
NK	Natural killer
NP	Nucleoprotein
nt	Nucleotides
OD	Optical density
ORF	Open reading frame
P	Phospo

PBS	Phosphate buffer saline
PCR	Polymerase chain reaction
p.t.	Post transfection
RACE	Rapid amplification of cdna ends
rAF	Recombinant AF2240-I
RBC	Red blood cells
RdRp	RNA dependent RNA polymerase
RE	Restriction enzyme
RIPA	Radio-immunoprecipitation assay
RNA	Ribonucleic acid
Rnase	Ribonuclease
rNDV	Recombinant Newcastle disease virus
RNP	Ribonucleoprotein
RPM	Revolution per minute
RT	Room temperature
RT-PCR	Reverse transcriptase-polymerase chain reaction
RVFV	Rift Valley Fever virus
SDS PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SPF	Specific-pathogen free
TAE	Tris-acetate- EDTA buffer
Taq	*Thermus aquaticus*
TBS	Tris buffered saline
TEMED	Tetramethylethylenediamine
µg	Microram
µl	Microlitre

µM	Micromolar
UTR	Untranslated regions
UV	Ultra violet
VLP	Virus like particles
w/v	Weight/volume

CHAPTER 1

INTRODUCTION

1.1 Background

Newcastle disease, caused by Newcastle disease virus (NDV) or avian paramyxovirus 1 is a poultry disease that affects chicken and many other known birds. It has resulted in severe economic losses to the poultry industry worldwide and remains a constant threat. The virus is a member of the genus *Avulavirus* of the family *Paramyxoviridae* (Alexander, 1997).

Different strains of viruses exhibit different pathogenicity and virulence. The contribution of specific proteins to the pathogenicity has been studied extensively and current progress has narrowed on the role of HN, F and a non-structural V protein. The HN protein allows the attachment of the virus on host cell membranes (Deng *et al.*, 1999), the cleavage activation of F gene and the removal of progeny virions from host cell. The F gene then mediates the fusion of viral envelope with host cell membrane (Lamb *et al.*, 2006). Finally the V gene acts as an alpha interferon antagonist. The genome is flanked by a leader and trailer region. Each gene has conserved regions at the beginning and the end of the genes for transcriptional control purposes. Located between the gene boundaries are the non-coding intergenic (ITG) sequences which vary in sizes (Figure 1.1) (Krishnamurthy and Samal, 1998).

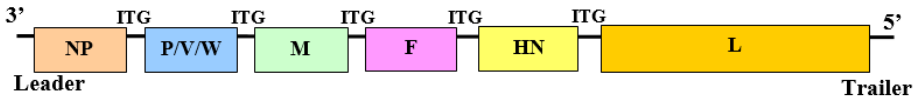

Figure 1.1 : NDV genomic representation.

Negative-stranded RNA virus genome of NDV encodes six genes nucleoprotein (NP), phosphoprotein (P), matrix (M), fusion (F), haemagglutinin-neuraminidase (HN) and large (L) gene. The genome itself is flanked by a leader and trailer region. The genes are separated by intergenes (ITG) which varies in lengths. All genes except for P encodes for one structural proteins from a single open reading frame (ORF). The P gene expresses the P protein and an additional of V and W protein by mRNA editing process during P gene transcription (Paldurai *et al.*, 2010).

Reverse genetics is a technique that enables the production of an infectious viral particle from complementary deoxyribonucleic acid (cDNA) plasmids. This technique has established a solid platform for research on negative stranded ribonucleic acid (RNA) viruses whereby the negative stranded RNA viruses lack the ability to initiate an infectious cycle unlike positive stranded RNA viruses (Romer-Oberdorfer *et al.*, 1999). This can be overcome by reverse genetics technique which allows the viruses to be recovered from plasmids, making it easier to manipulate the negative stranded viral genome (Schnell *et al.*, 1994). Such modifications allow the genomes of these viruses to be manipulated for further molecular understanding of the virus, its pathogenicity and virulence.

Prior to this, the vaccination programmes for NDV depended on live attenuated lentogenic strains. The newly emerging NDV strains are somehow able to overcome the effectiveness of live attenuated vaccines. The reverse genetics system therefore has allowed for the production of a more stable and efficacious attenuated vaccine due to better understanding of its virulence. Even though attenuated vaccine provide substantial protection from the disease, virus shedding is not completely prevented and disease can still occur in vaccinated birds. There still exists a need to continue to develop better vaccines to protect birds from both the disease and the infection to reduce virus shedding (Miller *et al.*, 2013). In addition, the current NDV vaccines are bivalent vector which would be able to offer protection against two diseases besides NDV such as avian influenza virus (AIV) and infectious bursal disease virus (IBDV) (Kanabagatte Basavarajappa *et al.*, 2014, Kim *et al.*, 2014, Liu *et al.*, 2015).

Besides being useful as a poultry vaccine, NDV possesses a natural ability to target and kill cancer cells leaving normal cells undisturbed (Russell *et al.*, 2012). Due to this property, NDV has become a favourite in virotherapy against cancer as it possess many advantages such as tumour selective replication and safety profile, its oncolytic potential and its immunostimulatory properties when compared to other oncolytic viruses (Fournier and Schirrmacher, 2013). Reverse genetics has boosted further the potential of NDV for the fight against cancer. Among the developments of NDV cancer vectors is the production of recombinant NDV with the expression of immuno-stimulators which allows and enhanced oncolytic property.

1.2 Problem Statement

The current study focuses on establishing a reverse genetics system for the local NDV strain AF2240-I. This strain is a viscerotropic velogenic genotype VIII strain. It was first isolated in Malaysia in the 1960's and is being used as a challenge virus in vaccine trials (Yusoff and Tan, 2001). The oncolytic study of this strain has been conducted by both *in vitro* and *in vivo* means. It has shown to act as a good apoptotic agent in several tumour lines such as 4T1 breast cancer cell line, MCF 7, WEHI-3B leukemic, brain tumour cell line U-87MG and HeLa cell line (Motalleb *et al.*, 2009, Othman *et al.*, 2009, Molouki *et al.*, 2010, Alabsi *et al.*, 2011, Ali *et al.*, 2011). Overall, it was demonstrated that AF2240-I possess

the ability to induce apoptosis in many tumour cell lines. Although various research are being carried out on negative stranded viruses in Malaysia, particularly NDV, there has been no development of a recombinant virus via reverse genetics method.

Prior to generating a reverse genetics system, it is essential that the complete genome sequence of AF2240-I to be established and this has yet to be done. Knowledge of the sequence would enable researchers to identify all restriction sites within the genome which ultimately will assist in the development of an antigenome cDNA, a crucial step in reverse genetics. Moreover, it was necessary to recognise the complete sequence for genome modifications to be conducted.

There has been a report indicating that recombinant viruses may revert mutation and lose its properties (de Leeuw *et al.*, 2003) after one passage. An important property of AF240-I is its velogenic virulence which as a lytic NDV strain, it is able to continuously produce infectious viral particles thereby leading to an amplification of the viral load; essential as an oncolytic virus (Schirrmacher and Fournier, 2009). Therefore, upon any successful rescue of a virus via reverse genetics, the virus must be ensured to be stable especially its pathogenicity.

1.3 Objectives

The objectives of this study are:

a) To sequence the full genome of NDV strain AF2240-I and perform *in-silico* characteristics analysis

b) To generate a reverse genetics system to rescue NDV strain AF2240-I.

c) To ensure the stability of pathogenicity of the rescued recombinant NDV strain AF2240-I

1.4 Significance of study

This research applies the principle of reverse genetics based on a synthetic NDV antigenome which was co-transfected with helper plasmids nucleoprotein (NP), phosphoprotein (P) and large protein (L). The virus rescue will be successfully performed in a shorter duration as compared to overlap PCR or restriction enzyme method. It is believed that this approach is important for the field of virology which is constantly subjected to new strains of viruses. Reverse genetics can be utilised as an important tool for the analysis of a negative stranded virus's genomic functions, development of novel poultry vaccines and oncolytic studies and allows to expedite the current related research that are taking place in Malaysia.

On an international level, despite increasing number of recombinant NDV strains being produced worldwide for animal vaccine and cancer research, an effective virotherapy has not yet been fully materialised (Buijs *et al.*, 2015, Cardenas-Garcia *et al.*, 2015). Virotherapy studies are currently being conducted to effectively improvise the application of NDV (Schirrmacher, 2005, Lorence *et al.*, 2007, Kim *et al.*, 2014). However, reverse genetics system must be applied for such studies and with the development of reverse genetics system in Malaysia, this matter can be helped to be addressed.

Recombinant NDV as an animal vector has been highly promising, but there can be significant advantages with the use of vaccines homologous to circulating strains such as improved potential to reduce environmental viral load, increased level of specific humoral antibodies, and increased survival rates after challenge. Such improvised animal vaccine can be produced with the application our developed reverse genetics techniques for Malaysian researchers.

In the present work, critical components such as the ratio of transfection plasmids, duration to harvest viral particles from transfected cells and types of allantoic fluid inoculum which may influence virus rescue were compared. By optimising the experimental parameters it will be possible to develop a robust and highly efficient system for the rescue of full-length AF2240 from cDNA. The results of the experimental variations upon AF2240 rescue can provide valuable guidelines for the generation of other negative strand RNA viruses from cDNA.

1.5 Hypothesis

a) The sequenced genome of AF2240 will have similarity up to 99% with previously published AF2240 NCBI sequences
b) The recombinant virus strain AF2240-I would be successfully rescued using a synthetically produced antigenome cDNA with helper plasmids expressing NP,P and L genes.
c) The rescued recombinant virus will stably maintain its pathogenicity as compared to its wild type after several passages.

REFERENCES

Abolnik, C., R. F. Horner, S. P. Bisschop, M. E. Parker, M. Romito and G. J. Viljoen (2004). "A phylogenetic study of South African Newcastle disease virus strains isolated between 1990 and 2002 suggests epidemiological origins in the Far East." Arch Virol 149(3): 603-619.

Alabsi, A. M., S. A. Bakar, R. Ali, A. R. Omar, M. H. Bejo, A. Ideris and A. M. Ali (2011). "Effects of Newcastle Disease virus strains AF2240 and V4-UPM on cytolysis and apoptosis of leukemia cell lines." Int J Mol Sci 12(12): 8645-8660.

Aldous, E. W. and D. J. Alexander (2001). "Detection and differentiation of Newcastle disease virus (avian paramyxovirus type 1)." Avian Pathol 30(2): 117-128.

Aldous, E. W., J. K. Mynn, J. Banks and D. J. Alexander (2003). "A molecular epidemiological study of avian paramyxovirus type 1 (Newcastle disease virus) isolates by phylogenetic analysis of a partial nucleotide sequence of the fusion protein gene." Avian Pathol 32(3): 239-256.

Alexander, D. J. (1997). Newcastle disease and other avian Paramyxoviridae infections.

Alexander, D. J., E. W. Aldous and C. M. Fuller (2012). "The long view: a selective review of 40 years of Newcastle disease research." Avian Pathol 41(4): 329-335.

Ali, R., A. M. Alabsi, A. M. Ali, A. Ideris, A. R. Omar, K. Yusoff and R. Saif-Ali (2011). "Cytolytic effects and apoptosis induction of Newcastle disease virus strain AF2240 on anaplastic astrocytoma brain tumor cell line." Neurochem Res 36(11): 2051-2062.

Auer, R. and J. C. Bell (2012). "Oncolytic viruses: smart therapeutics for smart cancers." Future Oncol 8(1): 1-4.

Bai, F., Z. Niu, H. Tian, S. Li, Z. Lv, T. Zhang, G. Ren and D. Li (2014). "Genetically engineered Newcastle disease virus expressing interleukin 2 is a potential drug candidate for cancer immunotherapy." Immunol Lett 159(1-2): 36-46.

Bai, F. L., H. Tian, Q. Z. Yu, G. P. Renl and D. S. Li (2015). "[Expressing foreign genes by Newcastle disease virus for cancer therapy]." Mol Biol (Mosk) 49(2): 195-204.

Balazs, D. A. and W. Godbey (2011). "Liposomes for Use in Gene Delivery." Journal of Drug Delivery 2011.

Ballagi-Pordány, A., E. Wehmann, J. Herczeg, S. Belák and B. Lomniczi (1996). "Identification and grouping of Newcastle disease virus strains by restriction site analysis of a region from the F gene." Arch Virol 141(2): 243-261.

Bauzon, M. and T. Hermiston (2014). "Armed therapeutic viruses - a disruptive therapy on the horizon of cancer immunotherapy." Front Immunol 5: 74.

Berman, H. M., J. Westbrook, Z. Feng, G. Gilliland, T. N. Bhat, H. Weissig, I. N. Shindyalov and P. E. Bourne (2000). "The Protein Data Bank." Nucleic Acids Res 28(1): 235-242.

Biebricher, C. K. and M. Eigen (2006). "What is a quasispecies?" Curr Top Microbiol Immunol 299: 1-31.

Biswas, M., J. B. Johnson, S. R. Kumar, G. D. Parks and S. Elankumarana (2012). "Incorporation of host complement regulatory proteins into Newcastle disease virus enhances complement evasion." J Virol 86(23): 12708-12716.

Blumberg, B. M. and D. Kolakofsky (1981). "Intracellular vesicular stomatitis virus leader RNAs are found in nucleocapsid structures." J Virol 40(2): 568-576.

Buijs, P., S. van Nieuwkoop, V. Vaes, R. Fouchier, C. van Eijck and B. Hoogen (2015). "Recombinant Immunomodulating Lentogenic or Mesogenic Oncolytic Newcastle Disease Virus for Treatment of Pancreatic Adenocarcinoma." Viruses 7(6): 2980-2998.

Cardenas-Garcia, S., D. G. Diel, L. Susta, E. Lucio-Decanini, Q. Yu, C. C. Brown, P. J. Miller and C. L. Afonso (2015). "Development of an improved vaccine evaluation protocol to compare the efficacy of Newcastle disease vaccines." Biologicals 43(2): 136-145.

Cattoli, G., A. Fusaro, I. Monne, S. Molia, A. Le Menach, B. Maregeya, A. Nchare, I. Bangana, A. G. Maina, J. N. Koffi, H. Thiam, O. E. Bezeid, A. Salviato, R. Nisi, C. Terregino and I. Capua (2010). "Emergence of a new genetic lineage of Newcastle disease virus in West and Central Africa--implications for diagnosis and control." Vet Microbiol 142(3-4): 168-176.

Chambers, P., N. S. Millar, R. W. Bingham and P. T. Emmerson (1986). "Molecular cloning of complementary DNA to Newcastle disease virus, and nucleotide sequence analysis of the junction between the genes encoding the haemagglutinin-neuraminidase and the large protein." J Gen Virol 67 (Pt 3): 475-486.

Collins, M. S., J. B. Bashiruddin and D. J. Alexander (1993). "Deduced amino acid sequences at the fusion protein cleavage site of Newcastle disease viruses showing variation in antigenicity and pathogenicity." Arch Virol 128(3-4): 363-370.

Connaris, H., T. Takimoto, R. Russell, S. Crennell, I. Moustafa, A. Portner and G. Taylor (2002). "Probing the sialic acid binding site of the hemagglutinin-neuraminidase of Newcastle disease virus: Identification of key amino acids involved in cell binding, catalysis, and fusion." J Virol 76(4): 1816-1824.

Courtney, S. C., L. Susta, D. Gomez, N. L. Hines, J. C. Pedersen, C. C. Brown, P. J. Miller and C. L. Afonso (2013). "Highly divergent virulent isolates of Newcastle disease virus from the Dominican Republic are members of a new genotype that may have evolved unnoticed for over 2 decades." J Clin Microbiol 51(2): 508-517.

Curran, J., J. B. Marq and D. Kolakofsky (1995). "An N-terminal domain of the Sendai paramyxovirus P protein acts as a chaperone for the NP protein during the nascent chain assembly step of genome replication." J Virol 69(2): 849-855.

Czegledi, A., D. Ujvari, E. Somogyi, E. Wehmann, O. Werner and B. Lomniczi (2006). "Third genome size category of avian paramyxovirus serotype 1 (Newcastle disease virus) and evolutionary implications." Virus Res 120(1-2): 36-48.

Czeglédi, A., D. Ujvári, E. Somogyi, E. Wehmann, O. Werner and B. Lomniczi (2006). "Third genome size category of avian paramyxovirus serotype 1 (Newcastle disease virus) and evolutionary implications." Virus Res 120(1–2): 36-48.

de la Torre, J. C. (2006). "Reverse-genetic approaches to the study of Borna disease virus." Nat Rev Micro 4(10): 777-783.

de Leeuw, O. and B. Peeters (1999). "Complete nucleotide sequence of Newcastle disease virus: evidence for the existence of a new genus within the subfamily Paramyxovirinae." J Gen Virol 80 (Pt 1): 131-136.

de Leeuw, O. S., L. Hartog, G. Koch and B. P. Peeters (2003). "Effect of fusion protein cleavage site mutations on virulence of Newcastle disease virus: non-virulent cleavage site mutants revert to virulence after one passage in chicken brain." J Gen Virol 84(Pt 2): 475-484.

de Leeuw, O. S., G. Koch, L. Hartog, N. Ravenshorst and B. P. Peeters (2005). "Virulence of Newcastle disease virus is determined by the cleavage site of the fusion protein and by both the stem region and globular head of the haemagglutinin-neuraminidase protein." J Gen Virol 86(Pt 6): 1759-1769.

Deng, R., Z. Wang, P. J. Mahon, M. Marinello, A. Mirza and R. M. Iorio (1999). "Mutations in the Newcastle disease virus hemagglutinin–neuraminidase protein that interfere with its ability to interact with the homologous F protein in the promotion of Fusion." Virology 253(1): 43-54.

Diel, D. G., L. H. da Silva, H. Liu, Z. Wang, P. J. Miller and C. L. Afonso (2012). "Genetic diversity of avian paramyxovirus type 1: proposal for a unified nomenclature and classification system of Newcastle disease virus genotypes." Infect Genet Evol 12(8): 1770-1779.

Domingo, E., J. Sheldon and C. Perales (2012). "Viral quasispecies evolution." Microbiol Mol Biol Rev 76(2): 159-216.

Dortmans, J., G. Koch, P. Rottier and B. Peeters (2011). "Virulence of newcastle disease virus: what is known so far?" Veterinary Research 42(1): 122.

Doyle, T. M. (1927). "A hitherto unrecorded disease of fowls due to filter-passing virus." J Comp Pathol 40: 144-169.

Elankumaran, S., D. Rockemann and S. K. Samal (2006). "Newcastle disease virus exerts oncolysis by both intrinsic and extrinsic caspase-dependent pathways of cell death." J Virol 80(15): 7522-7534.

Feng, H., D. Wei, G. Nan, S. J. Cui, Z. N. Chen and H. Bian (2011). "Construction of a minigenome rescue system for Newcastle disease virus strain Italien." Arch Virol 156(4): 611-616.

Fournier, P. and V. Schirrmacher (2013). "Oncolytic Newcastle Disease Virus as Cutting Edge between Tumor and Host." Biology 2(3): 936.

Freiberg, A., L. K. Dolores, S. Enterlein and R. Flick (2008). "Establishment and characterization of plasmid-driven minigenome rescue systems for Nipah virus: RNA polymerase I- and T7-catalyzed generation of functional paramyxoviral RNA." Virology 370(1): 33-44.

Galinski, M. S. and S. L. Wechsler (1991). The molecular biology of the *Paramyxovirus* genus. The *Paramyxoviruses*. D. W. Kingsbury. New York, NY, USA, Plenum Press: 41-72.

Garten, W., T. Kohama and H. D. Klenk (1980). "Proteolytic activation of the haemagglutinin-neuraminidase of Newcastle disease virus involves loss of a glycopeptide." J Gen Virol 51(Pt 1): 207-211.

Ge, J., G. Deng, Z. Wen, G. Tian, Y. Wang, J. Shi, X. Wang, Y. Li, S. Hu, Y. Jiang, C. Yang, K. Yu, Z. Bu and H. Chen (2007). "Newcastle disease virus-based live attenuated vaccine completely protects chickens and mice from lethal challenge of homologous and heterologous H5N1 avian influenza viruses." J Virol 81(1): 150-158.

Gould, A. R., E. Hansson, K. Selleck, J. A. Kattenbelt, M. Mackenzie and A. J. Della-porta (2003). "Newcastle disease virus fusion and haemagglutinin-neuraminidase gene motifs as markers for viral lineage." Avian Pathol 32(4): 361-373.

Hamaguchi, M., T. Yoshida, K. Nishikawa, H. Naruse and Y. Nagai (1983). "Transcriptive complex of Newcastle disease virus. I. Both L and P proteins are required to constitute an active complex." Virology 128(1): 105-117.

Herczeg, J., S. Pascucci, P. Massi, M. Luini, L. Selli, I. Capua and B. Lomniczi (2001). "A longitudinal study of velogenic Newcastle disease virus genotypes isolated in Italy between 1960 and 2000." Avian Pathol 30(2): 163-168.

Herczeg, J., E. Wehmann, R. R. Bragg, P. M. Travassos Dias, G. Hadjiev, O. Werner and B. Lomniczi (1999). "Two novel genetic groups (VIIb and VIII) responsible for recent Newcastle disease outbreaks in Southern Africa, one (VIIb) of which reached Southern Europe." Arch Virol 144(11): 2087-2099.

Holmes, D. E. and S. A. Moyer (2002). "The phosphoprotein (P) binding site resides in the N terminus of the L polymerase subunit of sendai virus." J Virol 76(6): 3078-3083.

Horikami, S. M., J. Curran, D. Kolakofsky and S. A. Moyer (1992). "Complexes of Sendai virus NP-P and P-L proteins are required for defective interfering particle genome replication in vitro." J Virol 66(8): 4901-4908.

Huang, Z., S. Elankumaran, A. Panda and S. K. Samal (2003). "Recombinant Newcastle disease virus as a vaccine vector." Poult Sci 82(6): 899-906.

Huang, Z., S. Elankumaran, A. S. Yunus and S. K. Samal (2004). "A recombinant Newcastle disease virus (NDV) expressing VP2 protein of infectious bursal disease virus (IBDV) protects against NDV and IBDV." J Virol 78(18): 10054-10063.

Huang, Z., S. Krishnamurthy, A. Panda and S. K. Samal (2001). "High-level expression of a foreign gene from the most 3'-proximal locus of a recombinant Newcastle disease virus." J Gen Virol 82(Pt 7): 1729-1736.

Huang, Z., S. Krishnamurthy, A. Panda and S. K. Samal (2003). "Newcastle disease virus V protein is associated with viral pathogenesis and functions as an alpha interferon antagonist." J Virol 77(16): 8676-8685.

Huang, Z., A. Panda, S. Elankumaran, D. Govindarajan, D. D. Rockemann and S. K. Samal (2004). "The Hemagglutinin-Neuraminidase Protein of Newcastle Disease Virus Determines Tropism and Virulence." Journal of Virology 78(8): 4176-4184.

Jang, J., S. H. Hong, D. Choi, K. S. Choi, S. Kang and I. H. Kim (2010). "Overexpression of Newcastle disease virus (NDV) V protein enhances NDV production kinetics in chicken embryo fibroblasts." Appl Microbiol Biotechnol 85(5): 1509-1520.

Janke, M., B. Peeters, O. de Leeuw, R. Moorman, A. Arnold, P. Fournier and V. Schirrmacher (2007). "Recombinant Newcastle disease virus (NDV) with inserted gene coding for GM-CSF as a new vector for cancer immunogene therapy." Gene Ther 14(23): 1639-1649.

Jemain, S. F. P. b. (1998). Sequence determination of the matrix gene in Newcastle disease virus strain AF2240, Universiti Putra Malaysia.

Kanabagatte Basavarajappa, M., S. Kumar, S. K. Khattar, G. T. Gebreluul, A. Paldurai and S. K. Samal (2014). "A recombinant Newcastle disease virus (NDV) expressing infectious laryngotracheitis virus (ILTV) surface glycoprotein D protects against highly virulent ILTV and NDV challenges in chickens." Vaccine 32(28): 3555-3563.

Khattar, S. K., P. L. Collins and S. K. Samal (2010). "Immunization of cattle with recombinant Newcastle disease virus expressing bovine herpesvirus-1 (BHV-1) glycoprotein D induces mucosal and serum antibody responses and provides partial protection against BHV-1." Vaccine 28(18): 3159-3170.

Khattar, S. K., Y. Yan, A. Panda, P. L. Collins and S. K. Samal (2009). "A Y526Q mutation in the Newcastle disease virus HN protein reduces its functional activities and attenuates virus replication and pathogenicity." J Virol 83(15): 7779-7782.

Kho, C. L., W. S. Tan and K. Yusoff (2001). "Sequence analysis of the nucleoprotein of a Malaysian heat resistant NDV strain: comparison with other members of Paramyxoviridae." Journal of Biochemistry Molecular Biology and Biophysics 5: 463-471.

Kho, C. L., W. S. Tan and K. Yusoff (2002). "Cloning and expression of the phosphoprotein gene of Newcastle disease virus in Escherichia coli." J Biochem Mol Biol Biophys 6(2): 117-121.

Kim, S. H., A. Paldurai, S. Xiao, P. L. Collins and S. K. Samal (2014). "Modified Newcastle disease virus vectors expressing the H5 hemagglutinin induce enhanced protection against highly pathogenic H5N1 avian influenza virus in chickens." Vaccine 32(35): 4428-4435.

Kim, S. H. and S. K. Samal (2009). "Role of Untranslated Regions in Regulation of Gene Expression, Replication, and Pathogenicity of Newcastle Disease Virus Expressing Green Fluorescent Protein." Journal of Virology 84(5): 2629-2634.

Kim, T. K. and J. H. Eberwine (2010). "Mammalian cell transfection: the present and the future." Anal Bioanal Chem 397(8): 3173-3178.

Kong, D., Z. Wen, H. Su, J. Ge, W. Chen, X. Wang, C. Wu, C. Yang, H. Chen and Z. Bu (2012). "Newcastle disease virus-vectored Nipah encephalitis vaccines induce B and T cell responses in mice and long-lasting neutralizing antibodies in pigs." Virology 432(2): 327-335.

Kortekaas, J., S. M. de Boer, J. Kant, R. P. Vloet, A. F. Antonis and R. J. Moormann (2010). "Rift Valley fever virus immunity provided by a paramyxovirus vaccine vector." Vaccine 28(27): 4394-4401.

Kraneveld, F. C. (1926). "A poultry disease in the Dutch East Indies." Nederlands-Indische Bladen voor Diergeneedkunde 38: 448-450.

Krishnamurthy, S., Z. Huang and S. K. Samal (2000). "Recovery of a virulent strain of Newcastle disease virus from cloned cDNA: Expression of a foreign gene results in growth retardation and attenuation." Virology 278(1): 168-182.

Krishnamurthy, S. and S. K. Samal (1998). "Nucleotide sequences of the trailer, nucleocapsid protein gene and intergenic regions of Newcastle disease virus strain Beaudette C and completion of the entire genome sequence." J Gen Virol 79(10): 2419-2424.

Kusumaningtyas, E., W. S. Tan, Z. Zamrod, M. Eshaghi and K. Yusoff (2004). "Existence of two forms of L protein of Newcastle disease virus isolates due to a compensatory mutation in Domain V." Archives of Virology 149(9): 1859-1865.

Lamb, A. R. and D. Kolakofsky (2007). Paramyxoviridae: the viruses and their replication. D. M. Knipe, P. M. Howley, E. D. Griffin *et al.* Philadelphia, Lippincott-Raven Press: 1449-1496.

Lamb, R. A. and D. Kolakofsky (1996). *Paramyxoviridae*: the viruses and their replication. Fields Virology. B. N. Fields, D. M. Knipe and P. M. Howley. New York, Lippincott-Raven: 1177-1204.

Lamb, R. A., R. G. Paterson and T. S. Jardetzky (2006). "Paramyxovirus membrane fusion: lessons from the F and HN atomic structures." Virology 344(1): 30-37.

Lardinois, A., M. Steensels, B. Lambrecht, N. Desloges, M. Rahaus, D. Rebeski and T. van den Berg (2012). "Potency of a recombinant NDV-H5 vaccine against various HPAI H5N1 virus challenges in SPF chickens." Avian Dis 56(4 Suppl): 928-936.

Lauring, A. S. and R. Andino (2010). "Quasispecies theory and the behavior of RNA viruses." PLoS Pathog 6(7): e1001005.

Li, M., P. T. Schmitt, Z. Li, T. S. McCrory, B. He and A. P. Schmitt (2009). "Mumps virus matrix, fusion, and nucleocapsid proteins cooperate for efficient production of virus-like particles." J Virol 83(14): 7261-7272.

Liang, R., D. J. Cao, J. Q. Li, J. Chen, X. Guo, F. F. Zhuang and M. X. Duan (2002). "Newcastle disease outbreaks in western China were caused by the genotypes VIIa and VIII." Vet Microbiol 87(3): 193-203.

Lien, Y. Y., J. W. Lee, H. Y. Su, H. J. Tsai, M. C. Tsai, C. Y. Hsieh and S. S. Tsai (2007). "Phylogenetic characterization of Newcastle disease viruses isolated in Taiwan during 2003-2006." Vet Microbiol 123(1-3): 194-202.

Liu, Y. L, Zhang, Y. M, Hu, S. L, Wu, Y. T, Liu, X. F, Long, J. X, Shi, H. Y, Zhang, X. R, Zhang, R. K (2005). "Generation of newcastle disease virus strain ZJI isolated from an outbreak in the goose using reverse genetics technique." Wei Sheng Wu Xue Bao 45(5):780-3

Liu, H., Z. Wang, Y. Wu, D. Zheng, C. Sun, D. Bi, Y. Zuo and T. Xu (2007). "Molecular epidemiological analysis of Newcastle disease virus isolated in China in 2005." J Virol Methods 140(1-2): 206-211.

Liu, Q., I. Mena, J. Ma, B. Bawa, F. Krammer, Y. S. Lyoo, Y. Lang, I. Morozov, G. N. Mahardika, W. Ma, A. Garcia-Sastre and J. A. Richt (2015). "Newcastle disease virus-vectored H7 and H5 live vaccines protect chickens from challenge with H7N9 or H5N1 avian influenza viruses." J Virol.

Liu, X. F., H. Q. Wan, X. X. Ni, Y. T. Wu and W. B. Liu (2003). "Pathotypical and genotypical characterization of strains of Newcastle disease virus isolated from outbreaks in chicken and goose flocks in some regions of China during 1985-2001." Arch Virol 148(7): 1387-1403.

Lomniczi, B., E. Wehmann, J. Herczeg, A. Ballagi-Pordany, E. F. Kaleta, O. Werner, G. Meulemans, P. H. Jorgensen, A. P. Mante, A. L. Gielkens, I. Capua and J. Damoser (1998). "Newcastle disease outbreaks in recent years in western Europe were caused by an old (VI) and a novel genotype (VII)." Arch Virol 143(1): 49-64.

Lorence, R. M., M. S. Roberts, J. D. O'Neil, W. S. Groene, J. A. Miller, S. N. Mueller and M. K. Bamat (2007). "Phase 1 clinical experience using intravenous administration of PV701, an oncolytic Newcastle disease virus." Curr Cancer Drug Targets 7(2): 157-167.

McGinnes, L. W. and T. G. Morrison (1986). "Nucleotide sequence of the gene encoding the Newcastle disease virus fusion protein and comparisons of paramyxovirus fusion protein sequences." Virus Res 5(4): 343-356.

Mebatsion, T., S. Verstegen, L. T. De Vaan, A. Romer-Oberdorfer and C. C. Schrier (2001). "A recombinant newcastle disease virus with low-level V protein expression is immunogenic and lacks pathogenicity for chicken embryos." J Virol 75(1): 420-428.

Millar, N. S., P. Chambers and P. T. Emmerson (1988). "Nucleotide sequence of the fusion and haemagglutinin-neuraminidase glycoprotein genes of Newcastle disease virus, strain Ulster: molecular basis for variations in pathogenicity between strains." J Gen Virol 69 (Pt 3): 613-620.

Miller, P. J., C. L. Afonso, J. El Attrache, K. M. Dorsey, S. C. Courtney, Z. Guo and D. R. Kapczynski (2013). "Effects of Newcastle disease virus vaccine antibodies on the shedding and transmission of challenge viruses." Developmental & Comparative Immunology 41(4): 505-513.

Miller, P. J., E. L. Decanini and C. L. Afonso (2010). "Newcastle disease: evolution of genotypes and the related diagnostic challenges." Infect Genet Evol 10(1): 26-35.

Miller, P. J., R. Haddas, L. Simanov, A. Lublin, S. F. Rehmani, A. Wajid, T. Bibi, T. A. Khan, T. Yaqub, S. Setiyaningsih and C. L. Afonso (2015). "Identification of new sub-genotypes of virulent Newcastle disease virus with potential panzootic features." Infect Genet Evol 29: 216-229.

Miller, P. J., D. J. King, C. L. Afonso and D. L. Suarez (2007). "Antigenic differences among Newcastle disease virus strains of different genotypes used in vaccine formulation affect viral shedding after a virulent challenge." Vaccine 25(41): 7238-7246.

Molouki, A., Y. T. Hsu, F. Jahanshiri, R. Rosli and K. Yusoff (2010). "Newcastle disease virus infection promotes Bax redistribution to mitochondria and cell death in HeLa cells." Intervirology 53(2): 87-94.

Morodomi, Y., M. Inoue, M. Hasegawa, T. Okamoto, Y. Maehara and Y. Yonemitsu (2013). Sendai Virus-Based Oncolytic Gene Therapy.

Motalleb, G., F. Othman, A. Ideris and A. Rahmat (2009). "Dissemination of Newcastle Disease Virus (NDV-AF2240) in Liver during Intratumoral Injection of Xenotransplant Breast Cancer in BALB/c Mice." Yakteh Medical Journal 11(3): 303-310.

Murphy, F. A., International Committee on Taxonomy of Viruses, , International Union of Microbiological Societies. Virology Division (1995). Virus taxonomy. Classification and nomenclature of viruses : sixth report of the International Committee on Taxonomy of Viruses, Springer-Verlag, 1995.

Nagai, Y., M. Hamaguchi and T. Toyoda (1989). "Molecular biology of Newcastle disease virus." Prog Vet Microbiol Immunol 5: 16-64.

Nagai, Y., H. D. Klenk and R. Rott (1976). "Proteolytic cleavage of the viral glycoproteins and its significance for the virulence of Newcastle disease virus." Virology 72(2): 494-508.

Nakaya, T., J. Cros, M.-S. Park, Y. Nakaya, H. Zheng, A. Sagrera, E. Villar, A. García-Sastre and P. Palese (2001). "Recombinant Newcastle disease virus as a vaccine vector." J Virol 75(23): 11868-11873.

Nayak, B., S. N. Rout, S. Kumar, M. S. Khalil, M. M. Fouda, L. E. Ahmed, K. C. Earhart, D. R. Perez, P. L. Collins and S. K. Samal (2009). "Immunization of chickens with Newcastle disease virus expressing H5 hemagglutinin protects against highly pathogenic H5N1 avian influenza viruses." PLoS One 4(8): e6509.

Othman, F., A. Ideris, G. Motalleb, Z. Eshak and A. Rahmat (2009). "Oncolytic effect of Newcastle disease virus AF2240 strain on the MCF-7 breast cancer cell line." Yakteh Medical Journal 12(1): 17-24.

Overbaugh, J. and C. R. Bangham (2001). "Selection forces and constraints on retroviral sequence variation." Science 292(5519): 1106-1109.

Paldurai, A., S. Kumar, B. Nayak and S. K. Samal (2010). "Complete genome sequence of highly virulent neurotropic Newcastle disease virus strain Texas GB." Virus Genes 41(1): 67-72.

Park, M. S., M. L. Shaw, J. Munoz-Jordan, J. F. Cros, T. Nakaya, N. Bouvier, P. Palese, A. Garcia-Sastre and C. F. Basler (2003). "Newcastle disease virus (NDV)-based assay demonstrates interferon-antagonist activity for the NDV V protein and the Nipah virus V, W, and C proteins." J Virol 77(2): 1501-1511.

Patton, J. T., N. L. Davis and G. W. Wertz (1984). "N protein alone satisfies the requirement for protein synthesis during RNA replication of vesicular stomatitis virus." J Virol 49(2): 303-309.

Peeters, B. P., O. S. de Leeuw, G. Koch and A. L. Gielkens (1999). "Rescue of Newcastle disease virus from cloned cDNA: evidence that cleavability of the fusion protein is a major determinant for virulence." J Virol 73(6): 5001-5009.

Peeters, B. P., Y. K. Gruijthuijsen, O. S. de Leeuw and A. L. Gielkens (2000). "Genome replication of Newcastle disease virus: involvement of the rule-of-six." Arch Virol 145(9): 1829-1845.

Phillips, R. J., A. C. R. Samson and P. T. Emmerson (1998). "Nucleotide sequence of the 5′-terminus of Newcastle disease virus and assembly of the complete genomic sequence: agreement with the “rule of six”." Arch Virol 143(10): 1993-2002.

Poch, O., B. M. Blumberg, L. Bougueleret and N. Tordo (1990). "Sequence comparison of five polymerases (L proteins) of unsegmented negative-strand RNA viruses: theoretical assignment of functional domains." J Gen Virol 71 (Pt 5): 1153-1162.

Porotto, M., Z. Salah, I. DeVito, A. Talekar, S. G. Palmer, R. Xu, I. A. Wilson and A. Moscona (2012). "The second receptor binding site of the globular head of the Newcastle disease virus hemagglutinin-neuraminidase activates the stalk of multiple paramyxovirus receptor binding proteins to trigger fusion." J Virol 86(10): 5730-5741.

Puhler, F., J. Willuda, J. Puhlmann, D. Mumberg, A. Romer-Oberdorfer and R. Beier (2008). "Generation of a recombinant oncolytic Newcastle disease virus and expression of a full IgG antibody from two transgenes." Gene Ther 15(5): 371-383.

Reichard, K. W., R. M. Lorence, C. J. Cascino, M. E. Peeples, R. J. Walter, M. B. Fernando, H. M. Reyes and J. A. Greager (1992). "Newcastle disease virus selectively kills human tumor cells." J Surg Res 52(5): 448-453.

Romer-Oberdorfer, A., E. Mundt, T. Mebatsion, U. J. Buchholz and T. C. Mettenleiter (1999). "Generation of recombinant lentogenic Newcastle disease virus from cDNA." J Gen Virol 80 (Pt 11): 2987-2995.

Romer-Oberdorfer, A., O. Werner, J. Veits, T. Mebatsion and T. C. Mettenleiter (2003). "Contribution of the length of the HN protein and the sequence of the F protein cleavage site to Newcastle disease virus pathogenicity." J Gen Virol 84(Pt 11): 3121-3129.

Rott, R. (1979). "Molecular basis of infectivity and pathogenicity of myxovirus. Brief review." Arch Virol 59(4): 285-298.

Rott, R. and H.-D. Klenk (1988). Molecular Basis of Infectivity and Pathogenicity of Newcastle Disease Virus. Newcastle Disease. D. J. Alexander, Springer US. 8: 98-112.

Russell, S. J., K.-W. Peng and J. C. Bell (2012). "Oncolytic virotherapy." Nat Biotech 30(7): 658-670.

Sakaguchi, T., T. Toyoda, B. Gotoh, N. M. Inocencio, K. Kuma, T. Miyata and Y. Nagai (1989). "Newcastle disease virus evolution: I. Multiple lineages defined by sequence variability of the hemagglutinin-neuraminidase gene." Virology 169(2): 260-272.

Salih, O., A. R. Ali and K. Yusoff (2000). "Nucleotide sequence analysis of the F protein gene of a Malaysian velogenic NDV strain AF2240." Journal of Biochemistry, Molecular Biology and Biophysics 4: 51-57.

Samal, S. K. and P. L. Collins (1996). "RNA replication by a respiratory syncytial virus RNA analog does not obey the rule of six and retains a nonviral trinucleotide extension at the leader end." J Virol 70(8): 5075-5082.

Sanchez-Felipe, L., E. Villar and I. Munoz-Barroso (2012). "alpha2-3- and alpha2-6- N-linked sialic acids allow efficient interaction of Newcastle Disease Virus with target cells." Glycoconj J 29(7): 539-549.

Sarfati-Mizrahi, D., B. Lozano-Dubernard, E. Soto-Priante, F. Castro-Peralta, R. Flores-Castro, E. Loza-Rubio and M. Gay-Gutierrez (2010). "Protective dose of a recombinant Newcastle disease LaSota-avian influenza virus H5 vaccine against H5N2 highly pathogenic avian influenza virus and velogenic viscerotropic Newcastle disease virus in broilers with high maternal antibody levels." Avian Dis 54(1 Suppl): 239-241.

Schirrmacher, V. (2005). "Clinical trials of antitumor vaccination with an autologous tumor cell vaccine modified by virus infection: improvement of patient survival based on improved antitumor immune memory." Cancer Immunol Immunother 54(6): 587-598.

Schirrmacher, V. and P. Fournier (2009). "Newcastle disease virus: a promising vector for viral therapy, immune therapy, and gene therapy of cancer." Methods Mol Biol 542: 565-605.

Schirrmacher, V., C. Haas, R. Bonifer, T. Ahlert, R. Gerhards and C. Ertel (1999). "Human tumor cell modification by virus infection: an efficient and safe way to produce cancer vaccine with pleiotropic immune stimulatory properties when using Newcastle disease virus." Gene Ther 6(1): 63-73.

Schnell, M. J., T. Mebatsion and K. K. Conzelmann (1994). "Infectious rabies viruses from cloned cDNA." EMBO J 13(18): 4195-4203.

Schutze, H., P. J. Enzmann, R. Kuchling, E. Mundt, H. Niemann and T. C. Mettenleiter (1995). "Complete genomic sequence of the fish rhabdovirus infectious haematopoietic necrosis virus." J Gen Virol 76 2519-2527.

Schutze, H., P. J. Enzmann, R. Kuchling, E. Mundt, H. Niemann and T. C. Mettenleiter (1995). "Complete genomic sequence of the fish rhabdovirus infectious haematopoietic necrosis virus." J Gen Virol 76 (Pt 10): 2519-2527.

Seal, B. S., D. J. King and J. D. Bennett (1995). "Characterization of Newcastle disease virus isolates by reverse transcription PCR coupled to direct nucleotide sequencing and development of sequence database for pathotype prediction and molecular epidemiological analysis." J Clin Microbiol 33(10): 2624-2630.

Seal, B. S., D. J. King, D. P. Locke, D. A. Senne and M. W. Jackwood (1998). "Phylogenetic relationships among highly virulent Newcastle disease virus isolates obtained from exotic birds and poultry from 1989 to 1996." J Clin Microbiol 36(4): 1141-1145.

Shobana, R., S. K. Samal and S. Elankumaran (2013). "Prostate-specific antigen-retargeted recombinant newcastle disease virus for prostate cancer virotherapy." J Virol 87(7): 3792-3800.

Snoeck, C. J., M. F. Ducatez, A. A. Owoade, O. O. Faleke, B. R. Alkali, M. C. Tahita, Z. Tarnagda, J. B. Ouedraogo, I. Maikano, P. O. Mbah, J. R. Kremer and C. P. Muller (2009). "Newcastle disease virus in West Africa: new virulent strains identified in non-commercial farms." Arch Virol 154(1): 47-54.

Snoeck, C. J., A. A. Owoade, E. Couacy-Hymann, B. R. Alkali, M. P. Okwen, A. T. Adeyanju, G. F. Komoyo, E. Nakoune, A. Le Faou and C. P. Muller (2013). "High genetic diversity of Newcastle disease virus in poultry in West and Central Africa: cocirculation of genotype XIV and newly defined genotypes XVII and XVIII." J Clin Microbiol 51(7): 2250-2260.

Steinhauer, D. A. and J. J. Holland (1987). "Rapid evolution of RNA viruses." Annu Rev Microbiol 41: 409-433.

Steward, M., I. B. Vipond, N. S. Millar and P. T. Emmerson (1993). "RNA editing in Newcastle disease virus." J Gen Virol 74 (Pt 12): 2539-2547.

Stobart, C. C. and M. L. Moore (2014). "RNA virus reverse genetics and vaccine design." Viruses 6(7): 2531-2550.

Susta, L., M. E. Jones, G. Cattoli, S. Cardenas-Garcia, P. J. Miller, C. C. Brown and C. L. Afonso (2015). "Pathologic characterization of genotypes XIV and XVII Newcastle disease viruses and efficacy of classical vaccination on specific pathogen-free birds." Vet Pathol 52(1): 120-131.

Susta, L., P. J. Miller, C. L. Afonso, C. Estevez, Q. Yu, J. Zhang and C. C. Brown (2010). "Pathogenicity evaluation of different Newcastle disease virus chimeras in 4-week-old chickens." Trop Anim Health Prod 42(8): 1785-1795.

Takimoto, T. and A. Portner (2004). "Molecular mechanism of paramyxovirus budding." Virus Research 106(2): 133-145.

Tan, W. S., C. H. Lau, B. K. Ng, A. L. Ibrahim and K. Yusoff (1995). "Nucleotide sequence of the haemagglutinin-neuraminidase (HN) gene of a Malaysian heat resistant viscerotropic-velogenic Newcastle disease virus (NDV) strain AF2240." DNA Seq 6(1): 47-50.

Tayeb, S., Z. Zakay-Rones and A. Panet (2015). "Therapeutic potential of oncolytic Newcastle disease virus: a critical review." Oncolytic Virotherapy Volume 2015:4 49-62.

Tessier, D. C., R. Brousseau and T. Vernet (1986). "Ligation of single-stranded oligodeoxyribonucleotides by T4 RNA ligase." Anal Biochem 158(1): 171-178.

Toro, H., W. Zhao, C. Breedlove, Z. Zhang and Q. Yub (2014). "Infectious bronchitis virus S2 expressed from recombinant virus confers broad protection against challenge." Avian Dis 58(1): 83-89.

Tsai, H. J., K. H. Chang, C. H. Tseng, K. M. Frost, R. J. Manvell and D. J. Alexander (2004). "Antigenic and genotypical characterization of Newcastle disease viruses isolated in Taiwan between 1969 and 1996." Vet Microbiol 104(1-2): 19-30.

Vidal, S. and D. Kolakofsky (1989). "Modified model for the switch from Sendai virus transcription to replication." J Virol 63(5): 1951-1958.

Vieira, J. and J. Messing (1991). "New pUC-derived cloning vectors with different selectable markers and DNA replication origins." Gene 100: 189-194.

Vigil, A., M. S. Park, O. Martinez, M. A. Chua, S. Xiao, J. F. Cros, L. Martinez-Sobrido, S. L. Woo and A. Garcia-Sastre (2007). "Use of reverse genetics to enhance the oncolytic properties of Newcastle disease virus." Cancer Res 67(17): 8285-8292.

Vignuzzi, M., J. K. Stone and R. Andino (2005). "Ribavirin and lethal mutagenesis of poliovirus: molecular mechanisms, resistance and biological implications." Virus Res 107(2): 173-181.

Wehmann, E., D. Ujvari, H. Mazija, M. Velhner, I. Ciglar-Grozdanic, V. Savic, G. Jermolenko, Z. Cac, E. Prukner-Radovcic and B. Lomniczi (2003). "Genetic analysis of Newcastle disease virus strains isolated in Bosnia-Herzegovina, Croatia, Slovenia and Yugoslavia, reveals the presence of only a single genotype, V, between 1979 and 2002." Vet Microbiol 94(4): 269-281.

Wei, D., B. Yang, Y.-l. Li, C.-f. Xue, Z.-n. Chen and H. Bian (2008). "Characterization of the genome sequence of an oncolytic Newcastle disease virus strain Italien." Virus Res 135(2): 312-319.

Xiao, S., B. Nayak, A. Samuel, A. Paldurai, M. Kanabagattebasavarajappa, T. Y. Prajitno, E. E. Bharoto, P. L. Collins and S. K. Samal (2012). "Generation by Reverse Genetics of an Effective, Stable, Live-Attenuated Newcastle Disease Virus Vaccine Based on a Currently Circulating, Highly Virulent Indonesian Strain." PLoS One 7(12).

Yachdav, G., E. Kloppmann, L. Kajan, M. Hecht, T. Goldberg, T. Hamp, P. Honigschmid, A. Schafferhans, M. Roos, M. Bernhofer, L. Richter, H. Ashkenazy, M. Punta, A. Schlessinger, Y. Bromberg, R. Schneider, G. Vriend, C. Sander, N. Ben-Tal and B. Rost (2014). "PredictProtein--an open resource for online prediction of protein structural and functional features." Nucleic Acids Res 42(Web Server issue): W337-343.

Yan, Y., S. N. Rout, S. H. Kim and S. K. Samal (2009). "Role of untranslated regions of the hemagglutinin-neuraminidase gene in replication and pathogenicity of Newcastle disease virus." J Virol 83(11): 5943-5946.

Yan, Y. and S. K. Samal (2007). "Role of intergenic sequences in Newcastle disease virus RNA transcription and pathogenesis." J Virol 82(3): 1323-1331.

Yu, L., Z. Wang, Y. Jiang, L. Chang and J. Kwang (2001). "Characterization of newly emerging Newcastle disease virus isolates from the People's Republic of China and Taiwan." J Clin Microbiol 39(10): 3512-3519.

Yu, Y., X. Qiu, D. Xu, Y. Zhan, C. Meng, N. Wei, H. Chen, L. Tan, S. Yu, X. Liu, A. Qin and C. Ding (2012). "Rescue of virulent class I Newcastle disease virus variant 9a5b-D5C1." Virol J 9: 120.

Yuan, P., R. G. Paterson, G. P. Leser, R. A. Lamb and T. S. Jardetzky (2012). "Structure of the ulster strain newcastle disease virus hemagglutinin-neuraminidase reveals auto-inhibitory interactions associated with low virulence." PLoS Pathog 8(8): e1002855.

Yusoff, K., N. S. Millar, P. Chambers and P. T. Emmerson (1987). "Nucleotide sequence analysis of the L gene of Newcastle disease virus: homologies with Sendai and vesicular stomatitis viruses." Nucleic Acids Res 15(10): 3961-3976.

Yusoff, K. and W. S. Tan (2001). "Newcastle disease virus: macromolecules and opportunities." Avian Pathol 30(5): 439-455.

Yusoff, K., B. T. Tey and W. S. Tan (1997). "Determination of the 3' terminal sequence of the HN genes of Newcastle disease virus isolates by direct nucleotide sequencing." Asia Pacific Journal of Molecular Biology and Biotechnology 5: 48-50.

Zamarin, D. and P. Palese (2012). "Oncolytic Newcastle disease virus for cancer therapy: old challenges and new directions." Future Microbiol 7(3): 347-367.

Zhang, X., H. Liu, P. Liu, B. P. Peeters, C. Zhao and X. Kong (2013). "Recovery of avirulent, thermostable Newcastle disease virus strain NDV4-C from cloned cDNA and stable expression of an inserted foreign gene." Arch Virol 158(10): 2115-2120.

Zhang, Y. and J. Skolnick (2005). "TM-align: a protein structure alignment algorithm based on the TM-score." Nucleic Acids Res 33(7): 2302-2309.

Zhao, K., Y. Zhang, X. Zhang, W. Li, C. Shi, C. Guo, C. Dai, Q. Chen, Z. Jin, Y. Zhao, H. Cui and Y. Wang (2014). "Preparation and efficacy of Newcastle disease virus DNA vaccine encapsulated in chitosan nanoparticles." Int J Nanomedicine 9: 389-402.

LIST OF PUBLICATIONS

Journal

Murulitharan, K., Yusoff, K., Omar, A.R., and Molouki, A. (2013). Characterization of Malaysian velogenic NDV strain AF2240-I genomic sequence; a comparative study. Virus Genes, 46(3):431-40.

Proceeding/Abstract

Murulitharan, K., Yusoff, K., Omar, A.R., Peeters. B.P.H., and Molouki, A. Recovery of a Malaysian Recombinant Newcastle Disease Virus Strain. Scientific Conference 2013 of World's Poultry Science Association (Malaysia) and World Veterinary Poultry Association (Malaysia) 30th November – 1st December 2013, Veterinary Medicine, UPM, Malaysia.

Murulitharan, K., Yusoff, K., Omar, A.R., Peeters. B.P.H., and Molouki, A. Recovery of a Malaysian Recombinant Newcastle disease virus strain. 10th Malaysia Genetics Congress. Advances in Genetics, Biotechnology and Genomics. 3rd – 5th December 2013. Palm Garden Hotel IOI Resort, Putrajaya.

Research Awards

Murulitharan, K., Yusoff, K., Omar, A.R., Peeters. B.P.H., and Molouki, A. Molecular Characterisation of Malaysian Velogenic NDV Strain AF2240-I: A Comparative Study. The 2nd International Symposium and Workshop on Functional Genomics and Structural biology (FGSB 2014). 21st – 24th January 2014. Mines Wellness Hotel, Seri kembangan Selnagor. Outstanding poster presentation.