Human Ebola Virus Infection In West Africa: Therapeutic strategies basing on available agents

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This article is dedicated to Dr. Lillian Lai Lan Fong, the founder of the Intensive Care Unit of Queen Elizabeth Hospital, Hong Kong.

The recent outbreak of human Zaire ebolavirus (EBOV) in West Africa countries such as Guinea, Liberia, Nigeria and Sierra Leone has resulted in 3069 infected patients with 1552 deaths, as of 28nd August 2014. Human EBOV haemorrhagic fever has a case fatality rate of up to 90%. Neither licensed vaccine nor specific therapy is available for the treatment of human EBOV infection. [1,2,3]

Type I Alpha/beta interferons (IFN-α/β), encoded by a single IFN-β and 13 homologous IFN-α genes in humans, represent an essential element of host defense against virus infection, including Ebola viruses.[4] Human Ebola virus infection is associated with robust IFN-α production, with plasma concentrations of IFN-α that greatly (60- to 100-fold) exceed those observed in other viral infections, but little IFN-β production.[5] Ebola virus, protected from the host interferon response by its encoded VP35[6,7,8,9,10] and VP24 protein[11,12,13], has produced a heavy viral load[14],

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cytopathic damages[15,16,17,18] and cytokine dysregulation in humans.[19,20,21] The efficient productive replication of Ebola virus inside monocyte and macrophages leads to massive release of proinflammatory cytokines/chemokines and reactive

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oxygen species[22] which in turn leads to diffuse endothelial cell dysfunction, disseminated intravascular coagulation [23, 24, 25, 26] and vasomotor collapse.[27,28,29,30,31,32,33] The infection of the antigen presenting dendritic cells and profound bystander apoptosis of lymphocytes impairs the development of adaptive immunity and Ebola virus specific $CD8^+$ T cells[34,35,36,37] important for the clearance of

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Ebola virus.[38,39]

Ebola virus is an enveloped filamentous RNA virus belonging to the family Filoviridae. The 18.9-kb linear, non-segmented, negative-sense, single-stranded RNA genome of Ebola virus encodes seven structural proteins and one non-structural protein. Ebola virus, being a RNA virus with limited coding capacity, has utilized the host's unique metabolic pathway for its viral entry and replication. Ebola virus entry into cells is initiated by the interaction of the viral glycoprotein 1 subunit (GP_1) with host cell surface TIM-1 receptors. Upon receptor binding, the virus is internalized into endosomes primarily via macropinocytosis. Within the acidified endosome compartment of the host cell, the heavily glycosylated GP_1 is cleaved to a smaller 19kDa fusogenic form by the low pH-dependent cellular proteases Cathepsin L (CatL) and B (CatB), exposing residues in the receptor binding site. This allows the binding of GP_1 to cholesterol transporter Niemann-Pick C1 (NPC1), a step in the late endosome phase essential for virus-host membrane fusion and viral entry.[40,41,42,43,44]

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Cells where NPC1 function has been biochemically disrupted or cells lacking NPC1 showed a resistance to EBOV infection. Subjects with Niemann-Pick type C1 disease were resistant to Ebola virus because of defects in the NPC1 protein. After complete fusion of the viral and host endosomal membranes, viral RNA and its associated proteins are released into the host cell cytoplasm. Once inside the cytoplasm of the host cell, Ebola virus suppress innate immune response via VP35 and VP24 protein and hijacks transcription and translation under the influence of RNA polymerase complexes leading to robust genome replication and the production of new virions. This distinct replication cycle of Ebola virus serves as attractive target for the development of therapeutic agents against Ebola virus. [11,45,46] Currently available therapeutic agents that are effective in targeting Ebola virus infection in cell or animal studies may include favipiravir, chloroquine, amiodarone, dronedarone, verapamil, clomiphene, toremifene and IFN-β. (Diagram)

(1) Favipiravir

Viral RNA polymerase inhibitor favipiravir which is registered in Japan for the treatment of influenza virus infection blocks the replication of many other RNA viruses.[47,48] Favipiravir is able to suppress the replication of EBOV in cell culture. Favipiravir, initiated at day 6 post EBOV infection, induced rapid virus clearance, reduced biochemical parameters of disease severity, and prevented a lethal outcome in 100% of mice lacking the type I interferon receptor.[49] Oral favipiravir at a twice-daily dosing for fourteen days is able to give 100% protection against aerosol Ebola virus infection in an immune-deficient mice model.[50,51]

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(2) Chloroquine

Anti-malarial drug chloroquine is able to increase the endosomal pH. An acidic endosomal environment is important for the pH-dependent activation of cysteine proteases CatB and CatL, the enzyme responsible for the cleavage of Ebola virus GP_1 essential for endosomal virus-host membrane fusion.[45,52,53,54] However, a recent study using CatB and CatL deficient mouse model for the study of EBOV infection demonstrate that CatB and CatL activity is not absolutely required for EBOV replication. EBOV glycoprotein cleavage seems to be mediated by a broader spectrum of proteases making therapeutic approaches targeting limited proteases unlikely to be beneficial to combat EBOV infections.[55]

(3) Cationic amphiphiles

Multiple cationic amphiphiles including amiodarone, dronedarone, verapamil, clomiphene and toremifene have been identified as potent inhibitor of EBOV entry in an NPC1-dependent fashion. Amiodarone, at concentrations that are routinely reached in human serum during anti-arrhythmic therapy (1.5-2.5μg/ml), is a potent inhibitor of filovirus cell entry through late endosomes (IC_{50} 0.25μg/ml for EBOV) by inducing a Niemann-Pick C-like phenotype. Significant inhibition was seen in most endothelial and epithelial cells (macrophage, monocyte, endothelial cells) except primary hepatocyte and fibroblast. The Inhibitory effect of amiodarone on EBOV entry was dose dependent and reversible upon removal of

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drug. Prolonged exposure to amiodarone will not lead to compensatory change in host cell. A similar inhibitory property was observed with the amiodarone-related agent dronedarone and the L-type calcium channel blocker verapamil.[46,56,57,58]

The anti-Ebola virus activity of clomiphene and toremifene is not dependent on its estrogen receptor antagonistic action but due to its ability to induce a Niemann-Pick C-like phenotype to inhibit viral entry at late endosome. Clomiphene and toremifene does not disrupt the interaction between primed GP_1 and NPC1 but mediate the entry block indirectly through NPC1 by targeting other endosomal/lysosomal proteins involved in the cholesterol uptake pathway whose function may be regulated by NPC1. Clomiphene and toremifene at 60 mg/kg every other day have been shown to produce a 90% and 50% survival respectively in EBOV infected mice compared with 100% mortality in the control group in an in vivo murine Ebola infection model. They are effective in both male and female mice.[46, 59] However therapeutic dose against EBOV cannot be achieved with oral clomiphene for ovulation induction in humans.[60 , 61 , 62] Therapeutic dose against EBVO with tolerable side effect can be achieved with toremifene at an oral dose used in human trial for the treatment of advanced

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carcinoma of breast.[63,64,65,66] Toremifene is well absorbed and 99% bound to plasma protein. Toremifene undergoes extensive liver metabolism and enterohepatic recirculation. The majority of a dose of toremifene is excreted as metabolites in faeces. The long half-life of toremifene may be due to both plasma protein binding and enterohepatic recirculation.[67,68]

(4) Interferon-beta

IFN-β is able to induce interferon-inducible transmembrane proteins production to restrict entry of Ebola virus.[69] Early postexposure treatment with IFN-β significantly increased survival time of rhesus macaques infected with a lethal dose of Ebola virus, although IFN-β alone failed to alter mortality. IFN-β treatment was associated with a trend towards lower plasma and tissue viral burden and proinflammatory cytokines production.[5]

There is a desperate need for a viable treatment protocol in Africa to engender hope to encourage people with symptoms and their close contacts to come to hospital to limit spread of the disease. This could also help in recruiting and maintaining adequate levels of hospital staffs who are at high risk of catching the diseases. WHO has advised that the use of experimental medications and vaccines under the exceptional circumstances of this outbreak is ethically justifiable. However, existing

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supplies of all these experimental medications are either extremely limited or exhausted.[1] Before these experimental agents are available, to combat such unprecedented global public-health crisis, alternative intervention that may be effective against Ebola virus should be explored in the management of human Ebola virus infection.

Ebola viruses have undergone a rapid mutation during its spread through humans. The virus amassed 50 mutations during its first month![70,71,72] Ebola virus is an enveloped filamentous RNA virus. RNA virus replication is highly error prone with nearly one viral mutation occurs during each cycle of replication. RNA viruses replicate with extremely high mutation rates and exhibit significant genetic diversity. This genetic and antigenic diversity allows the viral population to evolve resistance to antiviral drugs and vaccines.[73,74] Therefore combination therapy are introduced in the treatment of RNA virus infection such as human immunodeficiency virus[75,76] and hepatitis C virus[77,78] to prevent the develop of drug resistance. Given the broad cell tropism and high replication rate of EBOV due to potent suppression of both innate and adaptive immune response of the host by its encoded VP35 and VP24 protein, patient with EBOV infection has an extremely high viral load. The selective pressure in the presence of high mutation rate and viral load during human EBOV infection

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make the evolution of EBOV viral strains resistant to a single drug inevitable. Therefore, a cocktail of currently available agents that are targeting different steps in the replication cycle of Ebola virus should be considered to suppress viral proliferation to prolong survival in order to allow the development of natural body immune defense against Ebola virus. The goal of a treatment regimen containing a cocktail of anti-viral medications targeting different cycles of EBOV replication is to achieve maximal suppression of viral replication to prevent the rapid develop of EBOV to Favipiravir, the currently available medication that has been shown to reduce the replication of EBOV.[79,80,81]

A proposed regime basing on animal and cell studies is attached. (Diagram 2) Amiodarone and favipiravir are affordable and stockpileable in oral preparations. These properties may be advantageous to the treatment of Ebola virus infection in resource-constrained geographical regions where outbreaks of filoviral infection frequently occur. In more affluent countries, toremifene can be added on the treatment regime to protect the liver cells because amiodarone has no protective action in the liver. However, both amiodarone and toremifene can increase QTc and increase the risk of *Torsades de pointes*. Therefore QTc should be monitored if dual therapy is being considered. The avoidance of intravenous administration will prevent needle prick injury in healthcare workers while caring for the infected patients. IFN-β may have potential as an adjunctive postexposure therapy for high risk exposure such as needle prick injury because the reduction in viral load and cytokine dysregulation coupled with optimal supportive therapy may improve the chance of survival of the host to allow the development of natural immunity to control the underlying Ebola virus infection.[5]

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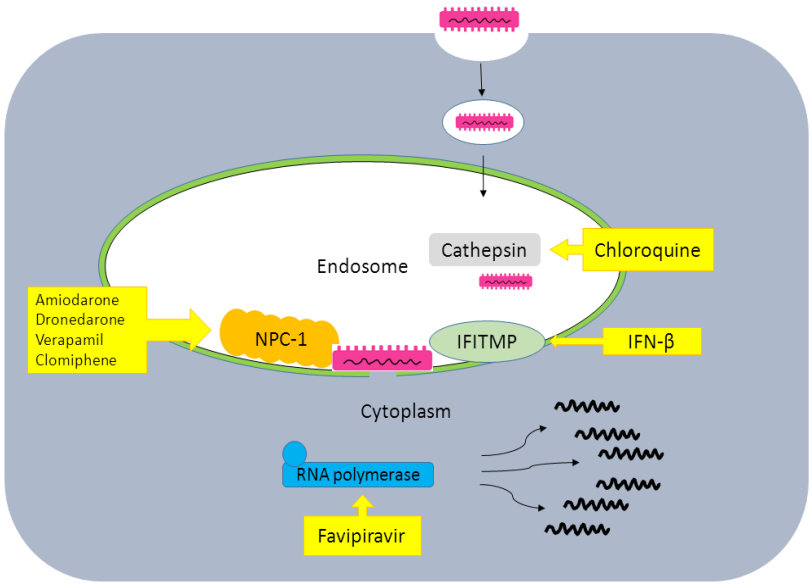

Medications	Mechanism of Action
Chloroquine[1]	Chloroquine leads to alkalinization of the late endosomes and prevents the acid pH-dependent cleavage of Ebola virus GP_1 by endosomal proteases cathepsin B and L.
Cationic amphiphiles - Amiodarone[1] - Dronedarone[1] - Verapamil[2] - Clomiphene	These agents induce a Niemann-Pick C-like phenotype and block the entry of Ebola virus through late endosomes.
Favipiravir	Favipiravir inhibit proliferation of Ebola virus through suppression of viral RNA polymerase.
Interferon- beta (IFN-β)	IFN-β is able to induce interferon-inducible transmembrane proteins (IFITMP) production to restrict entry of Ebola virus. IFN-β may reduce viral load and pro-inflammatory cytokine production.
1: Chloroquine, Amiodarone and Dronedarone administration is associated with an increased risk of QT prolongation and *Torsades de pointes*. 2: Verapamil should be avoided in patient with hypotension.	

Diagram 1: Schematic diagram showing the replication cycle of Ebola virus and the site of action of currently available therapeutic medications against Ebola virus infection.

Therapeutic Strategies Basing on Available Agent For Ebola Virus Prophylaxis and Treatment

Ebola Virus	Available Agent
Prophylaxis [1]	Amiodarone
Post Needle Prick Injury Prophylaxis	IFN-β + Amiodarone (macrophage, monocyte & endothelial cell) ± toremifene (liver) [2,3] + Favipiravir
Treatment	Amiodarone (macrophage, monocyte & endothelial cell) + toremifene (liver) [2,3] + Favipiravir + supportive care + correction of coagulopathy + early nutritional support

1. 1ml of blood may contain 10 [9-10] virions in terminally ill patient and pin-prick injury may lead to injection of over 1 million virions. Prophylactic therapy may prevent our macrophage, monocyte and endothelial cells immediately from infection after needle prick injury and allow time for consideration of IFN-β and Favipiravir therapy.
2. Amiodarone is unable to protect liver cells from Ebola virus infection.
3. Both amiodarone and toremifene can increase the risk of QT prolongation and *Torsades de pointes*

Diagram 2: Proposed therapeutic strategies basing on available agents for the prophylaxis and treatment of Ebola virus