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STRATEGIES FOR LEAD MOLECULE GENERATION AND SCREENING FOR EBOLA

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ABSTRACT

Ebola Virus Disease is a complex, highly infectious and fatal pandemic. While several groups across the world continue to work towards strategies to manage the disease, there is a need to simultaneously target all known and viable pathways in order to accelerate the process of developing a set of interventions. The following article, is concerned with providing an outline of all possible strategies that, if adopted could yield promising results. The process of intervention development has had a promising start with the identification of viable targets within the disease pathway. A combination of high throughput screening of known antivirals against key proteins, and computer aided design of molecules that bind to key proteins involved in the transcription process and applying pharmacophore mapping to the derived parameters could result in the development of an active molecule to arrest Ebola Virus proliferation.

KEY WORDS: Ebola Virus, Drug Development, Drug Discovery, Targets, Drug Repositioning, High throughput screening, Combinatorial chemistry.

INTRODUCTION

Much of the past two years has been spent trying to understand the epidemiology, disease pathway and etiology of a disease unprecedented in scale and complexity. As of now, while the WHO debates on measures such as expanded access of vaccines and the ethical use of unregistered interventions, attempting to linearize the management of individuals affected from Ebola Virus Disease remains the only widely followed protocol . As the disease continued to sweep across Liberia, Guinea, Sierra Leone, Nigeria, the United States and Mali. (BBC, 18th November 2014), it is important, now more than ever, to work on all known targets and strategies for intervention development simultaneously and collaboratively.

Key Requirements, A Reframed Problem Statement

For the purpose of finding a viable drug target it is important to keep in mind parameters such as selecting a drug combination with high potential for yielding clinical success within the efficacy–toxicity spectrum. Even a highly intelligent and complex pathogen is vulnerable to toxicity, which may often be a result of high dosage of a molecule. This may often result in a positive and promising proof of concept outcome but lose out during toxicity profiling.

Proposed Scheme

The onus of a molecule gaining success as an intervention is largely dependent on the selection of targets. It is for this purpose that the application of network analysis to indentify the most crucial paths of information flow within the disease pathway should be incorporated from an initial stage. This would involve screening not only molecules but also targets which rank higher in terms of influence in the disease network. Enhancement of these approaches may emerge from effectively integrating computational systems biology with pharmacodynamic systems analysis. Coupling genomics, proteomics, and metabolomics databases with systems pharmacology modeling may aid in the development of disease-specific networks.

Genome wide genetic screening could be first employed to primarily indentify molecules that are crucial for Ebola's Virulence, for example, Nieman Pick C1, a host protein that binds to viral glycoprotein and is essential for infection was identified using the same method.

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Proposed Strategies for Lead Generation: A Combination of Targets, Pathways and Known Molecules with Inhibitory Properties

VP-40 Inhibition

The product of the third gene within the Ebola Virus Genome, VP40, is located beneath the viral envelope where it helps to maintain structural integrity. It has also been associated with late endosome development and likely mediates filovirus budding due to its ability to induce its own release from cells in the absence of all other viral proteins [1]. ISG15 inhibits Ebola VP40 VLP budding in an L-domain-dependent manner by blocking Nedd4 ligase activity [2]. VP 40 may be considered a potential drug target,and a receptor based method for in silico drug deisgn based modification of ISG 15 could generate a likely candidate.

Si RNA Profiling

Si RNA profiling not only helps identify hotspots within the viral genome but also help target proteins crucial to its proliferation. It is thus possible to modulate of expression of ebola viral proteins using si RNA (sequence specific gene silencing). The seven known proteins of this virus can all be considered potential targets. It is also important to note that matrix proteins in the Ebola virus, which are also known as moonlighting proteins , as they adopt entirely different structures for its different jobs(as a hexamer in the virion structure (PDB entry 4ldd), as an octamer that binds to RNA and regulates viral transcription (PDB entry 1h2c), and as a dimer involved in transport of the protein (4ldb)), would be valuable targets, mainly because the virus' dependence on these proteins is greatest. [3]

The mechanism involved in this case is the dicer dependent endonucleolytic procedure for production of si RNA, executed by Argonaute protein .The administration of this intervention can be designed with respect to efficacy, safety and viability challenges and strategies may be required to handle the difficulty in delivering effector molecules

Selective Interruption of Clathrin Mediated Endocytosis

This strategy would involve the synthesis or selection of a novel small molecule that inhibits Ebola virus entry into cells. Pitstop 2 was identified as an inhibitor of the interaction of amphiphysin with the amino terminal domain of clathrin, and shown to inhibit clathrin dependent endocytosis in cells. Receptor based approaches could also be used, since the structure of the target is known. Also siRNA in conjunction with high throughput screening could help in the identification of potent targets within the host, that could selectively prevent clathrin mediated endocytosis for the pathogen [4-5]

Selective Inhibition of Macropinocytosis

A Dynamin dependent pathway is responsible for the internalization of Ebola virus into host cells, as it is responsible for glycoprotein mediated toxicity. Targeting and selectively blocking dynamin activity could interrupt the internalization of the virus. This can be achieved by Dynasore and dynole molecules. In order to achieve desired selectivity, in order that only macropinocytosis involved in the uptake of virus is interrupted, analogs of dynoles can be generated to target the late stage mediators of the pathway, especially the molecules which interact directly with the pathogen glycoprotein. An assay could be used to confirm the selective inhibition of this pathway. Brefeldin A [6] was shown to inhibit glycoprotein mediated cell detachment and consequent cell damage.

Degradation of Viral Nucelocapsid (Antiviral Repurposing and Combinatorial Chemistry Approaches)

It has been previously demonstrated that nucleoproteins, together with the minor matrix protein VP24 and polymerase cofactor VP35, are necessary and sufficient for the formation of nucleocapsid-like structures .It seems feasible to attempt to synthesize or screen for repositioning of molecules that target VP24 and VP 35 proteins in order to arrest the formation of nucleocapsid like structures. [7]

Proteases that have shown high degradative potential in Marburg viruses (close relative) seem likely candidates and other protease activity based antivirals could be repurposed in this case.Combinatorial chemistry approaches could also help in the synthesis of analogs of molecules that have propensity for viral nucleocapsid degradation and a high throughput nucelocapsid degradation assay could help in screening for activity. Pseudotyping and replicones in assays would be a viable approach for screening for activity in assays, in order to counter the risk of exposure.

Cathepsin L and B Inhibitors

Ebola virus and SARS virus both employ cathepsin L activity for viral entry and cathepsin L is strongly implicated in the processing of the Ebola virus glycoprotein. [8] Calpeptin (L), Leupeptin hemisulphate(L),E-64-D (B and L), Chymostatin [9] are known inhibitors of Cathepsin activity . Screening of these entities and their analogs for activity using high throughput screening could yield a prospective candidate.

Inhibition of Vesicular Acidification

Low pH is essential during the formation of lipid rafts that accompany micro and macro pinocytosis. [10] Moreover Cathepsin activity is also known to occur in a low pH. Disruption of the required pH gradient and consequently interrupting uptake is another useful strategy. Bafilomycin was found to be an inhibitor of vesicular acidification and interruption of membrane cholesterol depletion, both of which are necessary for entry and fusion of ebola glycoprotein.

Prevention of Release of Virion Attached glycoprotein

Ebola virus replication overwhelms protein synthesis of infected cells and host immune defenses. The GP forms a trimeric complex, which binds the virus to the endothelial cells which is essential for the uptake of the virion into the cell. TNF-α-converting enzyme (TACE) can release the virion-attached GP (critical in the EBOV lifecyle) through a cleavage site proximal to the transmembrane anchor. TIMP-3, a known modulator of TACE activity, can be used as a skeletal structure for the production of analogs that can inhibit TACE activity [11].

Inhibition of RAS Signalling Pathway

Reactivation of RAS signaling pathway in the presence of interferons is required for robust Ebola virus protein synthesis. An activated Ras/Raf/Mek cascade, even in the presence of interferons is responsible for the propagation of virus [12]. Screening for molecules that can disrupt the synergistic functioning of elements downstream of the Ras-singalling pathway could be a feasible strategy. Ras inhibitors frequently used in cancer therapy could be potentially repositioned for screening for inhibition of Ras reactivation.

Microtubule Disruption

The trafficking of Ebola virions from the cell surface to the appropriate acidified vesicular compartment for fusion is heavily dependent on both microtubule and microfilament function. Entry and fusion of Ebola virus glycoprotein pseudotypes were seen to be impaired in the presence of the microtubule-disrupting agent nocodazole. [13] Agents that impaired microfilament function, including cytochalasin B, cytochalasin D, latrunculin A, and jasplakinolide, also inhibited Ebola virus GP-mediated entry and fusion. Further exploration into analogs of these molecules and design and modification of analgos to improve specificity for virion microtubules is required.

Phosphoinositide-3 Kinase Pathway

Phosphoinositide-3 kinase (PI3K) pathway is responsible for functions such as cell growth, migration, survival, and vesicular trafficking [14]. Inhibitors of PI3K were seen to significantly reduce infection at an early step during the replication cycle and can be considered for further screening as likely drug candidates [15]. These inhibitors include the lead generation and screening strategy could include identification and screening of all possible inhibitors of PI3K from Ebola Virus and Marburg virus family.

CONCLUSION

While developing strategies to combat pandemics such as these, time is of essence. The one molecule per target strategy, while offering greater clarity in terms of mechanism of action and a greater scope for structural optimization, would prolong the process of drug development, which would greatly reduce the impact of the intervention. The strategies listed above, singularly or in combination could potentially help in an exponential increase in the number of leads, which when evaluated through high throughput screening could result in a number of lead candidates. What is needed, is an organized collaborative approach to study the biology of the pathogen and actively indentify the dependencies on the host and at the same time increase the number of hit molecules converted into leads.

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