Genetic diversity-independent neutralization of pandemic viruses (e.g. HIV), potentially pandemic (e.g. H5N1 strain of influenza) and carcinogenic (e.g. HBV and HCV) viruses and possible agents of bioterrorism (variola) by enveloped virus neutralizing compounds (EVNCs)

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Summary Genetic diversity and hypermutation contribute to difficulties in developing a vaccine against viruses like HIV and influenza. There are currently no known immune correlates of protection against HIV. This has made the development of a vaccine against HIV that would provide sterilizing immunity in the near future an impossible task. The abandonment of a recent AIDS vaccine human trial due to a failure to elicit a protective sterilising immune response confirms that empirical attempts to develop a vaccine may result in failures. Also the difficulty in predicting the next pandemic strain of influenza may make it difficult to respond rapidly should there be an outbreak. Therefore, it is time to explore broad spectrum agents that can target either the lipid portion of the envelope or the sugar moieties of the glycoproteins or the rafts (regions within viral and cell envelopes where a higher concentration of the glycoproteins exist). Broad spectrum agents that can serve as disrafters or neutralize the viral infectivity by binding to the envelope lipid or sugar moieties will not be affected by the vagaries of hypermutation of surface antigens. This is because the post-translation modification is a host function. Presented here is a review of recently reported agents present in pomegranate juice (polyphenols, beta-sitosterol, sugars and ellagic acid) and fulvic acid, described here as the envelope virus neutralising compounds (EVNCs) and

Abbreviations: EVNCs, enveloped virus neutralising compounds; HA, haemagglutinin; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; KSHV, kaposi sarcoma herpes virus.

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complex molecules like lectins and mucins. Pomegranate juice was previously reported to inactivate HIV and further shown by our group to inactivate influenza, herpes viruses and poxviruses. A formulation consisting of fulvic acid, a complex mixture of compounds was previously reported to render vaccinia virus, HIV and SARS virus non-infectious. Recently, both fulvic acid and pomegranate juice have been shown to inactivate genetically diverse strains of influenza including H5N1, further confirming the broad spectrum nature of these agents. How EVNCs will be used in developing a vaccine achieving sterilizing immunity or prophylaxis needs to be researched.

A network of researchers have been investigating the antiviral properties of a formulation called Secomet V, whose active ingredient is fulvic acid, which is a complex mixture of compounds arising from decomposing organic matter. Antiviral activity of fulvic acid containing Secomet V against poxviruses and SARS has been demonstrated in our laboratory and that of a German group [10]. The bioactive fulvic acid found in the formulation was separated by ultrafiltration using a 3000 Da filter and column chromatography and found to be in the range of 200–600 Da; with the peak activity observed around fractions corresponding to 113, 226 and 452 Da indicating that the active compounds maybe repeats of a monomeric unit of 113 Da (Kotwal, unpublished). The mechanism of action and structure of fulvic acid and bioactive compounds from pomegranate juice are currently being fully elucidated but hemagglutinating activity is inhibited indicating that fulvic acid possibly blocks entry of the virus by interacting with the sugar or lipid moiety or both of surface glycoproteins of enveloped viruses. Previously, pomegranate juice was shown to provide an HIV-1 entry inhibitor [14]. Our studies suggest that the bioactive molecule renders the virus non-infectious by inhibiting entry into the cells. Once a virus enters a cell, the agent has no effect on viral replication. Short- and long-term toxicity studies in vitro and in vivo show that the bioactive agent is safe. Physical and chemical analyses show that the formulation is acidic (around pH 2) and heat stable (survives autoclaving), and that the levels of iron and arsenic are well below the permissible levels. In order to test the hypothesis that the formulation containing fulvic acid or pomegranate juice can neutralize the infectivity of a wide range of genetically diverse strains of a given enveloped virus, possibly by interacting with sugar chains on the viral surface protein and serving as an entry inhibitor, we tested its activity against influenza A/HK/x31 (H3N2), influenza A/Vietnam/1203/04 (H5N1), and a reassortant x3 containing the NS gene segment of an H5N1 isolate (Sanger, Sandbulte, Webby and Kotwal, unpublished). The reassortant x3 was described before [15]. All viruses were inactivated in a dose-dependent manner when treated for 5 min at room temperature with the fulvic acid containing formulation or for the same period at 37°C with pomegranate juice. To obtain further evidence for the antiviral action of fulvic acid, mice were intranasally administered a normally highly lethal dose of A/x31 that had been treated for 5 min with fulvic acid. Control mice received an equivalent dose of mock-treated virus. Mice that received the treated virus showed no signs of disease, whereas the mock-treated virus produced extensive weight loss (due to diminished water consumption) and 75% mortality. In addition, the duration of the survival of the infected untreated...
mice mimics the duration of the period of survival from fever to death in H5N1 infected humans [16].

We suggest that compounds with antiviral activity from fulvic acid and from pomegranate juice neutralize the infectivity of diverse enveloped virus, and a number of subtypes of a given enveloped virus, indicating potential for development as a treatment option that can be broadly effective against pandemic viruses like HIV potentially pandemic viruses like influenza and carcinogenic viruses like HBV and HCV. Although it remains to be proved that the carcinogenic viruses and potentially dangerous bioweapon like variola can be neutralized by the EVNCs, one can predict with a fair bit of confidence that demonstration of routine experimental neutralization of a live attenuated vaccinia virus, vGKS [17] with the greatest structural robustness compared to all known enveloped viruses and similar to variola can be extrapolated to the carcinogenic hepatitis viruses and variola. These findings also raise the possibility that certain plant acids, sugars and complex carbohydrates may have an application in the production of inactivated whole viral vaccines as well as micobicides (see Fig. 1). The EVNCs could be administered orally in concentrated capsules or administered intravenously after extensive research into the bioavailability and proper well conceived clinical trials. Until this can be accomplished, universally available pomegranate juice or the pomegranate fruit known for centuries could be consumed regularly and in significant amounts, may provide benefit against enveloped viral infections but should not be seen as a substitute for other well established means of infection prevention such as effective vaccines against HBV, seasonal influenza and condoms against HIV. The EVNCs in the body fluid could neutralize viruses in the blood stream and elicit an immune response to the neutralized authentically folded virus particle and thus facilitate development of a potentially protective immunity [18].

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