Commentary

Automated Aptamer Development May Represent the Last and Best Line of Defense against Proverbial "Doomsday" Pathogens

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Abstract | This commentary addresses the perceived need for automated robotic development of aptamers or aptamer conjugates with associated mass production to provide rapid passive immunity for large human populations in the event of a major pandemic or infectious disease doomsday scenario.

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Introduction

The frequency of emerging infectious disease (EID) outbreaks around the globe including the 2014 Ebola crisis in West Africa which spread to the United States for the first time, a number of potentially lethal viruses such as Chikungunya, Dengue, West Nile, MERS and SARS as well as the newest carbapenem-resistant nosocomial bacterial scourges CRE and KPC (Limbago et al., 2011) have again alerted the public and governments to the very real potential of an uncontrollable "doomsday" pathogen. While such apocalyptic "Andromeda strain" pathogens may seem like the incredible stuff of science fiction novels and many EIDs are manageable from a public health standpoint, it is true that viruses can evolve very quickly and cross species barriers making another influenza pandemic a virtual certainty. In addition, the transfer of drug resistance conferring plasmids among bacteria, threaten to throw mankind back into the pre-antibiotic "dark ages." It is also true that many successful pathogens kill their hosts quite quickly before the host has a reasonable chance to mount an immune response or before human society would have any chance to develop even a prototype vaccine.

Aptamer-Based Passive Immunity

Given that passive immunity for deadly diseases such as Ebola with human immune serum and the triple monoclonal antibody ZMapp has demonstrated efficacy against at least the Zaire strain of Ebola (Furayama et al., 2016), it follows that a method for even more rapid passive immunity should be developed as a last and perhaps best line of defense for mankind in the event that a "superbug" threatens mankind with extinction. The ideal technology may well be automated or robotic Systematic Evolution of Ligands by Exponential (SELEX) nucleic acid aptamer development in which a human would never need to interact with the pathogen directly except in a BSL-4 laboratory. SELEX essentially represents an artificial immune system in a tube. Kary Mullis of PCR fame recognized this fact when he founded Altermune, LLC (recently sold to Centauri Therapeutics, Ltd.) based on aptamer-alpha gal conjugate binding to ~ 1% cir-

culating anti-alpha gal antibodies in serum to act like surrogate antibodies for passive immunity (Kristian et al., 2015). The United States National Aeronautics and Space Administration (NASA) has also recognized the potential for automated aptamer development to be used as a prophylactic or therapeutic countermeasure for astronauts, if they ever face deadly pathogenic microbes in outer space where medical assistance would be limited to the spacecraft (Dobler and Maki, 2005; Schmidt and Goodwin, 2013). And while NASA has considered the more exotic artificial nucleotide-based X-aptamers (Schmidt and Goodwin, 2013) for diagnostic use, X-aptamers would likely be immunogenic if used in vivo. Unmodified DNA and RNA aptamers are generally considered to be nonimmunogenic (Bruno, 2013, 2015), although some rare cases of immune system responses have been reported (Avci-Adali et al., 2013).

The author also has developed aptamer-Fc and aptamer-C1q conjugates to opsonize encapsulated bacteria (Bruno et al., 2009) and kill Gram negative antibiotic-resistant bacteria by invoking the classical complement system to inflict fatal lesions on the bacteria (Bruno, et al. 2008; Bruno, 2013). And numerous investigators have now published about DNA and RNA aptamers which can bind and possibly treat other multi-drug resistant bacteria including recalcitrant *Mycobacterium tuberculosis* by inhibition of key enzymes and virulence factors (Baig et al., 2015; Chen et al., 2007, 2012, 2013; Pan et al., 2014).

Aptamers have also been shown to inhibit the progression of Ebola (Binning et al. 2103), SARS coronaviruses (Jang et al., 2008; Shum and Tanner, 2008), various influenza viruses (Cheng et al., 2008; Choi et al., 2011; Gopinath and Kumar, 2013; Jeon et al., 2004; Kwon et al., 2014; Shiratori et al., 2014; Suenaga and Kumar, 2014; Wongphatcharachai et al., 2013; Zavyalova and Kopylov, 2016; Zhang et al., 2015), and arboviruses (Bruno et al., 2012; Chen et al., 2015). It is particularly interesting that both Bruno et al. (2012) and Chen et al. (2015) arrived at very similar G-quartet-like structures in the putative binding segments of their highest affinity anti-Dengue aptamers, attesting to general convergence of the SELEX process performed by two different laboratories and suggesting that robotic SELEX automation should yield a definitive set of ideal aptamers for passive immunity in the future, if ever properly implemented. With regard to the most recent Zika virus outbreaks, passive immunity via simple aptamer binding offers hope for inexpensive, yet effective, blockage or inhibition of the virus in pregnant women to possibly prevent the associated microcephaly in infants.

Automated Robotic SELEX with Possible Simplifications and Aptamer Mass Production

Robotic methods for rapid aptamer development using commercially available robotic systems have existed for over a decade (Eulberg et al., 2005; Lee et al., 2005; Hünniger et al., 2014; Wochner et al., 2007). Improvements or simplifications of the SELEX process such as MonoLEX (Nitsche et al., 2007) and capillary electrophoresis (CE)-based SELEX (Hamedani and Müller, 2016) or other high-throughput SELEX techniques (Dausse et al., 2011) could further speed the robotic aptamer development process. Another major virtue of developing aptamers for passive immunity is that the identity of any targeted "doomsday bug" would not even be required. One can develop aptamers against any target without knowledge of its actual identity. Aptamers can be developed against whole viruses, bacterial cells or subcellular components from lysates or extracts immobilized on magnetic microbeads to develop aptamers that will antagonize pathogen attachment, penetration or replication in the host. Scale up of the successful inhibitory or blocking aptamers to the gram level could be problematic and most likely impossible if performed by chemical synthesis. However, asymmetric PCR or other isothermal enzymatic DNA and RNA synthesis methods could provide a cost-effective answer to mass production of aptamers or antibody-like aptamer-protein (-C1q or -Fc) conjugates to emulate antibodies for passive immunity (Bruno, 2013, 2015). If proteins or polyethylene glycol (PEG) are conjugated to the 3' end, the resulting conjugates will exhibit enhanced nuclease resistance in serum and greater pharmacokinetic lifetimes (Dougan et al., 2000; Keefe et al., 2010). The author has developed a simple method to go from aptamer PCR amplicons to aptamer-3'-protein conjugates for mass production of such artificial "antibodies" as well (Bruno and Crowell 2008; Bruno, 2013).

The Future

While it may seem too futuristic at present to conceive of such robotic aptamer or aptamer-protein conjugate systems to combat pandemics, the individual technol-

ogies already exist and are rather well-developed. It would merely require the political or commercial will and appropriate funding of government or private industry to integrate and implement such a system. As with most human endeavors, such a project will probably not take shape until the microbial world threatens man with extinction to the point that mankind sees its own demise on the horizon and decides to develop automated aptamer generation systems to save lives during "blitzkrieg" epidemics or pandemics due to EIDs or previously unknown pathogens. Perhaps, however, such robotic aptamer development systems can evolve from private industry if aptamers prove themselves versus comparable antibodies in the diagnostics and therapeutics markets, thereby incentivizing industry to further explore aptamer development for commercial products.

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