Fighting Ebola with ZMapp: spotlight on plant-made antibody

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Currently, a large Ebola endemic is ongoing in West Africa. According to the World Health Organization, as of August 19, the viral outbreak has led to 2240 suspected and confirmed cases and 1229 deaths in four countries: Guinea, Liberia, Nigeria, and Sierra Leone (<http://www.cdc.gov/vhf/ebola/outbreaks/guinea/index.html>).

Ebola viruses are the infectious agent causing the Ebola virus disease (EVD). They belong to the genus *Ebolavirus* of the Filoviridae family and have been categorized into five known species: Ebola virus (EBOV, formerly Zaire Ebola virus), Bundibugyo virus (BDBV), Sudan virus (SUDV), Taï Forest virus (TAFV), and Reston virus (RESTV). EBOV was first identified in 1976 and named after the Ebola River where the first known epidemic occurred. Among the five *Ebolavirus* species, RESTV only infects non-human primates whereas the other four species can cause fatality between 30% to 90% in human. The 2014 Ebola outbreak is caused by Zaire EBOV, whose fatality rate is as high as 90%.

EBOV mainly infects endothelial cells, mononuclear phagocytes and hepatocytes. After infection, the virus uses multiple mechanisms to evade the host immune system. It causes massive damage to internal tissues and organs, such as blood vessels and the liver, and ultimately death. EBOV encoded glycoprotein (GP) and its secreted form (sGP) play an essential role in the pathogenesis of EVD.

Direct contacts with body fluids of infected individuals are considered to be the main route of EBOV transmission (<http://www.cdc.gov/vhf/ebola/transmission/index.html>), whereas aerosol infection has not been reported clinically,

despite it has been demonstrated in experimental infection in monkeys. Upon infection, early symptoms including fever, muscle pain, diarrhea, vomiting will appear between 2 and 21 days, followed by late symptoms and signs such as haemorrhagic diathesis, delirium and shock, with death often occurring 6–9 days after the onset of symptoms. Because of the high transmissibility, severity, as well as high mortality associated with EBOV, it has long been recognized that effective vaccines and treatments against EBOV should be developed to protect public health, maintain social stability, and reduce economic losses. However, neither prophylactic vaccine nor therapeutic drug for EBOV is commercially available.

In early August 2014, an experimental drug, called “ZMapp”, was used to treat two American medical workers who had contacted the deadly EBOV during treating Ebola patients in Liberia. Both of them claimed that they felt stronger after receiving ZMapp. They have since recovered miraculously from severe EVD and were discharged from hospital on August 21 (<http://edition.cnn.com/2014/08/21/health/ebola-patient-release/index.html>). This is the first time that a drug has shown therapeutic efficacy against Ebola in human. ZMapp has since received tremendous public attention and it was later used to treat four other Ebola patients: one Spanish priest and three African doctors. Except for the 75-year-old Spanish priest who died despite the ZMapp treatment, the other three were reported to have improved significantly (<http://www.ibtimes.co.uk/ebola-outbreak-three-african-doctors-receiving-zmapp-drug-show-remarkable-signs-improvement-1461744>).

ZMapp is an experimental drug co-developed by Mapp Biopharmaceutical, Inc. (San Diego, CA, USA) and Defyrus

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Inc. (Toronto, Canada). It is a combination of three “humanized” monoclonal antibodies (mAbs) against the EBOV GP protein. One of the three antibodies was selected from components of Mapp Biopharmaceutical’s MB-003 and Defyrus’ ZMAb monoclonal antibody cocktails (<http://en.wikipedia.org/wiki/ZMapp>). The uniqueness of ZMapp is that its component antibodies are produced in plants, specifically *Nicotiana benthamiana*. Mapp Biopharmaceutical states that the plant is capable of expressing pharmaceutical proteins using indoor cultivation under tightly controlled conditions and that this process is rapid and scalable to mass production. Due to the apparent success of ZMapp, spotlight is swiftly turned on plant-made antibody.

The first demonstration of mAb production in plants was reported in 1989 [1]. This proof-of-concept study used the transgenic technology, which involves delivery of genes of interest into plant cells, subsequent integration of these genes into the parental plant chromosome, and selection of stable transgenic plants. Many mAbs have since been expressed in transgenic plants, constituting the first wave of plant-made antibody studies. However, a number of issues associated with this expression strategy, including low expression levels, long timeframes to generate transgenic plants, undesired glycosylation patterns, and concerns on spreading transgenes into the environment, hindered further product development.

The second wave of plant-made antibody studies came after the development of high-yield transient plant expression systems, which are based on “deconstructed”, replication-competent viral vectors, such as magnICON [2] and Gemini [3]. These vectors contain elements required for viral replicon formation. Once delivered into plant cells via *Agrobacterium* infiltration, these vectors are able to assemble into replicons, which undergo automatic replication and result in a high copy number of RNA molecules encoding the desired antibody. High levels of mAb expression, up to 0.5 g kg⁻¹ fresh weight, could be achieved. This replicon-based transient expression appears to be the most rapid process for mAb production with a timeframe of 5–8 days from DNA delivery via *Agrobacterium tumefaciens* to harvested cells expressing mAbs. In addition, the production process is readily scalable to mass production. Moreover, the process does not involve gene integration into plant genome and thus eliminates the risk of spreading of the recombinant genes to other plants by pollination. Finally, the entire production process can be completed indoors under tightly controlled conditions and therefore meet the requirements for biological product manufacturing. Indeed, Kentucky BioProcessing (Owensboro, KY, USA) has built

a facility for manufacturing mAbs in *Nicotiana* under Good Manufacturing Practices conditions. It is worth noting that “ZMapp” was actually manufactured by Kentucky BioProcessing under a contract from Mapp Biopharmaceuticals, Inc.

Wildtype plants are able to glycosylate proteins of interest. However, their glycans carry residues of xylose and fucose in a non-mammalian linkage, which may affect pharmacokinetics and enhance immunogenicity of mAbs in humans. To overcome this problem, transgenic *N. benthamiana* plants with fucosyl- and xylosyl-transferase knocked down were generated. MAb produced in these glycomodified plants had mammalian-like glycans with more homogeneous glycoforms than that produced in CHO cells [4]. It has been shown by scientists of Mapp Biopharmaceutical that mAbs expressed in the glycomodified *Nicotiana benthamiana* plants had superior anti-EBOV efficacy in animal models to the counterpart produced in CHO cells [4,5].

With advances in plant biotechnology, manufacturing mAbs in plants now has multiple desirable attributes, including high yield, short production time, scale-up capacity, relatively low cost, and desired glycosylation. Plant-made mAbs are particularly attractive as biodefense agents for rapid response to a bioterror event or newly emergent and re-emergent disease outbreaks. Clearly, ZMapp has set a successful example for the use of plant-made mAbs in such an emergency situation. Clinical trial with ZMapp is now being planned. It is reasonable to expect that the first plant-made antibody will soon come to the market.

- 1 Hiatt A, Cafferkey R, Bowdish K. Production of antibodies in transgenic plants. *Nature*, 1989, 342: 76–78
- 2 Giritich A, Marillonnet S, Engler C, van Eldik G, Botterman J, Klimyuk V, Gleba Y. Rapid high-yield expression of full-size IgG antibodies in plants coinfecting with noncompeting viral vectors. *Proc Natl Acad Sci USA*, 2006, 103: 14701–14706
- 3 Huang Z, Phoolcharoen W, Lai H, Piensook K, Cardineau G, Zeitlin L, Whaley KJ, Arntzen CJ, Mason HS, Chen Q. High-level rapid production of full-size monoclonal antibodies in plants by a single-vector DNA replicon system. *Biotechnol Bioeng*, 2010, 106: 9–17
- 4 Zeitlin L, Pettitt J, Scully C, Bohorova N, Kim D, Pauly M, Hiatt A, Ngo L, Steinkellner H, Whaley KJ, Olinger GG. Enhanced potency of a fucose-free monoclonal antibody being developed as an Ebola virus immunoprotectant. *Proc Natl Acad Sci USA*, 2011, 108: 20690–20694
- 5 Olinger GG Jr., Pettitt J, Kim D, Working C, Bohorov O, Bratcher B, Hiatt E, Hume SD, Johnson AK, Morton J, Pauly M, Whaley KJ, Lear CM, Biggins JE, Scully C, Hensley L, Zeitlin L. Delayed treatment of Ebola virus infection with plant-derived monoclonal antibodies provides protection in rhesus macaques. *Proc Natl Acad Sci USA*, 2012, 109: 18030–18035