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ASX Announcement

China presentation of BioDiem's liver treatment technology

Highlights

- BioDiem's vaccine program targeting liver disease underway at University of Canberra
- Researchers successfully establish new system designed to deliver drugs specifically to the liver
- Poster showing research results presented at China conference on Hepatitis B
- Treatments for viral hepatitis and liver cancer represent large markets where new effective therapies are required.

Melbourne, 24 October 2013: Australian infectious disease therapy and vaccine development company BioDiem Ltd (ASX: BDM) has announced that a poster showing results from its hepatitis vaccine program, which has successfully achieved an important milestone towards developing treatments for liver diseases as previously announced, was presented at the 2013 International Meeting on Molecular Biology of Hepatitis B viruses in Shanghai, China. This meeting is organized by the Hepatitis B Foundation and is the premier annual international conference on Hepatitis B. The international meeting covers all aspects of the biology of hepatitis B and hepatitis D, as well as the latest developments in antiviral therapies against these two viruses. Attendees include those working in many areas related to viral hepatitis such as research into how to treat infected patients and pharmaceutical companies developing therapies or vaccines related for the disease.

Researchers at the University of Canberra have developed a system designed to target the liver. This could be used to aim drugs or other therapies directly at the liver to treat liver-specific diseases such as viral hepatitis and liver cancer, for example. An advantage of this targeting could be that smaller dosages of currently used therapies could be given to liver-disease patients. This could result in higher cure rates and/or fewer dose-related side effects. As the system has been successfully designed, work is progressing to establish the range of potential drugs or therapies that could be delivered to the liver by this technology. This liver targeting technology is licensed exclusively (worldwide) to BioDiem and is patent-protected.

"The opportunity to present the University of Canberra research at this international conference in China is well-timed as we look to engage with commercial partners to continue the development work of this liver-targeting technology" said BioDiem CEO Julie Phillips.

An estimated 4.4 million Americans and 112 million Chinese are living with chronic hepatitis, most of whom do not know they are infected. Viral hepatitis is the leading cause of liver cancer (HCC) and the most common reason for liver transplantation. Liver cancer in men is the fifth most frequently diagnosed cancer worldwide, and is the second leading cause of cancer-related death in the world. Over 40 per cent of all cases of HCC occur in the People's Republic of China, which has an annual incidence of 137,000 cases. The global hepatitis market was estimated to be $3,276m in 2009, representing a compound annual growth rate (CAGR) of 3.1% between 2001 and 2009. The market is anticipated to reach revenues of approximately US$5,977m by 2016, growing at a CAGR of 9% between 2009 and 2016. The chief reason for its growth is the large chronic carrier hepatitis population primarily Hepatitis B and C.

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About BioDiem Ltd

BioDiem (ASX: BDM) is an ASX-listed biopharmaceutical company developing vaccines and antimicrobials targeting treatment and prevention of infectious diseases and related cancers. The lead technology is the LAIV (Live Attenuated Influenza Virus) used for seasonal and pandemic influenza vaccines and is given intranasally. A therapeutic hepatitis vaccine project targeting hepatitis D and B is underway at the University of Canberra. BioDiem's antimicrobial, BDM-I, is in preclinical development for fungal and bacterial diseases, also schistosomiasis, tuberculosis and protozoal infections. The SAVINE (scrambled antigen) technology is in development for tuberculosis and also EBV-related disease including nasopharyngeal cancer. BioDiem's retinal product, BDM-E, in development for retinitis pigmentosa is available for outlicence.

About BioDiem's Liver-Targeted Technology

The vector, is based on the Hepatitis D virus (HDV) which is a small, enveloped RNA virus requiring the envelope proteins of a helper virus, Hepatitis B virus (HBV), for further particle formation. HDV can only infect hepatocytes and produce virus particles in cells that are co-infected with HBV. Based on this natural tropism for the liver and the successful generation of replication competent recombinants this should enable the delivery of biologically active molecules to the liver.
For additional information, please visit www.biodiem.com

About University of Canberra

The University of Canberra has a dynamic, innovative and collaborative research culture with a focus on applied research in areas aligned with the needs of our local community as well as national and international research priorities. The University of Canberra's researchers deliver breakthroughs that help solve real-world problems, particularly in the areas of governance, environment, communication, education and health.

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UNIVERSITY OF CANBERRA

Towards the Development of a Hepatitis D Virus-Based, Highly Liver-Specific Gene Delivery Vector

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Abstract

The *Hepatitis D virus* (HDV) is an extremely small RNA virus that requires the envelope proteins of a helper virus, *Hepatitis B virus*, for particle formation. It has repeatedly been suggested that HDV would make an excellent liver-specific gene delivery vector. However, all previous attempts to generate recombinant, replication competent viruses that carry substantial insertions have failed. Here we report the development of a new platform technology that enables genetic manipulations while maintaining replication competency. The patented technology uses 'stabilising' sequences that are partially complementary to an inserted sequence-of-interest and that are positioned 'opposite' the first insertion site to restore the typical rod-like secondary structure of the HDV genome/anti-genome. The use of 'stabilising' sequences has allowed us to engineer replication competent viruses with up to 78 additional nucleotides. Northern blot analysis revealed that the newly generated viruses replicate at levels similar to those of wild-type viruses. Our results indicate that it may soon become possible to transform HDV into a highly liver-specific gene delivery vector. Furthermore our findings will guide others in the manipulation of the HDV genome for both basic and applied research purposes.

Acknowledgements

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Results

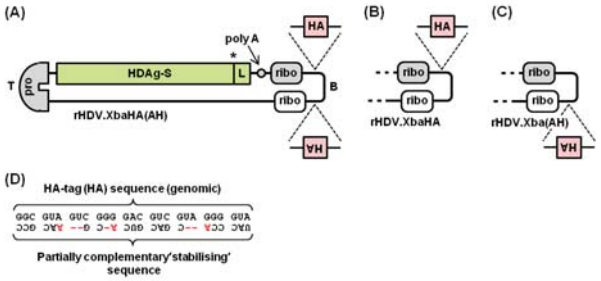


FIG. 1. Insertion of sequences at the 'bottom' of the rod. **(A, B and C) Schematic representations of the recombinant viruses rHDV.XbaHA(AH), rHDV.XbaHA and rHDV.Xba(AH), respectively. Relative positions of known genomic and anti-genomic features are shown, including those of the promoter sequence(s) (pro), the open reading frame for the short (S) and long (L) form of the hepatitis delta antigen (HDAg), the polyadenylation signal sequence (poly A), and ribozyme (ribo) sequences (white and grey boxes). The asterisk indicates the position of an editing site that can extend the open reading frame. Letters 'T' and 'B' indicate the top and bottom of the rod. (D) Artificially edited HA-tag nucleotide and partially complementary 'stabilising' sequence, as inserted in the recombinant viruses described above.**

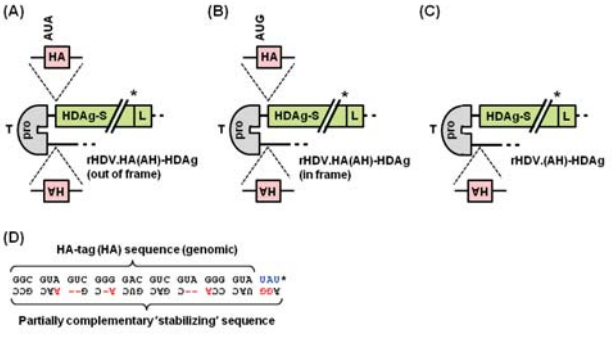


FIG. 2. Insertion of sequences upstram of the HDAg open reading frame. **(A, B and C) Schematic representations of the recombinant viruses rHDV.HA(AH)-HDAg (out of frame), rHDV.HA(AH)-HDAg (in frame) and rHDV.(AH)-HDAg, respectively. (D) Artificially edited HA-tag nucleotide and partially complementary 'stabilising' sequence, as inserted in the recombinant viruses described above.**

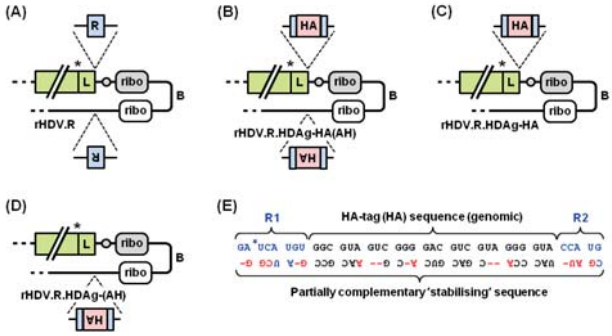


FIG. 3. Insertion of sequences downstream of the HDAg open reading frame. **(A, B, C and D) Schematic representations of the recombinant viruses rHDV.R, rHDV.R.HDAg-HA(AH), rHDV.R.HDAg-HA and rHDV.R.HDAg-(AH), respectively. Note that all 4 viruses are identical except for the insertion of heterologous sequences consisting of the remnants of a much larger insertion a HA-tag and/or partially complementary sequences (blue and pink boxes). (E) Artificially edited HA-tag nucleotide sequence and partially complementary 'stabilising' sequence (flanked by remnant sequences described above), as inserted in the recombinant viruses shown in (B, C, and D).**

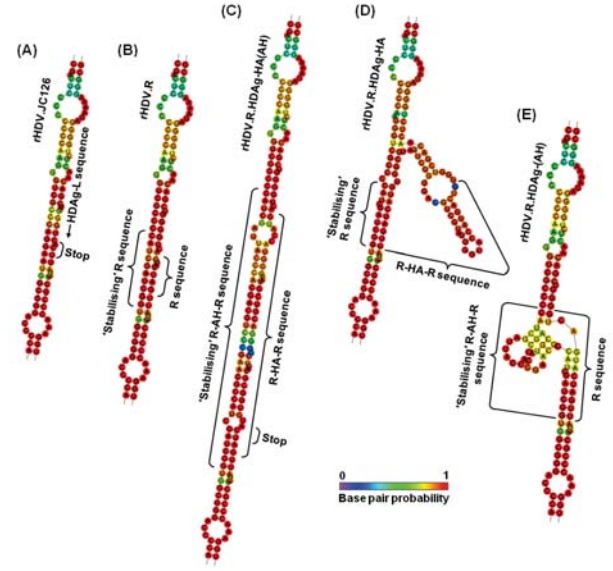


FIG. 4. RNA secondary structures. **(A, B, C, D and E) Predicted minimum free energy structures and base pair probabilities for genomic RNA sequences at the end of the HDAg open reading frame of rHDV.JC126, rHDV.R, rHDV.R.HDAg-HA(AH), rHDV.R.HDAg-HA and rHDV.R.HDAg-AH, respectively. Structures and probabilities were calculated using the RNAfold server (http://rna.tbi.univie.ac.at/) at the Institute for Theoretical Chemistry, University of Vienna (Austria). Note that inserting a sequence-of-interest along with a partially complementary, 'stabilising' sequence maintains the rod-like structure of the virus genome, whereas the insertion of either of these two sequences alone destroys it.**

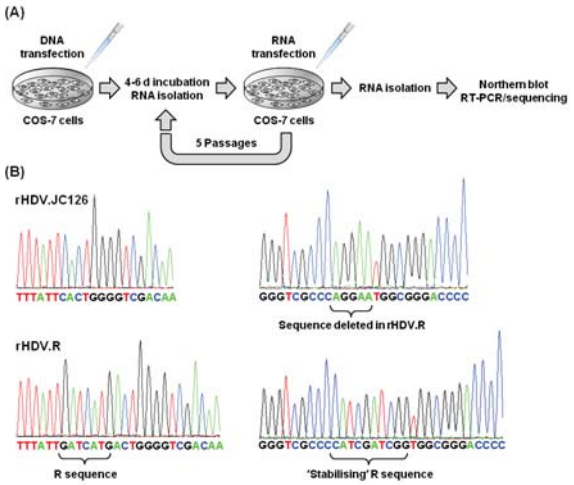
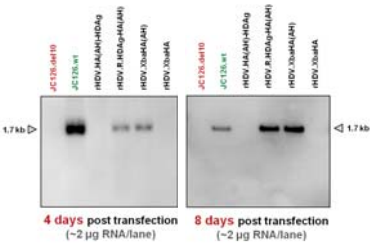


FIG. 5. Genetic stability of recombinant viruses carrying heterologous sequences. **(A) Experimental set-up. (B) Electropherograms showing DNA sequences obtained from HDV-specific RT-PCR products. Recombinant HDV RNAs were passaged, RNA was reverse transcribed using random hexamers, cDNAs spanning the insertion site of heterologous sequences were PCR amplified using HDV-specific primers and amplicons were sequenced using different set of primers.**

FIG. 6. Northern blot analysis. **African green monkey kidney fibroblast (COS-7) cells were transfected with plasmids encoding over-length (1.2-fold) copies of various recombinant HDVs. Total RNA was isolated 4 or 8 days after the transfection and RNA samples were separated and blotted according to standard protocols. HDV-specific RNA was detected by chemiluminescence using a digoxigenin (DIG)-labeled riboprobe corresponding to the HDV nucleotides 1620-429, alkaline phos-phatase (AP)-conjugated anti-DIG antibodies and the ImageQuant LAS4000 digital imaging system.**



Conclusions

We tested several sites in the HDV genome for their suitability to carry additional sequences. While inserting sequences upstream of the HDAg sequence destroyed virus replication, the insertion of additional sequences along with appropriately positioned 'stabilising' sequences were tolerated immediately downstream of the HDAg coding sequence and at the 'bottom' of the rod. These results indicate that it may soon become possible to transform HDV into a highly liver-specific gene delivery vector capable of expressing recombinant peptides, proteins and/or pharmaceutically active RNAs to treat liver-related diseases.