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Groundbreaking first nano molecular drill crosses cell membranes to safely deliver small molecules, biological therapeutics and nucleotide-based drugs

CyGenica Limited has developed a new class of engineered cell-penetrating protein as a drug delivery system, creating a drilling mechanism which can penetrate target cell membranes and safely deliver a range of cargoes including cancer drugs, antibiotics, gene therapy products and therapeutic nucleic acids.

The GEENIE delivery platform is a proprietary negatively charged engineered protein molecule that translocates through the cell membrane using a helical mechanism. It can deliver its payloads, including genome editing compounds such as CRISPR-Cas9, gRNA and DNA, directly to the targeted nucleus without harming the cell.

It can be programmed to recognize cell type receptors, achieving selective delivery to targets such as HER2+ expressing cells and glioblastoma. GEENIE can penetrate the membranes of mammalian cells (90% of cells take up within one to three hours), drug-resistant bacteria, yeast and plant cells. In vitro and in vivo mice studies have so far shown its novel entry mechanism leads to a lack of toxicity and very low immunogenicity.

The platform is robust and scalable. The production cost of this simple recombinant protein is less than $1/microgram compared to an estimated cost of $400/microgram for viral delivery devices, particularly in genomic medicine.

"The challenge of delivering drugs for cancer and genetic therapies, be it genes, RNAs or CRISPRs across cell membranes without damaging the cells and triggering an adverse immune response remains a complex hurdle in the pharmaceutical industry," said Nusrat Sanghamitra, founder and CEO of CyGenica Limited, which has offices in India and Ireland. "Our groundbreaking GEENIE technology is a molecular drill that acts as a nanomachine and tunnels through the cell membrane to deliver multitudes of cargoes in an efficient and targeted manner without any toxicity and minimum immunogenicity. This will revolutionize drug delivery and lead to better patient outcomes."

Summary of toxicity data

In vitro data showed no cell death in millimolar concentration in more than 12 cell lines and in vivo data showed single dose GLP grade toxicity in mice (6 male and 6 female) 28 days in 1800 mg/kg of body weight (which is >25× the expected dose in humans). Two male mice were sick on day 4. All other mice were healthy throughout the study. No organ toxicity was found. All other physiological parameters were correct.

In ex vivo data, in blood cells of healthy human volunteers, multiple dose studies were done. Cytokine response assay was carried out and minimal inflammatory response was found.

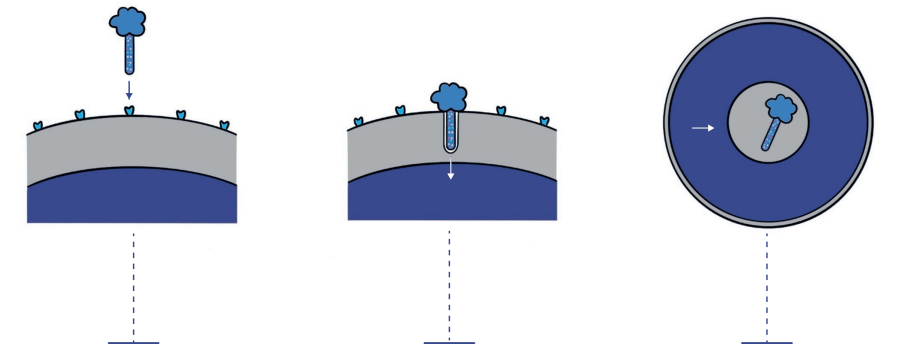

Fig. 1 | CyGenica's three-step delivery strategy.

Delivering lower doses of cancer drugs

With a PhD in chemistry and 15 years' research experience including at Leiden, Oxford and Kyoto universities, Sanghamitra's original focus was to reduce drug toxicity and dosage. She started synthesizing new water-soluble copper complexes in a search for an alternative for Cisplatin, a commonly used anticancer metallodrug. While the complexes killed cancer cells, high toxicity was associated with the most active compounds.

During this time, Sanghamitra's father was diagnosed with cancer and underwent debilitating chemotherapy.

Refocusing her research field to protein engineering, biophysics and cell biology, Sanghamitra had a major breakthrough with the discovery of a new function of an engineered protein. It performed as a nano molecular drill, transporting molecular cargoes through the cell membrane. An idea-to-POC Biotechnology Ignition Grant in India helped prove her hypothesis that crossing the living cell membrane could improve efficacy of cancer drugs and enable reductions in doses.

Sanghamitra describes current chemotherapy treatment as 'carpet bombing' which kills cancer cells as well as normal healthy cells. The toxic side effects reduce quality of life for some 18 million cancer patients worldwide.

Preclinical trials which conjugated the anticancer drug Cisplatin with GEENIE (creating CyPlatin) demonstrated that the drug retained fully efficacy with only 10% of the standard dose, thus significantly reducing side effects.

GEENIE's cell entry mechanism

GEENIE has a predominantly non-endocytic uptake mechanism. This is demonstrated by the internalization of GEENIE labelled with green florescent dye in red blood cells, which doesn't have an endocytic machinery. Endocytosis inhibition studies proved that more than 60% of GEENIE internalizes by an endocytosis independent mechanism ~40% is dependent on a micropinocytosis mechanism. CyGenica's delivery strategy (Fig. 1) involves three steps:

- **Step 1:** Target specificity by recognizing cell type receptors
- **Step 2:** Entry through target cell membrane
- **Step 3:** Entry to the nucleus of the target cells

"Given the fact that cancer is a complex disease, too much molecular targeting may not be medically flawless. My hypothesis is that if we effectively take everything inside the target cell by a non-toxic delivery system, we may reduce the dose thus leading to some reduction of toxicity or side effects. So, along with the approach of targeted therapy, I

think reducing the dose is essential." Sanghamitra said. "This technology could be a boon to public healthcare systems globally by bringing down the economic and social costs of managing cancer."

Intracellular delivery of gene editing tools

Recent developments in genome editing technologies, based on engineered or bacterial nuclei, have opened up the possibilities of directly targeting and modifying genomic sequences in almost all eukaryotic cells.

Genetic therapeutics will become the next-generation therapeutic solution for diseases such as cancer as they are more targeted and based on fewer treatments. "In futuristic terms, you edit the gene and the patient is cured in one or a few doses," Sanghamitra said. "It reduces the trauma of chemotherapy and multiple hospital admissions."

Of these technologies, CRISPR (clustered regularly interspaced short palindromic repeats) has captured much attention. It enables parts of the genome to be edited by the removal, addition or alteration of genetic material in the DNA sequence.

Two key molecules are involved in CRISPR-Cas9 therapeutics. The Cas9 enzyme is a scissor protein which cuts the target DNA at a specific location so it can be altered. The guide RNA (gRNA) is a pre-designed RNA sequence which guides the Cas9 to the correct part of the genome.

A major challenge to developments in CRISPR-based therapeutics is the inefficiency and unsafe delivery of the gene editing toolbox such as CRISPR-Cas9 and nucleotides. Existing delivery mechanisms such as electroporation, viruses and nanoparticles are extremely expensive ($300,000–$1 million) and carry risks including high cell death, no cell selectivity, immunogenicity, carcinogenicity and toxicity.

GEENIE has carried out successful tests on the intracellular delivery of CRISPR-Cas9, gRNA and plasmid DNA (2.4 kb size) into breast cancer cells, delivering them efficiently and in a non-toxic manner for less than $1 per microgram.

In gene editing tests, GEENIE was used to deliver CRISPR-Cas9/gRNA as ribonucleoprotein (RNP). Sanger sequencing was performed by a third party (GenOmbio) and analyzed with Synthego's ICE (Inference of CRISPR Edits) tool (Fig. 2). The tool identifies the percentage of the genome that has been successfully modified with insertions or deletions (indels) and then characterizes the sequence and abundance of each particular indel. Twenty per cent indel was observed.

While viral delivery systems have achieved editing efficiency of 80 to 90%, non-viral delivery systems have so far achieved 40 to 60%. CyGenica is working on strategies to improve efficiency and believes it will achieve more than 40% within a year.

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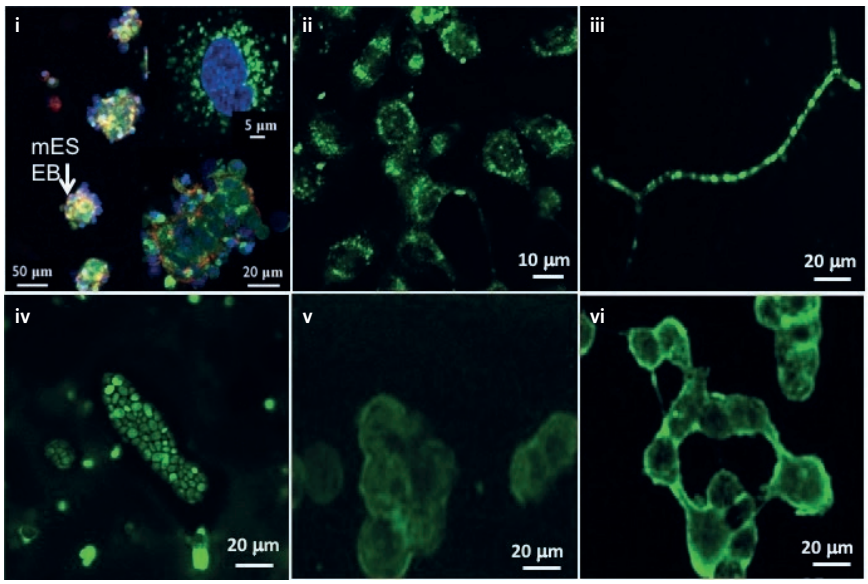

Fig. 2 | Intracellular delivery of various molecular cargoes into different types of cells by GEENIE. i) Fluorescent dye ATTO520 into mouse embryonic stem (mES) cells; ii) fluorescent dye ATTO520 into microglial cells; iii) fluorescent dye ATTO520 into the drug resistant strain of Pseudovibrio AD37; iv) fluorescent dye ATTO520 into the yeast cells *Sachromyces sp.*; v) Cas9-GFP into MCF7 cells; vi) gRNA-ATTO520 into MCF7 cells.

CyGenica has also succeeded in engineering a construct of GEENIE that effectively binds to small interfering RNA (siRNA). Sanghamitra believes future studies would establish GEENIE as a system to deliver siRNA by knocking down barriers and silencing key genes to achieve a desired therapeutic effect.

Fighting superbugs

The development of antimicrobial resistance in bacteria is a huge public health problem. Key issues are the antibiotic drugs' inability to cross the cell membrane, and the efflux pumps which can drain antibiotic agents from bacterial cells.

CyGenica has established that GEENIE can enter the cell membrane of resistant bacteria and is not thrown out by the efflux pump.

"The size of our conjugate is much bigger than the size of the active site of the drug expulsion pump," Sanghamitra said. "We haven't proven the hypothesis with a drug, but we have proven that GEENIE is able to carry molecules to the resistant strains. This is important, because if we can make existing antibiotics work for resistant strains, that is a big contribution. It's the equivalent of discovering a new antibiotic, because that takes more time than making the existing antibiotics work better."

Live cell tracking

Another GEENIE composite, CyGlo (GEENIE with any fluorescent dye) is a reagent to observe living cells under the fluorescence microscope:

- It is simple to use—add the recommended volume of reagent, incubate at 37°C for 1 hour, wash with PBS and image.
- There are no complications and no requirements for serum-free media.
- Equally efficient in labelling cancer cells, neurons (microglial cells, SH-SY5Y cells) keratinocytes, fibroblasts, stem cells, bacteria and yeast cells.
- Can be customized to any color required.

Innovation awards and partnerships

Sanghamitra and CyGenica have taken a number of top Indian and international innovation awards in the past few years. Sanghamitra is a Leaders in Innovation Fellow 2021 at the Royal Academy of Engineering, London. The company became a member of the Mayo Clinic Innovation Exchange program in the US in 2020, has won the Most Innovative Product Award from Enterprise Ireland, was a finalist in the International Innovation Challenge at the University of Massachusetts Medical School and won third prize at the She Loves Tech Global Startup Competition in Beijing.

Indian awards include an Emerging Biotech Startup, an Innovation Growth Program award, a Top Innovator gold award, a National Award for a Technology Startup and the Cancer Innovation Challenge.

Collaborators include the University College Cork and TSSG at Waterford University of Technology in Ireland, cLab Ventures in London, and Bioneeds and IISER in India. $2.3 million funding has been raised.

CyGenica's goal is to produce very robust proof of concept or preclinical trials with one or two molecules. It will then seek strategic partnerships with multiple partners on specific disease models to progress clinical trials. Of particular interest are pharma and biotech companies with a focus on genome editing therapeutics in rare cancers, glioblastoma and HER2-positive breast cancer.

CyGenica has applied for two international patents, one of which is this year entering national phase in the USA, Europe, China, India, Canada and Korea. Sanghamitra plans to move the company to the Boston, USA, area, and eventually list it on the NASDAQ.

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