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CAR T-cell therapy: a revolutionary alternative for cancer immunotherapy

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Chimeric Antigen Receptor T-cell therapy (CAR T-cell therapy) is a type of immunotherapy in which a patient's own T-cells are used to treat cancer. It's a revolutionary new treatment alternative for certain types of cancer. In this process, a patient's T cells are genetically modified to specifically recognize and attack cancer cells. The T-cells are extracted from the patient's blood, and then modified in the laboratory by introducing a new gene that produces a chimeric antigen receptor (CAR) on the surface of the T-cells. The CAR is designed to recognize a specific antigenic protein on the surface of the cancer cells. The modified T-cells are then multiplied *in vitro* to create millions of CAR T-cell copies. Finally, the CAR T-cells are infused back into the patient's bloodstream where they can specifically locate, recognize, and destroy the cancer cells.

CAR T-cell therapy has shown remarkable successes in treating some types of blood cancers, such as acute lymphoblastic leukemia (ALL) and certain types of non-Hodgkin's lymphoma. However, it is still a relatively new and complex treatment, and there are potential side effects. The major side effects include cytokine release syndrome (CRS), a potentially life-threatening immune reaction, which can cause flu-like symptoms, fever, and low blood pressure, and neurotoxicity, which can cause confusion, seizures, and other neurological symptoms. Hence, close monitoring and management of these side effects are crucial for ensuring the safety and efficacy of the treatment.

The first chimeric receptor was designed by Gross and his co-workers in the year 1989 at the Weizmann Institute of Science in Israel (Gross *et al.*, 1989). The chimeric antigen receptors are modular synthetic receptors that consist of four main components: (1) an extracellular target antigen-binding domain, (2) a hinge region, (3) a transmembrane domain, and (4) one or more intracellular signalling domains. The antigen binding domain confers CARs the required specificity to the target antigen. The hinge region provides flexibility to overcome steric hindrance and allows the antigen-binding domain to access the targeted epitope. The transmembrane domain helps to anchor the CAR to the T-cell membrane and is relevant for CAR-T cell function as it has been shown to influence the CAR expression level and stability. The intracellular signalling domain can also greatly affect the functional activity of CARs. The most common component of the intracellular domain is CD3ζ which delivers the first signal for T-cell activation and function. However, there is also a need to incorporate concomitant co-stimulatory signals (CD28 or 4-1BB) as the second signal, which is critical for increased secretion of cytokines like IL-2 as well as multiplication and persistence of T-cells.

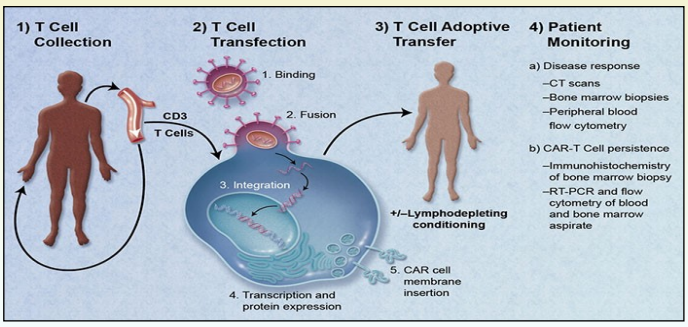

Image: Schematic representation of the procedure of CAR T-cell therapy
Image Source: Jacobson, C.A. and Ritz, J. (2011). *Blood* **118** (18): 4761–4762

Various types of cancer cells have different sets of antigens. Therefore, each kind of CAR T-cell therapy has to be developed to recognize a specific type of cancer antigen. Hence, a CAR T-cell therapy developed for one type of cancer will never be effective against another type of cancer. The U.S. Food and Drug Administration (FDA) has so far approved several CAR T-cell therapies and many more are in the process of development. The approved therapies include Axicabtagene ciloleucel (Yescarta™) and Lisocabtagene maraleucel (Breyanzi®) for B-cell lymphoma, Brexucabtagene autoleucel (Tecartus®) for Mantle Cell Lymphoma (MCL), Ciltacabtagene autoleucel (Carvykti™) and Idecabtagene vicleucel (Abecma®) for multiple myeloma, and Tisagenlecleucel (Kymriah®) for acute lymphoblastic leukemia.

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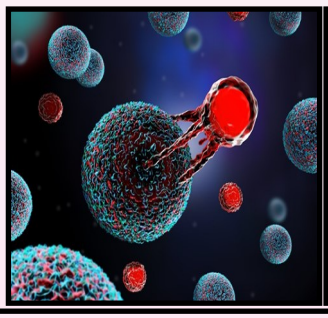

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Despite being a promising therapeutic alternative against cancer, CAR T-cell therapy has limitations in its use primarily due to important side effects and the prohibitive cost involved. Besides the cost of procedures and facility, the acquisition cost of CAR T-cell itself is between $373,000 to $475,000 per infusion (Fiorenza *et al.*, 2020) and the therapies being carried out in a standard inpatient environment, the additional cost for these facilities is around $79,466 to $85,267 (Lyman *et al.*, 2020). Another important limitation of CAR T-cell therapy is its potential side effects; one of the serious side effects is cytokine release syndrome (CRS), in which cytokines released by the CAR T-cells may cause an acute systemic inflammatory syndrome characterized by fever and multiple organ dysfunction. In case such an eventuality occurs in a patient, its cost of treatment ranges from $30,000 to $56,000 per patient (Hernandez *et al.*, 2018).

Despite its remarkable potential as observed in the clinical trials so far, most patients participating in CAR T-cell trials have only been followed for a relatively short time. Although data obtained from such trials have shown highly effective early responses to the therapy, the sustenance of these responses may be ascertained only when the trial participants are followed over a longer term. Further trials involving larger study samples including paediatric as well as adult patients, and evaluation of the responses over more extended periods will help understand the real impact of this novel cancer therapy and the ways to reduce or avoid its serious side effects in future.

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Inauguration of Biotechnology Lab by Dr. Probodh Borah at Royal Global University, Guwahati sponsored by ALSBT Hub.

Dr. P. Borah, Coordinator, ALSBT Hub receiving recognition as a Fellow of the National Academy of Veterinary Sciences (FNAVS) in 2022 at Nagpur

International Research Highlights

- **A new genome editing technology for insertion of large fragment of DNA into plant genome** : Edition of genome is a very important technique in modern biotechnology. It involves insertion or deletion of target sequence in the genome of a plant or animal. In a major development in this field, a new technology has been reported for insertion of a large fragment of DNA into the plant genome. A research paper published recently in Nature Biotechnology reported precise insertion of a DNA fragment of 11.5 kb into the plant genome using this novel method, PrimeRoot editors.
  Source: *Nature Biotechnology*: 2023 Apr 24. **doi:**10.1038/s41587-023-01769-w.

- **Vaccine printer for production of thermostable m -RNA vaccine:** Shelf life of a vaccine is an important criterion determining the success of mass vaccination programmes. In the vaccination against COVID-19, the world has realized the need of a stable vaccine which can be taken to remote places without deteriorating the quality of the vaccine. In a landmark research article published in Nature Biotechnology, a novel vaccine delivery system has been reported. The technology has been named as microneedle vaccine printer (MVP) which can very efficiently and quickly pack mRNA (vaccine candidate) in lipid spheres. The resultant product has been found to be immunogenic and stable at room temperature for up to 6 months. The research group involved has claimed that their technology will be very helpful in implementing mass vaccination programmes especially in resource poor third-world countries, where cold chain maintenance is a major challenge for efficacy of vaccination.
  Source: *Nature Biotechnology*. 2023 Apr 24
  doi:10.1038/s41587-023-01774-z.

- **"DNA" The Ultimate Hard Drive:** A team led by George Church, a synthetic biologist at Harvard Medical School in Boston, created a DNA information-archiving system that uses no cells at all. Instead, an inkjet printer embeds short fragments of chemically synthesized DNA onto the surface of a tiny glass chip. To encode a digital file, researchers divide it into tiny blocks of data and convert these data not into the 1s and 0s of typical digital storage media, but rather into DNA's four-letter alphabet of A, C, G, and T. Each DNA fragment also contains a

digital "barcode" that records its location in the original file.

Reading the data requires a DNA sequencer and a computer to reassemble all of the fragments in order and convert them back into digital format. The computer also corrects for errors; each block of data is replicated thousands of times so that any chance glitch can be identified and fixed by comparing it to the other copies.

Source: https://www.science.org/content/article/dna-ultimate-hard-drive.

- **Artificial intelligence for development to vaccine:** Artificial intelligence (AI), now a days, has been applied to various domains of science and the latest addition in the list is vaccine development. In an article published in Frontiers in Immunology, a group of researchers has reported the development of a T-cell based vaccine against SARS-CoV-2 infection using artificial intelligence. They claim that by using AI, potential mutant strains of the virus can be predicted even before they show up and vaccines can be kept ready beforehand. **Source:** *Frontiers in Immunology*.2023 Apr **doi:**10.3389/fimmu.2023.1166546. eCollection 2023.

(Compiled by Dr. Luit M Barkalita)

Sanctioning of research projects under "DBT-ALSBT Hub Microgrant" scheme

A total of 11 research proposals in different areas of biotechnology and allied sciences were sanctioned out of total 135 proposals received from young researchers below 45 years of age across the Northeastern Region. A total amount of Rs. 49,58,600.00 was released as the first-year grant for the research projects sanctioned for 2-year duration. The beneficiaries included researchers from seven institutions of the region, *viz*., Gauhati University, ICMR-RMRC Dibrugarh, Royal Global University, Guwahati, SBMS College, Sualkuchi, College of Agriculture, Jorhat, College of Veterinary Science, Guwahati and Darrang College, Tezpur.

Executive Committee meeting for ALSBT Micro grant scheme.

Expert committee meeting for DBT-ALSBT Micro grant scheme.

First review meeting of sanctioned projects under Micro grant scheme.

National Research Highlights

- The Indian vaccine maker company 'Bharat Biotech' has generated India's first intranasal vaccine **iNCOVACC®** to combat COVID-19. It is a replication-deficient adenovirus vectored vaccine which contains the spike protein gene of SARS-CoV-2. The vaccine has been approved by Drugs Controller General of India (DCGI) for restricted emergency use as a heterologous booster dose in 18 years and older adults. **Source:**https://www.bharatbiotech.com/intranasal-vaccine.html
- A new probiotic bacterium *Ligilactobacillus salivarius* F14 was discovered by researchers from the Institute of Life Sciences (ILS), Bhubaneswar. The bacterium was found in the gut of a tribal community of Odisha. This bacterium exhibits the health-benefit characteristics of probiotics with potential immunomodulatory and antimicrobial ability. **Source:** *World Journal of Microbiology and Biotechnology*, 39: 171. (2023) https://doi.org/10.1007/s11274-023-03626-z
- A research team from Tata Memorial Centre along with doctors from 10 other institutes have developed a new cancer treatment intervention with **Lignocaine**, a common, inexpensive, and local anaesthesia that could significantly increase the survival rate and cure rate of breast cancer patients. The effective anti-cancer property of lignocaine was demonstrated after 11 years of study in 1600 breast cancer patients. **Source:** https://tmc.gov.in/index.php/en/pressr
- The Serum Institute of India and Department of Biotechnology, Govt of India have jointly developed the first indigenous quadrivalent Human Papillomavirus vaccine (qHPV), '**CERVAVAC'** to fight cervical cancer. **Source:** https://pib.gov.in/ PressReleaseP age.aspx? PRID=1856034
- Zydus Cadila in collaboration with DBT-BIRAC, Govt. of India has developed the World's first COVID-19 DNA vaccine '**ZyCoV-D'** against SARS-CoV-2. It is a needle-free vaccine and approved for emergency use from the age of 12 years and above. **Source:** https://pib.gov.inPressRelease-age.aspx? PRID=1747669

(Compiled by Dr. Lukumoni Buragohain)

Research and Achievement of ALSBT Hub during 2022-2023

- A DNA vaccine (pCI-Cap) was generated against Porcine Circovirus 2 which exhibited a promising immune response as well as it has shown significant protection in a challenge study conducted in the mice model.
- Spatio-temporal variations in the gut metagenome of indigenous Pati ducks (*Anas platyrhynchos domesticus*) of Assam have been studied and a consortium of probiotic isolates have been recovered which showed promising probiotic efficacy in both *in vitro* and *in vivo* assessments.
- Four immunogenic peptides from the extracellular loops of the most immunogenic outer membrane protein (PagN) of *Salmonella* Typhi were selected by immuno-informatics tools, synthesized and evaluated for their immuno-protective efficacy in the mice model and found that the synthesized peptides alone or in combination with Vi-capsular antigen could induce humoral immune response and confer protection to challenge infection with virulent *Salmonella* Typhi.
- A polymerase spiral reaction (PSR) assay has been standardized to detect the African Swine Fever virus (ASFV) genome in clinical samples and the process of its validation has been initiated.
- Whole genome sequencing of ASFV strains circulating in seven North-Eastern States has been completed and phylogenetic analysis revealed that all the strains belong to genotype II of the virus.
- Polymorphic DNA mutations in mitochondrial genes encoding ND1, CO1 and CYTB associated with canine malignant tumours have been identified.
- Molecular characterization and genotyping of biofilm-producing staphylococci associated with bovine mastitis have been done. A combination of 5% povidone iodine (betadine®) and 5 mg/ml of chitosan was suggested as an alternative to using high concentration of an antiseptic for sanitization of the udder surface of milch cows to get rid of biofilm-producing *S. aureus* frequently associated with subclinical mastitis.
- A non-toxic form of recombinant beta toxin of *Clostridium perfringens* was expressed in *E. coli* and a combination of the recombinant beta toxin protein with calcium phosphate nanoparticles as an adjuvant was found to elicit better antibody response in the mice model compared to its combination with the conventional oil adjuvant.
- Anti-*Clostridium perfringens* bacteriophages have been isolated and characterized. The lytic properties of the bacteriophage have been studied in different physico-chemical conditions. Further work for production of a high-titre phage preparation and NGS-based characterization of the lytic

Major Research Focus of ALSBT Hub, Assam

Viral pathogens associated with porcine reproductive disorders with special reference to Porcine Circovirus 2 (PCV2): Molecular characterization, and development of rapid diagnostics and new generation prophylactics .

Salient Achievement of Advanced Level State Biotech Hub, Assam

- Dr. Probodh Borah, Project Coordinator of the ALSBT Hub has been recognized as a Fellow of the National Academy of Veterinary Sciences (FNAVS) in 2022 in the annual convocation of the Academy held at Nagpur on 20th June 2022.
- Dr. Nikhil Ch. Nath, Co-PI of ALSBT Hub visited University of East London, UK from March 15 to September 10, 2022 for advanced research training with a fellowship from Assam Agricultural University under National Agricultural Higher Education Project (NAHEP)
- Dr. Probodh Borah, Project Coordinator, ALSBT Hub visited Dhaka and Chittagong, Bangladesh to act as a Resource Person for training programmes on '*In-silico* approaches for genomics and proteomics research' organized by Bangladesh Livestock Research Institute (BLRI), Savar, Dhaka, Bangladesh from 15-16 October 2022
- Dr. Probodh Borah, Project Coordinator, ALSBT Hub acted as a Resource Person in Workshop on 'Proteomics, Comparative Genomics, Drug Discovery and Bioinformatics' at University of Science & Technology, Chittagong (USTC) on 17th October 2022 . Also, delivered a guest lecture on 'Computer-aided drug designing' at Asian University for Women (AUW), Chittagong Bangladesh, on 18th October 2022 .

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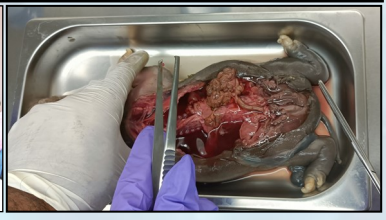


Activities of ALSBT Hub during 2022-2023

Hands on training programmes organized during 2022-2023

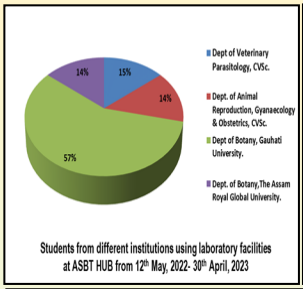
Sl No.	Title of Training	Duration and Date	No.	Level of participants
1.	Gene cloning, protein expression and next generation sequencing data analysis	7 days, 12th -18th July, 2022	12	Teachers, Research Scholars, Scientists
2.	Nucleic acid based techniques for detection and molecular typing of microbes	7 days, 15th -21st November, 2022	15	Teachers, Research Scholars
3.	Molecular cloning for production and purification of recombinant proteins	7 days, 21st – 27th February, 2022	13	Teachers, Research Scholars, Students

Outreach programmes conducted during 2022-2023

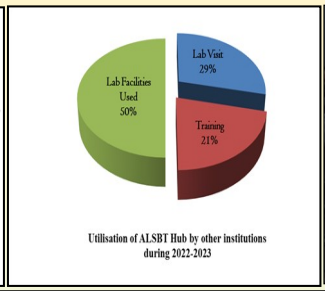
Sl No.	Title of Outreach Programme	Date	No	Level of participants
1.	"Scientific Pig farming for entrepreneurship development in North bank plain zone of Assam" At Krishi Vigyan Kendra, Lakhimpur, Assam	8th-9th September , 2022	36	Pig farmers and entrepreneurs
2.	"Scientific management of commercial Pig farms with special reference to biosecurity" at Krishi Vigyan Kendra, Nagaon, Assam	19th-20th December, 2022	49	Pig farmers and entrepreneurs
3.	"Advances in management and biosecurity of commercial Pig farms" in collaboration with Tamulpur Sub –divisional administration at Tamulpur, Assam	7th-8th February, 2023	82	Pig farmers, VFA & Veterinary Officers

DBT-ALSBT Hub 'Online Lecture Series'

Sl. No.	Date	Topic	Speaker
1.	3rd August 2022	Progress towards antiparasitic vaccines and what immunological lessons they can teach us	**Dr. Robin Flynn**, Project Manager, Graduate Studies, South East Technological University, Ireland cum Honorary Senior Lecturer, University of Liverpool, UK
2.	22nd December, 2022	*In silico* approach for understanding the molecular mechanism of diseases	**Dr. Reshmi Ramakrishnan**, Postdoctoral Research Fellow, Structural Biology and Engineering Unit King Abdullah University of Science and Technology, Jeddah, Saudi Arabia



Students from different institutions using laboratory facilities at ASBT HUB from 12th May, 2022- 30th April, 2023



Utilisation of ALSBT Hub by other institutions during 2022-2023

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