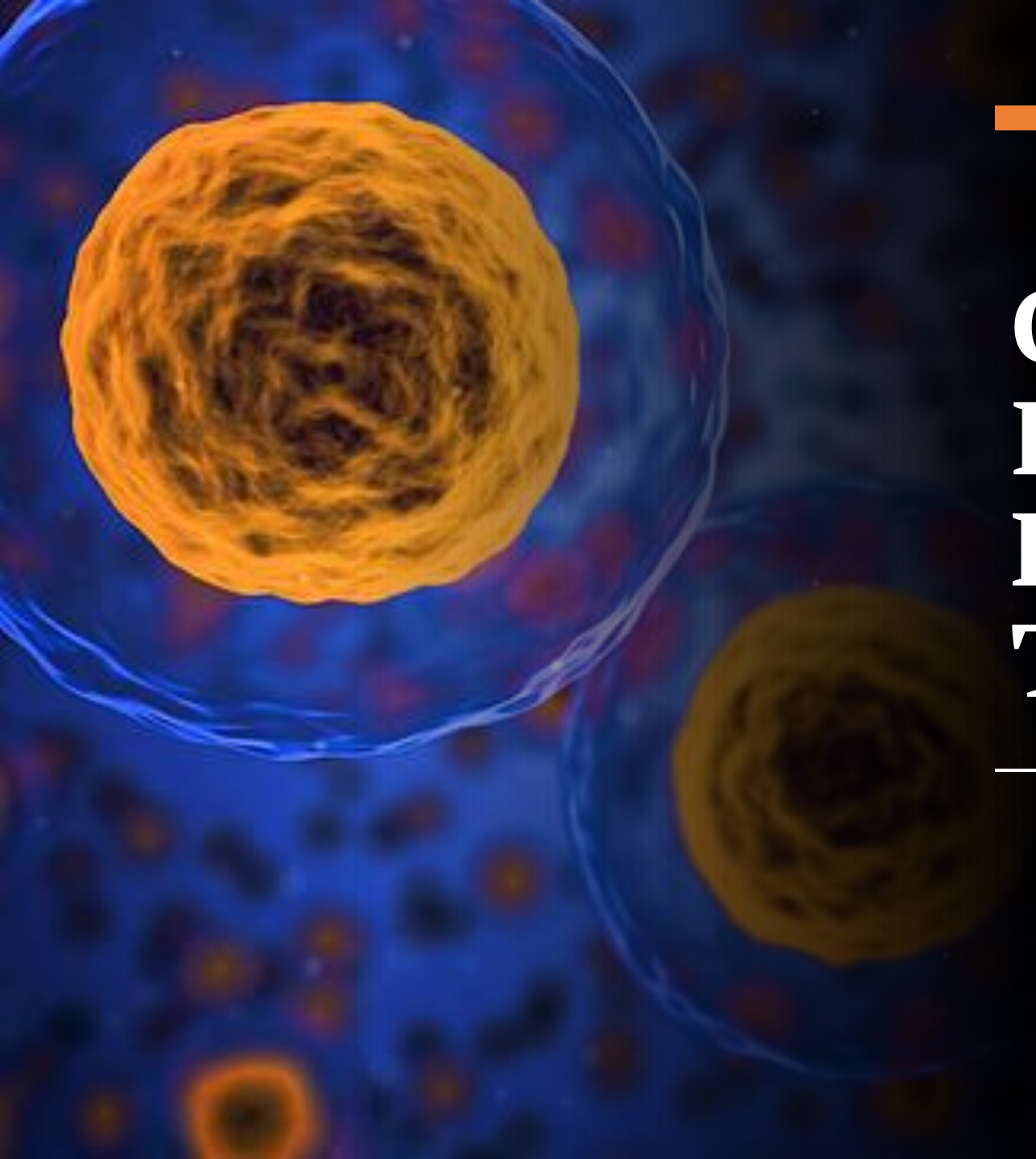


Cytokine Induced Killer Therapy

Introduction

Cytokine-induced killer cells were first discovered in 1991 [1] and are a heterogeneous population of CD8+ T cells, which were generated from human peripheral blood lymphocytes (PBLs) and simply expanded ex vivo via incubation with an anti-CD3 antibody, interferon-γ (IFN-γ), and interleukin (IL)-2. They can kill tumor cells mediated by FasL and perforin [2].

CIK cells can lyse cancer cells in an MHC-unrestricted manner through activating NK cell receptors such as DNAX accessory molecule-1, NKp46, NKG2D, and NKp30 [3-5]. In addition to the direct killing effect of CIK on cancer cells, they can also regulate the immune function by secreting various cytokines. A lot of studies have indicated that after stimulation by tumor cells, the levels of pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α, IFN-γ, and IL-2 secreted by CIK cells are significantly upregulated [6], and these cytokines further enhance systemic antitumor activity and induce a Th1 immune response.

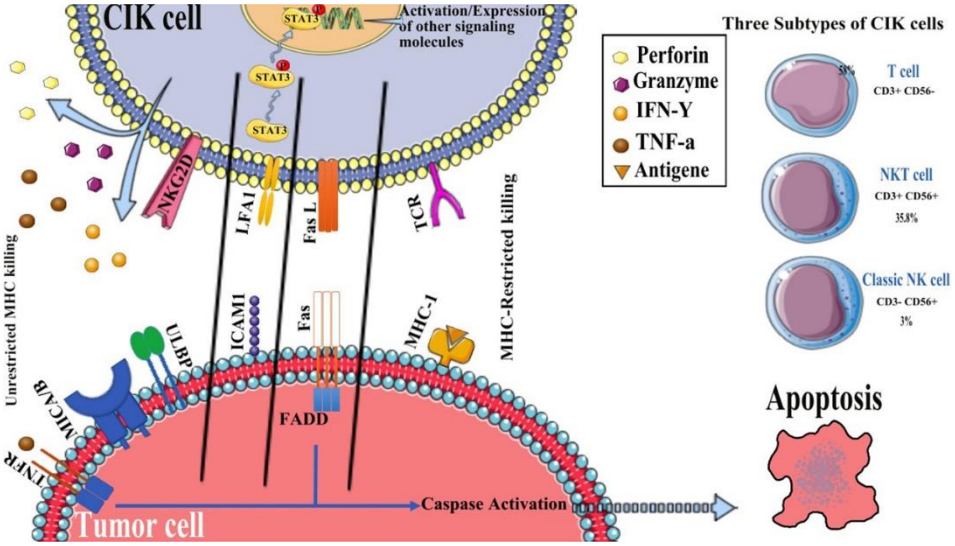


Figure 1: Possible mechanisms of CIK cell cytotoxicity. Being a mixture of cells, CIK includes T cells (CD3+CD56−), NK-T cells (CD3+CD56+), and NK cell population (CD3−CD56+).

Mechanism of Action

There has been less information about the cytotoxicity of CIK cells and its exact mechanism has not been completely elucidated. Blocking monoclonal antibodies against leukocyte function-associated antigen-1 and intercellular adhesion molecule-1 inhibits the cytotoxic effect of CIK cells. As a result, CIK cells have a cell-to-cell contact mediated by tumor cell lysis. [7].

The molecule that seems to play the most important role in tumor recognition by CIK cells is probably the natural killer group 2 member D (NKG2D) receptor. NKG2D is a member of the c-type lectin-activating receptor family that is evolutionarily conserved and is located within the NK gene complex on human chromosome 12p12-p13 [8]

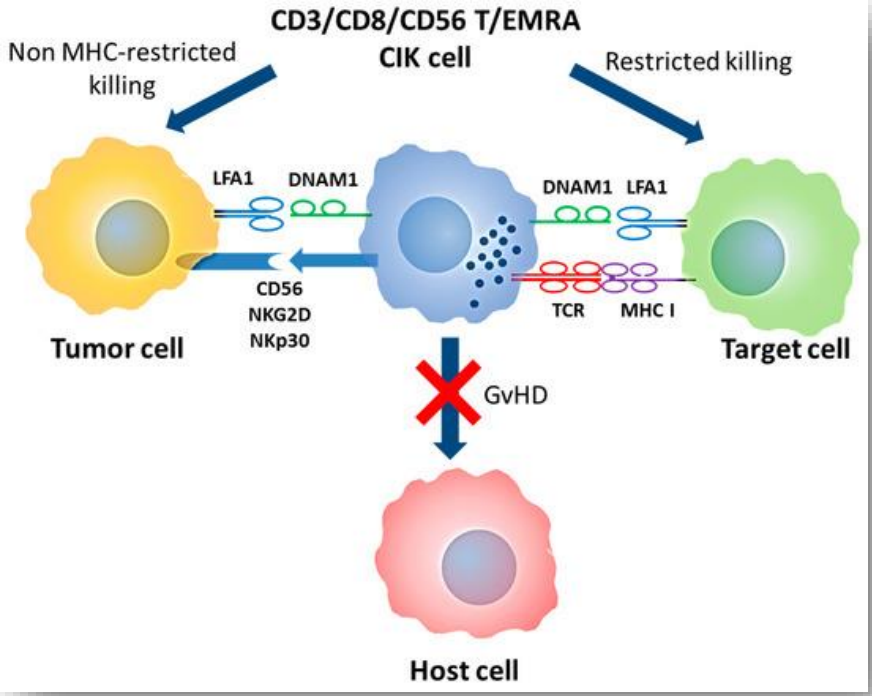


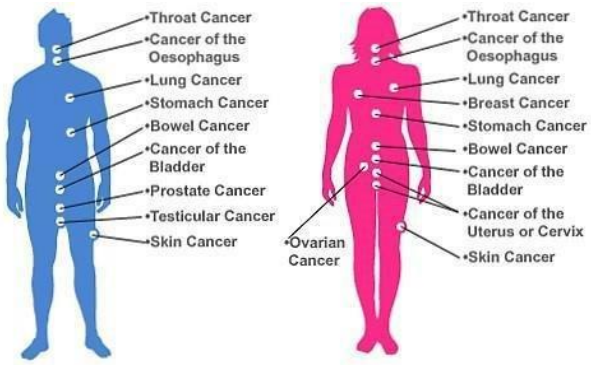
Figure 2: Double T/NK specificity of CIK cells and mechanism of action. CIK cells are T-EMRA lymphocytes that maintain the original TCR specificity and acquire NK-like cytotoxicity. In both cases, DNAM1 and LFA1 are required for target binding while NK-like cytotoxicity is mediated by CD56, NKp30 and NKG2D molecules.

Cancer Types

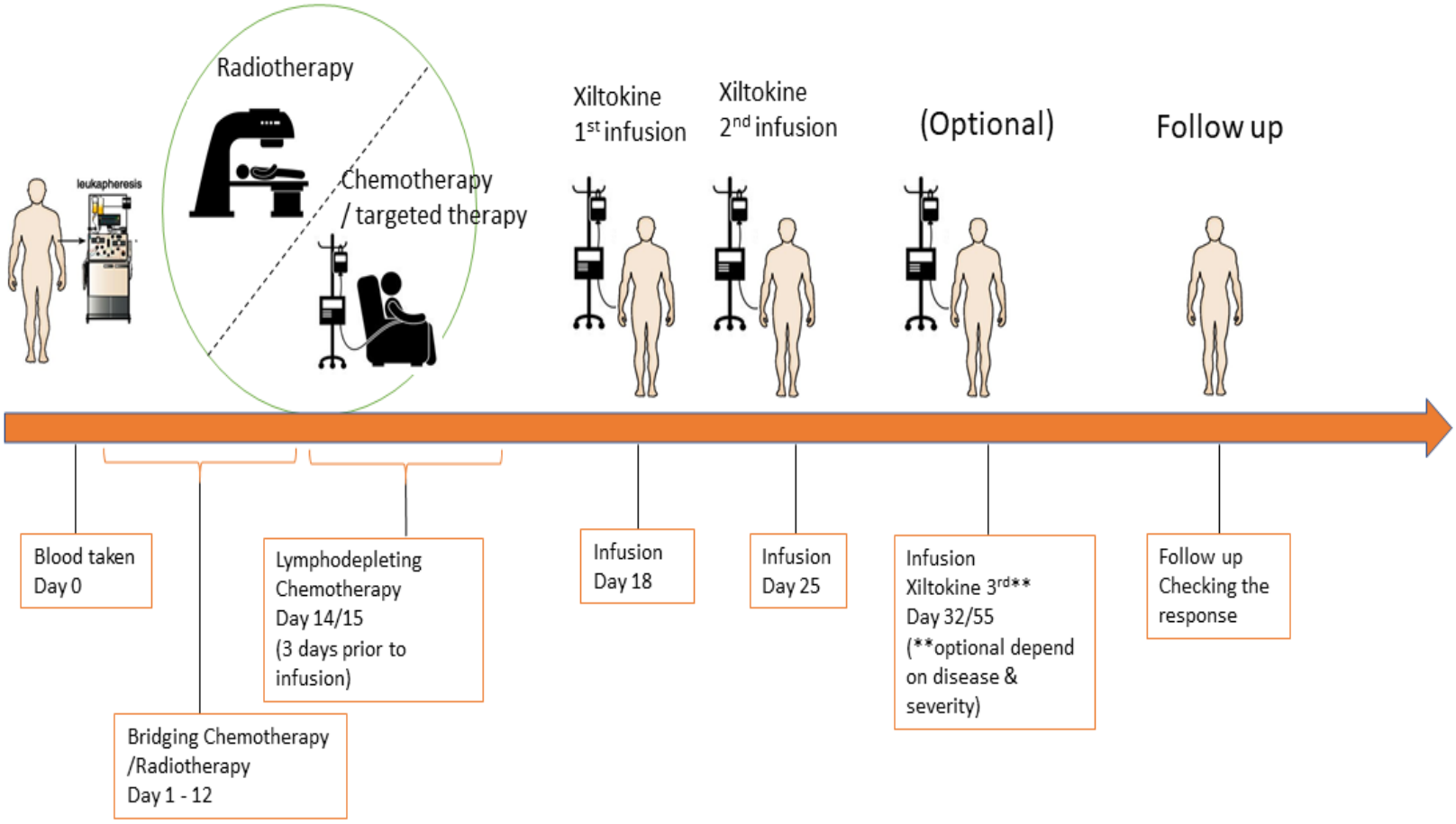
In cooperation with the Tungs' Taichung Metro Harbor Hospital, and various private hospitals in Malaysia, our group participated in application of various cell-therapies (DC, CIK, DC-CIK) in a variety of solid cancer treatment projects, that were approved by both the Ministry of Health and Welfare of Taiwan and the Ministry of Health of Malaysia.

The cell therapy applications include 22 types of stage 4 solid cancers, including the cancer types listed in the table below [ref: MOHW, Taiwan].

- Adrenal Cancer
- Biliary Tract Cancer
- Bladder Cancer
- Brain Malignant Tumor
- Breast Cancer
- Cervical Cancer
- Colorectal Cancer
- Endometrial Cancer
- Esophageal Cancer
- Gastric Cancer
- Germ Cell Cancer
- Head & Neck Cancer
- Kidney Cancer
- Liver Cancer
- Lungs Cancer
- Neuroendocrine Cancer
- Ovarian Cancer
- Pancreatic Cancer
- Prostate Cancer
- Soft Tissue Sarcoma
- Skin Cancer
- Thyroid Cancer



Treatment Flow



Application

For autologous use only. Patients will receive at least 2 cycles of CIK cells infusion with one-week intervals between each cycle. 5~7 x10^9 cells were suspended in 240mL normal saline and administered via intravenous infusion within 30-45 minutes.

The immune cell therapy program can be combined with other treatment methods, including anti-inflammatory drugs (NSAID) and antipyretic analgesics to relieve the patient's discomfort. Or combined use of radiation therapy, targeted therapy, chemotherapy, immune checkpoint inhibitor therapy, and so on to improve the overall anti-cancer efficacy. All concomitant treatment methods must be evaluated by the operating specialist's professional clinical experience, and the relevant adverse reactions of concomitant treatment should be observed at any time.

All patients were followed up after discharge, including blood routine examination and PET CT/CT screening every 3 months for the first 2 years, 6 months for the next 3 years, and yearly thereafter from the fifth year.

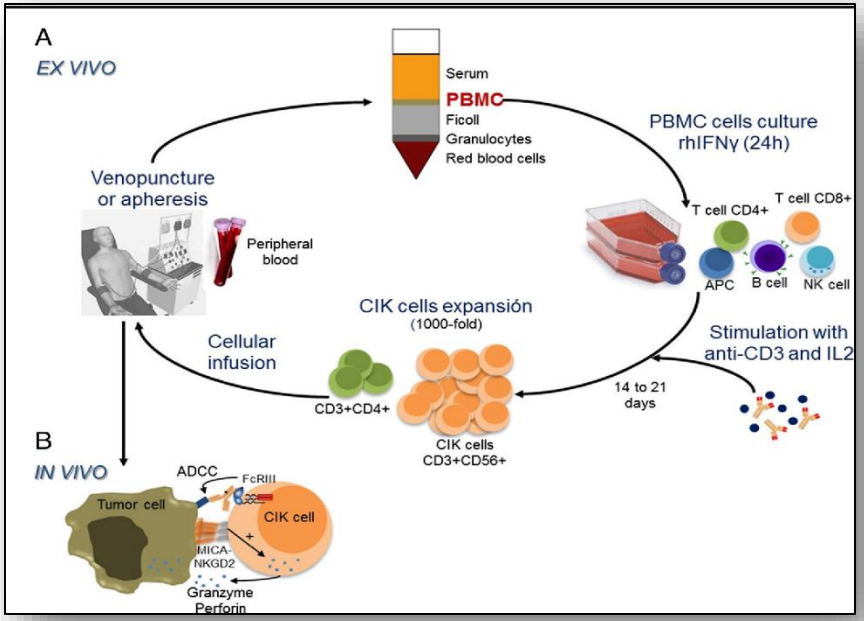
Possible Side Effects

According to the results of clinical trial literature, the most common adverse reactions of CIK cell therapy are mostly mild symptoms such as fever and pain. Although the frequency is incredibly low, the symptoms will resolve spontaneously without treatment. However, each patient may have special reactions (idiosyncrasy) due to individual differences and special constitutions. If nausea, vomiting, diarrhea, or shock occurs after the cell reinfusion, the cell reinfusion should be stopped and treated according to the specialist's instructions.

Adverse Effect	Descriptions
Grade 1	The operating physician needs to place the patient in a comfortable space and observe until the adverse event resolves spontaneously or gives appropriate physical support (ice compress, drinking warm water, sleep, etc.).
Grade 2	The operating physician immediately places the patient and observes whether the adverse event tends to ease or become more serious. If it tends to be relieved, physical support is given to accelerate the resolution of the adverse event; if it tends to be serious, evaluate the severity of the patient and give appropriate drug support (antipyretic, analgesic, steroid, etc.). Continue observation and drug support until the adverse event stabilizes or resolves. Suspend this course of treatment and enter the observation period and reassess whether to continue treatment after the cause is found out.
Grade 3	The operating physician must immediately take emergency measures and administer medication support (antipyretic, analgesic, steroids, pressure boosters, electrolyte infusion... etc.) according to the patient's symptoms. Arrange to be hospitalized and continue treatment and slow medication reduction until the symptoms are completely resolved. The patient withdraws from this course of treatment, terminates the cell reinfusion, and arranges for routine medical care for the patient.
Grade 4	The operating physician must immediately be sent to the emergency department to take first aid measures and provide life support (intratracheal intubation, steroids, pressure boosters, electrolyte infusion, etc.) according to the patient's symptoms. Arrange to the intensive care unit as soon as possible and continue treatment until the symptoms are completely stable or resolved. The patient withdraws from this course of treatment, terminates the cell reinfusion, and arranges for routine medical care for the patient.

Laboratory Processing

This picture illustrates the production of CIK cells in the laboratory. The patient's PBMC cells will be harvested by apheresis in hospital and sent directly to our laboratory in Malaysia via a professional courier service (World Courier). Once received, the PBMC sample will be prepared for isolation and performed cell counting. The cells are then incubated at 37^0C for 24 hours, under 5% CO_2, 95% relative humidity using serum-free medium containing 1000 U/ml IFN-g. After 24 hours, anti-CD3 antibody (at a final concentration of 100 ng/ml), 1 ng/ml IL-1a and 1000 U/ml recombinant human IL-2 is added to the medium. After 14 days of culture, autologous CIK cells are suspended in 240 ml normal saline. The cells are then transferred into a clinical grade cell culture bag. The cell products were tested according to the current Chinese Pharmacopoeia, such as microbial contamination testing being carried out to ensure the reliability, PCR based method for the mycoplasma detection to rule out contamination and quantification of bacterial endotoxin by kinetic turbidimetric methods. After passing the QC, the cell product will be cryopreserved and shipped to the Hospital. After recovery in hospital, the cells are ready for infusion.



Good Tissue Practice Lab

The facility is designed with regulations that are stricter than any Good Tissue Laboratory (GTP) regulations to avoid risks of environmental contamination including multiple gowning procedures (primary and secondary changing rooms, biologically safety areas, and limited access to qualified persons. The facility complies with the FDA high standard secondary dressing facility.

24 hours-Uninterruptible Power System-maintaining cells under carefully controlled conditions.

- **The lab was equipped with independent power generators.**
- **UPS battery backup supplies for all lab instruments.**

SITE SPECIFICATIONS

It has a cleanliness level of 10,000, a "positive pressure, constant humidity, constant temperature" high-standard dust-free independent laboratory, and a complete control mechanism, which can provide various process facilities for cell therapy.

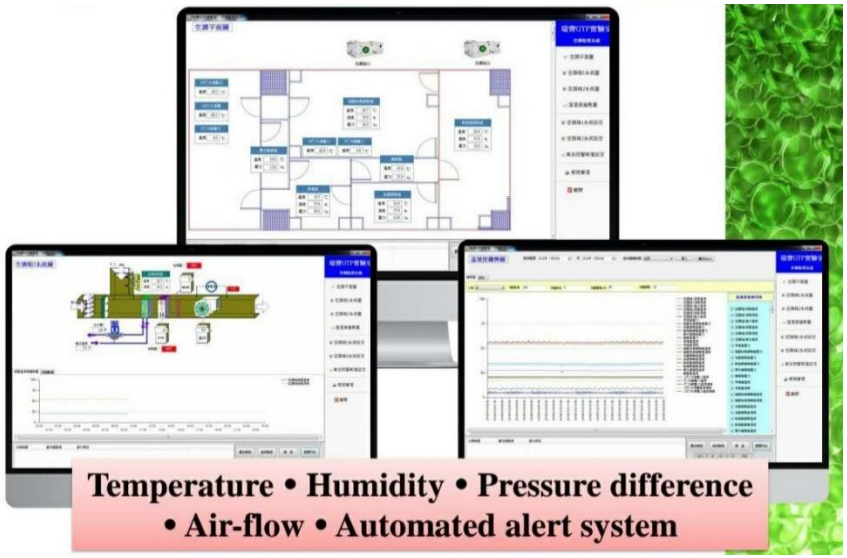
CLEANLINESS

With a cleanliness of 10,000 and a complete control mechanisms, it can provide various process facilities required for cell therapy.

STRICT REQUIREMENTS

Adhere to the use of high-quality manufacturer-sourced reagents, high-end equipment, and full-process serum-free culture to improve the effectiveness and safety of human cell tissue operations. Strict operation procedures, manufacturing procedures, storage conditions, effective time and records are established for the acceptance/feeding of human cell tissues, acceptance or return, distribution, and destruction or disposal.

Good Tissue Practice Lab

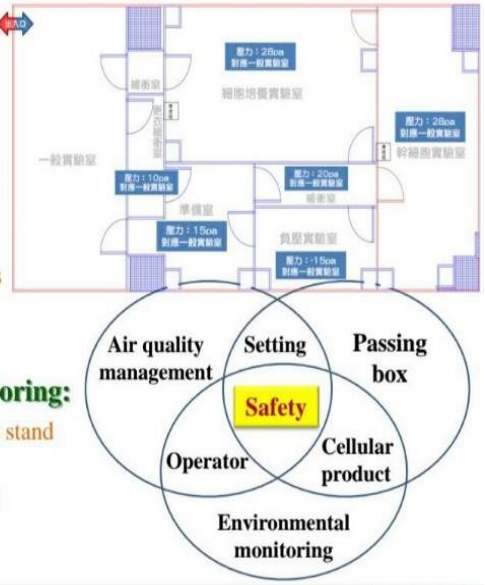


GTP requirements:

- Requirements for facilities
- Environmental control
- Equipment
- Supplies & Reagents
- Recovery
- Processing and Process controls
- Labelling controls
- Storage

Air qualification and monitoring:

- Air-handling system in separate stand alone units
- Filter-based stand alone system



Strict standards for final products testing according to the current Chinese Pharmacopoeia.

Bacteria
Microbial contamination testing being carried out to ensure the reliability.

Mycoplasma
Validation of a PCR-based method for the mycoplasma detection to rule out contamination.

Endotoxin
Quantification of bacterial endotoxin by turbidimetric technique.

Cryopreservation
Cellular products can be frozen and stored. It's important to be able to track each product.

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Our Focus

We invent and produce new technology and new substances for treatment of cancers, viral infections, antibiotic resistant bacterial infections, and other incurable diseases.

Our Results

Our products achieve results in treatments of many incurable diseases for which treatments are not available or no results can be reached.

Our Prices

The costs of our cancer immunotherapies are the lowest in the industry and significantly lower compared to any other competitor, while at the same time, our results are second to none.

THANK YOU

VAMOS BIOTECH (SHANGHAI) CO., LTD
2F, Building #5, Lin Gang Fengxian Industrial Park,
1800 Xin Yang Road, Feng Xian District,
Shanghai 201413, P.R. China

Tel: +86 213 712 3366
Email: info@vamos-biotech.com
www.vamos-biotech.com