Clinical Flowchart for CAR-T Treatment

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Flowchart

The flow chart describes the process of providing CAR-T Immunotherapy to patients, from initial selection of patients, through the production of the CAR-T for infusion, to post-treatment monitoring and efficacy assessment.

Flow Chart	Responsibility
1. Identify suitable patients for CAR-T therapy	Oncologist/ Hematologist
2. Assess patient's baseline status	Oncologist/ Hematologist
3. Apheresis at hospital	Hospital Apheresis Unit
4. PBMC isolation for CAR-T production	Vamos Biotech
5. Conditioning chemotherapy	Oncologist/ Hematologist
6. CAR-T infusion	Oncologist/ Hematologist
7. Monitor adverse effects and manage patients	Oncologist/ Hematologist
8. Efficacy assessment	Oncologist/ Hematologist

1.	Patient selection	CAR-T therapy is indicated for 1. Refractory or Relapsed B-cell precursor acute lymphoblastic leukemia (ALL) 2. Refractory or Relapsed B-cell lymphoma including diffuse large B-cell lymphoma (DLBCL), primary mediastinal large B-cell lymphoma, high grade B-cell lymphoma, and DLBCL arising from follicular lymphoma 3. Refractory or Relapsed Multiple Myeloma 4. Refractory or Relapsed Hodgkin's lymphoma
2.	Patent's baseline status	Ensure patient's condition is optimal prior to CAR-T therapy • Patient's tumor expressed the appropriate marker, eg CD19 • Performance status ECOG ≤1 • Absence of disease complication or chemo-toxicity such as infection, active GVHD, hyper-leukocytosis, severe extra medullary disease • Last dose of chemotherapy and/or steroid is at least 2 weeks ago; when in doubt, do T cell activation test. • Patient's tumor burden is low if possible. In patents with uncontrolled or accelerating tumor burden, induction chemotherapy should be performed first. Apheresis should be done prior to commencing the chemotherapy.
3.	Apheresis at hospital	A patient undergoes apheresis at a hospital site (Apheresis unit) to obtain peripheral blood mononuclear cells (PBMC). The apheresis procedure is conducted in "MNC" mode using the site's apheresis equipment (eg. Terumo's OPTIA Apheresis System) to collect 100 to 200 ml of apheresis product (AP) At the end of the Apheresis procedure, deliver the 100-200ml of AP in its collection bag (the bag is supplied as part of the apheresis kit) to the Vamos Lab within 8 hours. The AP should be packaged in a Cooler box packed with icepack to maintain its temperature at 2-8°C.
4.	PBMC isolation for CAR-T production	The PBMC isolation will commence on the same day the Apheresis product is delivered to the Vamos Lab. The isolated PBMCs will be cryo-preserved in liquid nitrogen storage tank until shipment to the Vamos laboratory for production of CAR-T in Malaysia. Production typically takes 10 to 14 days.

5.	Conditioning chemotherapy	If the CAR-T cell production is satisfactory to produce an adequate dose (0.5-1.5x10^6 cells/Kg Body weight), start conditioning chemo four days (Day -4) prior to anticipated CAR-T administration (Day 0). The patient shall start receiving a non-myeloablative but lymphodepleting chemotherapy regimen comprising Fludarabine 25 mg/m^2 per day on Day -4, -3 and -2 and Cyclophosphamide 500 mg/m^2 per day on Day –2 prior to CAR-T treatment.
6.	CAR-T infusion	The Vamos Lab will supply the CAR-T in a plastic infusion bag of 100 ml, containing between 150 and 225 million CAR-T cells. The plastic infusion bag is cryopreserved at -196 degrees Celsius, shipped in a dry shipper and equipped with a data logger. The package is shipped by Word Courier and is delivered directly to the hospital. The thawing procedure is simple and requires approximately 7 hours. After thawing, the treating physician administers the CAR-T cells intravenously via peripheral vein over 10-20 minutes and using the same infusion bag that was used to transfer the CAR-T cells to the hospital.
7.	Monitor the patients for adverse effects	Known adverse effects are: (a) **Cytopenias**: This occurs in almost all patients due to prior chemotherapy and the conditioning chemotherapy. Watchful waiting as most all patients will recover within a week or 2, though in some cases this may be prolonged (>1 month). In the event of febrile neutropenia, treatment according to current guideline. (b) **B cell aplasia**: Watchful waiting as the clinical course is benign, and all patients will recover from B cell aplasia when the effect of CAR-T has waned over time (after about 6 months). If there is suspicion of systemic infection due to this, then the patient's serum IgG level will be low (hypo-gammaglobulinemia) which will respond to IV immunoglobulin replacement therapy. (c) **Cytokine Release Syndrome (CRS).** This will occur 1 to 12 days after CAR-T administration in all patients (most will have mild grade 1 to 2 CRA). More severe (grade 3) CRS occurs in a few patients who will require intensive supportive care. Symptomatic CRS may be reversed with Tocilizumab (IL-6 receptor-blocking antibody) at dose of 8 mg/kg BW for adults, 12 mg/kg BW if < 30 kg BW, and up to a maximum dose of 800 mg with dosing interval of at least 8 h and. Life-threatening CRS (grade 4) can be revered using Corticosteroids or Cetuximab, which will also completely abrogate the therapeutic effect of CAR T therapy. (d) **Neuro-toxicity**. Diagnosis is entirely clinical. The most common clinical manifestation is global encephalopathy, other reported symptoms include seizures, hallucinations, aphasia dysgraphia. There is no specific treatment. Symptoms are usually brief, self-limited and resolve over several days without intervention.

8.	Efficacy assessment	At 3 months post-treatment, treating physician shall assess efficacy as appropriate for the tumor treated such as bone marrow aspirate (BMA) for Leukemias and Myeloma, imaging for Lymphomas. At 6 months post-treatment, treating physician may consider BMA to collect 12ml of marrow specimen in 3 EDTA tubes for qPCR test to assess CAR-T persistence

Abbreviations

AE	Adverse effect or event
ALL	Acute Lymphoblastic Leukemia
AP	Apheresis product
BMA	Bone marrow aspirate
BMP	Bone marrow puncture
BW	Body weight
CAR-T	Chimeric Antigen Receptor T Cells
CD	Cluster of differentiation
CNS	Central Nervous system
COA	Certificate of Analysis
CRES	CAR-T related Encephalopathy syndrome
CRS	Cytokine release syndrome
CSF	Cerebrospinal fluid
CT	Computed tomography
DFS	disease free or event free survival
EMD	Extra medullary disease
FACS	Fluorescence-activated cell sorting, same as Flow-cytometry
FBC	Full Blood Count
FCI	Flow-cyto-immuno-phenotyping
FCM	Flow-cytometry
HSCT	haematopoietic stem-cell transplantation, aka bone marrow transplant
IHC	Immunohistochemistry
IPT	Immuno-pheonotyping
ISH	In situ hybridization
IT`	Intrathecal
IV	Intravenous
LP	Lumbar puncture
MNC	Mononuclear cells
MRD	minimal residual disease
MRI	Magnetic resonance imaging
NGS	Next Generation sequencing
PBMC	peripheral blood mononuclear cells
PCR	Polymerase chain reaction
Ph+	Philadelphia translocation with BCR–ABL1 fusion
PRN	Pro re nata; as circumstances may require
QC	Quality control
Rx	Treatment
TRM	Treatment related mortality
TWC	Total white count