International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: SJIF: 5.995
Available Online at www.journalijcar.org
Volume 7; Issue 2(E); February 2018; Page No. 9919-9923
DOI: http://dx.doi.org/10.24327/ijcar.2018.9923.1655

Research Article

CAR-T ERING THE TECHNOLOGY: A NEW REVOLUTIONARY IMMUNE TROJANS AGAINST CANCER

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A R T I C L E I N F O

Article History:
Received 7th November, 2017
Received in revised form 13th December, 2017
Accepted 3rd January, 2018
Published online 28th February, 2018

Key words:
CAR-T , Lymphoma, neurotoxicity, immune therapy, ScFv, CD19,CD20, TLS, MS

A B S T R A C T

New way to recombinantly express specific tumor associated epitopes on the surface of T cell works as immune Trojans called CAR s or simply Chimeric Antigen Receptors. CAR s are recombinant protein like monoclonal antibodies. This technology is promising and a targeted therapy with great enthusiasm. CAR- T cell have shown potential application against the resistant, metastatic cancer. The review focus on understanding the CAR therapy, strategies, challenges and its imaginable and futuristic approach.

INTRODUCTION

Radiation and chemotherapy are non surgical traditional therapy which is outdated and ineffective. The revolutionary approach like immunotherapy based on mAbs, dendritic cells, ribozymes, cytokines, co stimulatory molecule, involvement of tumour suppressor gene, multidrug resistant gene, pro drug activation and combinatorial therapy are still evolving with effective outcomes. We focus on the new hoagie in the science of oncology called as CAR s which act as a strong immune Trojans against non haematological cancer, leukemia etc. where patient's own cell is used and reengineer them to fight malignancies. CAR s show an adoptive T cell response. T cell are immune fighter circulate throughout the body attacking abnormal cell. Reengineers cell from patient are sometimes called as "living drug." They are chimeric because engineered using different components to get desired T cell. The CAR protein added to the surface of engineered T cell and function as receptors and these receptor look for the compatible antigen on the cancer cell and encountering it. Reengineered T cells are primary warheads for this technology.

Understanding the car- t cell

CARs are chimeric antigenic receptor and mostly similar to monoclonal antibodies. Structurally CARs contains an extracellular domain (ECD) which has single chain variable domain (ScFv). It also has transmembrane domain (TD) linked with ECD by spacer region. This structure enhances the flexible nature and binding ability of the CARs. Intracellular domain comprises of co stimulatory domain (CSD) and a signalling domain(SD). Signalling domain shows downstream immunological avalanche. When the ECD binds to the tumour antigen, CAR is activated resulting in to the T cell cytotoxicity response. The CARs ECD contain a fragment of tumour specific monoclonal antibody called as ScFv. ScFv used in the trial for the B cell malignancies is the one directed against the cell surface protein CD19. Non B cell expression of CD19 is limited[3]. The degree of peak levels of CAR-T cell varies and maximum response between 1 to 14 days after infusion. Clinical trial datas and reports shows high efficacy encouraging results in relapsing and lasting absolution reported on chronic lymphocytic leukemia and non Hodgkin lymphoma. However, the technology is associated with various snag and hindrance including the cytokine release syndrome, neurotoxicity, macrophage activating syndrome, Bcell aplasiaetc. Evaluating the safety and studying the efficacy of CAR-T cells re initiative with the potential streamline the manufacturing process and a new targeted therapeutic window against global malignancies.

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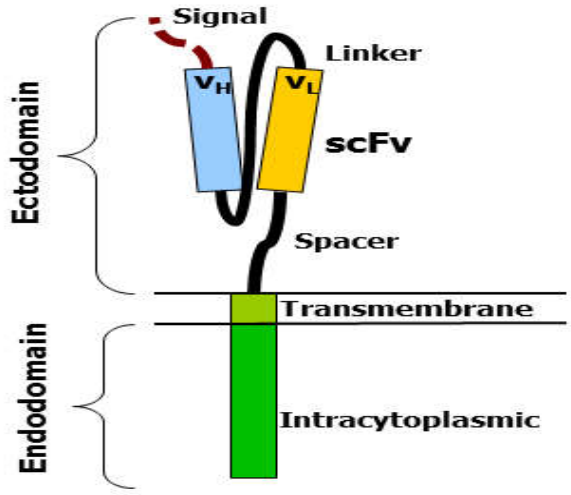

Figure 1 structural integrity of CAR T cell

Car T Cell Based Therapy And The Technology Insight

CAR-T cell therapy is one mode of immunotherapy and new fenestral to the science of hematologic oncolytic studies. Complete clinical trial for the treatment of glioblastoma, metastatic colorectal cancer were reviewed and examine for the study of safety, efficacy and the potential benefit of regional delivery system[4]. CAR T cell being produce to target tumour are very specific and little concern of off target is reported. Transduction of additional co stimulatory receptor, chimeric CD receptor, inhibitory CARs are expressed transiently. These modification have been put forward for clinical trials and proof to be promising [5-11] in actual context Regional delivery for chimeric antigen receptor for cancer treatment. CAR-T cell therapy have witnessed a new wave of scientific progress towards oncolytic studies. Modifications with chimeric antigen receptor T cell with tumour specific cytotoxicity and thus induce antitumor immunity against the malignancies [12].

Generating the Car-T Therapy

The monoclonal antibodies have light chain and heavy chain variable region which is kept to bind the tumour associated antigen of interest and single chain fragment variable region (ScFv) is generated from it. We clone the ScFv region sequence into an expression cassette containing the sequence of transmembrane domain and intracellular domain. The plasmid made through transfection process, is then introduce to group of T cells which then produce pool of T cells with express domain of full length CAR. The resultant T cell having TAA ScFv on its cell surface with intracellular immune signalling domain is referred as CAR- T cell. this CAR-T cell have been applied for treatment of various malignancies by using TAA ScFv's to locate and kills the cancer cell. We have to measure the pharmacokinetics and pharmacodynamics of CAR-T cell to check the success rate of those pre clinical trials. It is just the beginning the CAR-T cell therapy and researchers need more and more clinical trials as promising futuristic approach. In order to accomplish those obstacles we need to make the anti adiotype mAbs.

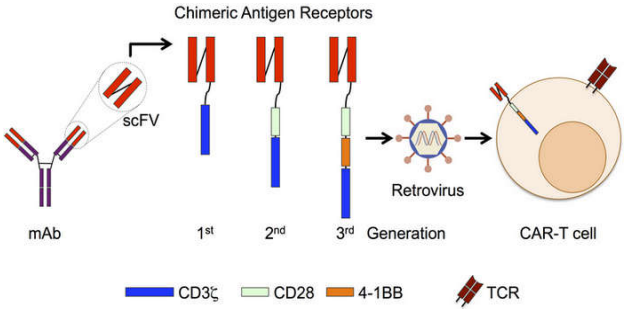

Figure 2 anti adiotype monoclonal antibodies for CAR T CELL IMMUNOTHERAPY [33]

Killing Actions of Car T Cell

CAR T cells follows MHC independant manner in recognising the specific tumour cell leading to the activation and execution of antitumor action[13]. On phosphorytlation of ITAM molecule after specific binding with TAA s and induce cytokine secretion, cytotoxicity and T cell proliferation [14]. Isolated CD8+ T cell evoke critical immune response against the tumour and CD4+ T cell Helps in enhancing the efficiency of CD8+ T cell mediated cytotoxicity[15-18]. CAR T cell follows two ways of cytotoxicity pathways: (1) secretion of perforin and granzyme granules (2) activation of death receptor signalling via Fas/Fas- ligand Fas L or TNF/TNF R. CD8+ T cell evade the tumor cell following these two pathways and CD4+ T cell eliminate the targeted tumour with perforin or granzyme granules. Apoptosis is believed to be another compensatory pathway in cytotoxicity process. For multiple amplification enhanced cytokine signalling, cytotoxicity and apoptotic induced death, the CAR T cells with multivalent signalling receptors are need to expressed extensively. CD40 modified T cell triggers the overproduction of proinflammatory cytokine like IL2, IFN gamma, IL12 and TNF[16].

Table 1 Some tumour associated antigen and molecular markers associated CAR T cell based therapy and its malignancies.

Markers	Markers associated malignancies	Tumour associated antigen	Disease associated with tumour antigen
CD19	No B-ALL,CLL,NHL	EGFR	Glioma,epithelial carcinoma
CD20	No B-ALL,NHL, FL	EGFRviii	Glioblastoma
CD22	No B-ALL,NHL,DLBCL	HER2	Ovarian cancer,breast cancer,glioblastoma
CD30	No NHL,HL	MSLN	Mesothelioma
CD33	No AML	PSMA	Prostate cancer
CD123	No AML	CEA	Neuroblastoma
RoR1	No B-ALL,CLL	GD2	Gliomna
IgLC	No CLL, NHL	IL13 ra2	Neuroblastoma, melanoma
BCMA	No MM	GD2	MPM
NKG2D	No AML,MM	FAP	glioma

B ALL : B cell acute lymphoblastic leukenmia
CLL: Chronic lymphocytic leukemia
NHL: Non Hodgkin lymphoma
HL : Hodgkin lymphoma
AML: Acute myeloid leukemia
DLBCL: diffuse large B cell lymphoma
MM: Multiple myeloma

Clinical and therapeutic advantages of CAR's T cell based therapy

Bypassing mechanism govern by cancer cell via down regulating the MHC1 molecule evading the immune signal is one therapeutic importance of CAR T cell[17]. CAR T cells ability to detect protein antigen, carbohydrate antigen, lipid antigen and collectively all the antigen recognised by antibodies is another technical benefit. CAR s are more flexible in compensating the down regulation of co stimulatory molecule. Promising advantage is the CAR T cells guard against cancer recurrence. CAR T cells may reside as memory or with memory cells in the body even long after infusion. CAR T cells can act as memory cells and guard against the cancer reccurance and thus benefits the long term remission therapies. It is one of the FDA approved therapy. FDA approved the treatment of acute lymphoblastic leukemia, diffuse large B cell lymphoma and life threatening cytokine releasing syndrome.

Potential clinical hindrance or snag of CAR T cell based therapy

Excess cytokine production after immune activation due to CRS may trigger uneasiness in patient like high fever, low blood pressure, poor oxygenation, delirium, confusion, seifure at the first onset of treatment. Metabolic implications could occur due to breakdown of dying cells called as tumorlysis syndrome (TLS). A condition of low B cell count or B cell absence even after first week of treatment known as B cell aaplasia occurs frequently. Most of the conditions mention above may be reversed. Patient death have been reported. Lastly a decomposer called as macrophage activation syndrome (MAS) or Lymphohistocytosis occurs due to macrophage activation therapy causing C reactive protein, D dimer, high level of ferretin and intense bleeding. Neural toxicity have been observe in most of the clinical trials like CD19 – directed CER T cell therapy and sometimes severity.

Forecasting the CAR T cell based immunotherapy

Saving the life of people is highly anticipated moments as stated by the Novartis CAR T therapy showing a promising durable response in lymphoma study. Out of 46 patient having DLBCL, 30% had a complete response showing no sign of detectable lymphoma and the rest 7 percent achieving a partial response. It is an indication of successful and promising therapy in the field of lymphoma. "The technology is spectacular; we are in amazing time and it's really just the beginning." Novartis feels. Safety efficacy, minimizing the toxicity, other clinical hindrance should be the active focus. Prediction, understanding and managing the risk associated with the complication before or after the onset of treatment will be the key player. Careful adjustment, regulation and timely responsive intellectual towards the infusion could be the control in futuristic approach. Eye on developing switchable or multi chain CAR s, co expression of proapoptotic gene with inducible CAR gene promotes for suicide system and for an effective disease control a must need of transient gene expression system. " TANDEM " CARs, affinity turned CARs focus on the high specificity of the CAR T technology remodelling the adequacy and persistence of this technology will become a fruitful effort in improving the CAR T therapy. Combinatorial therapy combining CAR t with chemo/radio; CAR T with immune cells based like NK cells, DC etc ; CAR Ting with PD1 blocking antibodies, armoured CARs with co expressing pro inflammatory cytokine IL12,IL15 and CARs sandwich with co expression CD19-CD12 will play an eventual critical role in improved version of this technology. Regulation of CAR T is also an important aspect. Use of fascinating gene editing tool to introduce genetic modification like selective deletion of endogenous T cell receptor. Tools like CRISPR, TALENS will have a possible control and help in more accuracy and specificity of targeting the tumour. Concern about off target may occur in minimizable percent.

Table 2 Some clinical trials on CAR T cell based immunotherapy

Cancer institute or hospital	Ground zero and qualifier	malignancies	Condition or degree of work	Clinical stage	Remarks
NCI	EGFRM11, NCT01454596	GLIOMA	Engaged	I	Retroviral vector and autologous Tcell
Shanghai cancer institute	EGFR,NCT02332693	GLIOMA	Engaged	I	Lentiviral vector
Duke university medical centre	EGFR VII, NCT02664363	GLIOMA	Not engaged but active	I	Dose escalation
Fuda cancerhospital Guangzhou	HER2, NCT02547961	BREAST CANCER	Engaged	II	Retrovirus vector
City of hope medical Centre	IL13Ra2, NCT00730613	GLIOBLASTOMA	Completed	I	Feasibility and safe
City of hope medical Centre	IL13a2,NCT02208362	GLIOBLASTOMA	Not engaged and going to start	I	Feasible and safe
University of Pensylvannia	MSLN,NCT01897415	PANCREATIC CARCINOMA	Completed	I	Transfected with chimeric anti mesothelinimmunoreceptor ss1
NCI	HER2,NCT009242	HER2+ve SARCOMA	Active but not engaged	I/II	Safe and feasible
Bayler college of Medicine	HER2,NCT01109095	GLIOMA	Completed	I	CMV specific cytotoxicity T cell
Roger Williams Medical centre	CEA,NCT01373047	GLIOBLASTOMA	Completed	II	Safe and feasible
UNZ	FAP,NCT02107963	Mesotheloma	Engaged	I	Lynmphodepletion

Bioinformatics tools like scoring analysis may reduce the off target in genome wide approach. However as an individual element in the scientific community I or we believe that after overcoming those obstacle limiting the technology and its application, world of oncology will refine a new therapeutic dynamics.

CONCLUSIONS

CAR T cells is considered as one of the living drug in the field of oncology. The evolution of adoptive therapy is growing fast and active clinical trials are in the process. This could pave the new way or strategies in combating one of the deadliest diseases known. The potential application is still evolving and the will reach a dimensionless elevation in future. The CAR T cell research is growing fast and moving is a swift pace in the targeting the blood cancer and some solid tumours. Clinical trials results are positive indicator of how successful the technology will be in future. What should keep in focus will be the regulation, developing new ideas and strategies for improving the safety efficacy and specificity of the process. Patients and families undergoing the therapy got immense support from the conducting authority and cancer research institute. The process still has a risk factor. However if weconsider the improved version in terms of specificity, safety and regulation, the technology will gain a new window in immunotherapy combating cancer.

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How to cite this article:

Hanjabam Joykishan Sharma *et al* (2018) 'Car-T Ering The Technology: A New Revolutionary Immune Trojans Against Cancer ', *International Journal of Current Advanced Research,* 07(2), pp. 9919-9923.
DOI: http://dx.doi.org/10.24327/ijcar.2018.9923.1655

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