Immunothrapy a Way to Treat Cancers

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ABSTRACT

The fields of immunology and oncology have been linked since several decades. Lot of advances occurred in field of oncology including insight about the intersection between immunology and oncology. This led to development of array of therapeutic drugs which can be used effectively with excellent results. It's very important to understand immunology of tumors / cancers to get insight about mechanism of action of these therapeutic agents. This review summarises basic immunology of Acute lymphocytic leukemis (ALL) and approach to immunothearpy for ALL.

Key words : Immunotherapy, Tumor immunology.

Introduction :

The fields of immunology and oncology have been linked since the late 19th century, when the surgeon William Coley reported that an injection of killed bacteria into sites of sarcoma could lead to tumor shrinkage[1]. Since that time, exponential advances in the understanding of the intersection between immune surveillance and tumor growth and development have led to broad therapeutic advances that are now being studied in all cancer types. The basic immunology and the various approaches to immunotherapy for ALL is discussed here.

The tumours develop surface antigens which are specific to the tumour which are detected by the immune competent cells and are killed by various mechanism. These tumour antigens are the products of mutated genes present in association with MHC 1. The products of Mutated Timor suppressor genes (P53), Oncogenes eg RAS, BCR-ABL, Or B cells showing Foetal stem cells antigen eg CD10,19 and CD20.

TUMOR IMMUNOLOGY :

Cell types involved in tumour recognition and rejection - An efficient and specific cytotoxic immune response against a tumour requires a complex, rapidly evolving interaction between various immune cell types in the adaptive and innate immune system.

- CD8+ lymphocytes and Th1/Th2 subclasses of CD4+ T lymphocytes are traditionally referred to as cytotoxic T cells and helper T cells. CD8+ and CD4+ edited by the immune cells lymphocytes initiate the distinction between self and non-self-antigens, through recognition at the "immune synapse. ***(Fig. 1)***
- Natural killer (NK) cells do not require antigen presentation by the major histocompatibility complex (MHC) for cytotoxic activity. In fact,

Fig. 1 : **Simplified immune synapse in T cells**

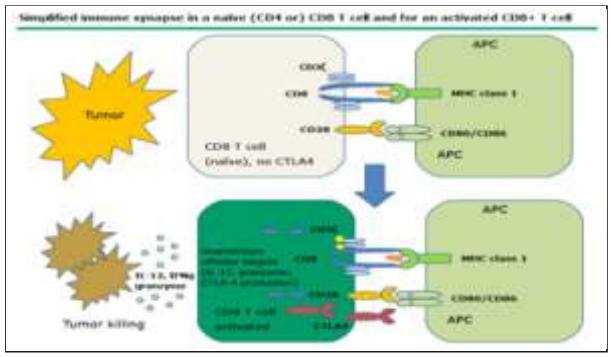
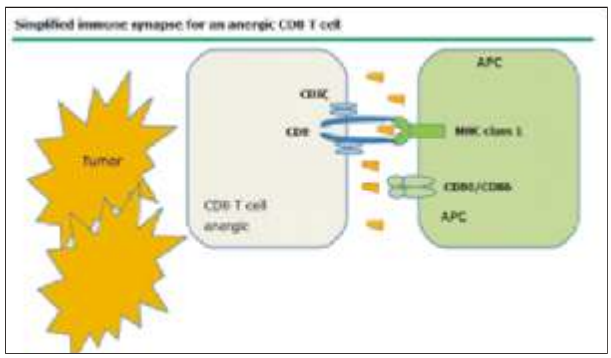


Fig. 2 : **Simplified immune synapse for CD8 cells**



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NK cells target cells with low MHC class 1 expression for destruction.

- Macrophages differentiate into at least two different phenotypes : M1 macrophages, which release interferon (IFN)-gamma and are responsible for phagocytosis, and M2 macrophages, which release cytokines such as IL-4, IL-10, transforming growth factor beta (TGF-beta), and dampen inflammatory responses and foster tolerance[3]

  The "immune synapse" - The most widely studied phenomenon in immunologic surveillance is the ability of T lymphocytes to distinguish self versus non-self-antigens, which are presented by antigen-presenting cells (APCs) such as dendritic cells. Overall, the cytotoxic activity of a CD8+ T cell is regulated by the presence and spatial orientation of a set of stimulating and inhibiting receptors whose expression is regulated by a myriad of cytokines. Together, this configuration is often referred to as the "immune synapse" ***(fig1).***

- The CD3 molecule, which encodes a nonvariable transmembrane protein complex with an intracellular tyrosine-based activation component relays surface signals to intracellular downstream effectors[4].

  The TCR binds specific short stretches of amino acids presented by MHC molecules MHC class 1 is expressed by all nucleated cells and is recognized by CD8+ T cells, while MHC class 2 molecules are constitutively expressed by APCs and are recognized by CD4+ T cells.

  For efficient activation of a naïve CD8+ T cell, its TCR must bind to a peptide presented by the MHC in the presence of a second set of costimulatory signals ***(fig. 1).*** This interaction leads to CD3 intracellular signalling that causes secretion of pro-inflammatory cytokines such as IL-12 and interferon gamma. In the absence of a costimulatory signal, a state of peripheral tolerance to the antigen ("anergy") develops ***(fig. 2)***[5].

  The most important costimulatory signal in naïve T cells is CD28, which binds to B7-1 and B7-2 (CD80/86) on the APC ***(fig. 1)***. This costimulatory process is tightly regulated by both "agonist" molecules (eg, GITR, OX40, ICOS) and inhibitory signals on both the APC and T cells, often collectively referred to as "immune checkpoint" molecules. Examples of co-inhibitory or "immune checkpoint" molecules include cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), programmed cell death-1 (PD-1), TIM3, and LAG3

THERAPEUTIC APPROACHES :

A number of therapeutic approaches are being studied to harness the immune system and control malignancy. We will discuss Acute lymphoblastic leukaemia as a prototype for treating cancer. The approach here is to get the tumor cell closer to chemotherapeutic drug eg Ionotuzumab or get the tumour cells close to T cells eg Blinatuzumab or Use genetically engineered cells to kill the tumour cells . (CAR T Cells)

Overall survival for acute lymphoblastic leukaemia (ALL) in children exceeds 85%. Improved survival primarily stems from decreased incidence of relapse, with very little improvement for more than 20 years in survival rates for children who relapse[6-8]. In contrast, overall survival for adults with ALL is quite poor (30% to 40%)[9-10], and relapsed ALL remains particularly challenging for all age groups, making it a leading cause of cancer deaths in children and carrying a dismal prognosis in adults.[6,8,11] Most children in first relapse will achieve a second complete remission (CR2), in contrast to the adult population, in which CR2 rates are 50% at best.[6,8,11] Even for patients who achieve CR2, those remissions are frequently not sustained.[6-8] With each subsequent relapse, achieving remission is harder and long-term survival is extremely poor. Refractory ALL is also challenging, with long-term survival close to 30%.[8] For those who do not achieve a remission. So there are challenges. With the current treatment protocols since year 2000 we have not moved an inch forward. Our mortality data (SEER IBMTR) continues to be >20% since 2006. The mortality graph does not seem to touch the X axis, With this pace it will take 150 years to touch the

bottom. Moreover the chemotherapy in relapsed and post-Transplant patients have a poor outcome Thus there is an unmet need to treat these patients with some alternative means.

We have seen the paradigm shift in precision therapies. The first wave which was generated in the first half of earlier century was in the form of chemical blockers viz Aspirin, Penicillin and Nitrogen mustard. The problem was side effects. The second wave of biological was generated in the last decade of last century. Rituxan has been extensively used. It was most natural to the body but the results were non-permanent. It was expensive with some side effects. The disease did relapse. The third wave which has generated in the current decade is of Immunotherapies, target therapies which are biological and seems to have permanent effect. These are curative and extremely expensive at present.

Immunotherapy in ALL

Inotuzumb It consists of an anti CD 22 monoclonal antibody attached to calecheamicin anticancer drug[12]. This drug has made a big wave in US as the drug has been successful in treating relapse refractory ALL[11]. This drug binds to CD 22 receptor on the cancer cell which gets internalised and is degraded in lysosomes where the antibody is separated from the calecheamicin which gets activated and binds to DNA causing intercalation and apoptosis of tumourcells. The first study in ALL (R/R) with 49 patients (6-80 years) showed overall response rate of 57%. 43% of Ph +ve and 20% of ALL with t4:11 showed complete response. 63% of 27 evaluable patients showed MRD negativity[13]. Subsequently Kantarjian et al also showed similar results (ORR 58%)[14].

Jabbour E[15] used this drug in elderly as frontline therapy in ALL where the usual treatment shows dismal results, Here the drug was used with mini Hyper C VAD and the results showed ORR 96% with 1 year PFS of 96%, Same authors[16] used this drug in Adults with R/R ALL and showed ORR of 71% and CR of 51%. Thus it was concluded that the drug can be used as an upfront treatment in elderly, Frail patients, and adults and children in Relapse refractory setting of acute lymphoblastic leukemia.

Bispecific T cell engagers : Here the concept is to get the tumor cell closer to Cytotoxic Killer T cells and use the power of T cells to kill the cancer cell . Conceptually, bispecific T cell engager antibodies (BiTEs) function as linkers between T cells and specific target antigens in an MHC-subtype independent manner. They consist of a protein fragment containing two separate single-chain variable regions. One end recognizes CD3, which is expressed on all T cells, and the other end recognizes the target antigen. BiTEs thus aim to induce cytotoxic T cell-mediated tumor eradication.

Because BiTEs are not MHC-specific, they can be administered to all patients regardless of human leukocyte antigen (HLA) type and do not require patient-specific processing. One consequence of this more broadly applicable approach is its relative lack of specificity in T cell recruitment when compared with the more labor-intensive method of adoptive T cell transfer. Because many different T cell subtypes express CD3. BiTEs recruit polyclonal cytotoxic T cells, Th1 & Th2 CD4+ cells, and Tregs.

The most well developed BiTE is blinatumomab, which has specificity for CD19 antigen found on many B cell malignancies and the Fc region of the CD3 receptor found on T lymphocytes. CD19 is universally present on ALL cells and hence is the target on one side and CD3 on the other side of the linker protein which targets the T cells. This drug has been used in treating relapse, refractory B cell ALL[17].

Phase II trial of the anti-CD19 bispecific T-cell engager blinatumomab shows hematologic and molecular remissions in patients with relapsed or refractory B-precursor acute lymphoblastic leukemia . Molecular remission was achieved in 70%[17]. Some of these patients underwent HSCT and survived for more than 2 years.

NicollaGökbuget[18] carried the first ever-international multicenter trial in ALL using MRD (minimal residual disease) positive as the criterion for inclusion. 116 MRD +ve patients >18 years (19-76) with a primary end point of MRD negativity after 1 cycle. 78% patients went into CR, the drug

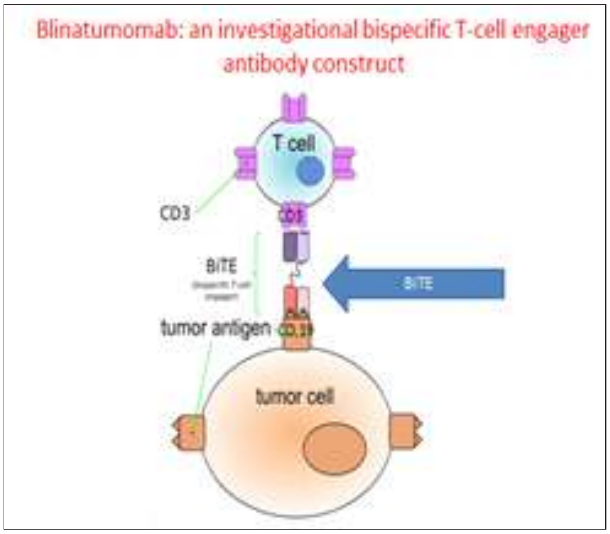


Fig. 3

was associated with adverse reactions almost in all with fatal reaction in 2 patients who showed subdural hematoma and pneumonia. The drug needed interruptions in 31% patients due to Cytokine release syndrome (CRS) and needed stopping in 17% patients. The results of this study were so good that US FDA approved it in Dec 2014.

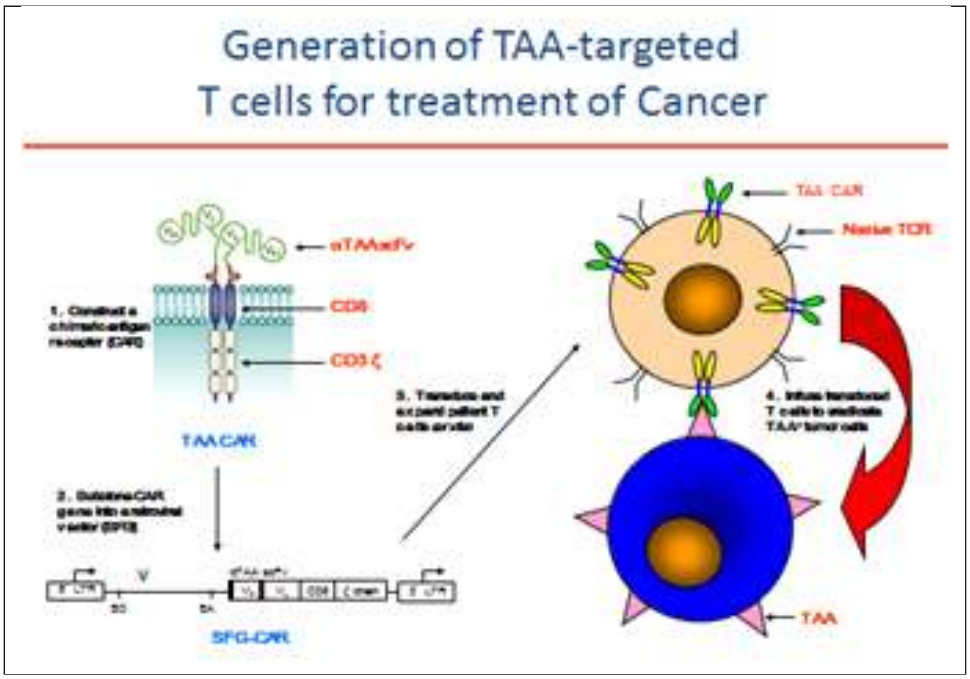
Pediatric patients 39 in number with ALL relapse and refractory status who were heavily treated prior to this, with multiple salvage therapies which also included patients relapsed after Bone Marrow transplantation received this drug. 12/39 achieved complete remission. 5 patients had complete MRD response. 2 patients became aplastic. 50% CR patients underwent HSCT, Thus this drug showed potentials of ant leukemia effect and opened a window for allo HSCT in those patients who were resistant to salvage therapy[19].

Manipulating T cells - Adoptive T cell transfer broadly refers to the practice of manipulating patient-specific T cells ex vivo to make them more reactive to specific antigens.

Chimeric antigen receptors[20,21,22] CARs were described more than 20 years ago for introducing tumor specificity in adoptive cell therapy. The intention was to teach the T cells to target and kill tumour cells. T Cells are armed with an Antigen specific monoclonal antibody. Here the T cells are engineered to develop specificity of B cells so that they can attach to the target antigen of interest on the tumour cells. These engineered T cells specifically attach to cancer cell of interest and use its power to kill the tumour cells .

In B Cell ALL the tumour antigen of interest is CD 19 which is present over Pro B, Pre B, immature, and mature B cell. Here the Single chain variable fragment of the monoclonal Ab CD 19 is linked to the zeta chain of CD3, an intracellular signalling domain of CD3 TCR complex. As a result, recognition of a specific cell surface antigen activates T cell response independently of MHC recognition. Various modifications can enhance CAR effector function, such as co-expression of

Fig. 4 : **T cell Engineering**



intracellular stimulatory domains such as CD28 or 4-1BB (CD137) or pro-effector cytokines such as IL-1. Thus there has been evolution in CAR designs. The first generation CARS were designed with antibody binding to CD3 zeta, activating the intracellular pathways (zap 70) leading to proliferation, survival and cytokine production. Second generation CARS were created by using CD28 or 41 BB, OX 40 costimulatory molecules making it more efficient in terms of signals for transcriptions.

In this form of treatment the patient undergoes leukapheresis. The lymphocytes are separated. Car Gene is inserted by using Viral vectors and are expanded exvivo and are injected back to patient. After Leukapheresis the blood sample needs to be sent to the factory where the T cell is are engineered and then sent back for injection. It takes 3 weeks for the procedure. It seems to be a personalised medicine very specific to the patient. CTL019 is the product manufactured by Novartis and costs $ 4,75,000/- for the entire treatment.

The first paediatric All[21] patient got treated at Penn Children Hospital Philadelphia, She was 7 years old ALL with 2nd relapse. She had no donor and hence was retreated to get her in remission. She did not go into remission, She was treated with these engineered T cells in April 2012, when she had blast cells of 60%. She received 3 infusions of CAR T cells. The total dose was CTL019 10^8CD3 + cells/kg (11% CARs). Following the infusion she became very sick due to immune reaction which was treated with IL6R Blocker. Later she continued to do well and is in remission till date .

As soon as these cells are injected, they seem to disappear from the blood and go into the tissues ,By Day15 they start reappearing in the blood. In the responding patient they are present for more than 6-18 months[22,23]. There number increases overtime and appears to be antigen driven. These cells have been tracked in CSF, The persistence of these cells long after injection can be tracked by QPCR. The longer they persist the patient seems to be in disease remission. The MRD testing shows disappearance of B cells which indirectly means persistence of CART cells in circulation.

CAR T cells have been studied most extensively in hematologic malignancies. Clinical trials targeting CD19, the pan-B cell antigen, have shown remarkable success in B cell acute lymphoblastic leukemia (B-ALL)[24] and pre-B-cell ALL[25]

A single centre, phase I/Ila trial of CTL019 showed complete and durable remissions in paediatric / young adult patients with relapsed / refractory (R/R) B-cell acute lymphoblastic leukaemia (B-ALL)[26]. These results were reproduced in a multicentre setting with 6-month interim analysis of the first multicentre phase II trial of an engineered cell therapy in leukaemia. 9 US sites participated in this single-arm phase II study in paediatric / young adult pts with R/R B-ALL. Here 29/35 patients underwent CAR T cell therapy. The ORR was 69%. 67.1% had no MRD and achieved deep molecular response.

CAR T CELL TOXICITIES[25,26,27]

Side effects are substantial in certain patients and include signs of the cytokine release syndrome such as fever, hypotension, altered mental status, and seizures, with some patients requiring intensive care. Cytokine release syndrome is turbulent and is related to tumor burden, Post CTL019 inj there is marked increase in IL6, TNFalpha, IL2R, GMCSF, CRP, Ferritin, IFN. The rise in all these correlates with Cytokine release syndrome (CRS). These appears to rise in those patient who have excessive disease burden. There is marked increase in IL6 that leads to turbulent CRS which needs IL6R antagonist Tocilizumab which effectively controls it. Similar CRS is seen in Blinatumumab related toxicity. This needs to be treated with Tocilizumab.

B cell aplasia means persistence of CAR T cells I circulation is observed in all the responding patients, This is managed with IV Ig replacement therapy.

Macrophage Activation syndrome (HLH / MAS) is associated with extraordinary high levels of ferritin, elevated D Dimers and Low fibrinogen. Bone marrow shows Hamatophagocytosis. This is entirely reversible with Cytokine blockade IL6R blocker Tocilizumab. Some patients develop significant confusion, aphasia as a feature of Neurotoxicity.

Summary :

The era has come when we need to have a decisive precision treatment for cancers. The Immunotherapy has a strong base which of seems to be more specific and will lead to a personalised treatment. Utilising the Power of T cells with specificity of B cells is the way to go now. This decade will witness lot many breakthroughs in cancer management.

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