closed) and Ph-like cases are being identified using a predictor described by our group (Chiaretti et al, BJH, 2018). In adult B-lineage ALL, Ph+ and Ph-like ALL account for 35–60% of cases, depending on age, making them the most prevalent genetic ALL subgroup.

https://doi.org/10.1016/j.htct.2020.09.012

SP 12

CAR T-cell in children all

Francesco Saglio[1,2]

[1] *Pediatric Onco-Hematology, Stem Cell Transplantation and Cellular Therapy Division, Regina Margherita Children's Hospital, Torino, Italy*
[2] *University of Turin, Turin, Italy*

Chimeric Antigen Receptor (CAR)-T cell therapy is emerging as one of the most powerful and promising therapeutictool for the treatment of malignat diseases. CAR-T cells are T-lymphocytes modified *in vitro* to harbor an artificial molecular construct (CAR) made by an extracellular domain consisting of a single-chain variable fragment (scFv) recognizing a specific tumor antigen joined to a transmembrane domain which is linked to the signaling unit CD3ζ and co-stimulatory units CD28 or 4-1BB of the T-cell receptor, making them capable to recognize and to kill tumor's cell in a HLA-independent manner.CAR T-cell therapy consists in the selection of patient's normal T-cells via leukapheresis, activation, transduction to express CARs using lentiviral or retroviral vectors, expansion of transduced cells and infusion of the final product back to the patient. After the CAR T-cells are infused back into the patient, the engineered cells proliferate, recognize and kill tumor cells bearing the specific antigen the CAR is directed against. Most of the current clinical trials have been with anti-CD19 CAR T-cells directed against the antigen CD19, mainly expressed by Acute Lymphoblastic Leukemia and B-cells Non Hodgkin Lymphomas.

In recent years US Food and Drug Administration (FDA) and European Medicine Agency (EMA) approved CD19 CAR T-cells in patients affected by relapsed and refractory ALL under the age of 25 years and this technology is moving from an experimental approach available for very selected patients treated in a small number of Centers to a standard-of-care therapy available almost worldwide.

The diffusion of this technology requires a re-definition of the role of all the other therapy options currently available including other forms of immuno-therapy as monoclonal antibodies, bi-specific monoclonal antibodies and, upon all, allogeneic hematopoietic stem cell transplantation (alloHSCT).

Until now data are limited, and the above-mentioned question is far from being answered but there are some observations derived from pivotal clinical trials that probably will help us in building future trials aimed to define this topic.

Another open question is represented by the persistence of these cells in the patients that is related to the definition of the need for patients responding to CAR-T cells to proceed to other therapies, especially to alloHSCT, to consolidate disease remission. Moreover CAR-T cells are characterized by some peculiar side effects as the Cytokines Release Syndrome or CNS toxicity that if are not properly detected and treated may lead to very severe consequences with a significant mortality rate.

Finally, some technological, practical and economical considerations need to be defined in order to extend the use of this technology worldwide, in respect to the other currently available therapies.

https://doi.org/10.1016/j.htct.2020.09.013

SP 13

Update on chimeric antigen receptor – T cells (CAR-T) CD19 therapy: the Sheba experience

Arnon Nagler

Chimeric antigen receptor (CAR) T-cell therapy for hematologic malignancies is a cutting edge therapeutic advancement which is leading the immunotherapy frontier and cancer therapy. CD19-specific CARs are the most commonly used. CD19 is expressed on the surface of most B-cell malignancies and thus can be used as a target for immunotherapies for ALL, and NHL. Phase II trials have showed that anti-CD19 CAR T-cell therapy can induce durable responses in patients with relapse/refractory (R/R) ALL and aggressive B cell NHL. Some of the AMLs with 8:21 translocation expressed CD19, as well. We initiated a single center program in which patients with R/R ALL and NHL were treated with academic produced anti-CD19 CAR T-cells (autologous T-cells expressing anti-CD19 CAR construct with CD28 co-stimulatory domain). Inclusion criteria were age between 1 and 50 years, failure of at least two prior therapeutic protocols, a CD3 count greater than 250/μL blood, absence of clinical signs of graft-versus-host disease and no immunosuppressive treatment. Depending on age, the minimal performance score was 50 on a Lansky scale or on a Karnofsky scale. Patients with prior CD19 directed therapies were eligible for the study. Lympho-depleting conditioning was inducted by fludarabine 25 mg/m^2 for 3 days and cyclophosphamide 900 mg/m^2 for 1 day, followed by infusion of 1–1.5 × 10^6 transduced CAR-T cells per kilogram weight. Primary endpoints of the study were production feasibility, patient safety and best overall response rates, documented 1 to 2 months after infusion. 93 patients with r/r B-cell malignancies. All patients were heavily pretreated. Three enrolled patients (3%) dropped out from the study due to clinical deterioration ($n = 2$) or failure to produce CAR-T cells ($n = 1$; absence of CAR-T cells in the infusion product). One patient was treated twice. Of the treated patients, 37 patients had r/r ALL and 53 patient's r/r NHL, including DLBCL ($n = 36$), Burkitt lymphoma ($n = 3$), PMBCL ($n = 7$), follicular lymphoma ($n = 4$), gray zone lymphoma ($n = 1$), mediastinal lymphoma ($n = 1$) and high-grade lymphoma ($n = 1$). The median age of pts with ALL was 17 ± 14 years and median age of those with NHL was 44 ± 15 years. Both, ALL and NHL patients received an average of three prior lines of therapy. Thirty-two of 90 patients (36%) received a stem cell transplantation(SCT) prior CAR-T therapy, including 17 allogenic or haloidentical SCT in patients with ALL ($n = 15$) and NHL ($n = 2$). Ten of 37 (27%) ALL patients received prior

therapy directed against CD19, such as blinatumomab and Inotuzumab. Clinical response was evaluated 1 to 2 months after CAR-T cell administration. One ALL patient died of sepsis before evaluation and one NHL patient is still awaiting his evaluation. Of 36 evaluated ALL patients, 24 (67%) achieved measurable residual disease (MRD) negative CR, 6 (17%) MRD positive CR and 5 patients (14%) progressed. One ALL patient with an initial response was treated a second time with CAR-T, but did not respond. Of 52 evaluated NHL patients, 32 (62%) achieved an objective response, including 16 complete remissions and 16 partial responses. Twenty (38%) patients had disease progression.[1,2] Notably, we recently show that CD19 CAR T-cells were able to induce remission in a patient with CD19+ AML with t (8; 21)(q22;q22.1) that relapsed 6 months post allogeneic transplant and failed re-induction. On day 28 post CAR-T CD19 infusion BM aspiration disclosed normal hematopoiesis with no excess blasts, full donor chimerism and lack of t (8; 21) by FISH confirming clinical and molecular remission.[3] We also assessed kinetic of cell phenotype on PBMCs of the CAR-T treated patients using multiparametric flow cytometry. The manufactured CAR-T products ($n = 9$) were also subjected to immunophenotypic analysis in order to elucidate the mechanisms of CAR-T cell trafficking and activity. We observed increased immunosuppressive phenotype as well as induction of T cell senescence/exhaustion in non-responding compare to responding patients.[4]

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https://doi.org/10.1016/j.htct.2020.09.014

SP 14

Treatment of sickle cell crises

Salam Alkindi

Sickle cell disease (SCD) is an inherited disorder prevalent in Sub-Saharan Africa, Middle East and parts of India. Its characterized by repetitive episodes of vaso-occlusive (VOC) process leading to recurrent painful episodes, hemolytic anemia and predisposition to infection. Sickle cell crises varies and this what brings patients to hospital including VOC leading to recurrent painful episodes, or organ specific complications such as acute chest syndrome, stroke, splenic sequestration, and many skeletal complications. Although the prognosis of patients with SCD has improved, however still these events contributes to decrease quality of life and increased risk of death. Also unfortunately, progress on the management of these acute complications is slow, and tended to be supportive including vaccination, use of antibiotics prophylaxis and blood transfusions. Better understanding of pathophysiology of the disease has allowed more accelerated progress on preventing these complications and development of more focused pharmacological therapies. Hemoglobin polymerization is a primary triggering event in the pathophysiology of the disease, leading to the sickling process, this usually ignite an inflammatory process/tissue ischemia and increased adhesions. This understanding of the pathophysiology has allowed scientist to develop drugs that interfere with these processes such as Voxeletor & Hydroxyurea (interfere with polymerization-both approved by FDA), L-glutamine and Omega 3 (interfere with inflammatory process and oxidative stress) and crizanlizumab and Tinzaparin (works by inhibiting adhesion molecules). This will allow patients and physicians the freedom for a number of therapeutic interventions including development of combinations protocols. SCD is very complex and require a drug with multi-faceted action such as Hydroxyurea and this is of the limiting factors in the new recently approved drugs, limiting the patients who can benefit from each of them. Further progress is also seen in the area of bone marrow transplant (including alternative donor pool) and gene therapy/gene editing.

https://doi.org/10.1016/j.htct.2020.09.015

SP 15

Secondary acute leukemia evolving from myeloproliferative neoplasm (MPN)

Tariq Mughal

The natural history of myeloproliferative neoplasms (MPNs), both Philadelphia-chromosome positive – [chronic myeloid leukemia(CML)] and negative – [essential thrombocythemia (ET), polycythemia vera (PV), and primary myelofibrosis (PMF)] has been well documented but the mechanism underlying the apparently inexorable progression from an initial, rather indolent or chronic phase (CP) to advance phase, a term including accelerated phase (AP) and blast crisis (BC) remains obscure. Most patients with MPNs present in the indolent phase, during which myeloid progenitor numbers are greatly increased in the bone marrow and blood. This phase may continue for as little as one year or as long as 20 years or more, but eventually it transforms into acute leukaemia (BC), in which an increasing proportion of blast cells are found in the marrow and peripheral blood. The risk associated with the development of advanced-phase disease differs depending on the MPN subtype and is influenced by a number of factors such as duration of disease, clinical factors, the presence of