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Coccidioidomycosis, immunoglobulin deficiency: safety challenges with CAR T cells therapy for relapsed lymphoma

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Treatment of patients with relapsed or refractory lymphoma may require allogeneic hematopoietic stem cell transplant (HSCT), but treatment of post-transplant relapse disease remains very challenging. Donor lymphocyte infusion and blinatumomab have been used with limited success for the treatment of relapse. Initial data on donor-derived CAR T cells has shown this modality to be safe and highly effective in various hematological malignancies. We present a case of a patient with highly refractory, transformed follicular lymphoma who failed both autologous and allogeneic HSCT. Patient achieved long-lasting complete remission with the use of donor origin CD19 CAR T-cell therapy, without any evidence of graft-versus-host disease flare. Our patient later developed disseminated coccidioidomycosis and persistent hypogammaglobulinemia. Immunotherapy using CD19 CAR T cells can be a highly effective salvage modality, especially in cases of focal lymphoma relapse. Long-term immunosuppression secondary to B cell lymphopenia, hypogammaglobulinemia, immunoglobulin subclass deficiency, fungal infections and other infectious complications need to be monitored and promptly treated as indicated.

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Transformed follicular lymphoma (tFL) and *de novo* indolent follicular lymphoma (FL) are incurable diseases with the use of routine chemotherapy, chemo-immunotherapy. 5-year overall survival is approximately 50% in such cases [1,2]. Histologic transformation of FL occurs at a rate of 2–3% per year [3]. t (14;18) and resultant overexpression of the antiapoptotic protein (i.e., BCL2) are genetic events originating at the precursor B-cell stage of development. Prognosis of patients with tFL is significantly inferior when compared with non-transformed FL. Relapse following an auto-HSCT and allo-HSCT remains a major challenge, donor lymphocyte infusion (DLI) and Blinatumomab, a bispecific antibody, have been used with limited success for the treatment of relapse disease. DLI use poses high risk of acute and chronic graft-versus-host disease (GVHD), about a third of patients receiving DLI can develop GVHD. There is risk of marrow aplasia with DLI, despite many side effects and limited efficacy it can lead to a small increase in median survival by 6 months [4,5]. Six to eleven percent of the treatment-related mortality from DLI is due to uncontrolled GVHD [5,6]. Blinatumomab, despite achieving a better median survival (9.8 months), is considered a bridge therapy in aggressive hematological malignancies and it is not curative in many cases [7]. Use of CD19 chimeric antigen receptor T (CAR T) cells therapy is a novel method of targeted immunotherapy currently in early phase trials for many hematological malignancies including relapsed lymphoma [8–11]. We present case of a patient with multiple relapses of tFL who failed both auto-HSCT and allo-HSCT. After regional relapse disease, patient achieved long-lasting remission with the use of donor origin CD19 CAR T cells. Long-term follow-up showed patient maintained remission but developed disseminated coccidioidomycosis and persistent hypogammaglobulinemia. We performed a comprehensive search (last updated search Nov 2016) on multiple

platforms (Medline/PubMed, Scopus, Embase, Cochrane CENTRAL, Web of Science, SCI-Expanded, WOS CPCI-S) and identified 4476 citations, only 80 non-Hodgkin lymphoma patients (NHL) could be identified who were treated with CAR T cells.

Long-term follow-up data on safety and efficacy in NHL patients who achieved complete response (CR) with CAR T therapy is still evolving. Randomized controlled trials, in principle, are superior but literature from such trials on long-term effects is limited. With the appreciation of the evolutionary value of case series feeding into single-arm trials, noncontrolled studies and finally leading to hypothesis-testing randomized trials, we present evidence from this single case report that focuses on long-term complications of hypogammaglobulinemia, immunoglobulin subclass deficiency, disseminated coccidioidomycosis and potential infection risks after CD19 CAR T therapy.

Case presentation

A 65-year-old Caucasian male was diagnosed as stage IVA composite FL/diffuse large B-cell lymphoma (DLBL) during September 2008. The patient presented with cauda equina syndrome, extensive lymphadenopathy, bone metastasis at L2, L4, S2, splenic enlargement and an elevated lactate dehydrogenase (LDH) level of 1153 IU/l. Patient was treated with eight cycles of chemotherapy using rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone (R-CHOP) and achieved first complete remission with a negative positron emission tomography (PET) scan performed during January, May and August of 2009. However, disease relapsed in December of 2009 with multiple skin nodules which was confirmed as diffuse large B-cell lymphoma on skin biopsy. Patient received three cycles of salvage rituximab, ifosfamide, carboplatin, etoposide (R-ICE) with partial response. Radiation therapy (2700 centigray [cGy] to the inferior clavicle mass and 2700 cGy to the right axillary mass) was given to PET avid areas in March 2010. Later, the patient underwent high-dose consolidation with auto-HSCT rescue using conditioning regimen with combination BCNU, Etoposide, AraC, cyclophosphamide in March 2010 and achieved second CR. Follow-up PET scans in May and July of 2010 were negative for residual disease.

Disease relapsed once again in October 2010, confirmed with a biopsy of the LN and a PET scan revealed subcutaneous nodules in multiple areas. He was treated with Rituxan, methotrexate and Ara-C during December of 2010 and achieved a partial response. The patient underwent an allo-HSCT following conditioning with Rituximab and 200 cGy (centi-gray) total body irradiation during February of 2011 and received HLA-matched granulocyte colony-stimulating factor mobilized peripheral blood stem cell transplant from sister as his donor. PET scan in August of 2011 confirmed a third CR. Patient developed GVHD of skin and mouth, received psoralen and ultraviolet A therapy for 11 sessions in April 2011 and GVHD later improved with topical steroids. Six months after achieving third remission, during February of 2012, the patient relapsed with a small area of abnormal PET uptake noted in the right scapula, upper abdomen and a small left inguinal lymph node (LN). At the same time, there was a flare of skin and oral chronic GVHD. Both relapse and cGVHD were treated with prednisone along with four doses of weekly Rituximab. Follow-up PET scan during July 2012 demonstrated mixed response. Subsequent monitoring revealed stable disease with small paratracheal LN and watchful waiting approach was adopted due to patient preference.

Later, local intensity-modulated radiation therapy to mediastinum was given during January of 2014 (4500 cGy) mainly due to persistent 18F-fluorodeoxyglucose (FDG) avid area on a PET scan. A repeat PET scan during March of 2014 showed new FDG avid subcarinal LN along with increase in activity and a number of multiple skin lesions involving the frontal scalp region (Figure 1A). A PET scan done during September 2014 showed progression of disease in the form of new FDG avid bilateral upper cervical LNs, and multiple FDG avid frontal scalp lesions that were shown to be cutaneous manifestation of systemic FL on biopsy. Considering highly refractory nature and post allo-HSCT relapse with measurable and biopsy-proven disease, he was selected as a candidate for therapy with CD19 directed CAR T cells. The patient enrolled in a clinical trial during the August of 2015, and underwent apheresis for T-cell collection. Before T-cell collection, the patient had 100% donor DNA in the bone marrow. The patient received preparatory 3-day cytoreductive chemotherapy using lymphodepleting cytoxan 60 mg/kg and fludarabine 25 mg/m². Pre-immunotherapy staging performed during August 2015 was positive for cervical, mediastinal and hilar lymphadenopathy along with multiple subcutaneous nodules. During the treatment session and up to 14 days postinfusion, he experienced low-grade fever with a mildly elevated C reactive protein, probably due to a cytokine-release syndrome (CRS), but there was no life-threatening hemodynamic instability. Post-therapy PET scan during September 2015 was consistent with remission and remarkable resolution of active lesions (Figure 1).

Figure 1. FDG-PET scan of skull before and after chimeric antigen receptor T-cell therapy. (A) Before CAR T. Showing two scalp area lesions with FDG-avidity. Right side lesion demonstrates an SUV of 10.7 while left side lesion demonstrate an SUV of 8.3. (B) After CAR T. Showing resolution of previously seen FDG-avid frontal scalp lesions.
CAR T: Chimeric antigen receptor T.

PET scan in December 2015 revealed new subcarinal FDG-avid adenopathy, fine needle aspiration showed spherules consistent with disseminated coccidioidomycosis and treatment with fluconazole 400 mg daily was started. A repeat PET in February 2016 showed interval resolution of FDG activity in the previously noted right lower paratracheal LN and decrease in FDG activity of subcarinal LN that are consistent with resolving infectious/inflammatory process. During 2016, the patient had multiple episodes of sinusitis and dry cough that was treated with antibiotics for upper respiratory tract infection. Computed tomography of the chest performed in June 2017 to investigate recurrent upper respiratory tract infection showed asymmetric pattern of interstitial and airspace disease with fibrosis and scattered additional subcentimeter nodules. In July 2017, bronchoscopy and fine needle aspiration of LN was performed showing recurrence of disseminated coccidioidomycosis. As of August 2017, the patient is still in CR for NHL and is getting re-treatment for his disseminated coccidioidomycosis. Case summary is given in Figure 2.

Discussion

CAR T cells are bioengineered to express receptors onto the surface against a specific antigen expressed by cancer cell, for instance CD19 or CD20 expressed by B-cell leukemia, CLL or NHL. CAR T cells with engineered receptor interact and lead to a cytotoxic response against target malignant cells. The first-generation CAR T cells were developed by Zillig Eshhar *et al.* in the 1980s [12]. Later generations of CAR T have undergone further development through augmentation of costimulatory signals. The second and third generation of CAR T cells currently in Phase I and II trials have clearly emerged as a novel immunotherapeutic modality. Early published data from CAR T clinical trials is very promising, although profound differences exist in response rates according to disease type and severity [13] among patients with acute lymphoblastic leukemia (ALL) and NHL [14]. The circulating pool of CAR T cells persists for many months and provides the body with living targeted immunity. CAR mediated T-cell target recognition is independent of both native T-cell receptor (TCR) and MHC presentation and depends on expressed antibody domain.

CAR T-cell therapy is not without clinically important side effects. The foremost is a systemic inflammatory response syndrome (SIRS)-like reaction. CAR T therapy is a state of augmented immunity and thus an effector response against cancer is associated with a systemic release of inflammatory cytokines like TNF- α/β , IL-6 and IFN- γ . Clinical features range from acute respiratory distress, hyperthermia, tachycardia and hypotension to multiorgan failure and death [15–18]. Such inflammatory response called cytokine-release syndrome (CRS) is observed usually in the first few weeks of CAR T therapy and in severe cases require treatment with immunosuppressive therapy like anti-TNF agents or steroids [19,20].

Figure 2. Summary of treatment with timeline.

The patient described in our case had extensive history of relapses, highly refractory disease and issues with cGVHD but after CAR T-cell therapy remains in long-lasting remission without a flare of GVHD. Before CAR T-cell harvesting, patient had 100% donor chimerism, hence T cells harvested from patient were of donor (sister) origin. CAR T-cell therapy is not yet approved by the US FDA for NHL, data on long-term effects is limited and evolving. First-generation CAR T cells had issues with decreased immune activation, limited efficacy and short duration of persistence. Second generation CAR T cells, with additional costimulation domains (CD28, CD137, or 4-1BB) showed superior results when compared with first generation. Adoptive CAR T cells have limited life span, are not considered curative and serve as a salvage, pre-emptive, prophylactic or bridge to Allo-SCT strategy.

B-cell depletion, resultant lymphopenia and hypogammaglobulinemia are predictable complications secondary to anti-CD19 nature of CD19-directed CAR T therapy. Data is emerging on the persistence of various constructs of CAR T cells in the body. As long as these effector cells are present in the body, they are expected to continue to stay active against B lymphocytes, hence B lymphopenia and prolonged hypogammaglobulinemia is an expected long-term complication. Long-term safety data of CAR T therapy on risk of viral, fungal, bacterial infections is scarce and prophylactic interventions need to be investigated. Due to lymphopenia, whether IgG quantitatively is reduced or any particular subclass (IgG1, IgG2, IgG3 and IgG4) is reduced disproportionately, and its impact on immune function and clinical significance in terms of increased risk of infections is not known. These IgG subclasses differ in their constant region, hinges, CH2 domains and are involved in binding to FcγR and complement. As a result, after CAR T therapy, patients with different subclass deficiencies will face unique challenges with triggering FcγR-expressing cells, phagocytosis or antibody-dependent cell-mediated cytotoxicity, and activation of complement cascade. Our patient had normal quantitative IgG level, normal IgG1 level but had deficiency of IgG2, IgG3 and IgG4 which is a unique finding in his case. Susceptibility to encapsulated bacterial infections is associated with IgG2 deficiency, we feel that IgG subclass deficiency potentially led to disseminated coccidioidomycosis in this case. Other possible risk factors in this case include prolonged lymphopenia, residency in the endemic area of south-western USA, history of immunosuppressive therapy with allo-HSCT, chronic GVHD and prior therapies like the use of CD20 targeting antibody Rituximab.

Research is underway to overcome the problem of 'on target – off tumor' efforts to develop highly selective CAR T cells against certain κ or λ light chain region of the malignant B-cell clones. Through extensive literature search, 80 NHL patients could be identified who were treated with CAR T cells. Regarding efficacy and toxicity date,

only half of these patients ($n = 40$) showed objective response and only 20% of treated cases showed CR, 30% showed partial response. Donor-derived CAR T therapy has shown to be safe and effective in a systematic review without significant GVHD effects [21]. Central nervous system toxicity has also been well described, other reported syndromes with CAR T use include macrophage activation syndrome and hemophagocytosis [22].

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In additions, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Informed consent disclosure

The authors state that they have obtained verbal and written informed consent from the patient/patients for the inclusion of their medical and treatment history within this case report.

Executive summary

- Chimeric antigen receptor T (CAR T) cell therapy is a novel immunotherapy currently in trials for many hematological malignancies. Relapsed patient even after allogeneic transplantation can achieve lasting remission with CAR T-cell use.
- Data on the use of donor-derived CAR T cells is emerging. Existing data reveal this therapy is effective without significant risk of flare of graft-versus-host disease (GVHD). This finding can build the foundation for relapse reduction strategies in future for patients undergoing allo-HSCT. Donor lymphocyte infusion is associated with GVHD flare and potentially can be replaced with antigen targeted donor derived CAR T cells, especially in the case of focal relapses with predominant donor chimerism.
- Following CAR T-cell therapy, data on long-term complications such as B lymphocyte depletion, quantitative immunoglobulin deficiency, IgG subclass deficiency and its impact on clinical risk of infections and best prophylactic strategy is emerging and needs further clinical studies with long-term follow-up.
- Disseminated coccidioidomycosis was diagnosed in our patient and possibly risk factors include prolonged lymphopenia, IgG quantitative immunoglobulin deficiency, subclass deficiency and residency in the endemic area of south-western USA. Other risk factors include history of allo-HSCT, GVHD and therapies like use of rituximab. Bacterial, fungal, viral infection risk need to be studied with long-term follow-up with emphasis on endemic areas for infections and individualized prophylactic strategies.

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