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Kidney Transplant T-Cell–Mediated Rejection Occurring After Anti-CD19 CAR T-Cell Therapy for Refractory Aggressive Burkitt-like Lymphoma With 11q Aberration: A Case Report

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Abstract

Post-transplant lymphoproliferative disorder is a growing complication of kidney transplantation (KT) associated with a dismal prognosis. Anti-CD19 chimeric antigen receptor (CAR) T-cell therapy is a game-changing treatment option modifying the outcome of refractory hematological cancers. Here, we report the case of a 40-year-old KT patient who developed a Burkitt-like Lymphoma with 11q aberration five years after KT. After three unsuccessful lines of chemotherapy, it was decided to treat the patient with anti-CD19 CAR-T cells as a salvage therapy. Three months after CAR-T cell infusion, she experienced a grade II-B T-Cell-mediated rejection with severe tubulitis (T3), slight interstitial inflammation (I1) and severe intimal arteritis (V2) with blood suffusion. Among T-cells infiltrating the graft, some of them expressed the anti-CD19 CAR. CAR-T cells within the graft and in blood samples were also evidenced by droplet digital PCR. KT function improved after corticosteroids treatment and remained stable. However, lymphoma progressed with a massive pulmonary mass leading to the patient's death 10 months after CAR-T cell infusion.

Keywords:

Kidney Transplant; Post-Transplant Lymphoproliferative Disorders; CAR-T cells; T-Cell-Mediated Rejection; droplet digital PCR

Introduction

Post-transplant lymphoproliferative disorder is an important complication of kidney transplantation (KT) that is associated with a poor prognosis. Anti-CD19 chimeric antigen receptor (CAR) T-cell therapy is an important new approach to treating hematological and other malignancies. We report the case of a 40-year-old KT recipient who developed a Burkitt-like lymphoma with a chromosome 11q aberration five years after KT. After standard chemotherapy proved unsuccessful, she was treated with anti-CD19 CAR-T cells as a salvage therapy. Three months after CAR-T cell infusion, she experienced a grade II-B T-Cell-mediated rejection with severe tubulitis (T3), slight interstitial inflammation (I1) and severe intimal arteritis (V2). Among T-cells infiltrating the graft, some of them expressed the anti-CD19 CAR. CAR-T cells within the graft and in blood samples were also evidenced by droplet digital PCR. Kidney function improved after corticosteroids treatment and remained stable, however, the patient died due to progressive lymphoma 10 months after CAR-T cell treatment.

Case Report

A 40-year-old woman with end-stage renal disease related to IgA nephropathy received in January 2013 a kidney transplant (KT) from a living donor with 4 HLA mismatches: 2 in class I and 2 in class II. There was no history of previous HLA sensitization events and no HLA antibodies were detected at the time of transplantation. Lymphocytotoxicity and flow cytometric crossmatch assays were both negative. After KT, her plasma creatinine concentration was 70 μ mol/L (0.8mg/dL) and remained stable for five years, without any complication or rejection episode. Her immunosuppressive regimen was an association of Tacrolimus (8mg twice daily, trough level 5.4ng/ml) and Azathioprine (75mg daily). Five years after transplantation (January 2018), the patient developed a high grade, Epstein-Barr virus negative, Burkitt-like Lymphoma with 11q aberration¹, revealed by a cervical

nodal localization, without extra-nodal involvement. Azathioprine was stopped and she received chemotherapy:

- First-line therapy based on four weekly rituximab infusions in February 2018 was a failure and extra nodal involvement appeared reaching breast nodes, thyroid, mediastinum, ovaries and the graft. Tacrolimus was stopped and only corticosteroids were continued (prednisone 20mg per day).

- Then she received a second-line chemotherapy with Rituximab, Vincristine, methotrexate, cyclophosphamide, doxorubicin and prednisolone (RCOPADM), with partial response. This treatment was completed in August 2018 with the following consolidation: rituximab, methotrexate, cytarabine (R-CYM), associated with intrathecal methotrexate plus cytarabine prophylaxis followed by vincristine, doxorubicin, cyclophosphamide, and prednisolone. Unfortunately, in September 2018, a Fluorodeoxyglucose–Positron Emission Tomography/Computed Tomography (PET/CT) revealed an ovarian and pulmonary relapse. An ovarian biopsy confirmed lymphoma progression.

- A third-line chemotherapy with rituximab, ifosfamide, carboplatin and etoposide was introduced, followed by an autologous stem cell transplantation with BEAM conditioning in February 2019. In May 2019, PET/CT revealed a new pulmonary and hepatic early relapse confirmed by liver biopsy.

It was then decided to treat the patient with anti-CD19 Chimeric Antigen Receptor (CAR) T-cells (Tisagenlecleucel, Kymriah®) as a salvage therapy. Patient displayed severe lymphopenia (0.3G/L) so corticosteroids were stopped at the beginning of June 2019 to allow optimal T lymphocyte collection by leukapheresis. She received bridging chemotherapy with rituximab and dexamethasone, and then a lymphodepletive conditioning with fludarabine and cyclophosphamide followed by CAR-T cell infusion in early July 2019. A grade 1 cytokine release syndrome and a severe pancytopenia occurred. Steroids (prednisone 20mg/day) were

reintroduced 10 days later as an anti-kidney transplant rejection treatment and then stopped at day 35 postinfusion to limit the risk of CAR-T cell apoptosis. During this period, KT function remained stable with a serum creatinine around 80 μ mol/L (0.9mg/dL). At day-30 after the infusion of CAR-T cells, PET/CT confirmed complete metabolic remission.

At day 81 postinfusion, she presented with an acute kidney injury with serum creatinine rising to 977 μ mol/L (11.1mg/dL) and a pancytopenia: hemoglobin: 7.2g/dL, white blood cells 0.7G/L and platelets 27 G/L. Laboratory findings revealed a 24-h urine total protein excretion of 2.0g, without leukocyturia or bacteriuria. There was no clinical or biological argument for a drug-induced nephritis: no recently introduced treatment, no clinical manifestation such as fever, rash or arthralgia, and no eosinophilia. BKvirus PCR was negative in blood. No donor-specific antibodies were found.

Because of the severity of KT dysfunction and strong suspicion of acute T-Cell-mediated rejection (TCMR), a first pulse of Methylprednisolone (500mg) was administered. After red blood cells and platelets transfusions, a percutaneous KT biopsy was performed.

Light microscopy examination revealed an active TCMR, grade II-B according to Banff 2019 classification, with severe tubulitis (T3), slight interstitial inflammation (I1) and severe intimal arteritis (V2) with blood suffusion (figure 1). Differential diagnoses were excluded: there was no eosinophil on the biopsy, no viral cytopathic changes, and SV40 immunostaining was negative.

To assess the accountability of CAR-T cells in the TCMR, several explorations were performed.

Chimeric Antigen Receptor (CAR) is composed of a single-chain variable fragment derived from an antibody (scFv), which is a fusion protein including the variable regions of the immunoglobulin heavy (V_H) and light (V_L) chain of an immunoglobulin². This scFv derived from a mouse IgG antibody targets human CD19^{3,4}. Human T-cells transduced with this anti-

CD19 CAR construct co-express two receptors: their own original TCR and the anti-CD19 CAR⁵.

Consequently, we aimed to identify the expression of the murine scFv on T-cells infiltrating patient's KT, using a goat polyclonal antibody directed against mouse IgG. As negative controls, we used three other KT biopsies with TCMR from other patients. Staining for CD3, CD4 or CD8 confirmed that mononuclear cells infiltrating the graft were T cells. Anti-mouse IgG staining was positive only on the patient's biopsy and negative in all three TCMR control biopsies (figure 1), showing that some infiltrating cells T cells expressed the anti-CD19 CAR scFv. Noteworthy, this type of polyclonal anti-mouse IgG staining has already been validated by several teams for identifying CAR-T cells in culture, in peripheral blood or in tissues^{6,7}.

Another negative control was performed using lymphoma tissue of the patient before CAR-T cell infusion. In this control, in which T cells were present, the anti-mouse IgG staining was negative. In order to confirm that CAR-T cells were present within the graft, we used a droplet digital PCR assay which enables the absolute quantification of the specific sequences encoding the CAR transgene⁸. This assay identified 5432 transgene copies/ μ g of gDNA extracted from a cryopreserved sample of the biopsy. CAR-T cells were also detected in patient's blood at different time points before and at the TCMR episode (figure 2). All together, these results indicate that CAR-T cells were present within the graft at the time of TCMR. To explore the possibility of a specific graft recruitment of anti-CD19 CAR-T cells by B cells, a CD19 staining was performed. This staining did not show the presence of any B cells, especially of any intragraft CD19-positive relapse of the lymphoma or of tertiary lymphoid structures. It was also negative on tubular epithelial cells.

After KT biopsy, the patient received 2 other infusions of methylprednisolone (500mg), followed by oral prednisone at a dose of 1mg/kg/day for one month and gradual discontinuation. Serum creatinine rapidly decreased to 134 μ mol/L (1.5mg/dL). A PET/CT

was performed on September 30, 2019 showing hepatic and pulmonary relapse of the lymphoma. The patient received salvage treatment with rituximab 375mg/m² on day 1 and lenalidomide 15mg/day orally on days 1-21 of repeated 28-day cycles between October 2019 and April 2020. During this period, with prednisone 20mg/day as the immunosuppressive regimen, serum creatinine remained stable. Unfortunately, lymphoma progressed with a massive pulmonary mass. The patient was also suspected of invasive pulmonary aspergillosis. She died despite Voriconazole treatment.

Discussion

Here, we report the case of a KT 40-year-old woman who underwent CAR-T cell therapy for a refractory Burkitt-like Lymphoma with 11q aberration, a rare but well described germinal center-derived lymphoma¹, that has already been reported after KT⁹. Four months later, an episode of TCMR grade IIB occurred, with potential contribution of CAR-T cells. There is a single report of CAR-T cell therapy in 3 solid organ transplant recipients by Krishnamoorthy et al¹⁰. None of these patients responded to CAR-T cells, with death occurring within three months post infusion. All patients presented with acute kidney injury occurring rapidly after CAR-T cell infusion, probably related to acute tubular necrosis secondary to cytokine release syndrome, and none showed biopsy-proven rejection.

This case brings up questions about the pathological process underlying this rejection and the potential involvement of CAR-T cells into the occurrence of TCMR, since our results indicate the presence of CAR-T cells within the graft. Importantly, KT biopsy was performed after the second pulse of corticosteroids, which could explain the only slight interstitial inflammation.

Several hypotheses can be raised concerning the mechanisms of TCMR in this patient.

First, an intra-graft CD19-positive relapse of the lymphoma could have led to local recruitment of CAR-T cells and KT lesions secondary to lymphoma cells lysis. However, such an hypothesis is not supported by the pathological findings and the clinical evolution of the

patient. Due to the absence of expression of CD19 on tubular epithelial cells and renal endothelium, a rejection process initiated through the interaction between the CAR-T cells and its CD19 target within the graft is also unlikely. While the stained cells had the morphological appearance of lymphocytes we were unable to perform double-staining to identify their lineage with absolute certainty.

A second hypothesis is the presence of alloreactive T-cells among the infused autologous CAR-T cell product. Activation of CAR-T cells co-expressing the CD19-targeting chimeric receptor together with an alloreactive TCR could be promoted through an intracellular CD3 ζ heterodimerization between the CAR and the TCR. Indeed, it has been reported that heterodimerization of endogenous and CAR-T associated CD3 ζ can occur, leading to full activation of T-cells expressing low affinity TCR through recruitment of the CD28 or 4-1BB domain of the CAR¹¹⁻¹⁵.

Alternatively, alloreactive untransduced T-cells, also potentially present in the CAR-T cell product, could have triggered TCMR. Finally, the few CAR-T cells observed within the graft may not have been directly involved in TCMR onset, but rather constitute bystander cells recruited locally through local inflammation during a rejection process mediated by the patient's unmanipulated depletion-resistant T cells.

Although we do provide evidence that CAR-T cells were present within the graft, no data are available that would support one of the proposed hypotheses. Nevertheless, context, timing between infusion and rejection and evidence of CAR-T cells presence within the graft raise the question of whether CAR-T cell therapy contributed to this KT grade IIB-TCMR.

In conclusion, we report a KT patient who experienced a rejection episode after anti CD19 CAR-T cell therapy for an aggressive Burkitt-like Lymphoma. Owing to the fact that CAR-T

cells indications and availability in centers are rapidly increasing, unexpected adverse events associated with this emergent therapy will probably be described, as this case of TCMR.

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Figure legends:

Figure 1: Active T-Cell-mediated rejection, grade II-B.

Upper panel: light microscopy, original magnification x 40, Masson trichrome. Severe tubulitis scored T3 (arrow), and severe epithelial injury.

Middle panel: immunohistochemistry, original magnification x 10, anti-mouse IgG staining. Mononuclear cells infiltrating the graft are CD3+ T-cells, with an aspect of tubulitis and interstitial inflammation. The anti-mouse IgG staining was positive on some cells of the patient's biopsy and negative on TCMR control biopsies from other patients, indicating that some cells were CAR-T cells expressing the mouse immunoglobulin-derived scFv of the anti-CD19 CAR. In the patient biopsy, there were 1769 CD3+ T cells, among which 776 were in tubule epithelium and 993 were interstitial. There were 8 intra-epithelial anti-mouse IgG+ cells (1.03% of intra-epithelial CD3+ cells) and 9 stained cells in the interstitium (0.91% of interstitial CD3+). By comparison, at 3 months post-infusion, usual proportion of CAR-T cells in peripheral blood of patients detected by flow cytometry and/or qPCR are around 2% of total CD3+ cells¹⁶⁻²⁰.

Bottom panel: immunohistochemistry, original magnification x 100, anti-mouse IgG staining. Two stained cells (arrow heads) next to tubular epithelial cells (arrow). The thin arrow points a distant lymphocyte that is comparable in size to the stained cells.

Figure 2: Anti-CD19 CAR-T cell kinetics in the patient's peripheral blood measured by Droplet Digital PCR (ddPCR). The ddPCR enables absolute quantification of the sequence encoding the CAR transgene. Maximal expansion occurred at day-14 following CAR-T cell infusion. The last available blood sample drawn at day 84, during the rejection episode, showed persistence of CAR-T cells.



