Monday, March 27th

09:00-10:30

Oral 3: Novel drugs and immunotherapies

Drug Resistant Lymphocyte Immunotherapy: Dose and Schedule Optimization

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Abstract

We have recently developed a combined chemotherapy and cell based gene therapy strategy for high-grade primary gliomas that we have termed Drug Resistant Immunotherapy (DRI). Specifically, this approach consists of activated MGMT-transduced γδ T cells and NK cells targeted to NKG2D ligands upregulated on the tumor during chemotherapy-induced stress following exposure to the alkylation agent Temozolomide (TMZ). While TMZ kills sensitive GBM cells and modifies the tumor microenvironment, MGMT genetic engineering enables cytotoxic function of stress-antigen targeted lymphocytes in high concentrations of chemotherapy. Pharmacokinetics of both oral and intravenous TMZ reveal a rapid increase in blood concentration followed by a gradual decline. We have separately shown a rapid upregulation of NKG2DL on TMZ-resistant GBM lines in vitro over the first 4 hours followed by a decline over 24h raising the question as to the effect of DRI during maintenance dose TMZ. We hypothesized that intermittent exposure to TMZ with a higher concentration can drive efficacy of DRI even in TMZ-resistant tumors. Intracranial (IC) glioma xenografts were established using either primary (P) or a TMZ-resistant clone (T) of human glioblastoma (GBM) xenolines X12 (Classical) and X22 (Mesenchymal). Two treatment schema were studied. In the first, tumor-bearing mice were treated twice weekly with intraperitoneal (IP) 60mg/kg TMZ and received intracranial (IC) of 1 x 10^6 MGMT-modified γδ T cells (DRI) on the alternating day between the TMZ injections. In the second schema, DRI-treated mice received TMZ on the same schedule but received intensified DRI 1 x 10^6 MGMT-modified γδ T cells IC concurrently with each TMZ injection. Control mice received MGMT-modified γδ T cells alone, TMZ alone or no therapy. Survival was assessed using Kaplan-Meier analysis. MGMT-modified γδ T cells alone did not improve survival over untreated mice for either tumor or treatment schema. In the first schema, both TMZ/TMZ alone and TMZ/DRI/TMZ significantly improved survival over untreated controls (p < 0.001) for X12P, while TMZ/DRI/TMZ increased median survival from 57 to 75 days over TMZ/TMZ. Median survival of X12T mice treated with TMZ/TMZ was not improved over untreated controls; however, TMZ/DRI/TMZ did improve median survival over untreated controls (p = 0.0147) but only marginally over TMZ alone (p = 0.0966). In the second schema, mice that received TMZ+DRI concurrently showed significantly improved median survival over TMZ alone with 80% long-term survivors. For X12T, DRI+TMZ improved median survival from 22 days to 38 days over untreated animals (p = 0.0004) and from 27 to 38 days over TMZ alone (p = 0.017) with 10% of animals showing long-term survival >119 days. Additionally, for TMZ-resistant tumors, Schema 2 showed significant improvement over Schema 1 (p = 0.001). TMZ eradicated tumors in all X22P animals. For X22T, Schema 2 significantly improved survival over untreated animals (20d vs. 27d; p = 0.0009) while Schema 1 did not (p = 0.0607), however neither Schema resulted in improved survival over TMZ alone. In summary, the combination of chemotherapy-induced stress antigen expression and targeted DRI significantly improves survival in tumor-bearing immunodeficient mice. The positive effect on survival is increased when with intensified DRI is given within the first 4 hours of each TMZ injection.

Disclosure of conflict of interest
Incysus, Ltd. Scientific Advisory Board ember and founding scientist
CD19-specific CAR T Cells with a Central Memory and Stem Memory Phenotype - Automated Generation in a Closed, GMP-compatible System from Peripheral Blood of Pediatric Patients with Acute Lymphoblastic Leukemia

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Abstract

Introduction: Chimeric antigen receptor (CAR) modified T cells targeting CD19 can induce sustained remissions in pediatric patients with relapsed and refractory B-precursor acute lymphoblastic leukemia (ALL) [1]. Essential preconditions for treatment efficacy and prevention of CD19+ relapse are proliferation and persistence of CAR T cells in vivo. Central memory T cells (Tcm) and stem cell-like memory T cells (Tscm) are known to be the best candidates for a sustained in vivo expansion after T-cell therapy with small cell doses [2]. Material and Methods: A protocol for generation of anti-CD19 CAR T cells in a closed and GMP-compatible system (CliniMACS Prodigy®) was established. Mononuclear cells derived from 100cc peripheral blood of pediatric ALL patients served as starting material. After separation for CD4+/CD8+ cells, T cells were activated with anti-CD3/CD28 beads and transduced with a lentiviral vector encoding the anti-CD19 single-chain variable fragment, a 4-1BB (CD137) co-stimulatory domain and the T cell receptor (TCR) zeta chain. After 12 days of IL-7/-15-based expansion, the final CAR T cell product was harvested from the device and analyzed for cellular composition, transduction rate and functionality by flow cytometry. Results: Despite small pediatric blood samples with low initial cell numbers and a broad variety in cellular composition including high counts of malignant cells and a rather exhausted phenotype, a robust T-cell composition was achieved on day five after activation with a mean of 63% CD4+ and 37% CD8+ T cells. Mean transduction rate was 30%. No malignant cells or B cells were detected in flow cytometric analyses of the final product. The vast majority of CAR T cells were of a Tcm (47%) and Tscm (44%) phenotype leading to a strong proliferative potential of more than 100-fold expansion. When co-cultured with CD19+ target cell lines or patient-derived autologous CD19+ B cells, CAR T cells showed effective cytotoxic functionality with only little background of the un-transduced control. At an effector to target ratio of 5:1 up to 80% of the CD19+ target cells were killed. A significant release of Interferon gamma (IFN-γ), Tumor necrosis factor (TNF-α) and Interleukin-2 (IL-2) confirmed a strong and target-specific Th1 response. Secretion of Interleukin-6 (IL-6) upon contact to the antigen was not detected. In addition, reduced sensitivity to inhibitory signals was documented by low expression of programmed cell death protein 1 (PD-1), cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and lymphocyte-activation protein 3 (LAG3).

Conclusion: Generation of CAR T cells from small pediatric blood samples was feasible in a closed, GMP-compatible and fully automated system. Despite variety of cell numbers, cellular composition and T-cell phenotype in the starting sample, a uniform T-cell product of Tcm and Tscm could be produced with a balanced CD4/CD8 ratio leading to high expansion potential, good functionality and reduced sensitivity to inhibitory signals.

References


Disclosure of conflict of interest

Miltenyi Biotec GmbH, Bergisch Gladbach, Germany, provided Franziska Blaeschke and Tobias Feuchtinger with reagents free of charge. Andrew Kaiser and Mario Assenmacher are employees of Miltenyi Biotec GmbH, Bergisch Gladbach, Germany.
Checkpoint blockade with pembrolizumab induce graft-versus-host disease for patients with refractory acute leukemia heavily treated after allogeneic transplantation

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1.Division of Hematology, Department of Internal Medicine and 2.Department of Laboratory Medicine, National Taiwan University Hospital, Taipei, Taiwan; 3.Graduate Institute of Clinical Medicine, National Taiwan University, Taipei, Taiwan; 4.Tai-Cheng Stem Cell Therapy Center, National Taiwan University, Taipei, Taiwan.

Abstract

Introduction: Refractory acute leukemia post allogeneic hematopoietic stem cell transplantation (allo-HSCT) carries a particularly grave prognosis. Immune checkpoint blockade with anti-PD1 antibody could theoretically induce graft-versus-host disease (GVHD) and possibly the graft-versus-leukemia (GVL) effect. Recently, anti-PD1 Nivolumab had shown activity against Hodgkin lymphoma relapse after allo-HSCT (ref 1), while checkpoint blockade with Ipilimumab had shown to induce marked immune reaction for patients relapse after allo-HSCT (ref 2). In this study, we aim to evaluate the treatment response and side effects of the anti-PD-1 antibody, pembrolizumab, in heavily treated patients with relapsed and refractory acute leukemia post allo-HSCT.

Materials & Methods: Between Sep 2015 and Nov 2016, nine adult patients received pembrolizumab as salvage therapy for refractory acute leukemia (6 AML, 3 ALL) post allo-HSCT at National Taiwan University Hospital. The baseline patient characteristics, treatment responses and side effects were retrospectively reviewed. Progression-free survival (PFS) was evaluated with the Kaplan-Meier survival analysis. The pilot use of Pembrolizumab in this population had been approved by the hospital Research Ethics Committee. Results: The median duration between allo-HSCT and the administration of the first dose of pembrolizumab in this study was 315 days (range 79-836). Before pembrolizumab administration, they had failed multiple lines of treatment after allo-HSCT (median 4, range 1-8), including repeated donor lymphocyte infusion, second allo-HSCT and chemotherapy, and only four of them had grade I acute GVHD. Pembrolizumab was given at the dose ranging from 1 to 1.6 mg/kg (7 patients received one dose, and 2 patients two doses). Immediate acute GVHD-like reaction occurred in all patients after pembrolizumab administration, including spiking fever (N=7, median 5 days, range 3-15 days), elevated hepatic enzymes (N=7), and skin rashes (N=7, 5 patients had >75% body surface area involved). No treatment related mortality was encountered. The overall response rate (ORR) was 44% (4/9), including two complete remissions (CR) (22%) and two partial remissions (PR). Regarding the two patients who ever achieved CR after pembrolizumab, one had developed Guillain-Barré syndrome (GBS) and immune-mediated esophageal stricture, which was controlled by plasmapheresis and endoscopic balloon dilation procedures, respectively; and the other one had extensive moderate chronic GVHD involving multi-organ. After a median follow-up of 3.2 months (range 0.5-15.5 months), three patients remain alive (one disease-free), while six had died of leukemia progression. The estimated 6-month PFS and was 26.7%, respectively. Conclusion: In this preliminary report, we observed that immediate and remarkable immune response, reminiscing aGVHD/cGVHD could occur after checkpoint blockade therapy. It should therefore be used with caution, especially for those with ongoing or severe GVHD. Some responsive patients could be seen even in these heavily pre-treated refractory patients, particularly in those with severe and special immune response.

Reference

Disclosure of conflict of interest

There is no conflict of interest to disclose.
Vaccines are safe and effective after T-cell depleted CD34-selected allogeneic hematopoietic stem cell transplantation (Allo HCT)

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1Adult Bone Marrow Transplant Service, Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY; 2Department of Hematology, Saint Antoine Hospital, Paris, France; INSERM UMRs 938, Paris, France; Université Pierre et Marie Curie, Paris, France; 3Department of Medicine, Weill Cornell Medical College; New York, NY; 4Department of Epidemiology and Biostatistics, Memorial Sloan Kettering Cancer Center, New York, NY

Abstract

Introduction: Myeloablative T-cell depleted (TCD) allogeneic HCT with CD34 selection is associated with decreased acute and chronic GVHD, and similar survival compared to unmodified grafts. However, there is delayed T-cell recovery and increased risk of infectious complications. Since this may also result in impaired vaccine response, we examined immune reconstitution and vaccine responses after TCD allo HCT. Methods: We retrospectively identified patients with hematologic malignancies from the institutional registry who received a first myeloablative TCD allo HCT between April 2012 and June 2015 and completed re-immunization per CDC guidelines. Patients were immunized based on immune recovery parameters: CD4 T >200 cells/μL, CD19 B cells > 50 cells/μL, IgG > 500 mg/dl, PHA > 50,000 cpm. Vaccine responses were determined based on comparison of pre- and post- vaccination titers. Patients were classified as responders, non-responders, immune by pre-vaccination titer, and not evaluable due to missing data (missing either pre or post-vaccination titers); descriptive statistics were used to summarize results for each vaccine. Adverse events due to vaccination were collected retrospectively. Total CD3, CD4, and CD8 T-cells, and naïve, central memory (CM), effector memory (EM), and effector subsets, as well as B cells were monitored by flow cytometry. Results: 77 patients met inclusion criteria (median age, 52 years; range, 23-71; 54% males). TBI was used in 22 patients (29%), and 64 (83%) received an 8/8 matched graft [24 related (31%) and 40 unrelated (52%)]. At 12 months post HCT, median CD3, CD4, CD8 T cell and B cell counts were 623, 284, 308 and 365, cells/μL, respectively. Effector memory cells were the predominant subset of CD4 (median 55.6%) and CD8 (median 40.2%) T cells (See Table 1 for details of immune subsets). Median time to vaccination was 15.2 months (7.6-34.3) after allo HCT. 68 patients completed the Haemophilus influenzae type b series vaccine and 80% of evaluable patients responded. Pneumococcal vaccination with Prevnar 13 was completed in 75 patients. All patients had lost their immunity prior to vaccination, but 40% responded to the vaccine. 72 patients completed the full Tdap vaccine series. Tetanus, diphtheria, and pertussis had respectively 75, 51 and 67% of responders among evaluable patients. Fewer patients received and completed the Hepatitis A and B vaccines. Responses occurred in 79 and 59% of evaluable patients, respectively. 63 patients received the Polio vaccine. With 81% retaining immunity, 100% of evaluable patients responded to the inactivated polio vaccine. No patients had any adverse events. Details of vaccine response are provided in Table 2. Conclusions: T cell recovery after myeloablative TCD allo HCT is characterized by early expansion of EM T cells and later rise in naïve T cells. While delayed compared to unmodified grafts, it attains normal values in most patients. Re-immunization with inactivated vaccines after myeloablative TCD allo HCT based on immune recovery is safe and effective, offering this population immunity to vaccine preventable diseases.
### Table 1: Immune reconstitution at one-year post CD34 selected allo HCT

<table>
<thead>
<tr>
<th></th>
<th>Cells/uL (Median, range)</th>
<th>% (median, range)</th>
</tr>
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<tbody>
<tr>
<td>B cells</td>
<td>365 (0-1349)</td>
<td></td>
</tr>
<tr>
<td>CD3</td>
<td>623 (97-5179)</td>
<td></td>
</tr>
<tr>
<td>CD4</td>
<td>284 (51-1047)</td>
<td></td>
</tr>
<tr>
<td>CD8</td>
<td>308 (22-4022)</td>
<td></td>
</tr>
<tr>
<td>CD4 naive</td>
<td>39 (2-240)</td>
<td>12.6 (0.4-65.6)</td>
</tr>
<tr>
<td>CD4 CM</td>
<td>42 (0-182)</td>
<td>19.5 (0-44.6)</td>
</tr>
<tr>
<td>CD4 EM</td>
<td>139 (5-475)</td>
<td>55.6 (1.8-92.2)</td>
</tr>
<tr>
<td>CD4 effector</td>
<td>13 (1-358)</td>
<td>3.6 (0.3-66.5)</td>
</tr>
<tr>
<td>CD8 naive</td>
<td>21 (1-191)</td>
<td>5.6 (0-63.0)</td>
</tr>
<tr>
<td>CD8 CM</td>
<td>5 (1-31)</td>
<td>1.5 (0-8.3)</td>
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<tr>
<td>CD8 EM</td>
<td>106 (3-1149)</td>
<td>40.2 (11.1-95.1)</td>
</tr>
<tr>
<td>CD8 effector</td>
<td>91 (6-1287)</td>
<td>29.6 (3-73.7)</td>
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### Table 2: Vaccine response post CD34 selected allo HCT

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Responder (%) evaluable</th>
<th>Non-responder (%) evaluable</th>
<th>Immunized (%) total</th>
<th>Not Evaluable (%) total</th>
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<tbody>
<tr>
<td>Haemophilus Influenza (n=68)</td>
<td>35 (80%)</td>
<td>9 (20%)</td>
<td>22 (32%)</td>
<td>2 (3%)</td>
</tr>
<tr>
<td>Pneumococcal (12) (n=75)</td>
<td>30 (40%)</td>
<td>45 (60%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Poliomyelitis (n=63)</td>
<td>4 (100%)</td>
<td>51 (81%)</td>
<td>8 (13%)</td>
<td></td>
</tr>
<tr>
<td>Tetanus (n=73)</td>
<td>27 (75%)</td>
<td>33 (45%)</td>
<td>4 (5%)</td>
<td></td>
</tr>
<tr>
<td>Diphtheria (n=72)</td>
<td>33 (51%)</td>
<td>32 (49%)</td>
<td>7 (10%)</td>
<td></td>
</tr>
<tr>
<td>B. Pertussis (n=72)</td>
<td>34 (67%)</td>
<td>17 (33%)</td>
<td>16 (22%)</td>
<td>5 (7%)</td>
</tr>
<tr>
<td>Hepatitis A (n=39)</td>
<td>22 (79%)</td>
<td>9 (23%)</td>
<td>2 (3%)</td>
<td></td>
</tr>
<tr>
<td>Hepatitis B (n=45)</td>
<td>20 (59%)</td>
<td>21 (41%)</td>
<td>2 (5%)</td>
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</table>

Disclosure of conflict of interest

None
**Nivolumab salvage therapy before and after allogeneic stem cell transplantation in relapsed/refractory Hodgkin Lymphoma**

El Cheikh, Jean; Massoud, Radwan; Abudalle, Imane; Haffar, Basel; Mahfouz, Rami; Kharfan-Dabaja, Mohamed; Jisr, Tamima; Mogharbel, Anas; Ibrahim, Ahmad; Baazarbachi, Ali

**Abstract**

BACKGROUND: Hodgkin’s lymphoma (HL), although considered a curable neoplasm in adults, could be associated with a very poor prognosis when refractory to primary induction therapy or when it relapses within 12 months from an autologous stem cells transplant (auto-SCT). The optimal treatment of patients with heavily pretreated/refractory HL is controversial. Options include: immunotherapeutic agents such as anti-CD30 Brentuximab (Bv), anti-PD1 antibody Nivolumab, Bendamustine (Benda), and allogeneic hematopoietic stem cell transplantation (allo-SCT). Several reports are available on safety and efficacy of Bv, and Benda before and after allo-SCT, 5 but data is scarce on the use of Nivolumab. We report a retrospective multicenter study to assess the outcomes of HL patients treated with Nivolumab pre or post allo-SCT. PATIENTS AND METHODS: This study was conducted in two major centers in Lebanon, the American university of Beirut Medical Center (AUBMC) and Makassed university hospital. We identified and analyzed the outcome and toxicity in 11 adult patients (nine pre-allo-SCT; two post-allo-SCT) with HL treated with Nivolumab peri-allo-SCT. The primary endpoint of the study was objective response rate (ORR). Secondary endpoints included successful bridging to allo-SCT, safety, and toxicity of Nivolumab. Nivolumab Pre-allo The median age at diagnosis was 28 years (range, 20-38). All patients relapsed post ASCT with a median time to relapse of 7 months (range, 1-24 months). Eight patients (89%) failed further salvage post ASCT, prior to initiation of Nivolumab, the remaining patient was refractory to Bv and Benda prior to ASCT so he was immediately salvaged with Nivolumab. The median number of treatment lines prior to Nivolumab and between ASCT and Nivolumab were 5 (range, 4-7), and 1 (range, 0-2) respectively. And the median follow up from Nivolumab initiation and from allo-SCT was 14 (range, 8-24) and 7 (range, 3-17) months respectively. All patients received a median of 8 cycles (range, 6-20) of Nivolumab. The treatment was well tolerated. One patient developed recurrent fever at every infusion. The ORR was 100% with three (33%) and six (67%) patients achieving CR and PR respectively. All of them proceeded with an allo-SCT immediately after Nivolumab. The median time between the last Nivolumab dose and allo-SCT was 44 days (range, 23-100). After a follow up of 7 months (range, 3-17) post allo-SCT, all patients are alive, seven (78%) in CR, and two (22%) in stable disease. Interestingly, none of our patients progressed post allo-SCT. The one-year Overall Survival (OS) and Disease free survival (DFS) have not been reached. Nivolumab post allo-SCT Two patients were treated with nivolumab for disease relapse post-allo-SCT. Two patients with refractory HL relapsed 10 and 9 months post allo-SCT Salvaged with Nivolumab 3mg/kg (1 dose) developed both a severe steroid refractory acute GVHD (10 and 28 days post therapy respectively). Both still in continuous CR at the last follow up. CONCLUSION: Albeit with the limits of a small observational retrospective study, our data suggests that Nivolumab can be an effective bridge to allo-SCT in patients with relapsed and refractory HL. Even though it does not affect stem cell engraftment, it may contribute to an increased incidence of acute GVHD when used pre and post allo-SCT.
Disclosure of conflict of interest

No conflict of interest to declare
Prospective phase II study of prophylactic low dose azacitidine and donor lymphocyte infusions following allogeneic hematopoietic stem cell transplantation for high risk acute myeloid leukemia and myelodysplastic syndrome.

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Abstract

Although allo HSCT is the only curative option for many pts with AML or MDS, relapse remains the main cause of morbidity and mortality. Post-transplant immune intervention with administration of pre-emptive DLI and/or hypomethylating agents such as 5-azacitidine (aza) is being investigated. We enrolled, in this phase II trial, pts with high risk AML (AML in CR1 with unfavorable cytogenetics, or in CR2, or refractory prior allogeneic transplantation) and MDS (MDS with intermediate-2 group and higher) to receive aza and DLI post-transplant as prophylactic treatment. The primary objective was to evaluate the relapse rate at 2 yrs, secondary objectives were to investigate DFS and OS at 2 yrs post-transplant, safety, incidence and severity of acute and chronic GVHD. Aza was scheduled to begin between d+56 and d+100 post-transplant at 32 mg/m²/d sc for 5 d every 4 w for up to a total of 12 cycles if the patient had not acute GVHD >1 or infection. The first DLI was started following 3 cycles of aza and discontinuation of immnosuppressive prophylaxis. Two other DLI were scheduled every 8 weeks after the 5th and 7th cycle of aza. The doses of DLI 1, 2 and 3 were respectively 5x106, 1x107, 5x107 CD3+cells/kg for related donor, and 1x106, 5x106, 1x107 CD3+cells/kg for unrelated donor. Sixty-one pts were pre-included prior transplantation, 30 pts were subsequently included, 20 pts with AML and 10 pts with MDS, median age 58 years (22-70). The status at transplantation was: CR1 = 16 pts (53%), CR2 = 6 pts (20%), refractory = 5 pts (16%), upfront transplantation for MDS = 3 pts (10%). Cytogenetics was normal or intermediate for 15 pts and unfavorable for 15 pts (namely 8 pts with complex karyotype). Conditioning was myeloablative for 11 pts, reduced for 19 pts (including 2 sequential). Donors were unrelated volunteers in 18 pts (60%). The time between allografting and first aza cycle was 66 d (38-93). The median number of cycles of aza administered was 5 (1-12) with 10 pts (33%) completing the 12 cycles. Forty two DLI were injected in 17 pts: 5 pts received 1 DLI, 2 pts received 2 DLI, 8 pts received 3 DLI. Two additional pts received 4 and 5 DLI because of a mixed chimerism. The first DLI was given at a median of 142 d (129-221) post-HSCT. Aza was well tolerated, but was discontinued in 20 pts: because of GVHD (n=11), relapse (n=5), GVHD/infection (1pt), sudden death due to heart failure (n=1), withdrawal of consent (n=2, one after 1 cycle and another after 5 cycles). Four months following transplantation, 24 pts (80%) demonstrated full donor chimerism (>95%) in CD3+ cells. Nine pts developed gr 1 to 3 acute GVHD (CI 31, 5%), 6 pts who did not receive DLI and 3 pts following DLI. Eleven pts developed chronic GVHD (4 limited, 7 extensive), 3 pts who did not receive DLI, 8 pts following DLI. Twenty patients are alive. With a median follow-up from the allotransplant for those alive of 36 months (range 12 – 46 months), the OS and PFS at 3 years are 66%. Causes of death were infection (n=1), relapse (n=8), sudden death due to heart failure (n=1). The median time to relapse was 5 months (2.5-9) and the CI of relapse at 2 years 28.1±8.5%. These results confirm that aza is well tolerated as a prophylactic treatment to reduce the risk of post-transplantation relapse. The incidence of GVHD following aza + DLI was not overwhelming. Further studies comparing preemptive therapy and no maintenance should clarify the place of preventive strategies.

Disclosure of conflict of interest

None
S1P modulator FTY720 regulates osteoclast precursor mobilization and targets osteoclastogenesis in multiple myeloma systemic xenograft model

Beider, Katia ¹; Rosenberg, Evgenia; Bitner, Hanna; Shimoni, Avichai ¹; Olam, Devorah ²; Weiss, Lola ²; Abraham, Michal ²; Peled, Amnon ²; Nagler, Arnon ¹

¹Sheba Medical Center, ²Hadassah University Hospital

Abstract

Introduction and aim: Sphingosine-1-phosphate (S1P), a lipid mediator enriched in blood, controls the dynamic migration of osteoclast (OC) precursors between the blood and bone. Bone disease is one of the hallmarks of multiple myeloma (MM) leading to substantial morbidity and disability. Bone lesions result from abnormally increased osteoclast (OC) formation and activation. Studying the underlying mechanism may help to develop new therapeutic targets to treat MM-associated osteolytic lesions. Previously we reported that S1P modulator FTY720 exhibits potent anti-myeloma effect in vitro and in vivo in novel disseminated xenograft model of MM. We report now the effect of FTY720 on localization and activation of OCs and their functional sequel in MM model. Methods: The in vitro effect of FTY720 was tested on OCs and OC precursors from healthy donors, and on bone marrow mesenchymal stromal cells (BMSCs). In vivo model of BM-disseminated human myeloma was used to evaluate FTY720 anti-MM and bone activities. Results: The generated OCs expressed the genes encoding for S1P1 and S1P2 receptors and enzyme SPHK1, tested by RT-PCR. Treatment with FTY720 significantly reduced in vitro formation of TRAP+ OCs, resulting in 90% inhibition following 1 µM treatment, and complete abolishment of OC formation at 2.5 µM FTY720 (p<0.0001). Furthermore, FTY720 significantly reduced the expression of genes associated with OC activation. Thus, expression of osteoactivin, cathepsin K, NFATc1, OSCAR, RANK, RANTES, MT1-MMP and MMP9 genes were significantly down-regulated in FTY720-treated OC cultures (p<0.001). In addition, FTY720 targeted the microenvironment components - MM cells and BMSCs, suppressing the expression of osteoclastogenic factors. Moreover, FTY720 altered the ability of myeloma and stroma cells to promote OC formation. Thus, FTY720 was able to disrupt the deleterious cross talk between the MM tumor cells and the OCs. Next, we evaluated the effect of FTY720 on OC activation in vivo taking advantage of our novel xenograft model of CXCR4-over-expressing MM cells that results in typical BM involvement by the MM cells accompanying with significant increase in number of TRAP+ murine OC. Treatment of MM-bearing mice with FTY720 (10 mg/kg) effectively targeted the MM cells in the BM milieu. Correspondingly, FTY720 significantly reduced mRNA levels of murine OC differentiation marker genes in BM, including those encoding cathepsin K, integrin β3, OSCAR, RANTES and RANKL (p<0.001). These effect correlated with increased numbers of circulating CD11c+ and F4/80+ monocytes, with OC precursors in both cell populations. To investigate whether OC precursor migration is affected by FTY720, we evaluated the in vitro migration of human monocytes toward CXCL12, well-known chemo-attractant of OC precursors. FTY720 completely blocked CXCL12-induced migration of CD14+ cells and significantly reduced their surface CXCR4 expression. These results suggest novel mechanism of action of FTY720, affecting both S1P and CXCR4 pathways, reducing the attachment of the OC precursors to the bone and thus leading to their mobilization to the blood. Conclusions: Our observations uncover novel roles of S1P pathway in OC formation and activation in MM, delineating a novel mechanism of action of FTY720 targeting OC formation and migration in vitro and in vivo and providing preclinical rationale for its therapeutic application in patients with MM bone disease.
Disclosure of conflict of interest

Nothing to declare
Decitabine enhances targeting of acute myeloid leukemia cells by umbilical cord blood CD34+ progenitor-derived NK cells in NOD/SCID/IL2Rnull mice

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Abstract

Introduction Combining NK cell adoptive transfer with hypomethylating agents (HMA) is an attractive approach for patients with acute myeloid leukemia (AML). While the direct anti-leukemic effects of HMA are recognized, the impact on the immune system in general and on NK cells in particular is not well established yet. Furthermore, data regarding their impact on NK cells are rather conflicting and mostly derived from in vitro studies. Here, we report a comparative study of azacitidine and decitabine in combination with NK cells generated ex vivo from umbilical cord blood (UCB)-derived CD34+ hematopoietic stem and progenitor cells (HSPC-NK cells). Materials and Methods CD56+Perforin+ HSPC-NK cells were generated under stroma-free conditions in the presence of StemRegenin-1, IL-15 and IL-12 as previously reported (1,2). These SR1/IL15/IL12 expanded HSPC-NK cells exert efficient in vitro cytolytic activity and IFN-γ production towards AML cells. Both HMA were tested for their potentiating effect on HSPC-NK cell mediated killing and targeting of AML cells in vitro and in THP1-bearing NOD/SCID/IL2Rnull mice. Used azacitidine and decitabine concentrations were based on clinical practice and plasma concentrations achieved in patients. Results In vitro, low dose HMAs had minor effect on HSPC-NK cell proliferation and viability. HSPC-NK cells remained phenotypically activated and only the frequency in KIR+ NK cells was increased by HMA treatment under proliferative conditions. In functional assays, the highest concentration of azacitidine diminished NK cell reactivity towards K562 cells. In contrast, decitabine did not influence HSPC-NK killing nor IFN-γ production capacity. Moreover, using AML cell lines and primary AML blasts, we showed that the effects of HMA and HSPC-NK cells were at least additive against AML. In vivo, while both agents exerted a significant effect on AML progression, the persistence of adoptively transferred HSPC-NK cells was not affected with sustained expression of activating receptors, up-regulation of NKP44 expression and remarkable KIR acquisition. Most importantly, only decitabine potentiated HSPC-NK cell anti-leukemic activity in vivo. Interestingly, besides upregulation of NKG2D and DNAM-1 ligands on AML cells, decitabine enhanced mRNA expression of inflammatory cytokines, perforin, and TRAIL in HSPC-NK cells. In addition, treatment resulted in higher numbers of HSPC-NK cells in the bone marrow compartment, suggesting that decitabine could positively modulate NK cell trafficking and tumor targeting. Conclusion Altogether, these data demonstrate that HSPC-NK cells and decitabine can potently cooperate to combat AML, and provide a strong rationale to explore this combination strategy to treat patients. Combining HSPC-NK cell adoptive immunotherapy with decitabine could serve as consolidating therapy for AML or even as bridge towards non-myeloablative allogeneic stem cell transplantation.

References


Disclosure of conflict of interest

No conflict of interest