Histiocyte predominant myocarditis resulting from the addition of interferon gamma to cyclophosphamide-based lymphodepletion for adoptive cellular therapy

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ABSTRACT

Background Adoptive cellular therapy (ACT) is a promising treatment for synovial sarcoma (SS) with reported response rates of over 50%. However, more work is needed to obtain deeper and more durable responses. SS has a ‘cold’ tumor immune microenvironment with low levels of major histocompatibility complex (MHC) expression and few T-cell infiltrates, which could represent a barrier toward successful treatment with ACT. We previously demonstrated that both MHC expression and T-cell infiltration can be increased using systemic interferon gamma (IFN-γ), which could improve the efficacy of ACT for SS.

Case presentation We launched a phase I trial incorporating four weekly doses of IFN-γ in an ACT regimen of high-dose cyclophosphamide (HD Cy), NY-ESO-1-specific T cells, and postinfusion low-dose interleukin (IL)-2. Two patients were treated. While one patient had significant tumor regression and resultant clinical benefit, the other patient suffered a fatal histiocytic myocarditis. Therefore, this cohort was terminated for safety concerns.

Conclusion We describe a new and serious toxicity of immunotherapy from IFN-γ combined with HD Cy-based lymphodepletion and low-dose IL-2. While IFN-γ should not be used concurrently with HD Cy or with low dose IL-2, IFN-γ may still be important in sensitizing SS for treatment with ACT. Future studies should avoid using IFN-γ during the immediate period before/after cell infusion.

Trial registration numbers NCT04177021, NCT01957709, and NCT03063632.

BACKGROUND

Synovial sarcoma (SS) is a soft-tissue malignancy with few available treatments, and overall survival is often less than 2 years when metastatic.1 2 However, SS maybe an ideal tumor type for adoptive cellular therapy (ACT), given frequent and homogenous expression of NY-ESO-1. Importantly, the NY-ESO-1 protein is routinely expressed only in testicular germ cells and in some cancers, but not in healthy adult tissues.3 4 Multiple trials targeting the A*0201 restricted NY-ESO-1 (157–165) epitope have demonstrated greater than 50% responses, with some showing durable and/or reinducible responses at the time of progression.5–7 Unfortunately, in almost all cases, patients who initially respond ultimately progress. We previously reported that SS has an immunologically ‘cold’ tumor microenvironment, with few infiltrating T cells, and low levels of gene expression related to antigen presentation, including constituents of the major histocompatibility complex (MHC).8 However, treatment of patients with SS with systemic interferon gamma (IFN-γ) increased MHC expression and increased T-cell infiltration in matched tumor biopsies in a phase 0 clinical trial.9

Here we describe a pilot study integrating weekly IFN-γ with ACT in the context of high-dose cyclophosphamide (HD Cy)-based lymphodepletion and low-dose IL-2. While the first patient (IFN#1) had an impressive regression of pulmonary tumors, the second patient (IFN#2) developed a fatal histiocytic myocarditis. Although we concluded that this conditioning regimen was not safe moving forward, IFN-γ could still play an important role in future immunotherapy trials; thus, future investigators must be aware of this toxicity.

METHODS

Patients, leukapheresis acquisition and clinical T-cell products

HLA typing of patient samples was performed at the Puget Sound Blood Center (PSBC). The interventional and pilot studies using
either NY-ESO-1-specific T cells or IFN-γ were registered with clinicaltrials.gov.

Leukapheresis was performed at either PSBC or at the University of Washington (UW) General Clinical Research Center if the patient expressed HLA A*0201, had an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, and if tumor biopsies stained positive for NY-ESO-1 in >25% of cells by immunohistochemistry (IHC). Subsequent processing of leukapheresed product was performed at FHCRC, including the cell processing facility (CPF), under current good manufacturing practice (GMP) guidelines. This form of ACT using peripheral blood as a source of antigen-specific T cells is known as endogenous T-cell (ETC) therapy and yields a T-cell product of near uniform specificity with central memory properties. ETC has been used in first-in-human studies targeting tumor-associated antigens with evidence of clinical efficacy.11–12

To facilitate detailed functional analysis, an aliquot of T-cell product was removed from the CPF and then expanded in the research laboratory. In vitro assays were used to assess recognition of target cells pulsed with various peptide concentrations and to assess cytokine production secondary to antigen stimulation. Cell surface phenotype and T cell receptor beta chain variable region (TCR Vβ) sequencing were performed on aliquots from the second expansion.13 Specificity of expanded T-cell products was confirmed using chromium release assays performed on day 12 of the rapid expansion protocol. T2 target cells were cultured alone for 2 hours or in media with target peptide; when relevant, the HLA A*0201+, NY-ESO-1+tumoraline, Mel A375 (A375), was also used as target cell.

Flow cytometry and functional analysis of antigen specific T cells

Cell surface phenotype of persisting cells was determined by co-staining with NY-ESO-1 tetramer (FHCRC, Seattle, Washington, USA) and fluorochrome-conjugated monoclonal antibodies (mAbs). Multicolor flow cytometry data were collected using a LSRII (Becton Dickinson, Franklin Lakes, New Jersey, USA) and FlowJo software. For staining, cells were fixed and stained for CD8 (BD Bioscience, Franklin Lakes, New Jersey, USA).

TCR Vβ sequencing

TCR Vβ sequencing and normalization was performed on suspensions of tumor-infiltrating lymphocytes, as well as paraffin-embedded specimens by Adaptive Biotechnologies (Seattle, Washington, USA). The number of total cells, T cells, T-cell fraction, the number of unique rearrangements, and clonality were calculated for each sample as previously described.14

Immunohistochemistry

Immunohistochemical analyses of myocardium for CD3, CD4, CD8, CD20, and CD68 expression were performed using an automated immunostainer (Bond III) and the following mAbs: CD3 (clone LN10, dilution 1:100; Novoceastra, Newcastle, UK), CD4 (clone SP35, dilution 1:25; Cell Marque, Rocklin, California, USA), CD8 (clone 4B11, dilution 1:200; Novoceastra), CD20 (clone L26, dilution 1:800; Dako, Glostrup, Denmark), and CD68 (clone KP-1, dilution 1:1000; Cell Marque). Tonsils were used as positive controls.

Immunohistochemical expression to confirm eligibility of patients for treatment with NY-ESO-1-specific T cells was performed at the UW Pathology Lab with mAb specific for NY-ESO-1 (1:100, E978 clone; Life Technologies, Grand Island, New York, USA), CD3 (clone LN10, dilution 1:200; Novoceastra), and MHC class I (1:8000, EMR8-5 clone; Abcam, Cambridge, Massachusetts, USA).

RESULTS

To test whether systemic weekly IFN-γ could be safely combined with ACT using NY-ESO-1-specific T cells, we designed a phase I trial using weekly IFN-γ (100 µg/m²) beginning 2 weeks prior to cell infusion and continuing for 2 weeks after the cell infusion. Lymphodepletion consisted of HD Cyclophosphamide (2 g/m²/day) on days −3 and −2, prior to the T cell infusion (day 0). To augment in vivo proliferation, low-dose IL-2 (250 000 U/m² every 12 hours) was administered for 2 weeks after the infusion (figure 1).

Patient 1

IFN#1 was a man in his mid-40s with intractable pain in his left shoulder. He visited a local emergency department, and an investigative chest X-ray showed a hilar mass. Subsequent CT of the chest, abdomen, and pelvis again demonstrated the hilar mass, along with a 2.5×3.6 cm pelvic mass. Biopsy showed diffuse expression of Bcl-2, vimentin, CD99, and neoplastic cells expressing TLE-1, indicative of SS. He received two cycles of doxorubicin and ifosfamide, with disease progression. Next, he received two cycles of trabectedin, again with disease progression (table 1).

Multiple brain metastases were identified, and the patient underwent gamma knife radiation. Given that his tumor demonstrated strong homogenous NY-ESO-1 expression and that he was HLA-A0201+, he was enrolled in the ACT trial.
| Identifier | Sarcoma subtype | Prior treatment | V gene | Cell dose ($\times 10^9$) | V$\alpha$ gene | CDR3$\alpha$-chain V$\beta$ gene | Best tumor response
|------------|----------------|-----------------|-------|---------------------------|----------------|-------------------------------|------------------------|
| IFN#1 SS A/I, trabectedin | Soft tissue, lung, brain | TRAV-9*02 TRBV12-3*01 | 22 | TRAβ*01 | CALSAVALGMCCI | CAVMGDNDRF | 32% lung
| IFN#2 SS A/I/vincristine, Rtx/Ifos | Lung | TRAV21*01 | 12.9 | TRAβ*01 | CAVMGDNDRF | TRBV12-30*01 | N/A

*part of dual alpha, only this alpha sequence was productive based on tetramer staining and functional assays.

IFN#2, second patient; IFN-γ, interferon gamma; IFN#1, first patient; Ifos, ifosfamide; N/A, not applicable; Rtx, rituximab; SS, synovial sarcoma.

Figure 2 (A) Persistence of transferred T cells as absolute values. Percent tetramer positive derived from peripheral CD8+ T cells. (B) CT scan showing pretreatment and post-treatment lung. (C) Forehead soft tissue mass. (D) Pretreatment lung (left) and post-treatment forehead mass (right) stained for NY-ESO-1. (E) Lung (left) stained for HLA-ABC, forehead mass (right) stained for HLA-ABC.

Despite the aggressive metastatic chemoresistant disease, 4 weeks after T-cell infusion, all six lung tumors showed a measurable decrease in size. A hilar lymph node decreased from 3.0×3.0 cm to 1.9×3.0 cm, and the small pleural-based lung nodules also decreased in size. The most dramatic response was in a large right upper lobe (RUL) tumor that previously received 3600 cGy of radiation 3 months prior to ACT. This mass was 10.8×8.1 cm prior to radiation and remained stable at 10.4×8.0 cm 3 months after radiation therapy. However, 4 weeks after T-cell therapy, the tumor decreased markedly in size to 6.9×5.3 cm, and by 10 weeks to 6.9×4.5 cm (figure 2B). Importantly, biopsy of lung lesion pretreatment and forehead lesion post-treatment were stained for NY-ESO-1 and MHC class I. The post-treatment sample showed both increased NY-ESO-1 staining (figure 2D) and increased MHC class I expression (figure 2E). No lung tumors (including subcentimeter nodules) had progressed 4 weeks after T-cell therapy. Subjectively, he reported a marked improvement in fatigue and respiratory status following ACT and even returned to work after missing weeks because of fatigue. He experienced common
self-limited toxicities of HD Cy (leukopenia, anemia, malaise, and mild mucositis), IFN-γ and IL-2 (fatigue, malaise, and myalgias), but no unexpected toxicities.

Prior to T-cell infusion, NY-ESO-1 tet+ T cells were undetectable in the blood (<0.01% of CD8+ cells). Four weeks after infusion, following complete reconstitution of the absolute lymphocyte and white blood cell counts and cyclophosphamide conditioning, NY-ESO-1 tet+ T cells represented 5% of all peripheral CD8+ T cells (figure 2A). At 10 weeks postinfusion, this cell population remained detectable.

Despite the marked response of the pulmonary metastases, the overall response was mixed as a soft tissue lesion on his forehead progressed 10 weeks after ACT (figure 2C). To investigate the lack of response at some tumor sites, his forehead lesion was excised. The pathology demonstrated homogenous staining of NY-ESO-1 by IHC, but few infiltrating T cells were found and the tissue lacked MHC class I expression (data not shown), which was thought to be responsible for the lack of response and may indicate that the impact of IFN-γ in the tumor microenvironment is not durable.

**Patient 2**

The IFN#2 was a man in his mid-40s who first noticed lower back pain. His pain progressed, and a small lump eventually developed. MRI at an outside hospital showed a right flank mass measuring 7.8×8.7×12.9 cm. Subsequent biopsy demonstrated poorly differentiated SS. He underwent six cycles of neoadjuvant doxorubicin with ifosfamide and vincristine, followed by surgical resection. He also received radiation and concurrent ifosfamide for two cycles after the surgical resection. Unfortunately, 2 years later during routine surveillance, lung metastases were discovered in both lungs. After discussing multiple treatment options, the patient elected for ACT.

Before trial enrollment, his poorly controlled type 2 diabetes was noted with a hemoglobin A1c of 10.1%, complicated by diabetic nephropathy and a baseline of 2 diabetes was noted with a hemoglobin A1c of 10.1%, complicated by diabetic nephropathy and a baseline.
Histological sections of the heart at autopsy demonstrate myocyte necrosis and marked mononuclear inflammation (A, H&E ×200). Immunohistochemical stains highlight a mixed population of inflammatory cells composed of predominantly CD68+ histiocytes (D) and few CD3+ T cells (B). CD20 stain does not demonstrate a significant B-cell population (C).

Figure 3

was made clinically relevant through IFN-γ-induced macrophage stimulation.

Although we conclude that IFN-γ should not be used concurrently with HD Cy or low-dose IL-2, IFN-γ may still play an important role in sensitizing SS for ACT. Interestingly, another recent ACT study showed the combination of interferon alpha and tumor-infiltrating lymphocyte (TIL) therapy, including Cy pretreatment in a similar dose, reinfusion of TILs, and postinfusion of IL-2 in a higher dose, was found to be safe in 12 patients in a published article by Andersen et al.26 However, future studies should avoid using IFN-γ during the immediate period around cell infusion and instead should consider alternative schedules, such as beginning IFN-γ weeks after cell infusion or at the time of progression.

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REFERENCES

1. Riedel PF, Jones RL, Italiano A, et al. Systemic anti-cancer therapy in synovial sarcoma: a systematic review. Cancers 2018;10. doi:10.3390/cancers10110417. [Epub ahead of print: 01 Nov 2018].

2. Moreau L-C, Turcotte R, Ferguson P, et al. Myxoid/round cell liposarcoma (MRCLS) revisited: an analysis of 418 primarily managed cases. Ann Surg Oncol 2012;19:1081–8.

3. Jungbluth AA, Antonescu CR, Busam KJ, et al. Monophasic and biphasic synovial sarcomas abundantly express cancer/testis antigen NY-ESO-1 but not MAGE-A1 or GCT. Int J Cancer 2001;94:252–6.

4. Pollack SM, Jungbluth AA, Hoch BL, et al. Monophasic and biphasic synovial sarcoma: a neoplastic entity revisited. Cancer 2012;118:4564–70.

5. Robbins PF, Morgan RA, Feldman SA, et al. Tumor regression in patients with metastatic synovial cell sarcoma and melanoma using genetically engineered lymphocytes reactive with NY-ESO-1. J Clin Oncol 2011;29:917–24.

6. Robbins PF, Kassim SH, Tran TLN, et al. A pilot trial using lymphocytes genetically engineered with an NY-ESO-1-reactive T-cell receptor: long-term follow-up and correlates with response. Clin Cancer Res 2015;21:1019–27.

7. D’Angelo SP, Melchiori L, Merchant MS, et al. Antitumor Activity Associated with Prolonged Persistence of Adoptively Transferred NY-ESO-1 T Cells in Synovial Sarcoma. Cancer Discov 2018;8:944–57.

8. Pollack SM, He Q, Yearley JH, et al. T-Cell infiltration and clonality correlate with programmed cell death protein 1 and programmed death-ligand 1 expression in patients with soft tissue sarcomas. Cancer 2017;123:3291–304.

9. Zhang S, Kohli K, Black RG, et al. Systemic interferon-γ increases MHC class I expression and T-cell infiltration in cold tumors: results of a phase 0 clinical trial. Cancer Immunol Res 2019;7:1237–43.

10. Li Y, Bleakley M, Yee C. II-21 influences the frequency, phenotype, and affinity of the antigen-specific CD8 T cell response. J Immunol 2005;175:2261–9.

11. Chapuis AG, Roberts IM, Thompson JA, et al. T-Cell therapy using Interleukin-21-Primed cytotoxic T-cell lymphocytes combined with cytotoxic T-cell lymphocyte antigen-4 blockade results in long-term cell persistence and durable tumor regression. J Clin Oncol 2016;34:3787–95.

12. Yee C, Lizee G, Schuenneman AJ. Endogenous T-cell therapy: clinical experience. Cancer J 2015;21:492–500.

13. Pollack SM, Jones RL, Farrar EA, et al. Tetramer guided, cell sorter assisted production of clinical grade autologous NY-ESO-1 specific CD8(+) T cells. J Immunother Cancer 2014;2:36.

14. Robbins HS, Srivastava S, Campero-Perez PV, et al. Overlap and effective size of the human CD8+ T cell receptor repertoire. Sci Transl Med 2010; 2:29ra64. 2.

15. Pollack SM, Ingham M, Spraker MB, et al. Emerging targeted and immune-based therapies in sarcoma. J Clin Oncol 2018;36:125–35.

16. Pollack SM, Lu H, Gnjatic S, et al. First-In-Human treatment with a dendritic Cell-targeting lentiviral Vector-expressing NY-ESO-1, LV305, induces deep, durable response in refractory metastatic synovial sarcoma patient. J Immunother 2017;40:1–306.

17. Somaiya N, Block MS, Kim JW, et al. First-In-Class, first-in-human study evaluating LV305, a dendritic-cell tropic lentiviral vector, in sarcoma and other solid tumors expressing NY-ESO-1. Clin Cancer Res 2019;25:5808–17.

18. Zhang S, Kohli K, Black G, et al. Systemic interferon gamma increases MCH class one expression and T-cell infiltration in cold tumors: results of a phase 0 clinical trial. Cancer Immunol Res 2019;8:1237–43.

19. Goldberg MA, Antin JH, Guinan EG, et al. Cyclophosphamide cardiototoxicity: an analysis of dosage as a risk factor. Blood 1986;66:1114–8.

20. Higgins AY, O’Halloran TD, Chang JD. Chemotherapy-Induced cardiomyopathy. Heart Fail Rev 2015;20:721–30.

21. Appelbaum FR, Strauchen JA, Graw RG, et al. Acute lethal carditis caused by high-dose combination chemotherapy. A unique clinical and pathological entity. Lancet 1976;1:58–62.

22. Katayama M, Imai Y, Hashimoto H, et al. Fulminant fatal cardiotoxicity following cyclophosphamide therapy. J Cardiol 2009;54:330–4.

23. Shanholz C. Acute life-threatening toxicity of cancer treatment. Crit Care Clin 2001;17:483–502.

24. Reifenberg K, Lehr H-A, Torzewski M, et al. Interferon-Gamma induces chronic active myocarditis and cardiomyopathy in transgenic mice. Am J Pathol 2007;171:463–72.

25. Torzewski M, Wenzel P, Kleinert H, et al. Chronic inflammatory cardiomyopathy of interferon γ-overexpressing transgenic mice is mediated by tumor necrosis factor-α. Am J Pathol 2012;180:73–81.

26. Andersen R, Borch TH, Draghi A, et al. T cells isolated from patients with checkpoint inhibitor-resistant melanoma are functional and can mediate tumor regression. Ann Oncol 2018;29:1575–81.