

Efficacy of Intravenous Cyclophosphamide Pulse Therapy for P-Glycoprotein-expressing B Cell-associated Active True Renal Lupus Vasculitis in Lupus Nephritis

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Abstract

True renal lupus vasculitis (TRLV), a vascular lesion usually associated with proliferative lupus nephritis (LN), is resistant to conventional treatments. The expression of P-glycoprotein (P-gp) on activated lymphocytes causes drug resistance. We herein report a patient with TRLV, minimal change LN, overexpression of P-gp on peripheral B cells, and accumulation of P-gp⁺ B cells at the site of TRLV. High-dose corticosteroids combined with intravenous cyclophosphamide pulse therapy resulted in clinical remission and the long-term normal renal function.

Key words: B cell, lupus nephritis, P-glycoprotein, renal vasculitis

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Introduction

Lupus nephritis (LN) is a serious complication in systemic lupus erythematosus (SLE), affecting about 30% to 50% of patients with SLE (1). The International Society of Nephrology and the Renal Pathology Society (ISN/RPS) 2003 classification (2), which focuses on glomerular lesions, provides useful pathologic information on the assessment of the severity and prognosis of LN and the selection of an appropriate treatment strategy. However, the high incidence of vasculopathy in LN (about 28% of renal biopsies) reduces the effectiveness of available therapies, thereby lowering the chance of a satisfactory renal outcome (3). Therefore, any direct treatments against renal vasculopathy should include an early diagnosis and the administration of effective therapies.

Among the vasculopathies in LN, true renal lupus vasculitis (TRLV) is a rare form of fibrinoid necrotizing vasculitis affecting small arteries without antineutrophil cytoplasmic autoantibodies (ANCA). TRLV is commonly associated with proliferative LN classified as class III or IV based on the WHO or ISN/RPS 2003 classifications and is known to be resistant to conventional treatments (3, 4). Among the

mechanisms of resistance to treatment in SLE patients, we previously reported that the overexpression of P-glycoprotein (P-gp) on activated lymphocytes in active SLE patients is associated with the active efflux of intracellular drugs, including corticosteroids, resulting in the development of P-glycoprotein-mediated multidrug resistance (5-7).

We herein report a case of P-gp-expressing B cell-mediated active TRLV associated with minimal change LN just after SLE onset and document the favorable renal prognosis following intravenous cyclophosphamide pulse therapy (IVCY).

Peripheral blood mononuclear cells (PBMCs) from the SLE patient were isolated by density gradient centrifugation. Staining and the flow cytometric analysis of the target molecules on PBMCs were conducted via standard procedures, as described previously (5, 6). MRK-16 (Kyowa Medex, Tokyo, Japan), a specific monoclonal antibody (mAb) against P-gp, was used for staining of P-gp on peripheral lymphocytes. The resected renal biopsy specimens were fixed in 15% phosphate-buffered formalin and embedded in paraffin. Serial sections 5 μm thick were stained with Hematoxylin and Eosin (H&E), Periodic acid-Schiff, or other appropriate immunohistochemical stains. For immunohistochemistry, the sections were incubated with a primary Ab against P-gp

(JSB-1, a murine mAb, dilution, 1:20; MONOSAN, Uden, The Netherlands), isotype-matched negative control antibody of P-gp (negative control mouse IgG1, dilution, 1:10; DAKO, Glostrup, Denmark), or CD19 (LE-CD19, anti-human mouse mAb, dilution, 1:50, DAKO). The sections were colorized in a solution consisting of 3,3'-diaminobenzidine (DAB) tetrahydrochloride, or in Vulcan Fast Red Chromogen Kit 2 (Biocare Medical, Concord, CA, USA). An immunofluorescent examination was conducted using frozen specimens sliced into serial sections 5 μ m thick.

This study was approved by the institution human subjective research committee, and informed consent was obtained from the patient.

Case Report

A 23-year-old woman developed general fatigue, a low-grade fever, and polyarthralgia in January 2010. On admission to our University Hospital in April 2010, she was diagnosed with SLE based on the criteria of the American College of Rheumatology (ACR), including arthritis, leukocytopenia (2,600/ μ L), positive antinuclear antibody (\times 1,280), and positive anti-double-stranded (ds) DNA antibody (28.7 U/mL). A clinical examination and laboratory tests showed a fever (body temperature 37.3°C), bilateral axillary and inguinal lymphadenopathy, a high erythrocyte sedimentation rate (ESR) (22 mm/h), and hypocomplementemia (C4 9 mg/dL, CH50 20 U/mL). The SLE disease activity index (SLEDAI) score was 9, and the disease activity was considered moderately high. Renal function tests were normal, with a normal urinalysis and normal creatinine level (creatinine 0.49 mg/dL, estimated glomerular filtration rate 127.22 mL/min/1.73 m², urinary protein excretion 0.04 g/day, urinary sediment of red blood cells <1/high power field, and urinary sediment of white blood cells <1/high power field). However, because hypocomplementemia and serological abnormalities [positive antinuclear antibody, positive anti-ds DNA antibody, and positive anti-Smith (Sm) antibody (64 IU/mL)] were observed, informed consent about renal biopsy was obtained from the patient, and a renal biopsy was performed. An examination of H&E-stained renal biopsy tissue showed normal glomeruli (Fig. 1A). However, marked deposition of complement and IgG in the glomeruli was noted in immunofluorescence staining (Fig. 1B). The glomerular lesion was classified as ISN/RPS class I lupus nephritis. However, 10 interlobular arteries were included in 7 resected specimens, and vasculitis was detected in 9 of them. Furthermore, necrotizing vasculitis was evident in three interlobular arteries. This included fibrinoid necrosis, prominent infiltration around the blood vessel wall with inflammatory cells including lymphocytes, and tears of the elastic laminae of the arteries (Fig. 1C).

We next investigated the reason for the renal small-vessel vasculitis. Before admission, the patient had not been using any drugs that could have induced necrotizing vasculitis in

small vessels (e.g., anti-thyroid agents, d-penicillamine, hydralazine, minocycline, or omeprazole). Furthermore, the patient had no symptoms suggestive of systemic vasculitis, and computed tomography (CT) angiography showed no abnormalities (e.g., stenosis or small aneurysm). Lastly, there were no significant abnormalities in the serum levels of ANCA or cryoglobulin, and the patient was free of infection and malignancy. Accordingly, the final diagnosis was established as TRLV with ISN/RPS class I lupus nephritis after the exclusion of all other forms of vasculitis, including drug-, cancer-, and infection-associated vasculitis, polyarteritis nodosa, ANCA-associated vasculitis, and cryoglobulinemic vasculitis. The involvement of other organs, including neuropsychiatric syndromes of SLE was ruled out based on the clinical presentation.

TRLV is often resistant to corticosteroid therapy and associated with a poor renal outcome (3, 4). To assess drug resistance and select the appropriate immunosuppressive therapy, we determined the P-gp expression on lymphocytes by flow cytometry on admission. The percentages of P-gp-positively stained CD4⁺ and CD19⁺ peripheral lymphocytes were 7.0% and 40.1%, respectively (Fig. 2A). We reported previously that only a small proportion of peripheral lymphocytes express P-gp in normal individuals (CD4⁺ lymphocytes: 4.9 \pm 2.1%, CD19⁺ lymphocytes: 3.1 \pm 3.1%, mean \pm SD) (6). Thus, the percentage of P-gp-expressing CD19⁺ lymphocytes was definitely higher in our SLE patient than in normal subjects. To confirm the involvement of P-gp-positive CD19⁺ lymphocytes in the foci of active vasculitis, serial sections of the renal tissue specimen were immunohistochemically stained for CD19 and P-gp. Marked infiltration of CD19⁺ lymphocytes and P-gp-positive lymphocytes was noted in the perivascular area of vasculitis; therefore, the P-gp⁺ CD19⁺ lymphocytes were suggested to have accumulated around the vasculitis (Fig. 2B, C). These findings further confirmed the involvement of P-gp overexpression on activated CD19⁺ lymphocytes in resistance to corticosteroids (5-7), in addition to the poor renal outcome associated with the pathological process of TRLV (3, 4).

Based on the above findings, intensive immunosuppressive therapy was required for P-gp⁺ B cell-associated active TRLV in the patient. We selected high-dose corticosteroids combined with cyclophosphamide, which is used for the initial induction of small- and medium-vessel vasculitis (8), and obtained informed consent from the patient. The patient was treated with biweekly IVCY (15 mg/kg/day) combined with oral betamethasone (equivalent to 1.0 mg/kg/day of prednisolone) (Fig. 3). Immediate clinical improvement was noted after two courses of biweekly IVCY, along with normalization of the ESR, complement and anti-ds DNA antibody, followed by clinical remission (SLEDAI score: 0). After six courses of IVCY, the resolution of bilateral axillary and inguinal lymphadenopathy was noted, together with a decrease in the ANA to \times 80. Subsequently, IVCY was switched to oral azathioprine as maintenance therapy, and the administration of corticosteroids was gradually reduced

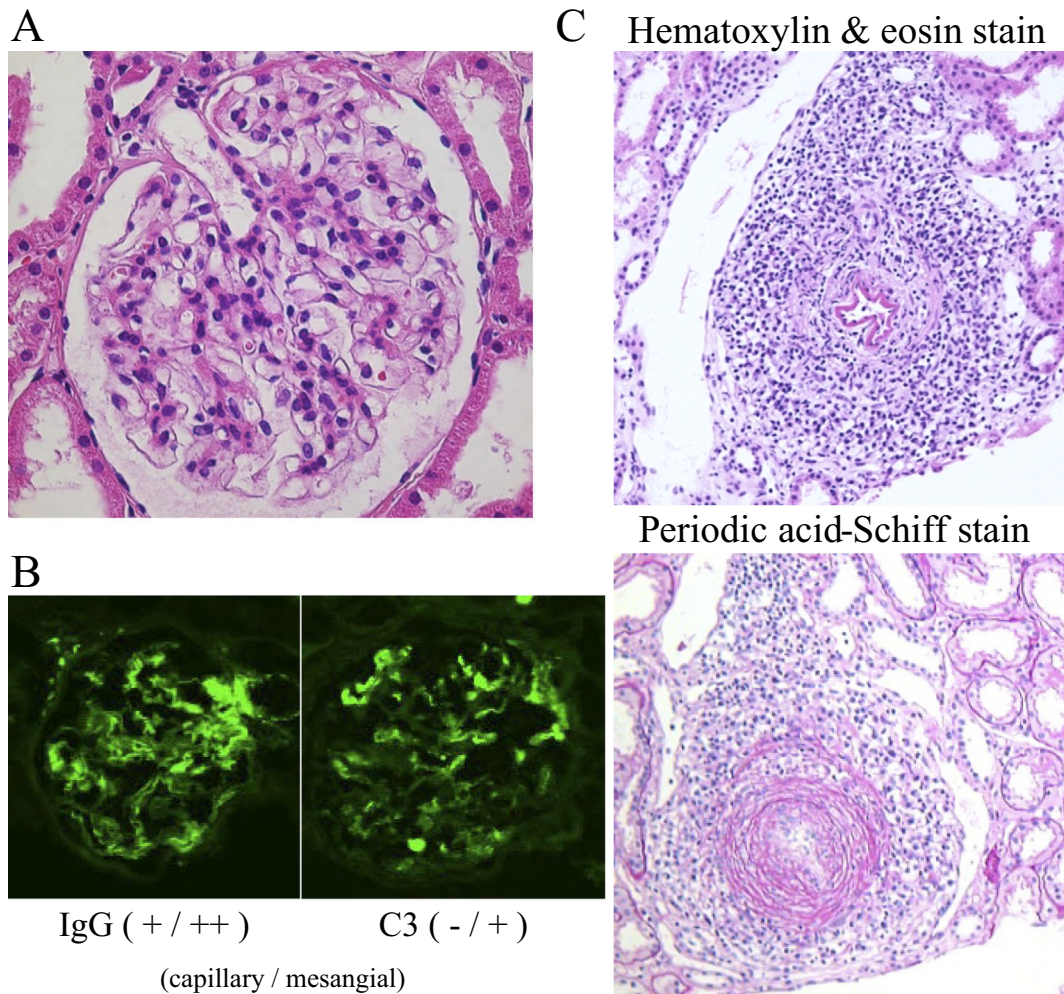


Figure 1. Renal biopsy on admission. **A:** Twenty-two glomeruli were observed. Note the lack of global sclerosis, thrombi, wire loop lesions, spike formation, fibrinoid necrosis or crescent [Hematoxylin and Eosin (H&E) staining, 200 \times]. **B:** Immunoglobulin G deposition in the capillary walls and mesangial areas, and C3 deposition in mesangial areas (immunofluorescence stain, 100 \times). **C:** Vasculitis of intralobular arteries with fibrinoid necrosis, inflammatory infiltrates, and destruction of the vascular wall (H&E staining or Periodic acid-Schiff stain, 100 \times).

and discontinued. Under such treatment, the clinical remission and normal renal function were maintained for more than three years (Fig. 3). Repeated biweekly IVCY (15 mg/kg/day) resulted in the development of leukocytopenia, but cessation of IVCY resulted in the normalization of the leukocyte count. The patient did not experience any severe side effects, such as severe infections, in relation to the treatment or during the observation period.

Discussion

TRLV is commonly associated with active proliferative nephritis with progression to renal dysfunction (3). However, TRLV in the present case represented an acute inflammatory phase with minimal mesangial lupus nephritis (ISN/RPS class I) and without clinically-evident renal dysfunction. Accordingly, TRLV was able to develop before progression to glomerular nephritis. A renal biopsy performed in the early stage of SLE onset allowed for the detection of active TRLV

before progression to glomerular nephritis.

Patients with Class I often show a good renal outcome, and accordingly, low-dose corticosteroid therapy is recommended (1). However, the criteria for ISN/RPS according to the 2003 classification do not include any vascular lesions (2). In this regard, Banfi et al. (3) reported that patients with lupus vasculopathy, including TRLV (5-year renal survival rate: 74.3%) had poorer renal outcomes than those without vascular lesions (5-year renal survival rate: 89.6%). Similarly, Wu et al. (9) reported that LN patients with renal vascular lesions (such as immune complex deposits or thrombotic microangiopathy) have poorer renal outcomes than those without renal vascular lesions. Descombes et al. (10) reported that four renal vasculitis patients treated with early and aggressive immunosuppressive therapy had good renal outcomes. The diagnosis of TRLV in the present case with moderate disease activity and ISN/RPS class I LN suggested a poor renal outcome. Although the optimum treatment strategy of TRLV has not yet been established, the

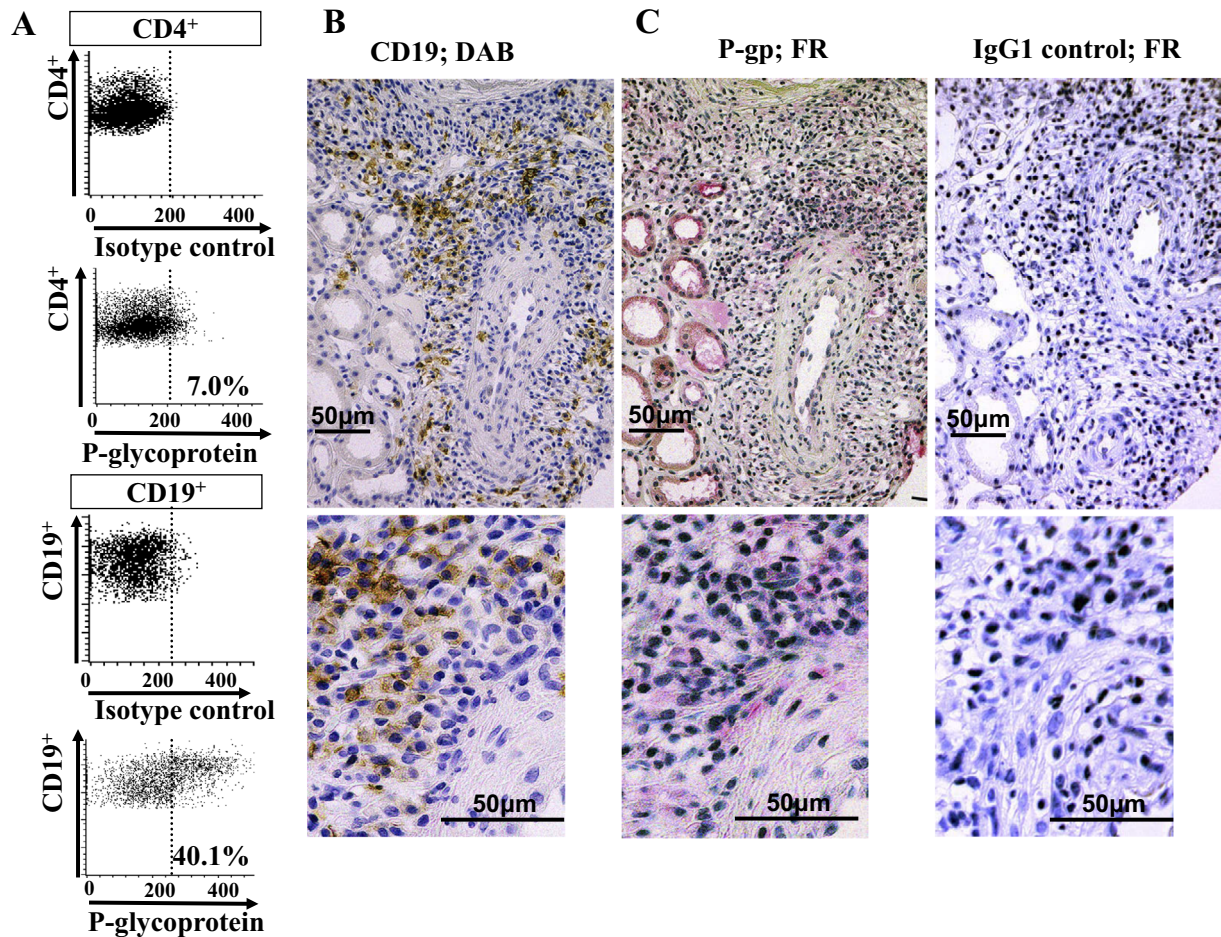


Figure 2. P-glycoprotein expression on peripheral CD19⁺ cells and infiltrated CD19⁺ cells around small vessels in the kidney. **A:** P-gp expression on CD4⁺ and CD19⁺ cells before treatment with prednisolone. The dotted line represents the gate set to discriminate negatively from positively stained cells as determined by control FITC-conjugated anti-mouse IgG Ab. The data represent the percentages of P-gp-positively stained CD4⁺ and CD19⁺ cells. **B:** Immunostaining for CD19⁺ lymphocytes using anti-CD19 monoclonal antibody (mAb) with 3,3'-diaminobenzidine (DAB) (brown color). **C:** Immunostaining for P-gp on lymphocytes using JSB-1 anti-P-gp mAb (P-gp) or isotype-matched negative control antibody immunoglobulin G1 (IgG1 control) with Vulcan Fast Red (FR) (red color). **B, C:** Nuclear counterstaining with hematoxylin.

combination of intensive immunosuppressants and corticosteroids has been recommended based on the poor renal outcomes associated with TRLV (4).

The present case showed expansion of peripheral P-gp⁺ B cells and accumulation of P-gp⁺ B cells in the perivascular lesion of small vessels. P-gp is a member of the ATP-binding cassette transporter superfamily and functions as an energy-dependent transmembrane efflux pump. The overexpression of P-gp on lymphocytes leads to a reduction in the intracellular concentrations of various drugs of P-gp substrates, including corticosteroids, and results in the development of P-gp-mediated multidrug resistance (11, 12). Certain cytokines, such as IL-2 and IL-6, can activate B cells and induce P-gp expression on B cells (7). The P-gp expression on B cells in SLE patients correlates significantly with disease activity. The activation of B cells by various stimuli in SLE patients with highly active disease can result in the development of P-gp-mediated multidrug resistance against

corticosteroids and failure to control the disease activity (7, 11, 12). Accordingly, the overexpression of P-gp on B cells in the present case represented steroid resistance.

The pathogenesis of TRLV parallels that of lupus vasculitis (4). B cells are involved in the pathogenesis of lupus vasculitis. The activation of the complement system by autoantibodies, e.g., anti-endothelial cell antibodies (13, 14), produced by activated B cells results in neutrophilic infiltration in vessel walls and vascular damage (15).

Regarding the pathogenic role of P-gp-overexpressing B cells in TRLV, these cells may be involved in the following respects: 1) Production of autoantibodies, resulting in the formation of immune-complex and activation of the complement system; 2) Direct infiltration of these cells into the perivascular lesion of small vessels. Thus, steroid-resistant activated B cells play an important role in the pathogenic immune response of TRLV, resulting in the development of steroid resistance and failure to control renal manifestations

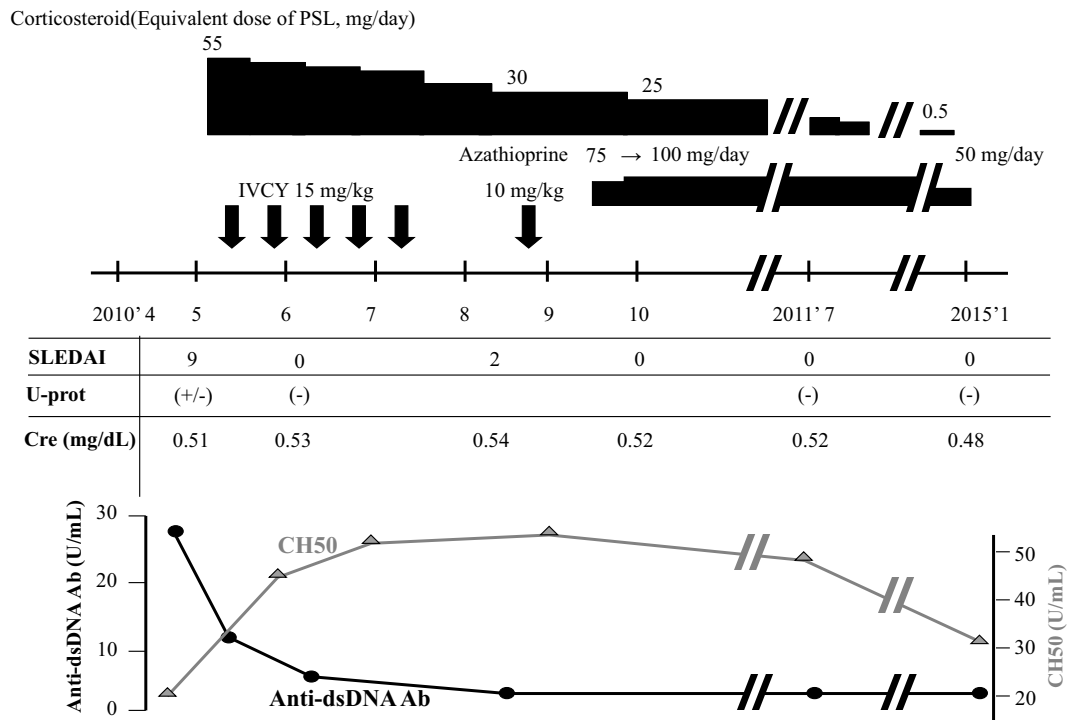


Figure 3. Clinical course. PSL: prednisolone, IVCY: intravenous cyclophosphamide pulse therapy, SLEDAI: systemic lupus erythematosus disease activity index score, U-prot: urinary protein, Cre: creatinine, CH50: 50% hemolytic complement activity, anti-ds DNA Ab: anti-double-stranded (ds) DNA antibody

with oral corticosteroid monotherapy.

IVCY, which is a potent inducer of remission in refractory lupus nephritis and ANCA-associated vasculitis (1, 8), is reported to bring about successful control of the disease activity by reducing the P-gp expression on lymphocytes in P-gp-mediated steroid-resistant SLE patients (5). In the present case, high-dose corticosteroid with IVCY resulted in immediate normalization of serological abnormalities, induction of clinical remission, maintenance of the normal renal function, and clinical remission for over three years, and allowed for the tapering of corticosteroids.

Taken together, the present findings highlight the importance of a renal biopsy in the early stages of SLE in patients with hypocomplementemia and high titers of autoantibodies, regardless of the severity of clinical renal dysfunction. The evaluation of renal vascular lesions, in addition to that of glomerular lesions according to the ISN/RPS 2003 classification, is essential in predicting the renal outcome and in selecting a treatment strategy. P-gp overexpressing-activated B cells play an important role in the pathogenesis of TRLV, resulting in the development of steroid resistance and poor renal outcomes. The early diagnosis of TRLV by a renal biopsy and the early induction of IVCY are recommended in order to improve the renal function and overall prognosis.

Author's disclosure of potential Conflicts of Interest (COI).

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