



Cutaneous sarcoidosis in a chronic hepatitis C patient receiving Peg-IFN and ribavirin therapy

Journal:	<i>Hepatology Research</i>
Manuscript ID:	HEPRES-12-0539.R3
Manuscript Type:	Case Report
Date Submitted by the Author:	n/a
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Category:	7. Viral hepatitis, 4. Immunology

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Title

**Cutaneous sarcoidosis in a chronic hepatitis C patient receiving Peg-IFN
and ribavirin therapy**

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Running Title: Sarcoidosis associated with Peg-IFN and RBV

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Abstract

A 61-year-old Japanese woman suffered from a small, painful, subcutaneous nodule on the sole of her foot that was 10mm across in diameter during pegylated interferon (Peg-IFN) and ribavirin (RBV) combination therapy for chronic hepatitis C. Skin biopsy revealed multiple noncaseating granulomas composed of epithelioid histiocytes with multinucleate giant cells, which was consistent with sarcoidosis. Ophthalmologic examination revealed uveitis. Thoracic computed tomography (CT) showed multiple bilateral hilar lymphadenopathies and a diffuse micronodular interstitial pattern of the lungs. Genetic analysis indicated a probable homozygous haplotype of *A*02:01-C*15:02-B*51:01-DRB1*16:02-DQB1*05:02* in human leukocyte antigen regions. The patient was observed carefully without any additional medication since no significant systemic symptoms were noted. Combination therapy was continued for 2 months afterwards. She was asymptomatic for over three years of follow up, and repeated hematological and biological investigations and chest CT showed improvement. In conclusion, clinicians should bear sarcoidosis in mind as a complication during Peg-IFN and RBV combination therapy. They should also be aware of the usually good prognosis of Peg-IFN-induced cutaneous sarcoidosis in order not to prematurely

discontinue a treatment necessary for liver disease; maintenance of Peg-IFN treatment may be advised with careful follow-up.

Keywords: pegylated interferon, ribavirin, sarcoidosis, human leukocyte antigen

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Introduction

Pegylated interferon (Peg-IFN) in combination with ribavirin (RBV) is the current standard of care for patients with chronic hepatitis C genotype 2, with the addition of a protease inhibitor for the treatment of genotype 1^{1,2}. Side effects are observed in almost 80 percent of patients treated with combination therapy¹ over the course of treatment, and the appearance of various skin conditions, such as eczematous and lichenoid eruptions and psoriasis, have been reported³. Cutaneous sarcoidosis in varying manifestations has also been reported to develop due to Peg-IFN and RBV combination therapy⁴⁻¹¹.

Sarcoidosis is a multisystem granulomatous disorder characterized by the presence of noncaseating granulomas in tissues. It typically appears in young to middle-aged women, but can affect both sexes and all age groups¹². Although the etiology of sarcoidosis remains uncertain, it is known that highly-polarized type 1 helper T (Th1) cells differentiated from activated CD4+ cells produce excessive interleukin-2 and interferon- γ , thus activating macrophages and leading to the formation of granulomas¹³. The disease is currently thought to be triggered by various genetic and environmental factors, and evidence of familial and ethnic clustering suggests the existence of a genetic predisposition to sarcoidosis¹⁴. Attempts to identify sarcoidosis

susceptibility genes have focused on those residing in the major histocompatibility complex (MHC), and particularly the human leukocyte antigen (HLA) genes¹³.

We herein describe a patient with an extremely rare homozygous HLA genotype who became complicated with cutaneous sarcoidosis during Peg-IFN and RBV therapy.

Case report

A 61-year-old Japanese woman was referred to our hospital by her primary care physician for treatment of hepatitis C virus (HCV) infection. She was suspected to have contracted HCV (genotype 1b) by a blood transfusion during childbirth 24 years prior. Serum HCV RNA was 5.2 log IU/mL, and serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) had both been consistently greater than 30 IU/L. Chronic hepatitis was histologically proven by liver tissue biopsy, which indicated scores of 3 for portal-portal bridging necrosis, portal inflammation, and fibrosis and scores of 1 for intralobular degeneration and focal necrosis, according to Knodell hepatitis activity index (HAI) classification¹⁵. Her single nucleotide polymorphism (SNP)

of interleukin 28B at rs8099917¹⁶ was T/T. She neither smoked nor habitually consumed alcohol. No history or findings of dermatological, pulmonary, or autoimmune diseases were noted. A chest X-ray before treatment showed no abnormal findings (Figure 1a).

Combination therapy was started with regular doses of Peg-IFN α 2b at 80 μ g s.c. once weekly and RBV at 600 mg/day p.o. (MSD. KK, Tokyo, Japan). Serum HCV RNA became undetectable in a TaqMan assay 12 weeks after the initiation of therapy. She showed occasional mild hematopenia without dose reductions of Peg-IFN or RBV. Ten months after treatment commencement, the patient complained of a small, painful subcutaneous nodule on the sole of her foot that was 10mm across in diameter (Figure 2). Combination therapy was continued for 2 months afterwards as no systemic symptoms were detected under close monitoring.

Skin biopsy of the nodule revealed multiple noncaseating granulomas composed of a compact aggregate of irregularly epithelioid histiocytes with multinucleated giant cells (**Figure 3**). Specific stains showed no evidence of bacterial, fungal, or mycobacterial organisms. Biopsy findings were interpreted as consistent with sarcoidosis, although the skin lesion disappeared around the

time of biopsy. Further examination was performed to evaluate systemic involvement. Laboratory studies demonstrated moderate leukocytopenia (2,800/ μ L), elevated lysozyme (10.5 μ g/mL; normal range: 5.0 to 10.2 μ g/mL), and normal angiotensin-converting enzyme (16.9 U/mL; normal range: 8.3 to 21.4 U/mL) and serum calcium (9.1 mEq/L). Her tuberculin skin test was negative, but ophthalmologic examination revealed uveitis. A chest X-ray (Figure 1b) and thoracic CT taken 2 months after combination therapy showed multiple bilateral, paratracheal, subcarinal, and hilar adenopathies (Figure 1d) and a diffuse micronodular interstitial pattern of the lungs (**Figure 1e**). Transbronchial lung biopsy revealed the presence of multiple noncaseating granulomas with multinucleated giant cells (Figure 4). The bronchoalveolar lavage fluid level of lymphocytes was elevated at 38.7% compared with macrophages (56.3%) and neutrocytes (5.0%) in a total cell density of $1.67 \times 10^5/\text{mm}^3$. An increased ratio of CD4 / CD8 cells of 2.33 was noted. Based on these findings, the patient was diagnosed as having sarcoidosis. She was observed carefully without any additional medication since no significant systemic symptoms were noted. A chest CT taken 20 months after combination therapy showed improvement (Figure 1f and 1g). She was also asymptomatic for over 3 years of follow-up, and

repeated hematological and biological investigations showed no exacerbation. A chest X-ray taken 40 months after combination therapy depicted no bilateral hilar lymphadenopathies (Figure 1c). She also achieved a sustained virological response.

Genotyping of the HLA and microsatellite markers in the HLA region

After obtaining informed consent, genomic DNA was extracted from peripheral blood using either the QuickGene-800 kit (FUJIFILM, Tokyo, Japan) or a standard phenol-chloroform method. HLA genotypes were determined by a Luminex multi-analyzer profiling system with a LAB type® SSO One Lambda typing kit (One Lambda, Inc. Canoga Park, CA). Additional polymerase chain reaction sequence-specific primers for SBT reactions were employed to determine high-resolution alleles. Determination of the number of repeat units in the 23 microsatellites (*D6S210*, *D6S265*, *C5-2-7*, *C3-2-11*, *C4-2-7*, *C2-4-4*, *C2-2-2*, *C1-3-1*, *C1-2-5*, *C1-4-1*, *MIB*, *MICA*, *C1-2A*, *TNFA*, *TNFD*, *D6S273*, *D6S2924*, *D6S2919*, *D6S2888*, *D6S2886*, *DQCAR*, *D6S2445*, and *M2-2-22*) in the HLA region was performed as described in a previous report^{17, 18}.

The patient's HLA genotype was *A*02:01/02:01*, *B*51:01/51:01*,

*C*15:02/15:02*, *DRB1*16:02/16:02*, and *DQB1*05:02/05:02*. As such, she was considered to be homozygous for the haplotype *A*02:01-C*15:02-B*51:01-DRB1*16:02-DQB1*05:02*. The frequency of this haplotype is 0.012% and very rare in a Japanese population. The alleles in all microsatellite loci were homozygous.

Genotyping of the IL-28B SNP (rs8099917) and BTNL2- SNP (rs2076530)

The patient was genotyped for *IL-28* (rs8099917) and *BTNL2* (rs2076530) polymorphisms using an SNP Genotyping Kit (Applied Biosystems, Tokyo, Japan), as previously reported¹⁹. The polymerase chain reaction was performed with a TaqMan Assay for Real-Time PCR (7500 Real Time PCR System; Applied Biosystems) following the manufacturer's instructions.

The patient's *IL-28* (rs8099917) allele was T/T and her *BTNL2* (rs2076530) allele was A/A.

Discussion

Peg-IFN and RBV therapy has numerous reported side effects that include hematologic disorders, flu-like symptoms, neuropsychiatric disturbances,

ophthalmologic disorders, glucose metabolism disruption, autoimmune disease exacerbation, dermatologic complications, hair loss, thyroid dysfunction¹, and even interstitial pneumonia in rare instances²⁰; almost all of which can be managed with supportive care. Sarcoidosis induced by combination therapy is also considered to be a comparatively rare complication, for which the highest annual incidence has been observed in northern European countries (5 to 40 cases per 100,000 people). The annual incidence in Japan ranges from 1 to 2 cases per 100,000 people²¹. The mechanism of Peg-IFN/RBV-related sarcoidosis remains elusive, but is believed to be related to pathophysiological and immunomodulatory causes. One possible mechanism is that interferon promotes cytokine synthesis by macrophage activation and the development and enhancement of Th1-mediated responses. Persistent HCV infection induces chronic liver damage through a Th1 immune type of response, and the antigenicity and viral persistence seen in chronic HCV infection may serve as a trigger for the development of clinical sarcoidosis in susceptible individuals, which is exacerbated by the exogenous use of INF-alpha^{22, 23}. In addition, RBV has the ability to inhibit viral RNA replication, and might contribute to the pathogenesis of sarcoidosis by inhibiting the production of Th2 type cytokines by

shifting the balance towards a Th1 response²⁴. However, RBV may act solely as a facilitator in Peg-IFN/RBV-related sarcoidosis induction because no such cases have been reported for RBV monotherapy^{9, 25}.

A total of 8 known cases complicated with cutaneous sarcoidosis during Peg-IFN and RBV combination therapy have been reported to date⁴⁻¹¹, but with no definite associations made with regards to age, gender, onset of sarcoidosis, or type of Peg-IFN (Table 1). Six cases^{5-8, 11} were described as cutaneous sarcoidosis without systemic lesions. Pulmonary disease accounts for the majority of the morbidity and mortality associated with primary sarcoidosis²⁶. Three cases, including ours, were of cutaneous lesions with pulmonary disease^{4, 9}. Since cutaneous sarcoidosis leads to physical impairment in only a minority of patients and is not life threatening, additional treatment is generally only indicated by considering the risks and benefits of treatment for patients with symptomatic, ulcerating, or progressively worsening skin disease. All reported sarcoidosis cases, along with ours, showed an improvement in disease activity within several months of finishing or discontinuing Peg-IFN and RBV therapy without immune-modulation therapy.

It is well established that sarcoidosis is associated with HLA class II

genes, especially HLA-DRB1 and HLA-DQB1, in several ethnic groups²⁷⁻³⁰. In Japanese sarcoidosis patients, a strong association with the HLA-DRB1* 08 allele has been reported²⁷. This is the first study to investigate HLA genotype in a patient with cutaneous sarcoidosis induced by Peg-IFN and RBV combination therapy. Interestingly, our patient showed no previously reported susceptibility HLA genotypes or haplotypes^{28, 31}. However, she had an extremely rare HLA haplotype (*A*02:01-C*15:02-B*51:01-DRB1*16:02- DQB1*05:02*) present in only 0.012% of the Japanese population. Moreover, she appeared to have a homogeneous HLA haplotype from the results of microsatellite and HLA genotyping, which is estimated to exist at a frequency of 1.4×10^{-6} % in Japan.

Recently, a single nucleotide polymorphism within the butyrophilin-like 2 gene (*BTNL2*) at rs2076530 has been implicated as a risk factor for sarcoidosis³². Located only 170 kb from the *HLA-DRB1* gene telomerically on chromosome 6, *BTNL2* is a member of the immunoglobulin superfamily with suspected costimulatory activities in T-cell activation on the basis of its amino acid homology with B7 molecules³³. The G/A transition of rs2076530 causes premature truncation of the protein, which disrupts membrane localization and downregulation of activated T cells (Th1)³⁴. As our analysis revealed the A/A

homozygote of the *BTNL2* rs2076530 polymorphism, the truncated protein caused by the rs2076530A allele may have led to an increased risk of sarcoidosis in this patient.

In conclusion, clinicians should bear sarcoidosis in mind as a complication during Peg-IFN and RBV combination therapy. They should also be aware of the usually good prognosis of Peg-IFN-induced cutaneous sarcoidosis in order not to prematurely discontinue a necessary treatment for liver disease. Maintenance of Peg-IFN treatment in such patients may be advised with careful follow-up.

Acknowledgements

We thank Trevor Ralph for his English editorial assistance.

Authors' disclosures of potential conflicts of interest

The authors indicated no potential conflicts of interest.

Table 1: Published cases of complicating cutaneous sarcoidosis in Peg-IFN and ribavirin combination therapy in English literature

Case	Age (yrs)	Gender	Genotype	Type of Peg-IFN	Peg-IFN ($\mu\text{g}/\text{week}$)	Ribavirin (mg/day)	Duration (weeks)	Onset (weeks)	Other sites of involvement	Therapy	Result
1 ⁴	63	Male	2	α 2a	135	800	48	45	lung	observation	resolved
2 ⁵	55	Male	1b	α 2a	150	1,200	48	40	-	observation	resolved
3 ⁶	44	Male	1a	α 2b	1.5 $\mu\text{g}/\text{kg}$	1,000	48	40	-	observation	resolved
4 ⁷	59	Female	2 and 4	α 2a	180	800	48	52 *	-	topical steroids	resolved
5 ⁸	60	Female	1	α 2a	180	800	24	24	-	discontinuation	resolved
6 ⁹	47	Female	N.D.	α	N.D.	N.D.	48	40	-	observation	resolved
7 ¹⁰	53	Female	N.D.	α	N.D.	N.D.	N.D.	N.D.	-	observation	resolved
8 ¹¹	54	Female	N.D.	α	1.4 $\mu\text{g}/\text{kg}$	1,000	N.D.	16	lung	observation	resolved
Our case	61	Female	1b	α 2b	80	600	48	40	lung, uveitis	observation	resolved

Abbreviations: N.D., not described; *, onset after Peg-IFN and RBV combination therapy

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Figure Legends

Figure 1. Chest X-ray before combination therapy showed no abnormal findings

(a). Chest X-ray taken 2 months after combination therapy showed bilateral hilar lymphadenopathies (white arrowheads) (b). Thoracic computed tomography (CT) taken at the same time clearly showed multiple bilateral, paratracheal, subcarinal, and hilar adenopathies (d) and a diffuse micronodular interstitial pattern of the lungs (e). Thoracic CT taken 20 months after combination therapy indicated improvement of bilateral hilar (f) and interstitial (g) lymphadenopathies. Chest X-ray taken 40 months after combination therapy depicted improvement of bilateral hilar lymphadenopathies (c).

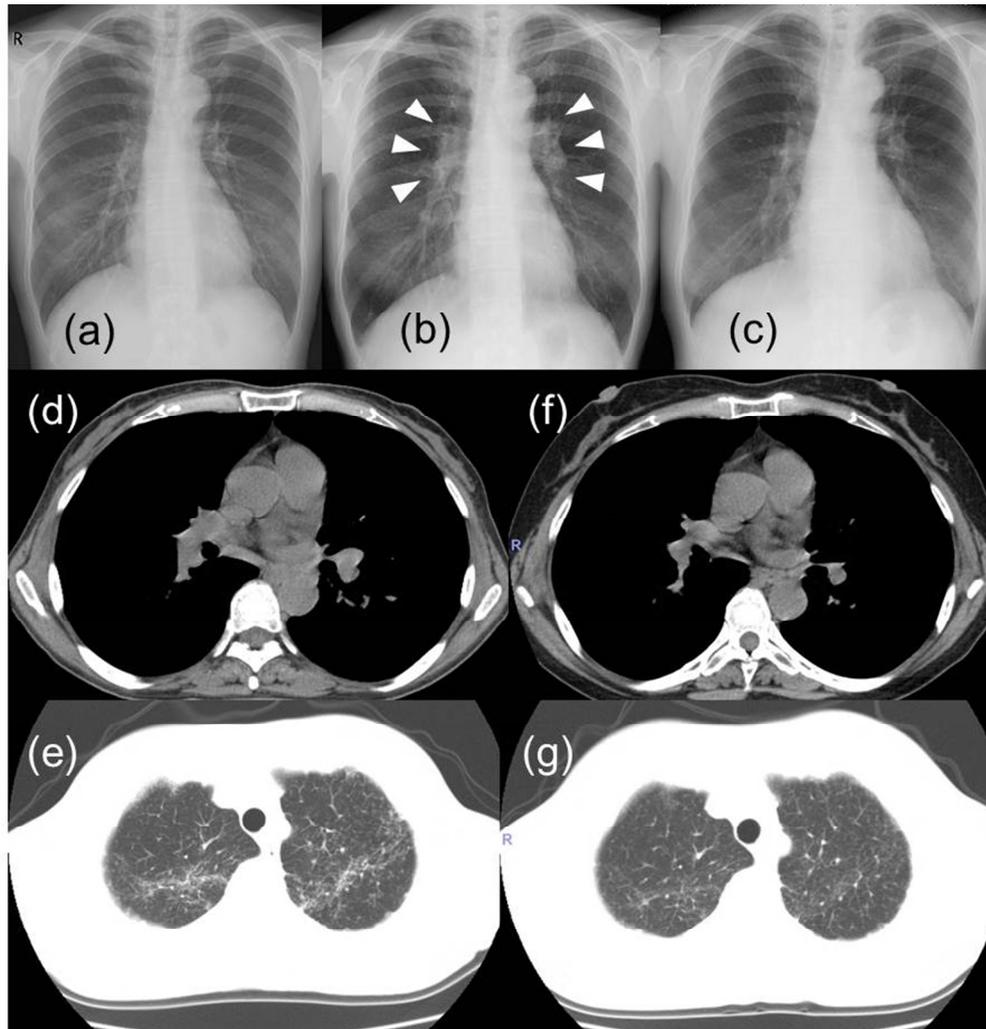
Figure 2. The patient developed a small, painful subcutaneous nodule (black circle) on the sole of her foot that was 10mm across in diameter 10 months after the initiation of therapy. The patient had no complaints about the sole of her foot before treatment.

Figure 3. Skin biopsy revealed multiple noncaseating granulomas composed of a compact aggregate of irregularly arranged epithelioid histiocytes

with multinucleated giant cells (Hematoxylin and Eosin staining $\times 100$).

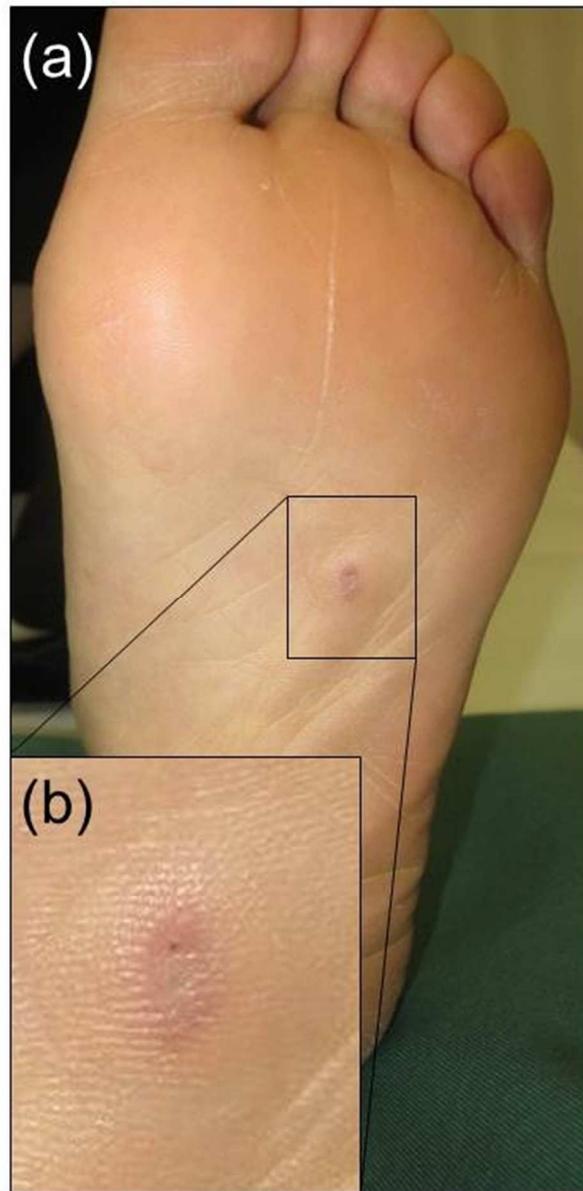
Figure 4. Transbronchial lung biopsy revealed the presence of multiple noncaseating granulomas with multinucleated giant cells (Hematoxylin and Eosin staining $\times 200$).

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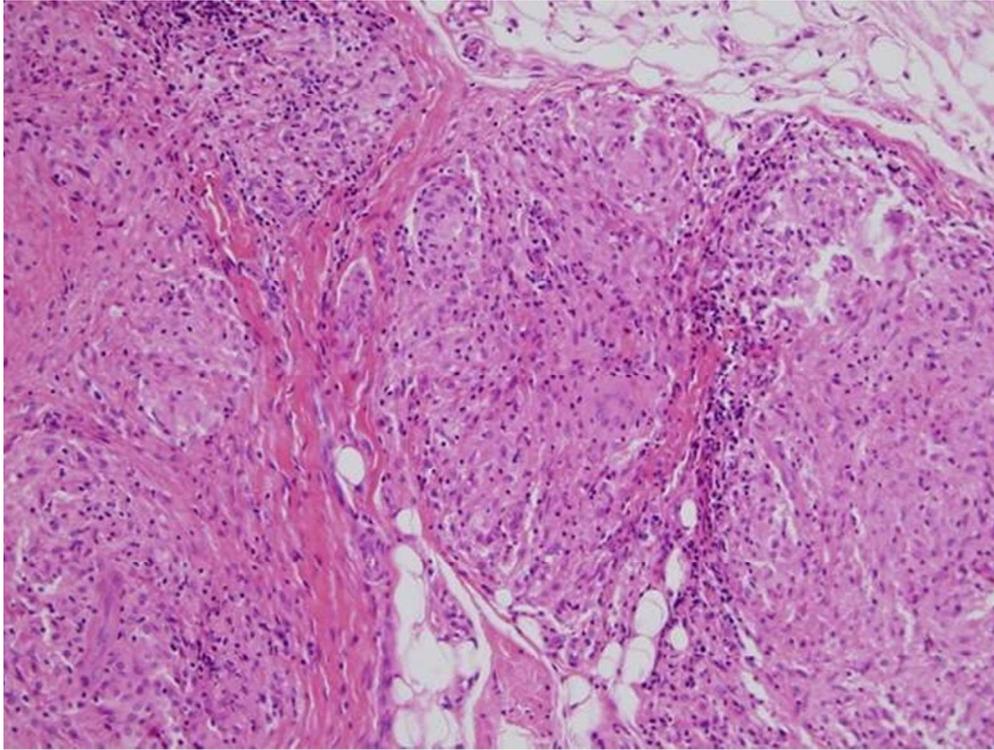


Chest X-ray before combination therapy showed no abnormal findings (a). Chest X-ray taken 2 months after combination therapy showed bilateral hilar lymphadenopathies (white arrowheads) (b). Thoracic computed tomography (CT) taken at the same time clearly showed multiple bilateral, paratracheal, subcarinal, and hilar adenopathies (d) and a diffuse micronodular interstitial pattern of the lungs (e). Thoracic CT taken 20 months after combination therapy indicated improvement of bilateral hilar (f) and interstitial (g) lymphadenopathies. Chest X-ray taken 40 months after combination therapy depicted improvement of bilateral hilar lymphadenopathies (c).

151x155mm (150 x 150 DPI)

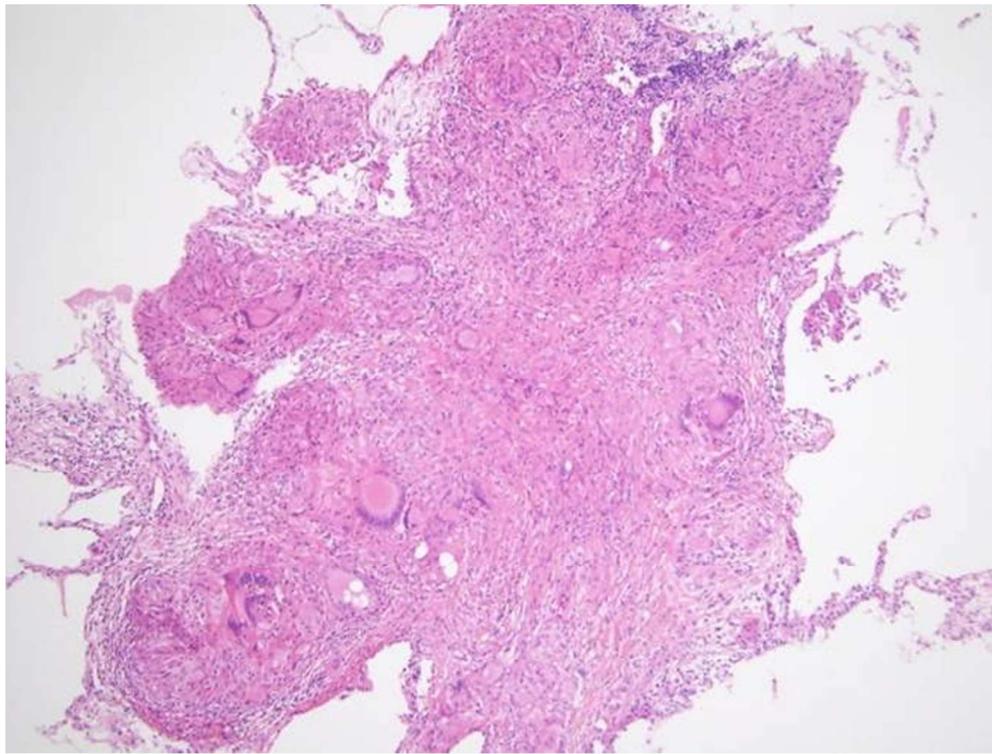


The patient developed a small, painful subcutaneous nodule (black circle) on the sole of her foot that was 10mm across in diameter 10 months after the initiation of therapy. The patient had no complaints about the sole of her foot before treatment.
67x128mm (150 x 150 DPI)



Skin biopsy revealed multiple noncaseating granulomas composed of a compact aggregate of irregularly arranged epithelioid histiocytes with multinucleated giant cells (Hematoxylin and Eosin staining $\times 100$).
105x79mm (150 x 150 DPI)

Only



Transbronchial lung biopsy revealed the presence of multiple noncaseating granulomas with multinucleated giant cells (Hematoxylin and Eosin staining $\times 200$).
112x84mm (150 x 150 DPI)

Only